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Disruption of Ion Homeostasis in the Neurogliovascular Unit Underlies the Pathogenesis of Ischemic Cerebral Edema

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Abstract

Cerebral edema is a major cause of morbidity and mortality following ischemic stroke, but its underlying molecular pathophysiology is incompletely understood. Recent data have revealed the importance of ion flux via channels and transporters expressed in the neurogliovascular unit in the development of ischemia-triggered cytotoxic edema, vasogenic edema, and hemorrhagic conversion. Disruption of homeostatic mechanisms governing cell volume regulation and epithelial/endothelial ion transport due to ischemia-associated energy failure results in the thermodynamically driven re-equilibration of solutes and water across the CSF–blood and blood–brain barriers that ultimately increases the brain’s extravascular volume. Additionally, hypoxia, inflammation, and other stress-triggered increases in the functional expression of ion channels and transporters normally expressed at low levels in the neurogliovascular unit cause disruptions in ion homeostasis that contribute to ischemic cerebral edema. Here, we review the pathophysiological significance of several molecular mediators of ion transport expressed in the neurogliovascular

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unit, including targets of existing FDA-approved drugs, which might be potential nodes for therapeutic intervention.

Keywords

Blood–brain barrier; Stroke; Ischemia; SUR1; Glyburide; Bumetanide; Ion channel; Edema; NKCC1; Tumor; Hemorrhage; TRPM4; Hypertonic

Background

Cerebral edema is a major cause of morbidity and mortality following ischemia in the brain. Ischemia causes an abrupt decline in the rate of oxidative metabolism in neurons, glia, and endothelial cells and, consequently, a rapid depletion of energy. The acute decline in cellular metabolism caused by hypoxia or anoxia inhibits energy-dependent mechanisms that are responsible for maintaining homeostatic solute gradients across neuroglial cell membranes, the vascular endothelium, and the choroid plexus endothelium. When these homeostatic mechanisms fail, edema results from a thermodynamically driven re-equilibration of solutes and water across cellular and endothelial barriers that ultimately increases the volume of the extravascular space of the brain. Edema causes swelling, which increases tissue pressure and may compromise capillary perfusion, thereby causing further ischemia.

The blood–brain barrier (BBB) ordinarily restricts paracellular diffusion across the endothelium and tightly regulates transcapillary transport, thereby providing the specialized environment crucial for proper neural function. The BBB is formed by endothelial cells that are joined by tight junctions [1–3]. However, the integrity of the BBB endothelium requires complex interactions with other cells. Astrocytes, which maintain close contact with the microvascular endothelial basement membrane via foot processes, are critical for maintaining the BBB [4–6]. In addition, microglia [7] and pericytes [8] play important roles. These anatomical and functional interrelationships have given rise to the concept that the actual functional unit of the BBB encompasses a larger ensemble of cells types—the “gliovascular unit” [5, 6]. Ischemic edema involves the pathological movement of solutes and water across the BBB into the extracellular space of the brain and therefore implies dysfunction of the gliovascular unit. This dysfunction is characterized by a progression from abnormal ionic homeostasis across gliovascular cell membranes to breakdown of the tight junctions and endothelial cell integrity that restricts paracellular flow across the BBB (vasogenic edema).

The pathogenesis of ischemic edema involves a stepwise series of events set in motion by failure of energy-dependent regulators of neuroglial cell oncotic homeostasis (Fig. 1). In the acutely energy-depleted state that results from ischemia, neuroglial ion pumps—particularly Na^+/K^+ -ATPase—can no longer regulate movement across the membrane. In addition, ischemia results in the deregulation of excitatory amino acid release, which activates channels that enable further ionic flux. Some cells undergo necrotic cell death and spill cellular contents into the extracellular space, further contributing to abnormal ionic gradients and movement. Thus, thermodynamically driven ionic movement occurs through secondary active transporters or ion channels that harness the potential energy in electrochemical gradients to drive ionic flux. As solutes—particularly Na^+ —flow down their concentration gradient into the intracellular space, oncotic pressure drives water through aquaporin channels and other pathways into neurogliovascular cells. This results in neurogliovascular cell swelling, or cytotoxic edema.

Cytotoxic edema represents a volume shift from the extracellular to intracellular space and so does not in itself cause parenchymal swelling. However, the influx of ions into neuroglial cells in cytotoxic edema depletes extracellular concentrations of these solutes, which subsequently disrupts the electrochemical equilibrium across the vascular endothelium. Thus, a concentration gradient is established between the intravascular and extracellular spaces. During this process, the permeability of endothelial cells is disrupted. For example, there is evidence that endothelial cells in ischemia activate transcriptional programs potentially involving activator protein-1, hypoxia inducible factor-1, specificity protein-1, and nuclear factor- κ B, which create new proteins enabling cation transport and disrupt the permeability of the endothelium [9]. This disruption causes ionic edema, in which solutes and water flow from the intravascular to extracellular space and contribute to the expansion of total extravascular brain volume. Importantly, ionic edema is characterized by a period where the regulation of ion and water conductance is disrupted, but the integrity of the BBB is maintained in that tight junctions and endovascular exclusion of macromolecules are both preserved [10–12]. As endovascular cells swell in the process of solute and water transfer, volume perturbations cause cytoskeletal rearrangements [13], and the toxic environment of the ischemic lesion causes cell death, thereby disrupting BBB integrity and leading to vasogenic edema. Ionic edema precedes vasogenic edema by about 4 h [12, 14]. In vasogenic edema, capillaries become fenestrated, tight junctions are disrupted, and reverse pinocytosis occurs to increase the transport of macromolecules into the brain parenchyma, thereby causing disruption of the BBB, solute influx, more edema, and, ultimately, hemorrhagic conversion.

