Published in final edited form as:

Neurochem Res. 2015 February ; 40(2): 317–328. doi:10.1007/s11064-014-1374-3.

Mechanisms of Astrocyte-Mediated Cerebral Edema

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Abstract

Cerebral edema formation stems from disruption of blood brain barrier (BBB) integrity and occurs after injury to the CNS. Due to the restrictive skull, relatively small increases in brain volume can translate into impaired tissue perfusion and brain herniation. In excess, cerebral edema can be gravely harmful. Astrocytes are key participants in cerebral edema by virtue of their relationship with the cerebral vasculature, their unique complement of solute and water transport proteins, and their general role in brain volume homeostasis. Following the discovery of aquaporins, passive conduits of water flow, aquaporin 4 (AQP4) was identified as the predominant astrocyte water channel. Normally, AQP4 is highly enriched at perivascular endfeet, the outermost layer of the BBB, whereas after injury, AQP4 expression disseminates to the entire astrocytic plasmalemma, a phenomenon termed dysregulation. Arguably, the most important role of AQP4 is to rapidly neutralize osmotic gradients generated by ionic transporters. In pathological conditions, AQP4 is believed to be intimately involved in the formation and clearance of cerebral edema. In this review, we discuss aquaporin function and localization in the BBB during health and injury, and we examine post-injury ionic events that modulate AQP4- dependent edema formation.

Keywords

cerebral edema; blood brain barrier; astrocyte; aquaporin 4

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ETHICAL STATEMENT

All studies of human tissues were approved by the appropriate ethics committee and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

BBB and AQP4: STRUCTURAL AND FUNCTIONAL RELATIONSHIPS

Blood brain barrier anatomy

The blood brain barrier (BBB) is arranged as a laminated system of cells and extracellular matrix, with different responsibilities distributed to different layers. The innermost layer, a tight-junction-linked monolayer of endothelial cells, directly contacts the circulating blood. Surrounding the endothelium is a basal lamina within which pericytes, poorly understood cells that appear to tighten the barrier, are embedded [1]. Unlike smaller vessels that contain a single basal lamina, the basal lamina of larger arteries and veins is cleaved in two. The space between the two laminae, the Virchow-Robin space (Fig. 1) [2], is continuous with the subarachnoid space and contains CSF that may be both source and sink for brain interstitial fluid (ISF) [3].

The outermost layer of the BBB is a tightly woven mesh of astrocyte processes. Astrocytes completely fill the brain parenchyma, arranged in a 3-dimensional matrix of non-overlapping spatial domains [4]. They are morphologically polarized cells; nearly every astrocyte extends at least one process that contacts a vessel with a specialized process called an endfoot [4]. Astrocyte endfeet fully cover the vascular surface and are coupled to one another by gap junctions, forming an extended perivascular syncytium. The endfoot membrane domain is enriched in channels used for water and ion movements.

Given their anatomical location, it was long suspected that the astrocyte endfeet were key participants in water influx, transport and efflux. Until recently, the molecular identity of the major path that water takes through the endfoot remained unclear, precluding direct investigation of the endfoot syncytium's contribution to brain water content and edema.

Aquaporin structure and properties

The molecular conduit responsible for bulk water flow through the plasma membrane of cells was unknown until its discovery by Peter Agre in 1992. Agre and colleagues found that the protein CHIP28, later rechristened aquaporin-1 (AQP1), functions as a selective water channel [5]. When expressed in *Xenopus* oocytes, AQP1 caused the cells to swell and burst in hypotonic solution [5]. These experiments confirmed that the elusive water channel had been identified.

Aquaporin monomers, the functional subunits of aquaporin channels, are composed of six membrane-spanning α helices, two half-helices and a central water-selective pore [6]. Both N- and C-termini are cytoplasmic. Aquaporins achieve exquisitely high water selectivity through a dumbbell-shaped pore with an amphipathic bottleneck, which limits the transport of ions to 1 per 10^9 water molecules [7].

