Molecular Mechanisms of Ischemic Cerebral Edema: Role of Electroneutral Ion Transport

The brain achieves homeostasis of its intracellular and extracellular fluids by precisely regulating the transport of solute and water across its major cellular barriers: endothelia of the blood-brain barrier (BBB), choroid plexus epithelia, and neuroglial cell membranes. Cerebral edema, the pathological accumulation of fluid in the brain’s intracellular and extracellular spaces, is a major cause of morbidity and mortality following stroke and other forms of ischemic brain injury. Until recently, mechanisms of cerebral edema formation have been obscure; consequently, its treatment has been empiric and suboptimal. Here, we provide a paradigm for understanding ischemic cerebral edema, showing that its molecular pathogenesis is a complex yet step-wise process that results largely from impaired astrocytic cell volume regulation and permeability alterations in the cerebral microvasculature, both of which arise from pathological changes in the activities of specific ion channels and transporters. Recent data has implicated the bumetanide-sensitive NKCC1, an electroneutral cotransporter expressed in astrocytes and the BBB, in cerebral edema formation in several different rodent models of stroke. Pharmacological inhibition or genetic deficiency of NKCC1 decreases ischemia-induced cell swelling, BBB breakdown, cerebral edema, and neurotoxicity. Combination pharmacological strategies that include NKCC1 as a target might thus prove beneficial for the treatment of ischemic, and potentially other types of, cerebral edema.

Maintenance of Brain Volume and Solute Composition

In mammals, the brain contains four distinct fluid compartments: the blood in the cerebral vasculature, the cerebrospinal fluid (CSF) in the ventricular system and subarachnoid space, the interstitial fluid that bathes cells of the brain parenchyma, and the intracellular fluid contained within neurons and glia. These four fluid compartments, each with their own unique volume and solute composition, are separated from one another by specialized cellular barriers that permit the selective flow of solutes and water from one compartment to the next. These barriers, such as the blood-brain barrier (cerebral endothelial cells), the blood-CSF barrier (choroid plexus), and the plasma membranes of glia and neurons, maintain the proper volume and solute composition of the different fluid compartments, which is critical for neurological function. Because the brain is contained in the inelastic skull, small changes in total brain volume can rapidly increase intracerebral pressure, resulting in herniation of intracranial contents, damage of structures, neurological injury, and death. Minor changes in the composition of ions in the brain’s extracellular or intracellular fluids can significantly affect the function of neurons, which rely on precise ion gradients across their plasma membranes to trigger the changes in membrane potential that underlie action potential generation and propagation. Hence, ion and water transport in the brain are tightly regulated.

Ischemic Brain Injury and Cerebral Edema

Ischemic stroke, the most common and serious form of ischemic brain injury, is a loss of neural function resulting from a critical reduction in cerebral blood flow, usually due to arterial occlusion by thrombosis or an embolus. Because brain metabolism is almost entirely dependent on the oxidation of glucose delivered by the blood, significant reductions in cerebral blood flow...
cause a rapid decline in tissue metabolism, which is followed by severe tissue damage. Currently, there is no effective therapy specifically directed toward ameliorating a common secondary consequence of ischemic stroke that contributes to its high morbidity and mortality: cerebral edema, or “brain swelling.”

At present, physicians use a battery of empirically derived drugs and maneuvers to decrease the high intracranial pressures that accompany cerebral edema after large ischemic strokes. These treatments include the external drainage of CSF, sedation, hyperventilation, osmotic diuretics such as mannitol or hypertonic saline, hypothermia, and, when intracranial pressures become refractory to medical management, decompressive hemicraniectomy, a relatively morbid yet oftentimes life-saving neurosurgical operation that removes part of the skull to allow “breathing room” for the edematous brain. An increased understanding of the normal mechanisms that govern ion and water transport in the brain, along with a more detailed knowledge of the cellular and molecular events underlying the formation of ischemic cerebral edema, will likely identify novel pharmacotherapeutic targets and treatment strategies.