Thus, ion transport plays a critical role early in the pathogenesis of malignant cerebral edema by enabling abnormal ionic flux into cells of the neuroglial unit, resulting in cytotoxic and ionic edema, which initiates a cascade of events leading to vasogenic edema and, possibly, hemorrhagic conversion. All cell types of the neuroglial unit are involved—ionic influx into neurons and glia cause cytotoxic edema of these cells and establish the gradient required for transcapillary solute transfer, and ionic flux through endovascular cells causes cytotoxic edema of these cells, ionic edema, and, finally, vasogenic edema and hemorrhagic conversion. Given the importance of pathological ionic movement in the initiation and progression of ischemic cerebral edema, ion transport proteins are thought to be potential targets to preempt the breakdown of ionic homeostasis causing cytotoxic and ionic edema, which ultimately results in vasogenic edema and hemorrhagic conversion. Several ion transport protein targets have been identified; in some, normal physiological activity becomes damaging in the setting of ischemia (e.g., Na^+/H^+ antiporter, NMDA receptor, acid-sensing ion channels, hemichannels, and volume-regulated anion channels), while in others, ischemia appears to stimulate activity or expression, which may have both functional and maladaptive effects (e.g., NKCC1, SUR1-TRPM4, other transient receptor potential channels, and aquaporins). Ion transport proteins with constitutive expression and activity likely represent a major mechanism by which acute ischemia results in early cytotoxic edema, and so are potential targets in an acute setting. In contrast, proteins whose expression and/or activity increases gradually following ischemia, such as SUR1-TRPM4 or NKCC1, are potential targets in the several hours following injury. Additionally, some channels are thought to be more important contributors of ionic flux in the ischemic core itself (e.g., SUR1-TRPM4), while others may be important in the penumbra (e.g., NKCC1). An understanding of the pathophysiology of all of these transporters may enable the development of therapeutic strategies that synergistically inhibit the formation of edema at distinct times and different areas around the ischemic core. Here, we review the pathophysiological significance and evidence of potential for therapeutic intervention on these ion channel transporter targets in the neuroglial unit.

Targets

NKCC1

The cation–chloride co-transporter NKCC1 (“sodium–potassium–chloride co-transporter 1”) is a member of the SLC12 gene family that mediates the influx of Cl^- , K^+ , and Na^+ in a $1\text{Na}^+:1\text{K}^+:2\text{Cl}^-$ stoichiometry. Although there are two iso-forms of the NKCC co-transporter, only NKCC1 is expressed in the central nervous system (CNS). It is located on neurons, glia, and the luminal face of both vascular and choroid plexus endothelial cells [15]. Flux through NKCC1 is secondary active transport driven by the transmembrane concentration gradient of Na^+ that is ordinarily maintained by the activity of Na^+/K^+ -ATPase. Ionic transport through NKCC1 is matched by water movement through aquaporins to maintain osmotic neutrality. The activity of NKCC1 plays an important role in sodium secretion and absorption, maintenance of intracellular Na^+ concentration, cell volume regulation, and maintenance of intracellular Cl^- concentration in gliovascular cells [16]. It is regulated via phosphorylation by kinases, which increase its activity, and phosphatases, which inhibit it. These regulatory mechanisms are an important part of the response to a decrease in intracellular Cl^- , hypertonic stress, increased intracellular Ca^{2+} , and β -adrenergic receptor stimulation.

In ischemia and other pathological conditions, NKCC1 becomes deregulated and contributes to excessive Na^+ accumulation within neuroglial cells, resulting in cytotoxic and ionic edema. In the gerbil hippocampus, NKCC1 immunoreactivity is significantly intensified 24 h after middle cerebral artery occlusion (MCAO) [17]. In the rat cerebral cortex and striatum, NKCC1 protein and mRNA levels increase following MCAO for 24 h after reperfusion [18, 19]. In the neonatal rabbit, NKCC1 mRNA and protein levels are elevated up to 48 h after hypoxic insult [20]. The post-ischemic increase in NKCC1 protein levels corresponds with the demethylation of the NKCC1 promoter region [21]. In addition to the increased transcript and protein levels, NKCC1 activity increases following ischemia via phosphorylation [19,22,23]. Recent evidence suggests that WNK and SPAK-OSR1 serine-threonine kinases are critical components of the NKCC1 regulatory pathway, although their role in ischemia is not fully understood [24, 25].