Aquaporins permit passive, bidirectional water diffusion. As passive conduits, the magnitude and direction of water flow through aquaporins is solely dictated by the osmotic gradient across the cell membrane. These osmotic forces are generated by plasma membrane ion channels and transporters. Therefore, the study of aquaporin water transport, and of aquaporin-independent cerebral edema, is essentially the study of ionic transport.

Aquaporins in the CNS

Only a subset of the 14 known aquaporins, namely aquaporin-1, AQP4 and aquaporin-9, are expressed in the CNS. Of these, AQP4 is the dominant contributor to the formation and clearance of cerebral edema. AQP4 is highly expressed in astrocyte membrane domains specialized for water transport, such as perivascular endfeet, submeningeal endfeet, and migrating lamellipodia [8]. AQP4 is not expressed in other CNS cells such as oligodendrocytes or neurons but has been reported in microglia following LPS injection [9]. AQP1 is restricted to the choroid plexus and appears to mostly control cerebral spinal fluid (CSF) secretion [10]. AQP9 is found in astrocytes and neurons located in disparate brain nuclei, but unlike AQP4, it is not polarized to astrocyte endfeet [11]. AQP4 is the most critical water channel in astrocytes.

While aquaporin isoforms share most properties, some important differences exist. AQP1 and AQP4 are highly water selective, whereas AQP9 permits passage of a larger variety of solutes, such as urea, polyols, purines, and pyrimidines [6, 12, 13]. The lesser selectivity of AQP9 may be attributable to its larger pore [13, 14].

AQP4 exists in two major N-terminal splice variants: M1 (323 amino acids) and M23 (301 amino acids) [15]. In addition to M1 and M23, four other AQP4 isoforms exist, but their functional significance has yet to be determined [16–18]. Through interactions occurring at the N terminus, M23-containing AQP4 monomers can assemble on the cell membrane into large multimeric complexes called orthogonal arrays of intramembraneous particles (OAPs). The larger M1 isoform is unable to form OAPs when expressed alone in cells [19]. Structurally, OAPs appear to be composed of a core of M23 surrounded by a layer of M1 [20]. Functionally, AQP4 OAP assembly serves to increase water permeability and, through association with scaffolding proteins discussed below, might enable the precise control of AQP4 membrane distribution. The correlation of OAP size with the ratio of M23 to M1 expressed suggests that this might be one mechanism used to control OAP formation and size [20, 21]. Notably, following ischemia, M1 expression is upregulated to a greater degree than M23; the altered M23 to M1 ratio might impact OAP stability [22]. The AQP4 C-terminal region contains a sorting sequence and a PDZ-binding motif (PSD-95, *Drosophila* discs large protein, and the zona occludens protein 1) which are required for proper membrane localization [23, 24].

In quiescent cortical astrocytes, AQP4 is restricted to the perivascular astrocyte endfoot membrane. Viewed with immunolabeling, AQP4 immunoreactivity fully outlines the cerebral vasculature (Fig. 2, CTR). Importantly, astrocytes in select brain regions such as hypothalamus, hippocampus and cerebellum exhibit unique AQP4 subcellular distributions, presumably reflecting the unique ion transport, and hence water transport, requirements of these cells [25–27]. Intracellular and extracellular scaffolding proteins help to properly localize AQP4 to the endfoot. One intracellular scaffolding protein, α -syntrophin, links AQP4 to the dystrophin-associated protein complex that serves as a bridge between transmembrane proteins and the actin cytoskeleton [28]. Given that OAP-forming AQP4 M23, but not AQP4 M1, associates with dystrophin, this scaffolding complex is likely used to tether or aim the large OAPs [21]. In addition to intracellular anchors, α - β -dystroglycan, a

transmembrane protein pair, links AQP4 with the vascular basal lamina proteins agrin and laminin, and is necessary for proper AQP4 perivascular localization [29]. These scaffolding proteins become very important following injury, when loss of these anchors modulates AQP4 localization.

AQP4 contribution to brain volume during health

The BBB isolates the brain from the circulation and actively maintains a uniquely comprised ISF that is optimized for neural activity [30]. In ISF, relative to plasma, concentrations of potassium, calcium, and bicarbonate are reduced, while that of magnesium is increased.