Brain Cell Volume Regulation

Over 70% of fluid in the central nervous system (CNS) is contained in the intracellular fluid compartment. Compared with the interstitial fluid and the CSF, the intracellular fluids have a much higher level of potassium and a much lower level of sodium and calcium. Because of these ion gradients, under physiological conditions, the flux of sodium and calcium into cells, and the flux of potassium out of cells, are balanced with efflux of these ions against their electrochemical gradients by active, energy-dependent ion pumps such as the Na+-K+-ATPase and the Ca2+-ATPase. The Na+-K+-ATPase prevents the intracellular accumulation of sodium ions, thus preventing an influx of solute and water that would result in cell swelling, the associated loss of cytoskeletal integrity, and osmotic cell death. The activity of the Na+-K+-ATPase also generates the electrochemical gradients necessary for secondary-active and passive ion transport processes. The intracellular fluid compartment is the first fluid compartment in the brain that is affected by ischemic insult. Derangements in the energy-dependent processes that regulate the volume and solute composition of the intracellular fluid are primary drivers behind cerebral edema (see below). As in other tissues, water is in thermodynamic equilibrium across the plasma membranes of all brain cells. As a result, the osmotic concentration of cytoplasmic (\(\psi_c\)) and extracellular (\(\psi_e\)) fluids is equal under steady-state conditions. Because cell membranes are freely permeable to water, changes in the intracellular or extracellular content of solutes establish transmembrane osmotic gradients (\(\Delta\psi\)) that result in the flow of water into or out of cells. This movement of water will continue until thermodynamic equilibrium is reached (that is, until \(\Delta\psi = 0\)). The membranes of animal cells are unable to generate enough hydrostatic pressure to counterbalance \(\Delta\psi\). As a result, changes in the intracellular or extracellular content of solutes alter this balance to cause a rapid decline in tissue metabolism, which is followed by severe tissue damage. Currently, there is no effective therapy specifically directed toward ameliorating a common secondary consequence of ischemic stroke that contributes to its high morbidity and mortality: cerebral edema, or “brain swelling.”

Under steady states, intracellular solute influx triggers and balances solute efflux and the removal of osmotically active solutes (anion shunt hypothesis). After this balance is disrupted by ischemic insult, the osmotic swelling occurs within seconds of the volume perturbation. Acute cell shrinkage is countered by regulatory volume increase, which triggers the activation of chloride and sodium-permeable ion carriers, thereby decreasing intracellular volume (see below). As in other tissues, water is in thermodynamic equilibrium across the plasma membranes of all brain cells. As a result, the osmotic concentration of cytoplasmic (\(\psi_c\)) and extracellular (\(\psi_e\)) fluids is equal under steady-state conditions. Because cell membranes are freely permeable to water, changes in the intracellular or extracellular content of solutes establish transmembrane osmotic gradients (\(\Delta\psi\)) that result in the flow of water into or out of cells. This movement of water will continue until thermodynamic equilibrium is reached (that is, until \(\Delta\psi = 0\)). The membranes of animal cells are unable to generate enough hydrostatic pressure to counterbalance \(\Delta\psi\). As a result, changes in the intracellular or extracellular content of solutes alter this balance to cause a rapid decline in tissue metabolism, which is followed by severe tissue damage. Currently, there is no effective therapy specifically directed toward ameliorating a common secondary consequence of ischemic stroke that contributes to its high morbidity and mortality: cerebral edema, or “brain swelling.”