Several mechanisms may be involved in the ischemia-induced increase in NKCC1 activity. The metabolic microenvironment in the ischemic core and penumbra may stimulate NKCC1. An in vitro study found that the hypoxia-induced increase in NKCC1 activity was also caused by pyruvate and glucose deprivation in normoxic conditions, suggesting that metabolic inhibition that results from hypoxia contributes to increased NKCC1 activity [23]. Arginine vasopressin also stimulates NKCC1 [23]. There is also evidence that dysregulated glutamatergic signaling stimulates NKCC1 in ischemic conditions. The activation of both ionotropic and metabotropic glutamate receptors stimulates inward ionic flux through NKCC1 via Ca^{2+} -dependent mechanisms, indicating that post-ischemic glutamatergic activity contributes to increased NKCC1 activity in neuroglial cells, which also may represent a mechanism for excitotoxicity [26–30]. The inflammatory response may also contribute to NKCC1 stimulation as interleukin-6 secreted by astrocytes in ischemia has been shown to increase co-transporter activity [31]. Finally, ischemia causes elevated extracellular concentrations of K^+ , which also increases flux through NKCC1 [28].

In neuroglial cells, increased activity of NKCC1 without compensatory activity of Na^+/K^+ -ATPase allows Na^+ influx that subsequently causes cytotoxic swelling as water concurrently flows into the cell via aquaporins to maintain osmotic neutrality. Genetic ablation of NKCC1 blocks hypoxia-induced cytotoxic swelling of astrocytes, demonstrating the importance of this co-transporter in this process [29, 32]. As cytotoxic edema of neuroglial cells establishes a sodium gradient across the vascular endothelium, NKCC1 expressed on

the luminal membrane enables Na^+ and Cl^- loading into endothelial cells. To complete the transcapillary movement into the extracellular space, Na^+/K^+ -ATPase on the abluminal membrane of these cells extrudes Na^+ into the interstitium, and water follows to generate ionic edema and tissue swelling. Thus, increased flux through NKCC1 contributes to post-ischemic cytotoxic edema and transcapillary flow of Na^+ and fluid into the extracellular space that causes ionic edema, making NKCC1 an attractive target to prevent post-ischemic edema.

NKCC1 is potently and specifically inhibited by low concentrations of the loop diuretic bumetanide, and several studies in rat models have demonstrated that bumetanide is useful in preventing ionic edema and neuroglial injury following focal ischemia. Intravenous bumetanide reduces in-farct volume and brain edema by about half [32–34] (Fig. 2). The post-ischemic increase in extracellular K^+ that stimulates NKCC1 is abolished by bumetanide [28, 29]. Post-ischemic treatment with bumetanide reduces glutamate-triggered Na^+ and Cl^- accumulation in neuroglial cells by more than 50 % [27, 32]. Bumetanide is safe [35] and produces relatively few side effects in humans [36]. Its inhibition of NKCC1 to modulate intracellular Cl^- concentrations is currently being explored as therapy for neonatal seizures, temporal lobe epilepsy, and other disorders in which abnormal Cl^- gradients are suspected to play a role [37], and two clinical trials are underway (ClinicalTrials.gov identifiers NCT00830531 and NCT01434225). Bumetanide is limited by low BBB penetration [38] and lack of specificity at high concentrations (e.g., [39]). Design of drugs with more specific inhibition of NKCC1 or better BBB penetration would overcome these limitations and may have enhanced therapeutic efficacy. Nevertheless, bumetanide, or another more selective NKCC1 inhibitor with better BBB penetration, has potential as therapy to preempt post-ischemic cytotoxic and ionic edema.

In the vascular endothelial cell, luminal NKCC1 plays a major role in solute uptake from the intravascular compartment, resulting in cytotoxic edema of these cells. However, to complete transcapillary movement of these solutes and accompanying water into the extracellular space, abluminal Na^+/K^+ -ATPase is required. In conditions of complete anoxia, cellular ATP is quickly depleted, so the activity of the Na^+/K^+ -ATPase is unlikely to be a major contributor of transmembrane ionic flux in this case. Therefore, it is more likely that transcapillary movement through the NKCC1- Na^+/K^+ -ATPase pathway is physiologically significant in areas of hypoxia but not anoxia, i.e., where there is some energy available to drive the Na^+/K^+ -ATPase and generate ionic edema, such as the penumbra of the injury or in reperfusion [40]. In much lower-energy environments that exist in the ischemic core, the absence of ATP enables transcapillary flow of ions through a different mechanism—the nonselective cation channel SUR1-TRPM4.

SUR1-TRPM4

TRPM4 (transient receptor potential—melastatin 4) is a member of the TRPM subfamily of transmembrane channels first identified by Launay et al. [41, 42]. Under pathological conditions, TRPM4 co-associates with SUR1 (sulfonylurea receptor 1) to form SUR1-TRPM4 channels [43]. The dual structure of the channel relates to its function; a regulatory subunit, SUR1 combines with a pore-forming subunit, TRPM4, to act as a functional unit (herein referred to as “SUR1-TRPM4”) [9, 44–48]. This 35-pS channel conducts inorganic monovalent cations, but is otherwise impermeable to divalent cations such as Ca^{2+} and Mg^{2+} [49]. Opening of the channel is only dependent on nanomolar concentrations of intracellular Ca^{2+} and is effectively prevented by the presence of intracellular ATP (EC_{50} , $\sim 1 \mu\text{M}$). While no direct modulator of the pore-forming TRPM4 subunit exists, the SUR1 regulatory subunit controls the receptor–channel complex as a whole and is blocked with high affinity and specificity by the sulfonylurea antagonist glibenclamide (EC_{50} , 48 nM) [45]. This has significant implications for not only individual cellular function and survival

but also regional and global brain homeostatic mechanisms including maintenance of the blood–brain barrier and inflammatory responses to injury.