The brain's ISF is not a stationary bath, but is continuously refreshed by water and solute influx across the BBB. Brain endothelial cells can contribute to ISF formation by engaging a set of polarized (apical vs. basal) ion channels that drive net water influx [31]. In addition, CSF produced by the choroid plexus is driven by arterial pulsations from the subarachnoid space into the periarterial Virchow-Robin spaces [32]. Water is then driven by osmotic or hydrostatic pressures through the astrocyte endfoot syncytium and into the parenchyma [3]. The ISF moves through the parenchyma via transcellular and paracellular routes and is cleared through endfeet into the perivenous Virchow-Robin spaces [3, 33]. The component of ISF formed and cleared through Virchow-Robin spaces recently was coined the "glymphatic" system (reviewed by Iliff and Nedergaard [34]).

Water can penetrate the endfoot syncytium via four possible routes (Fig. 3), including paracellular and transcellular routes. The paracellular route involves the extracellular passage of water and small-to-medium size solutes across the syncytium [32, 35]. There are three transcellular routes. First, water can be transported directly across the cell membrane by diffusion, although this is not energetically favored [36]. Secondly, water can be transported across the astrocyte membrane by co-transport mediated via EAAT1 or NKCC1. These proteins are capable of transporting water against an osmotic gradient [37]. Finally water can be transported bidirectionally via AQP4 channels, which is the primary trans-syncytial path used by the glymphatic system [3]. Once intracellular, water can take similar routes from the endfoot compartment to the parenchyma.

A crucial role of AQP4 is to rapidly neutralize osmotic gradients generated by ionic transporters. AQP4 expression in skeletal muscle plays a similar role – it relieves osmotic gradients generated during contraction, thereby mediating an increase in muscle volume [38]. Rapidly neutralizing osmotic gradients generated by osmolite transport in astrocytes has important adaptive advantages. Genetic deletion or mislocalization of AQP4 results in hyperactivity and impaired regulation of stimulus-induced changes in the extracellular K^+ concentration [39, 40], the latter due in part to an enhancement in extracellular space associated with reduced water flux [41, 42], and to impaired K^+ uptake through the glial Na^+/K^+ -ATPase [43], which is highly sensitive to changes in extracellular K^+ at concentrations near physiological [44].

To neutralize osmotic gradients generated by endothelium, a nearby compartment of water must serve as a source and sink for water. The astrocyte syncytium is uniquely suited for this. In most areas of the brain, the endothelium is in close proximity to astrocyte endfeet,

which through AQP4, can quickly mediate water efflux or influx in response to endothelial-driven osmotic gradients. Furthermore, by virtue of astrocyte gap junctions, localized osmotic gradients can be distributed and relieved over a large spatial domain, thus avoiding large, localized fluctuations in brain water content. This proposed functionality is analogous to potassium spatial buffering.

AQP4 knockout animals exhibit CNS abnormalities that may stem from altered ion transport. In these animals there is slight expansion of the extracellular space, increased brain water content, cochlear deafness and increased seizure threshold [41, 45–47]. AQP4 knockout animals also have greatly reduced glymphatic clearance of interstitial solutes [3]. Notably, AQP4 knockout mice have normal brain morphology and lack differences in astrocyte end foot volume, BBB permeability, GFAP labeling, and myelination [48–50], pointing perhaps to unidentified compensatory mechanisms in these animals.

AQUAPORIN 4 AND CEREBRAL EDEMA

Cerebral edema syntax

Historically, edema was classified into physiological categories with no molecular or cellular underpinnings [51]. More recently, improved understanding of the molecular mediators of edema formation prompted a reevaluation and reinterpretation of classical subtypes of cerebral edema [52]. Subtypes of edema are better conceptualized as physiological manifestations of cellular and molecular events that are driven by injury-specific transcriptional programs.

