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 neuronal 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 intracerebral 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 fol-
lowed by severe tissue damage. Currently, there is no
effective therapy specifically directed toward ameliorat-
ing a common secondary consequence of ischemic
stroke that contributes to its high morbidity and mortal-
ity: 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,
the 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 mech-
anisms 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 pharmacother-
apic 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 fluid has 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 Cl⁻-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 trans-
port process. The intracellular fluid compartment is the
first fluid compartment in the brain that is affected by
ischemic insult. Derangements in the energy-depend-
ent processes that regulate the volume and solute com-
position of the intracellular fluid are the primary drivers
behind cerebral edema (see below).

As in other tissues, water is in thermodynamic equi-
librium across the plasma membranes of all brain
cells. As a result, the osmotic concentration of cyto-
plasmic (Vc) and extracellular (Vc) fluids is equal
under steady-state conditions. Because cell mem-
brane ions are actively maintained by changes in the
intracellular or extracellular content of solutes
establish transmembrane osmotic gradients (∆Ψm)
that result in the flow of water into or out of cells. This
movement of water will continue until thermodynam-
ic equilibrium is reached (that is, until ∆Ψm = 0).

The membranes of animal cells are unable to generate
enough hydrostatic pressure to counterbalance ∆Ψm. As
a result, the osmotic concentration of cytosolic
water must be kept at the extracellular concentration
level. In the brain, the osmotic concentration of extracellular
solute is equal to the intracellular concentration
of solute. Therefore, the osmotic concentration of
cytosol is the same as the osmotic concentration of
extracellular solution.

FIGURE 1. Molecular mediators of cell volume regulation

The homoeostatic 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 the bumetanide-sensitive NKCC1 cotransporter, resulting in the influx of sodium (with chloride and potassium) and water, thereby increasing cell vol-
ume. 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⁻ cotrans-
porters and swelling-activated potassium and chloride channels. Ischemia-triggered increases in NKCC1 and other ion carriers can cause cytosolic
edema or anisotonic cell swelling. Figure from Ref. 33 and used with permission.
The cell membrane is a permeability barrier that would resist the osmotic flow of water into or out of the cells, causing cells to swell or shrink. Under physiological and pathophysiological conditions, osmotic changes in the extracellular fluid and levels of potassium, sodium, chloride, and calcium, which can alter membrane potential that provides an electrical counter-permeability of barrier.

Membrane depolarization is particularly threatening to neuronal cell structural and functional integrity, thereby altering the permeability of barrier.

Ischemic injury, failure of RVD to decrease cell swelling or inappropriate 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 neuronal cells (which does not require active blood flow) to the subsequent development of ionic and vasogenic edema (which occur once ischemic tissues 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, choroid plexus, and neuronal 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, 30). 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
that promote the transendothelial passage of fluid into the extracellular space. Because astrocytes outnumber neurons 20 to 1 in humans, 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, 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 voltagedependent, 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 promote neuronal and glial injury and death in part by triggering an influx of sodium, chloride, and water, resulting in extensive cell swelling.

Immediate glutamate-induced brain edema can be blocked by application of a specific sodium channel blocker bumetanide or chloride transport inhibitors. In addition to the brain edema resulting from increased sodium and chloride permeability in blood-brain barrier (BBB) endothelial cells, there is evidence that elevated sodium in the cerebral extracellular space can be blocked by application of a specific sodium channel blocker bumetanide or chloride transport inhibitors.

Ionic and vascular edemas resulting from blood-brain barrier (BBB) permeability change.