FIGURE 1 Molecular mediators of cell volume regulation

The homeostatic counter-responses that maintain normal cell volume are mediated by changes in the activity of ion transporters and channels, which occur within seconds of the volume perturbation. Acute cell shrinkage is countered by regulatory volume increase, which triggers the activation of chloride and sodium-permeable ion carriers, thereby decreasing intracellular volume. Other sodium influx pathways like the Na+/H+ exchanger NHE1, working in concert with the Cl-/HCO3- exchanger, are also involved. Acute cell swelling is countered by regulatory volume decrease, which involves the cellular loss of chloride and potassium via the activation of the K+Cl- cotransporter and swelling-activated potassium and chloride channels. Ischemia-triggered increases in NKCC1 and other ion carriers can cause cytotoxic edema or isotonic cell swelling. Figure from Ref. 33 and used with permission.
The epithelial cells of the choroid plexus and the endothelial cells of the choroid plexus are reperfused (43) (FIGURE 2). Cytotoxic, ionic, and vasogenic edema arise from ischemia-induced permeability changes in the brain’s cellular barriers. These permeability changes, in turn, result from the pathological stimulation or transcriptional upregulation of ion channels and transporters in the blood-brain barrier, choroidal plexus, and neuroglial cells.

**Cytotoxic edema: pathological cell swelling due to disruptions in cell volume regulation**

The initial, and to some extent the predominant, type of edema following ischemia is "cytotoxic" edema (17, 50). Cytotoxic edema promotes the intracellular accumulation of osmotically active solutes that not only cause cell swelling but also lead to the alteration of ionic gradients by restoring the volume of the cell. The \( F_v \) generates the driving force for the flow of water across the cell membrane, thereby restoring the volume of the cell. The \( F_v \) is then dissipated as equilibrium is achieved.

The homeostatic counter-responses that maintain normal cell volume are mediated by changes in the activity of ion transporters and channels, which occur within seconds of the volume perturbation (33) (FIGURE 1). Acute cell shrinkage is countered by RVI, which triggers the activation of the bumetanide-sensitive NKCC1 cotransporter, resulting in the influx of sodium (with chloride and potassium) and water. This action is anticipated to increase cell volume and has been shown to do so in several glial lines in vitro (see Refs 25, 46). Other sodium influx pathways like the Na’/H’ exchanger (NHE), working in concert with the CI’/HCO3’ exchanger, are also involved. To avoid needless expenditure of ATP, cells concurrently inhibit ion efflux pathways, like potassium channels and transporters. Acute cell swelling is countered by RVD, which involves the cellular loss of chloride and potassium, in part, via the activation of the K’-Cl cotransporters and swelling-activated potassium and chloride channels. Ischemia-triggered increases in NKCC1 and other ion carriers can cause cytotoxic edema or isosmotic cell swelling.

In the setting of ischemic injury, failure of RVD to decrease cell swelling or inactive activation of mediators that orchestrate RVI can result in cell swelling, which impairs the structural integrity of cells via disruption of the actin cytoskeleton. Swelling of vascular endothelial cells decreases cerebral perfusion, causing ischemia and infarction. Swelling of the epithelial cells of the choroid plexus and the endothelial cell blood-brain barrier can compromise their structural and functional integrity, thereby altering the permeability of barrier.

**Ischemia-Induced Cerebral Edema: Cytotoxic, Ionic, and Vasogenic Components**

Classic dogma has stressed the importance of mechanical disruption of the blood-brain barrier and the resulting formation of vasogenic edema fluid in the development of cerebral edema following ischemic or traumatic brain injury. Recent data has challenged this concept. Another paradigm holds that the development of cerebral edema is a complex yet stepwise process that stems first from the cytotoxic edema of neuroglial cells (which does not require active blood flow) to the subsequent development of ionic and vasogenic edema (which occur once ischemic tissues are repurposed) (43) (FIGURE 2). Cytotoxic, ionic, and vasogenic edema arise from ischemia-induced permeability changes in the brain’s cellular barriers. These permeability changes, in turn, result from the pathological stimulation or transcriptional upregulation of ion channels and transporters in the blood-brain barrier, choroidal plexus, and neuroglial cells.
that promote the transendothelial passage of fluid into the extracellular space. Because astrocytes outnumber neurons 20 to 1 in the brain, the uptake of solute into astrocytes is primarily responsible for cytotoxic edema. Solutes are transported into neuronal cells by primary and secondary active transport processes and passive transport. Due to the paucity of ATP in ischemic cells, secondary active transport (e.g., via cotransporters), which utilizes energy stored in ionic gradients established by primary active transport (such as the Na+-K+ ATPase), and passive transport (via ion channels) are the primary mechanisms by which cells accumulate solute during cytotoxic edema. As ATP is depleted from cells with the worsening of ischemia, passive ion transport processes may predominate in presynaptic tissues with reduced but not depleted levels of ATP.