The SUR1-TRPM4 channel is not constitutively present in the CNS, but rather is expressed de novo following hypoxic injury. This unusual expression pattern was first discovered in reactive astrocytes isolated from a relatively hypoxic layer following stab injury and foreign body implantation [45, 49]. Subsequently, further experiments (both in vivo and in cell culture) have identified the expression of SUR1 following CNS injury [44, 50]. This has been validated in several rodent models of cerebral ischemia [44, 51]. After permanent arterial occlusion, 3 h is required before *Abcc8* mRNA (encoding for SUR1) is increased 2.5-fold, and a full 8 h is required before SUR1 protein expression is increased 2.5-fold [44]. The up-regulation of SUR1 is first observed only in microvascular endothelial cells, but is eventually evident in neurons [52]; by 24 h, SUR1 is upregulated in all members of the neuroglial unit in the affected tissue [44]. Notably, the upregulation of SUR1 takes place without the upregulation of the inward rectifier potassium ion channel (Kir6.2 protein or *Kcnj11* mRNA) otherwise known to be associated with SUR1, demonstrating the association of SUR1 with TRPM4 after ischemic injury [44].

Several studies have demonstrated the importance of the SUR1-TRPM4 channel in the formation of cytotoxic, ionic, and vasogenic edema. Activation of the channel results in the net influx of cations, driving the osmotic influx of water, thereby causing cellular swelling [44, 45, 49]. In cell culture, ATP depletion induces a strong inward current that depolarizes the cell completely to 0 mV. Cells subsequently undergo oncotic cell swelling and, ultimately, non-apoptotic, propidium iodide-positive oncotic cell death. This oncotic cell death is significantly inhibited by glibenclamide, highlighting the role of SUR1-TRPM4 in this process [44].

Post-ischemic upregulation and activation of SUR1-TRPM4 leads to excess sodium influx and cytotoxic edema in all cells of the neuroglial unit, including endothelial cells [45, 49]. As SUR1-TRPM4 is expressed on both luminal and abluminal endothelial cell membranes, upregulation and activation of this channel enables transcapillary movement of ions (and, consequently, water) and so is sufficient to cause ionic edema. Additionally, as endothelial cell volume is perturbed, there is a reorganization of the actin cytoskeleton and weakening of intercellular tight junctions, leading to an increase in blood–brain barrier permeability and vasogenic edema [13]. Increased BBB permeability and oncotic death of endothelial cells may play a role in the progression of ischemic edema to hemorrhagic conversion [9]. Consistent with this hypothesized mechanistic sequence, one study has shown that significant disruption of the endothelial actin cytoskeleton that develops following ionic edema is reduced by SUR1 inhibition with glibenclamide [53]. Treatment with glibenclamide ameliorates edema formation and reduces symptomatic hemorrhagic transformation [54]. Thus, SUR1-TRPM4 upregulation and activation plays an important role in post-ischemic cytotoxic, ionic, and vasogenic edema in ATP-depleted areas of the injury, and inhibition of SUR1-TRPM4 with glibenclamide can attenuate these effects.

Targeted inhibition of the SUR1 subunit to mitigate the development of brain edema formation and hemorrhagic transformation following ischemic stroke has been well studied in various small mammal models. SUR-TRPM4 blockade with glibenclamide not only diminishes edema formation but also confers a significant survival advantage [44, 51, 55–57] (Fig. 3). Gross pathological examination following ischemic injury demonstrates better functional tissue perfusion and outcomes with SUR1 inhibition; for example, significantly more cortical sparing was seen in animals treated with glibenclamide compared to controls. It is likely that edema reduction facilitates leptomeningeal collateral blood flow (and therefore perfusion to the penumbra), one of the key determinants of clinical outcome in

humans with large vessel occlusion [58]. Treatment with glibenclamide uncouples the correlation between stroke volume with outcome, presumably secondary to a reduction in edema [56]. Glibenclamide treatment is associated with reduced subcortical (ventral pallidum) necrosis, reduced neuronal loss, and reduced pathological calcium deposition [55]. In a rat model of malignant cerebral edema, treatment with glibenclamide was associated with not only decreased swelling but also reduction in hemorrhagic transformation [54]. There is even evidence that glibenclamide offers better neurological outcomes than craniotomy to relieve edema following ischemic injury [59].

Glibenclamide is a common oral anti-diabetic agent that has been used for more than 40 years and has a good safety profile and tolerability associated with long-term use [60, 61]. Retrospective analyses of patients taking glibenclamide for diabetes suffering acute ischemic stroke revealed that these patients were more likely to experience a major neurological recovery compared to controls not taking the drug [62]. Given these findings, several clinical trials exploring the use of glibenclamide for the prevention of edema following ischemic stroke and other brain injuries are ongoing or anticipated. One trial nearing completion is a prospective, multicenter, open-label, phase IIa study of glyburide in patients with severe anterior circulation ischemic stroke to prevent cerebral edema (ClinicalTrials.gov identifier NCT01268683). Pharmacological manipulation of SUR1-TRPM4 is a promising method of attenuating post-ischemic edema and improving outcomes after stroke.