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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 intravascular space and the extracellular 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 the paracellular pathways that limit the paracellular movement of solute and fluid between endothelial cells, the values of $K_o$ and $K_h$ 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 ischemic edema, $K_o$ is increased due to an increased transport of ions and water from the vascular compartment to the extracellular space, which occurs once transendothelial permeability alterations are triggered in the blood-brain barrier (55). 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. Iodic 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 ($P_i$), and two distinct filtration coefficients, the hydraulic conductivity ($K_h$) and the osmotic conductivity ($K_o$). 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 ($P_{osm}$), 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 restitution of ischemic tissue occurs during reperfusion. In contrast, the SGLT1-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 SGLT1-regulated NCCa-ATP channel opens, triggering the ATP-dependent 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% (42). In addition to NKCC1 and SGLT1-regulated NCCa-ATP channels, non-voltage- or ligand-gated sodium channels on the luminal and subendothelial 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 brain-blood 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 physical disruption of capillaries is not the primary mechanism involved. Endothelial cell swelling due to cytotoxic edema (see above), actin polymerization-dependent interendothelial gap formation, tight junction breakdown, and enzymatic degradation of endothelial cell basement membranes are all mechanisms that have been proposed to account for changes in endothelial permeability accompanying vasogenic edema (7). Ischemia-induced factors such as thrombin have been shown to cause gaps in the blood-brain barrier by triggering endothelial cell retraction following focal ischemic episodes like stroke and intracerebral hematoma (28). VEGF, also induced by ischemia, disrupts the physical integrity of endothelial tight junctions and promotes the formation of vasogenic edema; VEGF inhibitors reduce vasogenic edema following reperfusion of ischemic tissues (52, 51). Vasogenic edema also results when the capillary basement membrane is breached via ischemia-induced matrix metalloproteinases, matrix metalloproteinase 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 cotransporter that transports chloride and sodium ions together. CCCs are intrinsic membrane proteins that transport chloride ions, together with sodium and/or potassium ions, across plasma membranes of cells. The stoichiometric coupling and directionality of the cations and chloride ions translocated by the CCCs results in an electrochemical equilibrium. Bumetanide is a specific inhibitor of NKCC1 at low concentrations (2-10 μM), with significant effects on NKCC2, an isoform most expressed in the thick ascending limb of Henle in the kidney's nephron. Hence, low-dose bumetanide is spared of the diuretic effects seen with other loop diuretics that target NKCC2 more specifically, like furosemide (Lasix).

NKCC1 is expressed on the basolateral membrane of secretory epithelial cells throughout the body; however, in microvascular endothelial cells of the BBB, NKCC1 is located in the apical (luminal) membrane, which transports sodium and chloride from the blood into the brain (see Ref. 15 for an in-depth review of 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 level of [Cl⁻]i in neurons, glia, BBB endothelial cells, and choroid plexus epithelial cells, thereby helping maintain cellular volume against changes of extracellular osmolality and intracellular solute content to prevent excessive swelling or shrinkage (25). The remaining sections of this review will specifically focus 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 transient middle cerebral artery occlusion (MCAO) and reperfusion, diuretics that target NKCC1 more specifically, like furosemide (Lasix), are known to reduce fluid accumulation, most likely as well as in vivo following middle cerebral artery occlusion and striatum (14). Bumetanide has been shown to reduce astrocyte swelling and the influx of sodium into ischemia-induced ischemic brain edema (11, 12), hydrochloric acid (HCl) and the ischemia-induced increase in intracellular sodium (13). Bumetanide reduces the formation of vasogenic edema after middle cerebral artery occlusion (MCAO) and 2 h of reperfusion (52, 51). Vasogenic edema, however, is not a limiting factor in the uterine artery ligation model (14) and the immunotoxic intraperitoneal injection of interstitial volume (51). Ischemia-induced vasoconstriction, which is characterized by the breakdown of tight junctions, is responsible for the increased permeability of the blood-brain barrier. Bumetanide reduces the formation of vasogenic edema by over 50% (42) and protects against ischemic injury (14, 27).

Consequences of Ischemic Brain Edema

Studies of the BBB employing in vivo imaging techniques have shown that brain edema reduces neural tissue viability and function in vivo. Ischemia reduces neural tissue viability and function in vivo. Ischemia reduces neuronal and astrocytic viability and function in vivo. Ischemia reduces neuronal and astrocytic viability and function in vivo. Ischemia reduces neuronal and astrocytic viability and function in vivo. Ischemia reduces neuronal and astrocytic viability and function in vivo. Ischemia reduces neuronal and astrocytic viability and function in vivo. Ischemia reduces neuronal and astrocytic viability and function in vivo.