The primary driver behind the formation of cytotoxic edema is the intracellular accumulation of sodium. This ion is usually more highly concentrated in the extracellular space than in the intracellular space due to the selective permeability of the plasma membrane and the activity of the Na+-K+ ATPase. However, ischemia triggers changes in the cell membrane that render it more permeable to the passage of sodium. Chloride follows the influx of sodium through chloride channels, and water follows via aquaporin water channels to maintain electrical and osmotic neutrality, respectively.

Different molecular mediators are responsible for the accumulation of intracellular solute during cytotoxic edema, including various sodium channels and transporters (43). These ion transport proteins are stimulated by factors associated with ischemia such as elevated levels of extracellular potassium and protons, inflammatory mediators, and excitatory neurotransmitters (see below). Constitutively expressed pathways, such as tetrodotoxin-sensitive sodium channels, and NHE and NKCC1 pathways that normally mediate sodium entry into cells have increased activities in response to these factors (2, 3, 5).

Excitatory amino acids like glutamate play a particularly important role in ischemic cell injury not only by triggering the excitotoxicity of neurons but also by stimulating the inward fluxes of sodium and chloride that promote cytotoxic brain cell swelling (46). Glutamate, normally released into nerve terminal synapses at millimolar concentrations, mediates excitatory synaptic transmission by binding to at least two classes of ion channel-coupled receptors, including N-methyl-D-aspartate (NMDA) and non-NMDA types like AMPA receptors. NMDA receptors are linked to voltage-sensitive, high-conductance cation channels that are permeable to both sodium and calcium. Normally, glutamate is rapidly cleared from synapses. However, microdialysis studies have shown that, after 30 min of ischemia, extracellular glutamate levels are increased by >150-fold due to impaired clearance. These high levels of glutamate produce neuronal and glial injury and death in part by triggering an influx of sodium, chloride, and water, resulting in extensive cell swelling.
Immediate glutamate-triggered cell swelling and injury can be blocked by the removal of extracellular sodium or chloride from the extracellular media in cell culture and can be attenuated by the administration of the NKCC1-blocker bumetanide, suggesting that sodium and chloride transport via NKCC1 is instrumental to glutamate-mediated cell swelling and excitotoxicity. In addition, ion channels not normally expressed in the brain have been shown to be transcriptionally upregulated following ischemic injury; examples include cation channels like TRP channels and the surly urea receptor 1 (SUR1) regulated NCCa-ATP channel. Opening of these cation channels allows sodium (and calcium) to enter cells. The net influx of cations with chloride via volume-regulated anion channels (VRAC) creates an osmotic force that drives the influx of water and causes cell swelling.

Ionic and vasogenic edema: fluid shifts resulting from alterations in endothelial permeability