Na⁺/H⁺ antiporter

Na⁺/H⁺ antiport by the Na⁺/H⁺ exchanger (NHE) serves a variety of functions in many different cell types throughout the body, including the regulation of intracellular pH, cell volume, cell proliferation, and transepithelial ion transport [63–66]. Of nine isoforms that have been identified in humans, five (NHE1–5) are expressed on plasma membranes and three (NHE1–3) are expressed in epithelial cells [67–72]. Early studies demonstrated that the Na⁺/H⁺ exchange inhibitors amiloride and EIPA reduce intracellular pH in cultured cerebral microvascular endothelial cells (CMECs), suggesting that NHE is expressed in this cell type [73, 74]. A subsequent study demonstrated that NHE1 and NHE2 are expressed on both luminal and abluminal membranes of the cerebral micro-vascular endothelium [75].

Several studies have demonstrated that Na⁺/H⁺ exchange plays a role in cytotoxic and ionic edema formation following ischemic injury. Na⁺/H⁺ exchange is increased across the BBB in ischemia [76]. Na⁺ influx into CMECs through NHE is stimulated by endothelin, which is increased in the brain during ischemia [77, 78]. H⁺ flux through NHE in cultured CMECs was stimulated by hypoxia, aglycemia, and arginine vasopressin, which are all associated with ischemia [75]. Genetic ablation of NHE reduces neuronal injury following transient ischemia [79]. Inhibitors of NHE reduce transepithelial Na⁺ transport into the extracellular space without affecting paracellular permeability, suggesting that flux through NHE plays a direct role in Na⁺ transport in the formation of cytotoxic and ionic edema [80, 81].

There is also evidence that pharmacological inhibition of NHE can reduce post-ischemic edema formation. The NHE inhibitor SM-20220 reduces cerebral endothelial dysfunction after 1–7 days of reperfusion following MCAO in the rat [82, 83]. Edema and neutrophil invasion of the area of ischemic injury are reduced by SM-20220 as well [84]. Another inhibitor, HOE-642, reduces infarct volume after 1–3 days of reperfusion following MCAO [85] and also reduces Na⁺ uptake and cerebral edema immediately following ischemia, before reperfusion [86]. Pretreatment with HOE-642 improves neurologic preservation in a cerebral ischemia–reperfusion model of hypothermic arrest [87]. Infarct volume was reduced in spontaneously hypertensive rats subjected to 4 h of ischemia by the NHE inhibitor dimethylamiloride [76].

Given these findings, pharmacological inhibition of Na^+/H^+ exchange may be a viable strategy to reduce the transcapillary movement of Na^+ and thereby prevent edema following ischemia. In addition to HOE-642, SM-20220, amiloride, and dimethylamiloride, a number of other compounds, such as SM-20550, BMS-284640, T-162559, and TY-12533, have been developed in efforts to inhibit individual isoforms of NHE more specifically, particularly NHE1 [88]. Blockade of NHE1 shows promising results in vivo, reducing infarct volume following focal ischemia in rats [84].

NMDA Receptor

Glutamate is the principal excitatory neurotransmitter of the central nervous system. Deregulated glutamatergic signaling is a major contributor of neuronal cell death in ischemia, a phenomenon known as “excitotoxicity.” One class of ionotropic glutamate receptor, the NMDA receptor channel, is ligand-gated by both glutamic acid and glycine simultaneously in a voltage-dependent manner. At physiological membrane potentials, Mg^{2+} blocks the channel. Depolarization removes Mg^{2+} and allows Na^+ , K^+ , and Ca^{2+} conductance. In ischemic injury, breakdown of the energy-dependent mechanisms to maintain resting membrane potential and dysregulation of glutamate release and reuptake stimulate uncontrolled glutamatergic signaling. Indeed, there is up to 500-fold increase in synaptic concentrations of glutamate and unregulated influx of Na^+ and Ca^{2+} into neurons during hypoxic or ischemic insult [89–91]. The resulting Na^+ and Ca^{2+} influx into the cell causes cytotoxic edema and activates Ca^{2+} -dependent mechanisms of cell death, including ATPases, proteases, lipases, caspases, and DNAses [92–94]. Intracellular Na^+ and Cl^- concentrations also increase [27] and could play a role in acute edema and excitotoxicity [95, 96].

Glutamate receptors exist on most neurons; however, neuroepithelial and glial cells also express NMDA receptors and play an important physiological role in regulating glutamatergic signaling [97, 98]. High levels of glutamate that result in ischemic stroke have been shown to disrupt human cerebral endothelial barrier integrity through NMDA signaling [99], suggesting the possible involvement of NMDA signaling in BBB breakdown that results in vasogenic edema. As such, antagonists of glutamate signaling have been explored as therapies for ischemic stroke. Although some are successful in the laboratory [100–102], early efforts to translate these findings to humans failed. Some have suggested that these early clinical trials were complicated by severe side effects associated with the complete blockade of glutamatergic signaling, and the trial design did not take into account the fact that the effect of glutamate switches from early neurotoxicity to late neuro-recovery following ischemia [103, 104].