Conclusion

In conclusion, the contribution of NKCC1 to brain fluid accumulation is important in the pathogenesis of ischemic edema, as outlined by the actions of bumetanide and other diuretics. This article provides a comprehensive overview of the role of NKCC1 in brain edema and its potential as a therapeutic target.
mechanisms for maintaining cellular edema following brain ischemia (52, 51). The apical base-transmembrane potential-driven, apical-to-basal, sodium-potassium co-transporter accompanies the net transmembrane movement of water. During early phases of ischemia, partial failure of Na+-K+ ATPase activity (due to neuronal excitotoxicity and oncotic cell death) increases the extracellular level of potassium, [K+]o (10, 8) (a few minutes of anoxia/ischemia raises [K+]o to ~40 mM); concurrently, the intracellular level of sodium increases as this ion moves down its electrochemical gradient across an increasingly permeable membrane.

In turn, the rise in extracellular potassium augments the osmotic effect of intracellular sodium by stimulating a secondary influx of potassium via cotransport mechanisms. In astrocytes, NKCC1 has been shown to play an important role in potassium uptake under high [K+]o conditions. In 75 mM [K+]o, NKCC1-mediated potassium influx during ischemia is completely abolished by either removal of extracellular calcium or blocking of L-type voltage-dependent calcium channels with nifedipine (47, 48).

These data suggest that NKCC1 activity is stimulated by ischemia-induced increases in NKCC1 activity: ischemia-induced, astrocyte-derived, interleukin-6 (IL-6) activates the brain microcapillarum, and the immunosuppressant FK506 (which blocks IL-6 upregulation in microglia and astrocytes) reduces infarct volume in a rat MCAO model (9).

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 NKCC1 in cerebral microvasculature, and the immunosuppressant FK506 (which blocks IL-6 upregulation in microglia and astrocytes) reduces infarct volume in a rat MCAO model (9).

Ischemia-induced increases in NKCC1 activity, similar to the mechanisms of acute stimulation of NKCC1 in response to hypertonicity or decreases in intracellular chloride, are generally associated with increased NKCC1 phosphorylation on serine 184 and serine 189. These changes in the phosphorylation status have been reported in rat cortical astrocytes at 2 h of OGD (29), and in response to hypoxia and AVP in cultured bovine cerebral microvascular endothelial cells (13). The protein kinases that mediate the ischemia-induced phosphorylation (and activation) of NKCC1 in the brain are currently unknown; however, several candidate kinases that regulate NKCC1 in other tissues, such as the WNK and SPAK/OSR1 serine-threonine kinases, are highly expressed in the brain and are therefore prime candidates (21, 11, 22). Indeed, Delpire and colleagues have already shown that SPAK/OSR1 are necessary for the proper regulation of NKCC1 in dorsal root ganglion neurons (16). This area of research will be an important topic of future study.

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

Studies of NKCC1 knockout mice, as well as studies employing in vitro vascular 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 50% in a nephrectomized rat transient 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 infarction 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 suggesting an ischemia-induced NKCC1 activation elicits excessive solute uptake (9, 37).

During early phases of ischemia, partial failure of the Na+-K+ ATPase and neuronal and glial damage (due to neuronal excitotoxicity and oncotic cell death) increases the extracellular level of potassium, [K+]o (a few minutes of anoxia/ischemia raises [K+]o to ~40 mM); concurrently, the intracellular level of sodium increases as this ion moves down its electrochemical gradient across an increasingly permeable membrane.
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 ischemic injury is largely mediated by NMDA ionotropic receptor-activated 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 ischemic injury is largely mediated by NMDA ionotropic receptor-activated 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.

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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, 28), and with adults medically intractable and surgically unresolvable temporary local edema associated with medial temporal lobe sclerosis or various cortical malformations (30, 23). These preliminary studies have shown the bumetanide might be used at doses that provide specific inhibitory action 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 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 secondarily active transport. Because of this, NKCC1 is important in the earlier stages of cerebral edema formation, when ischemia has 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 halted 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 the SUR1-regulated NCCa-ATP channel might yield incomplete inhibition of edema. However, the absence of all, using a combination regimen of bumetanide plus glibenclamide, might be a particularly attractive therapeutic option. This is a testable hypothesis that can be the subject of future research.

References

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