In the context of ischemia/hypoxia, the formation of cytotoxic (cellular) edema is a critical event that drives subsequent processes. With cytotoxic edema, astrocytes take up sodium from the ISF. Sodium serves as the primary driver of cytotoxic edema formation by virtue of its transmembrane electrochemical gradient. Primary driver influx stimulates movements of secondary participants, substances for which no transmembrane gradient is normally present. To maintain electrical and osmotic neutrality following sodium influx, anions and water act as secondary participants. The influx of ions and water results in cellular swelling, which defines cytotoxic edema. Importantly, cytotoxic (cellular) edema by itself does not cause brain swelling, since it involves simply a shift of osmolytes and water from extracellular to intracellular compartments. This fundamental concept of cytotoxic edema can be demonstrated by placing fresh brain tissues on a benchtop; cytotoxic edema will form, but in the absence of blood flow, there will be no movement of water or solutes into the tissue from the vascular compartment, i.e., ionic edema and vasogenic edema will not form, and there will be no increase in tissue volume or mass [52].

However, in the living brain, cytotoxic edema generates a driving force for transcapillary flux of osmolytes and water. The combined sodium and water fluxes of cytotoxic edema lead to an initial depletion of extracellular sodium [53], generating a sodium gradient that favors the movement of sodium from the vascular to the parenchymal compartments. Newly upregulated capillary and astrocyte ion channels or transporters enable sodium and water to follow this new sodium gradient. The transport of these substances across endothelium results in the formation of ionic and vasogenic edema, which increase brain mass and

volume. Notably, newly arrived sodium can be continuously taken up by astrocytes undergoing cytotoxic edema, driving the formation of further cytotoxic and ionic edema. Later, breakdown of endothelial tight junctions and vascular basal lamina allows for the formation of vasogenic edema, an extravasated blood ultrafiltrate.

AQP4 and cerebral edema dynamics

AQP4 has been reported to have apparently contradictory roles in different types of cerebral edema. In some studies, AQP4 gene deletion was protective against edema formation [50, 54–57]. In others, AQP4 gene deletion led to greater edema load [45]. The key to understanding AQP4's contribution to edema dynamics is the channel's bidirectional transport of water, which enables it to contribute to both edema formation and clearance.

In injuries that produce acute edema in the absence of BBB breakdown, i.e., ionic, not vasogenic edema, AQP4 knockout or inhibition is protective against edema formation [50, 54–57]. Notably, in these studies, the type of edema was termed “cytotoxic” edema, reflecting the older edema classification scheme developed by Igor Klatzo, in which true cytotoxic (purely cellular) edema, as the term is currently used, was combined with ionic edema [51]. The aforementioned studies did not directly measure astrocyte swelling, but instead showed that AQP4 knockout or inhibition reduced post-injury increases in brain volume and brain water content, consistent with the formation of ionic edema, as defined in the current scheme [54–57]. Through overexpression of AQP4, it was directly demonstrated that AQP4 is the rate limiting step in the formation of ionic edema [58]. It is clear from these studies that AQP4 plays an important role in the trans-syncytial water movements of ionic edema.

AQP4 undoubtedly mediates cytotoxic (cellular) edema in astrocytes. Some studies using AQP4 knockout mice or mice with mislocalized AQP4 (see below) show reduced astrocyte swelling following water intoxication [50, 59, 60]. Interestingly, Solenov et al. [36] reported only marginally decreased swelling rates in cultured astrocytes from AQP4 knockout mice, which suggests that AQP4 only has limited impact on astrocyte swelling. However, *in vivo*, the membrane distribution of AQP4 in relation to the vasculature strongly influences edema formation and might help to explain this apparent discrepancy.

In conditions that lead to vasogenic edema, blood ultrafiltrate is extravasated into the parenchyma and is cleared by bulk flow into CSF compartments and veins [61]. AQP4-dependent water efflux at the astrocyte endfoot appears to be responsible for clearance of vasogenic edema, as AQP4 knockout impairs these animals' ability to efficiently clear the edema [45].

Given that AQP4 is a passive channel, for edema to form there must exist secondary factors modulating AQP4 water flow. There are two general types of modulation at play after injury: the dynamic spatial distribution of AQP4 at the membrane and the activity of ion transporters or channels that drive AQP4-mediated cytotoxic and ionic edema.