Newer compounds with fewer side effects are being developed and tested for efficacy in reducing edema and neurotoxicity by inhibition of glutamatergic signaling following ischemia in the hopes that our enhanced understanding of glutamate signaling during injury may guide better trial design with safer drugs. These studies show promising early results. Memantine, an NMDA channel blocker [105] that is commonly used to treat other neurological disorders and has a well-established safety profile, reduces neuronal dysfunctions following ischemia in vitro [106] and reduces edema following focal cerebral ischemia in vivo [107]. Polycyclic cage amines are a novel class of NMDA antagonists that act at the polyamine sites of the receptor without significant side effects and reduce edema and neuronal death in focal ischemic injury [108, 109]. These and other compounds may prove useful in reducing edema following ischemia that results from glutamatergic signaling.

Acid-Sensing Ion Channels

Acid-sensing ion channels (ASICs) are hydrogen ion-gated cation channels that open when pH falls below a physiological value, 7.4. They are also activated by membrane stretching, arachadonic acid, and decreased extracellular Ca^{2+} [110–112] and pharmacologically blocked by amiloride. Four genes code six different ASIC subunits that are expressed in the central and peripheral nervous systems [113, 114]. Some studies have shown high levels of ASIC1a expression at synapses of the CNS, where local pH occasionally falls under physiological conditions [115], while others have shown more diffuse localization throughout neuronal bodies, dendrites, and axons. They are expressed in many areas of the adult rat brain, including the cerebral cortex, hippocampus, and cerebellum [116]. They are permeable to Na^+ , and the ASIC1a subunit is permeable to Ca^{2+} [117]. Ischemia and hypoxia result in uncontrolled generation of lactic acid, leading to a significant reduction in tissue pH that stimulates ASICs [118, 119]. Some studies have suggested that tissue pH falls as low as 6.0 in the ischemic core [120]. Arachadonic acid, membrane stretching, and abnormal Ca^{2+} gradients are also associated with ischemic injury and stimulate ASICs. At least some Ca^{2+} influx in ischemic injury occurs through ASIC1a in a glutamate-independent mechanism [121]. This Ca^{2+} influx likely activates a host of intracellular pathways that ultimately lead to cell death following ischemia-induced activation of ASIC1a. The ASIC2a subunit is potentially involved since ischemia induces its expression in the rat brain [122].

ASIC1-null mice experience significantly less acidosis-related toxicity and have a lower infarct volume [121]. Furthermore, neurons treated with pharmacological ASIC1a inhibitors also experience less acidosis-related toxicity [121]. As a result, ASICs are suspected to play a role in transmembrane ion flux and the development of cytotoxic edema and may be potential therapeutic targets. In addition to amiloride, ASICs are inhibited by psalmotoxin-1 [123], NSAIDs [124], and zinc [125], suggesting the candidacy of these compounds in ischemic stroke to inhibit pathological ionic flux through ASICs. However, a more detailed understanding of the role of ASICs in the central and peripheral nervous system is needed before translation to humans can be achieved.

Other TRP Channels

In mammals, 28 different transient receptor potential (TRP) channel genes have been identified [126]. These channels are almost ubiquitously expressed in the body, and different iso-forms and splice variants vary in their activating stimuli, patterns of expression, and selectivity of ions [127]. The family has six subfamilies: TRPC, TRPV, TRPM, TRPP, TRPML, and TRPA. These channels respond to pH, osmolarity, membrane stretching, or Ca^{2+} levels by allowing the movement of Ca^{2+} , Na^+ , or other cations. Genetic analysis of the promoter regions of TRPC1–7 and TRPM1–8 indicates that these channels may become upregulated in ischemia, suggesting a role for ionic movement through these channels in the generation of cytotoxic edema [128]. Consistent with this, TRPM2 mRNA [129] and TRPC4 protein levels [130] are upregulated after MCAO in rats. In cell culture, TRPM2 and TRPM7 may have a role in the development of abnormal Ca^{2+} gradients [131]. The TRPV1 subunit is widely expressed in the brain and may be involved in hypoxic neurodegeneration [132, 133].

Given these findings, some early studies have explored blocking TRP channels as a strategy in preventing post-ischemic damage. Inhibition of TRPM2 prevents oxidative stress-induced neuronal death [134–136]. Capsaicin [137, 138] and rimonabant [139] provide neuroprotection that is at least partially attributable to antagonism of TRPV1. Pharmacological and RNA interference blockade of TRPM7 prevents Ca^{2+} influx after

hypoxia and glucose deprivation [140, 141]. Thus, targeting TRP channels may prove useful in preventing post-ischemic ionic flow and resultant edema and neurotoxicity.

Hemichannels

Gap junctions are formed by two hemichannels embedded in adjacent cell membranes and are found in all cells of the neurogliovascular unit [142]. In ischemic injury, there is a hypothesized increase in the number of hemichannels exposed to the extracellular space, i.e., disconnected from its counterpart in another plasma membrane [142–144]. Because they represent an unregulated pathway for ionic flux across cell membranes, they may play a role in the development of cytotoxic and ionic edema in ischemia; indeed, ischemic conditions open neuronal gap junction hemichannels that contribute to massive depolarizing currents [145]. However, the role of hemichannels in injury is unclear, particularly those expressed in astroglial cells in ischemic tissue. Two theories have been proposed to define the role of hemichannels on astrocytes in ischemic injury—the “good Samaritan” effect and the “bystander” effect [146].