Cytotoxic edema of brain cells does not by itself increase the net volume of the brain unless cerebral blood flow is reestablished, because cytotoxic edema is merely the redistribution of fluid from the brain’s extracellular to intracellular space. For an actual increase in brain volume to occur, additional fluid must be added to the brain’s extracellular space. The movement of ions and water into cells from cytotoxic edema results in the depletion of these constituents from the extracellular space (34, 45). Newly established gradients for sodium and other osmotically active solutes between the extracellular and intracellular space are the driving forces for the transendothelial movement of edema fluid across the blood-brain barrier. However, the stored potential energy of these ionic gradients cannot manifest into solute and water movement until the permeability of cerebral endothelial cells to ions and water, and the relative “tightness” of tight junctions that limit the paracellular movement of solute and fluid between endothelial cells, the values of $K_u$ and $K_p$ are typically close to zero. Thus little to no transendothelial movement of solute and water occur, and edema does not develop in the extracellular space. However, with ionic edema, $K_P > 0$ due to an increased transport of ion and water transport pathways like the NKCC1, the SUR1-regulated NCCa-ATP channel, and aquaporin water channels (9, 42, 32). Because $K_u$ remains close to zero, $J_f$ is positive, and transendothelial fluid transfer occurs, resulting in the accumulation of ionic edema fluid. With vasogenic edema, both $K_u$ and $K_p > 0$, with the increase in $K_p$ due to the degradation of endothelial cell tight junctions via molecules like prothrombin, VEGF, and matrix metalloproteins (35).

Ionic edema is the earliest phase of endothelial dysfunction triggered by ischemia and precedes vasogenic edema by ~6 h (4). Due to the cytotoxic edema of neurons or oligodendroglial cells, the brain’s extracellular space is depleted of ions and water, generating gradients that drive the movement of solute and water across the extracellular space, which occurs once transendothelial permeability alterations are triggered in the blood-brain barrier (51). Such increases in permeability in endothelial cells are usually due to the increased activity and/or expression of ion transport proteins triggered by ischemia or associated toxic metabolites. Ionic edema fluid is protein poor because tight junctions of the blood-brain barrier are intact. Because endothelial cells, unlike neurons and astrocytes, do not express voltage-gated sodium channels, the secondary active cotransporter NKCC1, expressed on the luminal (blood) side of the endothelium, plays an important role in the formation of ionic edema by loading sodium and chloride into cells. The sodium pressure ($P_s$), interstitial osmotic pressure ($O_i$), and two distinct filtration coefficients, the hydraulic conductivity ($K_p$) and the osmotic conductivity ($K_u$). The driving forces for the movement of fluid movement are the hydrostatic pressure ($P_h$) generated by the pumping of the heart, and osmotic pressure ($O_i$), which is potential energy stored in the electrochemical gradients between the plasma and interstitial fluid (43).
inside capillary cells is then expelled into the brain's extracellular space by the activity of the Na+-K+-ATPase, which is expressed on the capillary cell abluminal membrane; chloride follows through anionic channels.

The contribution of NKCC1 to ionic edema is important in the early stages of fluid accumulation, when ischemic tissues are still being perfused, because ATP is needed to drive the activity of the Na+-K+-ATPase, which establishes the inwardly directed sodium gradients required for NKCC1 activity. NKCC1's role in sodium and chloride accumulation may also be relevant when energy restoration of ischemic tissue occurs during reperfusion. In contrast, the SIRI-regulated NCCa-ATP channel is normally absent but transcriptionally upregulated with ischemic injury. With severe ischemia-associated ATP depletion, which occurs in the later stages of the pathogenesis of cerebral edema, NKCC1's role is halted and the SIRI-regulated NCCa-ATP channel opens, triggering the ATP-independent passive transport of water and solute. Inhibition of this channel with low doses of glibenclamide reduces the formation of ionic edema after ischemia by over 50% (4). In addition to NKCC1 and SIRI-regulated NCCa-ATP channels, non-voltage- or ligand-gated sodium channels on the luminal and adhimal membranes of brain capillaries provide sodium influx and efflux pathways, respectively, for the passive diffusion of sodium down its electrochemical gradient from the blood and into the brain's extracellular space. Chloride and water follow via chloride channels and aquaporins (e.g., aquaporin-1), respectively.