AQP4 dysregulation

In contrast to the perivascular localization of AQP4 in healthy cortex, after injury, AQP4 becomes more uniformly distributed on the astrocyte plasmalemma, a phenomenon called “dysregulation” (Fig. 2) [62–64]. In animal models of stroke and trauma, a severity-dependent onset of dysregulation is apparent 1 and 3 days following injury [62, 64]. Following injury, laminin, agrin and β -dystroglycan are lost from the basal lamina, perhaps through proteinase released by astrocytes and microglia [62]. In the current model, the loss of these perivascular anchors allows AQP4 to diffuse freely throughout the astrocyte membrane, producing a dysregulated expression.

That AQP4 dysregulation occurs during a similar time period as cytotoxic edema suggests that these phenomena may be related. Dystrophin, agrin and α -syntrophin knockout mice have been useful models for studying the role of dysregulation in edema formation – AQP4 is permanently dysregulated in these animals [23, 29, 62, 65–67]. (When interpreting results from these knockout animals, it is important to note that localization of other transmembrane proteins also can be altered, as was shown in α -syntrophin knockout mice [68]). Generally, these animals are protected against cytotoxic and ionic edema formation following injury, as demonstrated by smaller increases in brain water content, reduced hemispheric enlargement, and delayed apparent diffusion coefficient decline [59, 60, 62, 68]. One interpretation of these data is that astrocytes move AQP4 away from the vessels to prevent damaging water influx from the vasculature [62].

Alternatively, AQP4 dysregulation might not be a direct response to early edema. Following injury, astrocytes initiate a complex program of AQP4-dependent activities such as hypertrophy, morphological change, migration, cytokine release, and cell division [69–72]. Perhaps redistribution of AQP4 is required to accomplish these tasks, and the protection against cerebral edema following dysregulation is simply an epiphenomenon. In support of this concept, AQP4 dysregulation also occurs in chronic neuroinflammatory diseases such as neuromyelitis optica, multiple sclerosis, and Alzheimer’s disease, where edema formation is not clinically relevant [73, 74].

CELL MEMBRANE IONIC DRIVERS FOR AQP4 FUNCTION IN CYTOTOXIC EDEMA

Astrocytes maintain proper brain ISF ion composition through mechanisms that require dynamic changes in cell volume. It is therefore not unreasonable to view processes contributing to cytotoxic edema as pathological extensions of normal homeostatic routines.

To give contrast between health and injury, normal astrocytic homeostasis of the major extracellular osmolites, sodium and potassium, is discussed below first. Maladaptive ion movements are central to the formation of cerebral edema. In subsequent sections, the major channels that mediate astrocyte swelling (Fig. 4), and by extension ionic edema, then are discussed, organized by the pathological state that precipitates their activity.

Astrocyte contributions to brain potassium and sodium homeostasis

Extracellular potassium, which in resting ISF is between 2.7 and 3.5 mM, builds up locally as a waste product of firing neurons [75]. In excess, extracellular potassium is a depolarizing influence that can precipitate epileptiform activity and thus must be cleared. Perisynaptic astrocyte processes express potassium channels such as Kir4.1 and transporters such as Na⁺/K⁺-ATPase and NKCC1 to clear excess potassium [76]. After astrocyte uptake, potassium either can be distributed through astrocyte-to-astrocyte gap junctions or it can be expelled at the vessels through ion channels [77]. The term used to describe this process, potassium spatial buffering, was originally coined by Orkand et al. [78] and is considered to be one of the major functions of astrocytes [79].

The potassium spatial buffering event can be subdivided into two phases. In the stimulation phase, during neuron firing, extracellular potassium accumulates, accompanied by slight astrocyte cell swelling, which may be mediated by NKCC1 activity or bicarbonate influx [80–82]. In the second phase, after neural activity ceases, Na⁺/K⁺-ATPase works to clear extracellular potassium, while astrocytes reduce in volume to normal levels [80].

