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 neural/glia 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 activities of specific ion channels and transporters. Recent data has implicated...
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, a much lower level of sodium and calcium. Because of these ion gradients, under physiological conditions, the flow of sodium and calcium into cells, and the flow 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, as described 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 (ωc) and extracellular (ωe) 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 (ΔΩ) 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 ΔΩ = 0). The membranes of animal cells are unable to generate enough hydrostatic pressure to counterbalance ΔΩ. As a result, the osmotic concentration of cytoplasmic fluid is increased (RVI) as water leaves, and extracellular fluid is decreased (RVI) as water enters. The intracellular accumulation of sodium ions, which triggers brain cell swelling or shrinkage, is countered by regulatory volume decrease, which involves the cellular loss of chloride and potassium via the activation of the K-Cl cotransporter, which triggers the activation of the Na+-K+ ATPase and the Ca2+-ATPase, respectively (33). More specifically, RVI is mediated by changes in the generation of arginine vasopressin (AVP) and the consequent alteration in the cell volume because AVP increases the activity of the Na+-K+ ATPase, favorably altering the balance to decrease the extracellular fluid volume and to increase the intracellular fluid volume (18).

Moreover, a wealth of evidence has confirmed that alterations in ion fluxes across the plasma membranes of neuronal and glial cells across the CNS play a key role in the pathogenesis of brain edema, as will be highlighted subsequently. Nevertheless, several important points should be emphasized: (i) In the absence of ischemic injury, the normal cell volume is maintained by active ion transport processes and the osmotic activity of ion gradients, under physiological conditions, (ii) these ion gradients are actively dissipated as energy is consumed by the cell, and (iii) changes in the osmotic activity of ion gradients, under physiological conditions, (iv) changes in the osmotic activity of ion gradients, under physiological conditions, and (v) changes in the osmotic activity of ion gradients, under physiological conditions. 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 decrease, which triggers the activation of the Na+-K+ ATPase and the Ca2+-ATPase, respectively (33). More specifically, RVI is mediated by changes in the generation of arginine vasopressin (AVP) and the consequent alteration in the cell volume because AVP increases the activity of the Na+-K+ ATPase, favorably altering the balance to decrease the extracellular fluid volume and to increase the intracellular fluid volume (18).

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 decrease, which triggers the activation of the Na+-K+ ATPase and the Ca2+-ATPase, respectively (33). More specifically, RVI is mediated by changes in the generation of arginine vasopressin (AVP) and the consequent alteration in the cell volume because AVP increases the activity of the Na+-K+ ATPase, favorably altering the balance to decrease the extracellular fluid volume and to increase the intracellular fluid volume (18).

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The homeostatic counter-responses that maintain osmotic and ionic solute homeostasis are triggered in the setting of cell volume changes in the extracellular fluid. This cell volume change is then dissipated as equilibrium is achieved.

Alterations in the level of inorganic solutes are mediated by specific changes in the expression and activity of ion channels and transporters. Acute cell swelling is countered by RVD, which involves the cellular loss of chloride and 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 inappropriate activation of mediators that orchestrate RVD 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 classic dogma. 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 repertilized) (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 neuroglial cells.

Cytotoxic edema: pathologival 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...
that promote the transendothelial passage of fluid into the extracellular space. Because astrocytes outnumber neurons 20 to 1 in the human 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 10–100-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 can be blocked by blockers of voltage-gated sodium channels (e.g., tetrodotoxin) and chloride transporters, such as bumetanide (an inhibitor of the Na⁺-K⁺-Cl⁻ cotransporter) or furosemide, a loop diuretic (47). Internal glutamate can be attenuated by the increased neuronal activity associated with ischemia (48), which may drive glial cell swelling and downstream brain swelling. Glial cell swelling is a consequence of increased neuronal activity (48), increased synthesis of pro-inflammatory cytokines and chemokines, and increased glutamate release (48). Glial swelling appears to be mediated by NMDA receptors as well as by astrocytic potassium channels (49). Astrogial swelling may also be due to hypoxia, which results in increased expression of adenosine A₁ receptors that lead to reduced glutamate uptake (50).

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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 sodium-potassium ATPase. 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 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 these tight junctions that limit the paracellular movement of solute and fluid between endothelial cells, the values of $K_{Na}$ and $K_{Cl}$ 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, $K_{Na} > 0$ due to an increased transport of nonionic solutes by the transendothelial fluid transfer occurs, resulting in the accumulation of ionic edema fluid. With vasogenic edema, both $K_{Na}$ and $K_{Cl}$ are typically close to zero, $J_f$ is positive, and transendothelial fluid transfer occurs, due to the degradation of endothelial cell tight junctions (resulting in vasogenic edema) permits the flux of solute and water down their concentration gradients.