After ionic edema, the second phase of endothelial dysfunction triggered by ischemia is vasogenic edema, which is characterized by the breakdown of tight junctions within the blood-brain barrier and an accumulation of fluid into the brain's interstitial space. It is unclear what causes the permeability changes during vasogenic edema. Although the newly created permeability pores are large enough to permit the passage of plasma-derived macromolecules, the pores do not allow the passage of red blood cells, suggesting that the pores do not exclude renin and kinins, a major vasoconstrictor, as well as renin and angiotensin-converting enzyme inhibitors reduce ischemia-induced or reperfusion-associated cerebral edema (14, 27).

Role of NKCC1 in the Pathogenesis of Ischemic Cerebral Edema

The bumetanide-sensitive NKCC1 is a cation-chloride cotransporter (CCT) of the SLC12 gene family. CCTs are intrinsic membrane proteins with a neutral charge that are abundant in the brain tissue. NKCC1 is expressed in the choroid plexus, the blood-brain barrier (BBB), the kidneys, and secretory epithelial cells throughout the body; however, its contribution to ionic edema is more specific to the brain. NKCC1 is highly expressed in microvascular endothelial cells of the BBB, which transports sodium and chloride from the blood into the brain (see Ref. 15 for an in-depth review of the science of brain edema). NKCC1 expression and basic transport physiology. In the choroid plexus, NKCC1-mediated flux also occurs in an apical-to-basolateral direction. Under physiological conditions, the activity of NKCC1 modulates the levels of [Cl–]i in neurons, glia, BBB endothelial cells, and choroid plexus epithelial cells, thereby helping maintain cellular volume across changes of extracellular osmolality and intracellular solute content to prevent excessive swelling or shrinkage (25). The remaining sections of this chapter will focus specifically on the changes in expression and activity of NKCC1 in ischemic brains and the role of NKCC1 in cytotoxic edema and the alterations in transendothelial capillary permeability. Genetic ablation or pharmacological inhibition of NKCC1 with a low dose of the furosemide-related diuretic bumetanide have shown protection in neurons, astrocytes, and brain endothelial cells against ischemic damage.

Mechanisms

In a rat model of ischemia, NKCC1 knockout reduces ischemia-induced edema as well as water and sodium accumulation in the brain and striatum (39). Inhibition of NKCC1 in rats decreases infarct volumes (39). NKCC1 expression is known to be increased in astrocytes (3, 4) and brain capillary endothelial cells (4), as well as in microvascular endothelial cells in the kidney (51). The precise mechanisms underlying the increased expression of NKCC1 in these cells are not well understood. The induction of NKCC1 may be due to an increase in the expression of the gene encoding NKCC1, or to a post-translational modification, or both. The induction of NKCC1 may be due to an increase in the expression of the gene encoding NKCC1, or to a post-translational modification, or both. The induction of NKCC1 may be due to an increase in the expression of the gene encoding NKCC1, or to a post-translational modification, or both. The induction of NKCC1 may be due to an increase in the expression of the gene encoding NKCC1, or to a post-translational modification, or both. The induction of NKCC1 may be due to an increase in the expression of the gene encoding NKCC1, or to a post-translational modification, or both.

Consequences of Ischemic Cerebral Edema

Studies of NKCC1 knockout mice employing ischemia-reperfusion injury models have revealed that NKCC1 knockout reduces neurologic injury and improves outcome in rats with ischemic brain injury. NKCC1 inhibition reduces the release of excitatory amino acids, such as glutamate, which are known to cause neuronal death after ischemic insults. The effects of NKCC1 inhibition are dose-dependent, with low doses of bumetanide having a protective effect on neuronal morphology, while higher doses of bumetanide are associated with toxicity. In the rat model of ischemia-reperfusion injury, NKCC1 knockout reduces neuronal damage and improves functional outcomes, as assessed by the rotarod test and the beam balance test. These results suggest that NKCC1 inhibition has the potential to improve neurologic outcomes in humans with ischemic brain injury. NKCC1 inhibition with a low dose of bumetanide also reduces infarct volume and improves functional outcomes in a rat model of ischemia-reperfusion injury. The precise mechanisms underlying the protective effects of NKCC1 inhibition are not well understood. The induction of NKCC1 may be due to an increase in the expression of the gene encoding NKCC1, or to a post-translational modification, or both.
Mechanisms of NKCC1 activation in ischemia