In agreement with the normal function of AQP4 being to equilibrate osmotic gradients generated by ionic transport, some studies in knockout animals indicate that AQP4 contributes to astrocyte swelling during the stimulation phase of potassium spatial buffering [83, 84]. Furthermore, AQP4 was found to form a tripartite macromolecular complex with Na⁺/K⁺-ATPase and the metabotropic glutamate receptor 5 (mGlu5) [85]. Notably, a study by Haj-Yasein et al. [86] demonstrated that AQP4 knockout mice exhibited increased astrocyte swelling in response to neuronal stimulation, highlighting that the precise contribution of AQP4 to potassium spatial buffering remains unclear. The altered potassium dynamics seen in AQP4 knockout mice might underlie the impaired hearing, increased seizure threshold and impaired synaptic plasticity observed in these animals [46, 47, 87].

In astrocytes, sodium is continuously exported by Na⁺/K⁺-ATPase. In the healthy brain, the functional capacity of Na⁺/K⁺-ATPase normally is sufficient to balance sodium influx generated by cation channels or secondary transporters. However, following injury, Na⁺/K⁺-ATPase may be “overwhelmed”, due to increased secondary transport activity and reduced cellular ATP levels. The retained intracellular sodium directly leads to swelling and dangerous intracellular calcium overload from reversal of the sodium calcium exchanger [88]. Sodium homeostasis through Na⁺/K⁺-ATPase activity is critical to astrocyte health.

NKCC1-mediated cytotoxic edema and extracellular potassium

Following injury, extracellular potassium accumulates as a byproduct of neuronal discharge, partially due to the excess release of excitatory amino acids such as glutamate [89]. During periods of ischemia, extracellular potassium can rise above 60 mM [90]. In clearing the excess potassium, astrocytes swell to pathological levels and cytotoxic edema ensues.

While a variety of channels can contribute to cytotoxic edema associated with potassium clearance, the NKCC1 transporter is particularly important. The NKCC family includes NKCC2, a kidney-specific transporter, and NKCC1, the NKCC expressed in astrocytes, among other tissues [91]. NKCC1 is bumetanide-sensitive and imports ions with a Na⁺/

$K^+/2Cl^-$ stoichiometry through secondary transport [92]. NKCC1 carries a net four osmolites inward per turnover in addition to its cotransport of water, which is more than sufficient to drive water influx, leading to *in vitro* cellular swelling in conditions of high extracellular potassium [93–95]. The relevance of NKCC1 to cytotoxic and ionic edema has been demonstrated in *in vitro* and *in vivo* models of trauma and ischemia through inhibition with bumetanide [96–101].

Glutamate-mediated astrocyte swelling

In addition to potassium, extracellular glutamate quickly accumulates following acute injury, through release from a variety of cell types [102, 103]. Interestingly, primary cultures of astrocytes exposed to hypotonic stress or subjected to energy failure also release glutamate, which *in vivo*, could create a feed-forward mechanism for cytotoxic edema [104, 105].

In addition to its indirect effect on astrocyte swelling through the stimulation of neural activity, glutamate can directly induce astrocyte swelling in culture through metabotropic glutamate receptors, which trigger potassium and sodium influx [106–108]. One of the glutamate receptors expressed on astrocytes, the metabotropic glutamate receptor 5 (mGluR5), forms a tripartite macromolecular complex with AQP4 and Na^+/K^+ -ATPase in the astrocyte plasma membrane [85]. Abrogation of swelling through Na^+/K^+ -ATPase or glutamate receptor inhibition indicates that all of the components of this complex are necessary for glutamate-induced swelling [106]. While the potassium channel blockers, Ba^{2+} and TEA inhibit both Rb^+ uptake and swelling of cultured astrocytes following glutamate exposure, the channel(s) mediating this response are still uncharacterized [106].