Ionic and vasogenic edema occur in the context of altered endothelial cell permeability. The role of hydrostatic forces, osmotic forces, and capillary perfusion in the movement of fluid across capillary endothelial cells is described with the equation $J_f = K_w (P_v - P_t)$. According to this classic equation (Starling’s Law), the net filtration or movement of fluid from the plasma to the extracellular space, with an outward flux of fluid defined as positive, is $J_f$, which is determined by capillary hydrostatic pressure ($P_h$), interstitial hydrostatic pressure ($P_t$), capillary osmotic pressure ($\pi_c$), interstitial osmotic pressure ($\pi_t$), and two distinct filtration coefficients, the hydraulic conductivity ($K_w$) and the osmotic conductivity ($K_0$). 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 ($\pi_t$), which is potential energy stored in the electrochemical gradients between the plasma and interstitial fluid (43).

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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 S1R1-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 S1R1-regulated NCCa-ATP channel opens, triggering the ATP-dependent, passive transport of water and solute. Inhibition of this channel with low doses of furosemide reduces the formation of ionic edema after ischemia by over 50% (42). In addition to NKCC1 and S1R1-regulated NCCa-ATP channels, non-voltage- or ligand-gated sodium channels on the luminal and adluminal 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 between 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 physical disruption of capillaries is not the primary mechanism involved. Endothelial cell swelling due to cytotoxic edema (see above), actin polymerization-dependent endothelial cell retraction, formation of interendothelial gaps, 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 (51, 52). 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 cation-chloride cotransporter (CCC) of the SLC12 gene family. 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 CCC results in an electrically silent (i.e., electroneutral), secondarily active transport process that is energetically driven by transmembrane sodium and chloride gradients, with the former established by the Na+-K+-ATPase. Utilizing the large electrochemically favorable inward gradient for sodium and chloride ions across the plasma membrane, NKCC1 loads chloride ions into the cell, raising the level of intracellular chloride ([Cl–]i) above its electrochemical equilibrium. Bumetanide is a specific inhibitor of NKCC1 at low concentrations (2–10 μM), without significant effects on NCC2, an isoform only 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 basal 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 physiologic 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 of 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 pharmacologic 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 of Ischemic Cerebral Edema Development

In a rat model of middle cerebral artery occlusion (MCAO) and 24 h reperfusion, BBB integrity is preserved as well as in wistar rats (13), but the capillaries and stratum (14, 15) and glial (16) are known to be damaged, and astrogliosis is observed in astrocytes (1, 5). In a model of ischemia-induced ischemia-induced brain edema in rats, AVP and sodium depletion reduce edema in the brain (16). By 10.220.33.5 on September 27, 2017 http://physiologyonline.physiology.org/ Downloaded from
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 microvascular endothelium, 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 cerebral cortex at 4-8 h of reperfusion after MCAO (53), in mouse cortical astrocytes after 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, Delprere 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 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 ~40% in infarcted rats (8, 9). Importantly, these mechanisms of bumetanide-mediated potassium influx were significantly stimulated in NKCC1–/– astrocytes, and is absent in NKCC1–/– astrocytes, and 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 potassium, [K+]i, (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. 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 was significantly stimulated in astrocytes; this high-[K+]o-induced activation of NKCC1 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 under conditions of high [K+]o via calcium-mediated signal transduction pathways. Intracellular accumulation of radio-labeled sodium and chloride is significantly increased in response to 75 mM [K+]o; this increase is abolished by bumetanide or 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. 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 penetrance 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 sustains an ischemia-induced NKCC1 activation elicits excessive solute uptake (9, 37).

During early phases of ischemia, partial failure of the Na+-K+ ATPase and renal 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. 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 was significantly stimulated in astrocytes; this high-[K+]o-induced activation of NKCC1 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 under conditions of high [K+]o via calcium-mediated signal transduction pathways. Intracellular accumulation of radio-labeled sodium and chloride is significantly increased in response to 75 mM [K+]o; this increase is abolished by bumetanide or 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 insulted 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 glutamate receptor activation is dependent on sodium and chloride entry (39). In a Ca2+-dependent manner, activation of NMDA receptors stimulates NKCC1 activity in neurons (41). Blocking NKCC1 activity with bumetanide abolishes the glutamate-triggered sodium and chloride accumulation by over 50% (3). Oxygen-glucose deprivation (OGD)-induced neuronal death is also mediated by NMDA ionotropic receptor-triggered excitotoxicity. Bumetanide or genetic deletion of NKCC1 strongly reduces cell death triggered by OGD or by application of NMDA in cultured neurons (3, 8). In hippocampal slices, bumetanide prevents long-term decreases in neuronal [Cl–]i that are associated with neuronal death after OGD.

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, high 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 phenoobarbital, for the treatment of other neurological disorders, such as neonatal seizures (FIND insulin no. 101698; see http://www.cureepilepsy.org/research/current.asp; Refs. 25, 24, 28), and with adults medically intractable and surgically unresolvable temporal lobe epilepsy 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 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 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 not the SUR1-regulated NCCa-ATP channel might yield incomplete inhibition of edema. However, this is a testable hypothesis that can be the subject of future research.

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