In a rat model of focal cerebral ischemia/reperfusion (2-h MCAO and 24-h reperfusion), NKCC1 transcripts and protein are significantly upregulated in cortical neurons as well as in whole brain lysates from rat cerebral cortex and striatum (53, 54). Elevated extracellular potassium and glutamate levels, which occur in cerebral ischemia, are known to stimulate NKCC1 activity in neurons and astrocytes (3, 47, 48). Cytokines may also be involved in ischemia-induced increases in NKCC1 activity: ischemia-induced, astrocyte-derived, interleukin-6 (IL-6) activates rat cerebral microvasculature, and the immunosuppressant FK506 (which blocks IL-6 upregulation in microglia and astrocytes) reduces infarct volume in a rat MCAO model (9).

In focal cerebral ischemia/reperfusion, strong upregulation of NKCC1 may occur. This cell swelling can result in a net increase of intracellular sodium and potassium, 

Consequences of NKCC1 activation in ischemia brain: cell swelling, cerebral edema formation, and neurotoxicity

Studies of NKCC1 knockout mice, as well as studies employing intracerebral bumetanide administration, strongly implicate a role for NKCC1 in ischemic brain edema and damage. Bumetanide potently reduces neuronal and astrocyte cell swelling, along with infarct volume, after 2-h MCAO followed by 24-h reperfusion, a well-known model of focal cerebral ischemia in rats (8, 9). Systematic administration of bumetanide has also been shown to reduce infarct volume and brain edema by 30% in a nephrectomized rat permanent ischemia model, thereby excluding renal effects of bumetanide (37). These studies have been corroborated genetically in NKCC1 knockout mice, which display an ~40% reduction in infarct volume and brain edema in the infarcted hemisphere in a transient ischemia model (6).

The role of NKCC1 in BBB endothelial cells deserves special mention. In the BBB, NKCC1 is expressed on the luminal side of microvascular endothelial cells. Here, NKCC1 can come in contact with systemically administered bumetanide. Because bumetanide may have less than ideal penetration across the BBB, the fact that intravenous administration of bumetanide has been shown to significantly decrease edema formation in the rat models of stroke highlights the important role for NKCC1 in mediating solute uptake from the vascular lumen (blood) into endothelial cells and consequently in ischemia-induced increases of extracellular sodium or blocking of L-type voltage-dependent calcium channels with nifedipine (47, 48). These data suggest that NKCC1 activity is stimulated under conditions of high [K+], via calcium-mediated signal transduction pathways. Intracellular accumulation of radiolabeled sodium and chloride is significantly increased in response to 75 mM [K+]o; this increase is abolished by bumetanide or by genetic ablation of NKCC1 (47, 48). Importantly, these mechanisms of NKCC1-mediated potassium influx during ischemia most assuredly take place during the early phases of ischemia, when some activity of the Na+-K+ ATPase is still present and ion gradients are not totally collapsed. High [K+]o-mediated stimulation of NKCC1 can result in cell swelling via a net increase of intracellular sodium, potassium, and chloride and accompanying water. High [K+]o causes cell swelling in NKCC1+ astrocytes, but is absent in NKCC1−/− astrocytes, and is abolished with bumetanide (47, 48). High [K+]o-induced astrocyte swelling is also observed in the rat optic nerve model (31). In inactivated nerves, light transmittance progressively increases with high [K+]o, causing cell swelling. Bumetanide can reversibly suppress this high [K+]o-induced cell swelling (31). In astrocytes, NKCC1 is also an important contributor to excitatory amino acid (EAA) release in response to high [K+]o. In accordance with this, astrocytes from NKCC1−/− mice exhibit absence of swelling and
decreased EAA release after high [K+]o (47, 48). The main mechanism in NKCC1-mediated EAA release appears to be that NKCC1-induced cell swelling activates the volume-expansion sensing outward rectifying anion channel (also known as the volume-regulated anion channel), through which EAA are released. Glutamate-mediated neurotoxicity plays an important role in neuronal damage after ischemic injury in the CNS. Acute excitotoxic neurodegeneration after ischemia is mediated by excitatory amino acids (EAA), which act on ionotropic glutamate receptors. These receptors include the N-methyl-D-aspartate (NMDA) and kainate receptors, which are important in the pathogenic complexity of the disease process.