The “good Samaritan” view suggests that astrocyte hemichannels play an important role in clearing solutes from the extracellular space that would otherwise cause cytotoxic edema of neurons, and so are neuroprotective in ischemia. Accordingly, connexin 43-null mice, which have impaired hemichannel expression, have increased infarct volume and neural damage after focal ischemia [147], and connexin-32 knockout increases neuronal vulnerability in transient global ischemia [148]. Connexin-43 has also been seen in human samples of ischemic stroke tissue, suggesting that hemichannels of connexin-43 monomers also play a role in human neuronal injury [149].

The “bystander” view holds that astrocytic hemichannels spill intracellular contents such as Na^+ , Ca^{2+} , or glutamate into the extracellular space and thereby contribute to the spread of ionic dysregulation that forms cytotoxic and ionic edema [146, 150]. In support of this theory, inhibitors of gap junctions decreased cell death after hypoxia in vitro [151, 152]. In vivo knockdown of connexin-43 reduced neurotoxicity in cerebral ischemia [151].

Some have interpreted conflicting results to suggest that flux through hemichannels is both neuroprotective and neurotoxic in various conditions [104]. This line of study is ongoing, and further work will be required to fully elucidate the role of hemichannels in ionic flux and edema formation in ischemia.

Volume-Regulated Anion Channels

Astrocyte swelling in ischemia activates physiological mechanisms of regulatory volume decrease, in which volume-regulated anion channels (VRACs) play a major role. In response to cell swelling, VRACs open, enabling outward Cl^- flow (that is coupled with outward K^+ and organic osmolyte flux through other channels) and causing osmotic cell volume decrease [153]. There is also evidence that VRACs have a number of other physiological roles, including regulation of vascular tone [154], regulation of cell proliferation [155], and apoptosis [156]. Despite its numerous physiological functions, the exact molecular identity of VRAC has not yet been identified. VRAC activation requires non-hydrolytic ATP binding [157, 158]. Some studies have demonstrated that ionic flux through VRACs are a major contributor of the release of excitatory amino acids, such as glutamate, that may contribute to further edema and excitotoxicity [159, 160]. Based on these findings, VRAC inhibition may be a potential target to prevent excitotoxicity, particularly from astrocytic swelling in cytotoxic edema.

A few studies have validated this hypothesis. Inhibition of mitochondrial respiration that occurs in hypoxia or ischemia provokes neuronal cell swelling through a VRAC-related

mechanism, indicating its involvement in ischemic edema [161]. VRAC inhibition decreases infarct size in MCAO [162] and inhibits glutamate release in ischemia in vivo [163]. Another kind of volume-activated channel has been characterized, known as the maxianion channel; simultaneous inhibition of both channels strongly inhibits glutamate release from astrocytes in ischemia in vitro [164]. Although these results are preliminary, they suggest potential value in targeting VRACs and VRAC-like channels in ischemic edema and subsequent neurotoxicity.

Aquaporins

Aquaporins are a class of water channels that are key regulators of transmembrane water conductance in cell types throughout the body, including those in the gliovascular unit. In cerebral edema, water conductance through aquaporins (AQPs) follows ionic flux through ion channels and transporters to maintain osmotic neutrality. Seven AQP isoforms have been identified in the human CNS with distinct localizations. The expression of these isoforms changes significantly following hypoxia [165]. Of these isoforms, AQP-4 is the most abundant and best characterized. It is expressed on capillary-facing astrocyte foot processes surrounding neurovasculature and in ependymal cells and is upregulated after focal ischemia [166]. The distribution of AQP-4 on fluid interfaces of the CNS correlates with its significant role in regulating the influx of cellular water associated with edema formation.

AQP-4 facilitates cytotoxic edema by allowing for trans-cellular water conductance, particularly in astrocytes. AQP-4-null mice are phenotypically normal, but exhibit less cerebral edema in ischemic stroke [167]. These results seem to be specific to cytotoxic edema as AQP-4 knockout appears to be disadvantageous in other conditions such as cortical freeze injury and brain tumors [168]. Other AQPs are suspected to also be important in the development of ischemic edema.

Given the importance of water conductance in the development of cerebral edema, AQPs are an attractive target. However, at present, there is no specific pharmacological way to block AQPs. Some compounds, such as carbonic anhydrase inhibitors, quaternary ammonium compounds, mercerial sulfhydryl compounds, lithium, silver, and gold, can inhibit AQPs [169–171], but are prohibitively toxic for use in vivo. Alternatively, AQP expression may be downregulated by manipulating upstream transcriptional mechanisms induced by ischemia. The pro-inflammatory cytokine IL-1 β , acting via nuclear factor B (NF-B), is a positive regulator of AQP-4 [172], so inhibiting NF-B is expected to reduce AQP-4 expression. Similarly, inhibition of hypoxia-inducible factor 1 α with 2-methoxyestradiol suppresses the expression of AQP-4 [173].