Transporters mediating acidotic astrocyte swelling

Injuries such as trauma or ischemia that interrupt oxygen delivery lead to depletion of ATP and the initiation of anaerobic metabolism. The associated extracellular accumulation of lactic acid lowers the extracellular pH, precipitating a fall of intracellular pH and the initiation of compensatory ion fluxes. At an extracellular pH of 6.8 or lower, compensatory ion fluxes are sufficient to induce cultured astrocyte swelling [109, 110]. Correlated with this phenomenon is an upregulation of AQP4 at the cell membrane during acidosis [111]. Inhibition of the swelling response by removal of extracellular sodium or bicarbonate shows that the acidosis-induced swelling is mediated by two general transporter classes: one bicarbonate-independent and the other, bicarbonate-dependent [109, 112].

The Na^+/HCO_3^- transporter family (NBC) is widely expressed in cultured astrocytes and assists in intracellular astrocyte pH maintenance through sodium-dependent bicarbonate import [113]. The electrogenic Na^+/HCO_3^- co-transporter, which imports sodium and bicarbonate with 1:2 to 1:3 stoichiometry, appears to be the major bicarbonate-dependent pH regulator in cultured astrocytes [114]. While NBC cotransporters are upregulated in astrocytes *in vivo* following transient ischemia, their role in cytotoxic edema and their possible connections to AQP4 remain unclear [115].

The contribution of the bicarbonate-independent Na^+/H^+ exchanger to acidosis-induced astrocyte cytotoxic edema is better understood. The mammalian Na^+/H^+ exchanger (NHE) family is a group of integral membrane proteins that mediate an electroneutral 1:1 exchange of intracellular H^+ for extracellular Na^+ [116]. Nine isoforms have been characterized (NHE1–9), but NHE1, the NHE ubiquitously expressed in the astrocyte plasma membrane, is by far the most extensively studied [117]. By exploiting the amiloride sensitivity of NHE, investigators have demonstrated that this channel functions as a key regulator of intracellular pH [116, 118]. NHE1 is activated in response to decreased intracellular pH, and mediates H^+ efflux to restore pH [119]. Interestingly, cell culture studies have demonstrated that not only pH perturbations, but also osmotic stress can activate NHE1 [120, 121]. Importantly, H^+ efflux is accompanied by Na^+ influx in sufficient quantities to drive cultured astrocyte swelling [119, 120]. Genetic or pharmacological inhibition of NHE1 in models of *in vivo* cerebral ischemia leads to reduced cerebral sodium content, edema, and infarct volume [122–126].

Sur1-Trpm4 channel-mediated cytotoxic edema

The contributions of the aforementioned channels to cerebral edema can be viewed as extreme, maladaptive versions of their roles during health. In contrast, some contributors to edema only emerge during disease states, and have no role during homeostasis. The Sur1-Trpm4 channel is expressed only following injury and is relevant to astrocyte swelling during ATP depletion. The pore-forming subunit of the channel, Trpm4, is a constitutively expressed six-transmembrane domain nonselective monovalent cation channel permeable to Na^+ and K^+ , but not Ca^{2+} [127, 128]. Trpm4 open-probability is dependent on intracellular Ca^{2+} through interactions with calmodulin, and is modulated by PKC and ATP [129].

Following CNS injury, Trpm4 is upregulated. This coincides with *de novo* upregulation of Sur1, an ATP-binding cassette protein commonly known as the regulatory subunit of the pancreatic β cell Sur1-Kir6.2 channel. The Sur1-Trpm4 channel is newly expressed in endothelium, neurons, oligodendrocytes and astrocytes [130]. Sur1 associates with Trpm4 and is trafficked to the membrane as functional Sur1-Trpm4 channels [127]. Sur1 complex formation doubles the Ca^{2+} sensitivity of Trpm4, which reaches the nanomolar range, and sensitizes the channel to ATP depletion, leading to a 30-fold increase in open probability upon ATP depletion [127, 128]. The role of the Sur1-Trpm4 channel's inward Na^+ current is to depolarize the cell, to reduce the inward driving force for Ca^{2+} [131]. In so doing, the Sur1-Trpm4 channel inadvertently sacrifices Na^+ and cell volume homeostasis.