Clinical Ramifications

Bumetanide is a relatively specific inhibitor of NKCC1 at low concentrations, with well established pharmacokinetic and pharmacodynamic properties in humans with few side effects (44). Thus the use of this diuretic, or better yet a specific inhibitor with known penetrance across the blood-brain barrier, might be beneficial for the treatment of ischemic cerebral edema. Pilot studies are already underway examining the efficacy of bumetanide, administered with phenobarbital, for the treatment of other neurological disorders, such as neonatal seizures (FDA IND no. 101690; see http://www.cureepilepsy.org/research/current..asp; Refs. 25, 24, 20), and adults with medically intractable and surgically unresectable temporal lobe epilepsy associated with hippocampal sclerosis in human temporal lobe epilepsy associated with hippocampal sclerosis (2, 15). Bumetanide is a relatively specific inhibitor of NKCC1 in the CNS, without a profound diuretic effect in the periphery. Although low doses of bumetanide specifically target NKCC1, and thus do not produce the diuretic effect seen with high doses of bumetanide (which inhibits the renal NKCC2 isoform), what is clearly needed is a specific NKCC1 inhibitor that has good CNS penetration across the BBB. Efforts to design such drugs are currently underway.

If lessons are to be learned from previous clinical trials of cerebral edema, the effects of agents that target a single molecule are likely to be ineffective, given the pathogenic complexity of the disease process. NKCC1 is constitutively expressed and indirectly requires ATP, which is used by the Na+-K+-ATPase to establish ionic gradients required for NKCC1's secondary active transport. Because of this, NKCC1 is important in the earlier stages of cerebral edema formation, when ischemia is not yet compromised ATP production. In this context, it might be valuable to couple an inhibitor of NKCC1 with an agent that targets the latter stages of edema formation, which occur largely in the absence of ATP. For example, the SUR1-regulated NCCa-ATP channel is normally absent but transcriptionally upregulated with ischemic ischemic injury. With severe ischemia-associated ATP depletion, which occurs in later stages of cerebral edema, NKCC1's role is halved and the SUR1-regulated NCCa-ATP channel opens, triggering the ATP-independent passive transport of water and solute. Because of their distinct functional and temporal roles in the pathogenesis of cerebral edema, blocking NKCC1 and not the SUR1-regulated NCCa-ATP channel might yield incomplete inhibition of edema. However, this blockage of both, using a combination regimen of bumetanide plus glibenclamide, might be a particularly attractive therapeutic option. This is a testable hypothesis that is subject of future research.

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because of their role in regulating Na+ entry into neurons. The SUR1–KCC3 complex, which is highly expressed in the cerebellum, is involved in maintaining neuronal Na+ homeostasis and excitatory GABABergic signaling during neuronal activity. The SUR1–KCC3 complex is thought to be involved in the regulation of K+ uptake via NKCC1 and swelling of astrocytes. A study on the role of Na-K-Cl cotransporter during focal cerebral ischemia suggests that inhibition of the Na-K-Cl cotransporter reduces edema formation and tissue damage after ischemic injury. A review of the role of NKCC1 in brain edema formation highlights the importance of this cotransporter in the development of cerebral edema during ischemia. In conclusion, the regulation of Na-K-Cl cotransporter activity is crucial for maintaining neuronal homeostasis and excitatory GABABergic signaling during neuronal activity.