Conclusions

The current understanding of the pathophysiological basis of ischemic cerebral edema suggests a stepwise progression from cytotoxic edema to ionic edema, vasogenic edema, and, ultimately, hemorrhagic conversion. This process is initiated and driven by the abnormal flux of ions through channels and transporters across membranes of the neurogliovascular unit. Many of these channels and transporters exhibit abnormal expression and activity following ischemia. As water follows these ions to maintain osmotic neutrality, cytotoxic edema occurs, which ultimately disrupts the permeability and structure of cells of the BBB. Because the pathological movement of ions through a host of channels and transporters in the neurogliovascular unit initiates the cascade of events that results in vasogenic edema and hemorrhagic conversion, they are potential targets for the prevention of edema following ischemia. By reducing the pathogenesis of cytotoxic and ionic edema by inhibiting flux through these channels and transporters, vasogenic edema and hemorrhagic

conversion may be attenuated or ameliorated. As we have discussed in this review, many of these targets show promising results.

Some of these targets, such as NKCC1 and SUR1-TRPM4, are inhibited by drugs already approved for use in other conditions and have well-established safety profiles. Ongoing and future clinical trials are exploring the off-label use of these therapies. For other targets, safe drugs have yet to be fully developed, and details of their involvement in ischemic edema are still unclear. Future work on these channels will be required to explore their viability as targets in humans. Nevertheless, the body of work we have reviewed here demonstrates the central role of abnormal ionic flux through channels and transporters of the neuroglial unit in the development of ischemic edema. By targeting these channels and transporters to limit this abnormal movement of ions, we may be able to better prevent ischemic edema and avoid its associated sequelae in the millions of patients that suffer cerebral ischemia.

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Fig. 1.

Illustration of the progression of various types of edema to hemorrhagic conversion. Under normal physiological conditions, Na^+ concentrations in the serum and extracellular space are in equilibrium, and energy-dependent mechanisms keep the intracellular Na^+ concentration low. Cytotoxic edema of neurons results from Na^+ entry into the cell via several transporters and channels. Some of these have constitutive activity (e.g., Na^+/H^+ antiporter, TRP channels), while others are stimulated or newly expressed in ischemia (e.g., NKCC1, SUR1-TRPM4). Ca^{2+} influx may also be driven by ASICs, TRPs, and VRAC. This depletes extracellular solutes, particularly Na^+ , and sets up a concentration gradient between the intravascular and extracellular compartments. Ionic edema results from cytotoxic edema of

endothelial cells as they express cation channels on luminal and abluminal membranes that allow transcapillary Na^+ movement into the extracellular space via newly expressed SUR1-TRPM4, NKCC1, and possibly other mechanisms. During this process, as neurogliovascular cells swell and undergo oncotic cell death, there is deregulation of glutamatergic signaling that increases glutamate concentration up to 500-fold in excitatory synapses, causing excitotoxicity. Furthermore, cell swelling and death expose hemichannels, which further contributes to abnormal ionic homeostasis. Vasogenic edema results from a degradation of endothelial tight junctions and capillary fenestration that allows extravasation of proteinaceous fluid. Ultimately, cytotoxic edema of neurogliovascular cells causes oncotic cell death. As endothelial cells die, there is a complete loss of capillary integrity, causing hemorrhagic conversion. Figure from Simard et al. [9], with permission

Fig. 2.

Bumetanide inhibition of cerebral edema in rats subjected to MCAO. **a** The apparent diffusion coefficient (ADC) is used to assess the degree of edema in the left and right hemispheres after MCAO in the left hemisphere in rats. Bumetanide (7.6 mg/kg) or vehicle was administered intravenously in a single dose 20 min before MCAO. The *vertical axis* shows the ADC ratio between the left (occluded) and right (non-occluded, control) hemispheres. A decrease in the ratio indicates edema of the left hemisphere. MCAO causes significant edema of the left hemisphere at all time points. Bumetanide significantly reduces the level of edema caused by MCAO at all time points ($p < 0.0001$). **b** Brain slices are from

rats subjected to 180 min of left MCAO (*left panel*) or 20 min of pretreatment with bumetanide followed by 180 min of left MCAO (*right panel*). Slices were stained with 2,3,5-triphenyltetrazolium chloride (*TTC*) to identify the infarct sizes. A large infarct is visible in the *left panel*, whereas the infarct is noticeably reduced in the *right panel* due to pretreatment with bumetanide. Figure from O'Donnell et al. [34], with permission

Fig. 3.

Glibenclamide reduces infarct volume, attenuates hemispheric swelling, and improves neurological functions in a rat model of permanent MCAO. **a** TTC-stained coronal sections from rats given either vehicle or glibenclamide in brains harvested 48 h after occlusion. Infarcts are shown as pale (unstained) regions involving the striatum and overlying cortex. The infarct area in glibenclamide-treated animals is substantially reduced. **b** Infarct volumes after 48 h of occlusion. Compared to vehicle alone, glibenclamide significantly ($p < 0.05$) reduced hemispheric infarct volumes (as a ratio to the size of the contralateral hemisphere). **c** Glibenclamide attenuates hemispheric swelling in MCAO. The extent of swelling was quantified as the ratio of the increase in edematous hemisphere volume compared to the contralateral hemisphere. Treatment with glibenclamide significantly ($p < 0.05$) reduces hemispheric swelling at 48 h. **d** Glibenclamide improves neurological function. Treatments with glibenclamide significantly ($p < 0.05$) decreased the NSS compared to the vehicle-treated group at 48 h after occlusion. Figures from Wali et al. [57], under the Creative Commons Attribution License