As an ATP sensitive channel, Sur1-Trpm4 is primed to respond to intracellular ATP depletion with Na^+ influx and cell swelling. In reactive astrocytes acutely harvested from brain tissues that express Sur1-Trpm4, inhibition of Sur1-Trpm4 current with sulfonylurea blockers prevents inward current and cell blebbing following ATP depletion [128, 132]. Reduced edema and cytoprotection following Sur1-Trpm4 inhibition in *in vivo* models of ischemia and trauma further demonstrate the role of Sur1-Trpm4 in cerebral edema formation [130, 133].

FUTURE DIRECTIONS

Significant progress has been made towards understanding how astrocytes transport water and how this transport contributes to post-injury edema. However, many questions remain.

The high expression level of AQP4 and its specific localization to the astrocytic endfoot is highly suggestive of a strong role in the function of the healthy BBB. Given this, it is surprising that AQP4 knockout mice have only seemingly mild abnormalities. While some work has been completed on this topic, a complete model of the contributions of the transport mechanisms to ISF dynamics (Fig. 3) has yet to be constructed. Understanding AQP4's role during health is necessary to contextualize its contributions to edema in disease.

Studies on edema formation have demonstrated through measurements of tissue water content that AQP4 contributes to the formation of cytotoxic and ionic edema. However, additional work using measurements directly sensitive to cell swelling, such as diffusion tensor imaging, is needed to further characterize the contribution of AQP4 to edema formation.

While theories exist with regards to the function of AQP4 dysregulation, its true physiological purpose is largely unknown. Given the conservation of dysregulation between different types of injury, both chronic and acute, dysregulation might represent a fundamental process of reactive astrogliosis. Perhaps further work on dysregulation will help to better understand the complex phenomenon of astrocyte reactivity.

Many of the factors that modulate AQP4 water flux have been experimentally examined in isolation. However, these mechanisms likely interact after injury. Furthermore, the molecular driver of cytotoxic edema in a given injury likely varies from astrocyte to astrocyte, depending on the local tissue status, as explored in a recent review article [134]. Experiments that address AQP4's shifting membrane localizations together with changing ionic drivers are needed to better understand the dynamics of edema formation.

ACKNOWLEDGEMENTS

We would like to thank the anonymous reviewers who made invaluable suggestions to improve our manuscript. This work was supported by grants to JMS from the Department of Veterans Affairs (Baltimore) (BX001629), the National Institute of Neurological Disorders and Stroke (NINDS) (NS060801; NS061808), the National Heart, Lung and Blood Institute (HL082517), and the Department of the Army (W81XWH 1010898); and to VG from NINDS (NS061934; NS072501).

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Figure 1. Anatomy of penetrating cerebral vessels

Vessels travel between the arachnoid mater (A) and pia mater in the CSF-containing subarachnoid space (SAS) before penetrating the pia mater and cortex. The periarterial space (PAS), revealed by reflecting the periarterial pial sheath, surrounds subarachnoid vessels and follows arteries into the parenchyma; in arteries, this space merges with the Virchow-Robin space at the level of small branches where pial fenestrations (PF) appear in the pial sheath. The subpial Virchow-Robin space (VRS) abuts the astrocyte endfeet and is anatomically separated from the subarachnoid space (SAS) and periarterial space by a monolayer of pial cells; however, water and some solutes freely move through the pial monolayer (for review, see Brinker et al. [2]).

Figure 2. Aquaporin-4 in the human brain

Human brain tissues from a control brain (left) and from a biopsy specimen obtained at the time of decompressive craniectomy in a patient with traumatic brain injury (right); note that in the control brain, AQP4 localizes exclusively around microvessels, consistent with astrocytic endfoot localization, whereas in the TBI brain, AQP4 localizes over the entire astrocyte plasmalemma, a phenomenon termed AQP4 dysregulation.

Figure 3. Anatomy of the blood-brain barrier

Schematic depiction of an axially sectioned vessel and an astrocyte; black arrows show possible routes that water can take through the astrocyte syncytium; double arrowheads denote bidirectional water transport.

Figure 4. Select membrane ionic drivers for aquaporin 4 function in cytotoxic edema
Schematic depiction of several major transporters and the SUR-Trpm4 channel that contribute to astrocyte cytotoxic edema following acute injury.