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

Molecular Mechanisms of Ischemic 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 hemiepithactomy, 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 water, which causes cell swelling or shrinkage. Ischemia-triggered increases in NKCC1 and other ion carriers can cause cytotoxic swelling is countered by regulatory volume decrease, which involves the cellular loss of chloride and potassium via the activation of the K-Cl cotransporter, resulting in the influx of sodium (with chloride and potassium) and water, thereby increasing cell volume. Acute cell shrinkage is countered by regulatory volume increase, which triggers the activation of 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, resulting in the influx of sodium (with chloride and potassium) and water, thereby increasing cell volume. Acute cell shrinkage is countered by regulatory volume increase, which triggers the activation of 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, resulting in the influx of sodium (with chloride and potassium) and water, thereby increasing cell volume.
Efflux of these solutes by active, energy-dependent mechanisms involves the Na+-K+-2Cl–/HCO3
exchange (33). Other sodium influx pathways like the Na+/H+ exchanger (NHE), working in concert with the Cl-/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 KCl cotransporters and swelling-activated potassium and chloride channels. Ischemia-triggered increases in NKCC1 and other ion carriers can cause cytotoxic edema or iso-osmotic cell swelling.

In the setting of 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 neuroglial 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 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.
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), 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 proteins, 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 can be blocked by the chloride channel blocker bumetanide (20). However, 

![Diagram showing the mechanisms of cytotoxic, ionic, and vasogenic edema contributing to brain swelling.](https://www.physiologyonline.org)
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/chloride cotransporter (NKCC1) channel. Opening of these channels allows sodium (and calcium) to enter cells. The net influx of ions 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 edemas: 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 intracellular and 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 is increased. As 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 \( K_{H} \) and \( K_{O} \) 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 ion edema, \( K_{H} \) and \( K_{O} \) are increased due to an increased transport of ions and water and transport pathways like the NKCC1, the SUR1-regulated NCCa-ATP channel, and aquaporin water channels (9, 42, 32). Because \( K_{H} \) remains close to zero, \( J_{v} \) is positive, and transendothelial fluid transfer occurs, resulting in the accumulation of ionic edema fluid. With vasogenic edema, both \( K_{H} \) and \( K_{O} \) are increased, with the increase in \( K_{H} \) due to the degradation of endothelial cell tight junctions (43). Due to the cytotoxic edema of neurons, the brain's extracellular space is depleted of ions and water, generating gradients that drive the movement of solute 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. 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 \( (\sigma_{s}) \), interstitial osmotic pressure \( (\pi_{i}) \), and two distinct filtration coefficients, the hydraulic conductivity \( (K_{H}) \) and osmotic conductivity \( (K_{O}) \) are altered.

Alterations in the hydraulic coefficients \( K_{H} \) and \( K_{O} \) underlie the formation of ionic and vasogenic edema. Because of the limited transcellular 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 \( K_{H} \) and \( K_{O} \) 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 ion edema, \( K_{H} \) and \( K_{O} \) due to an increased transport of ion and water and transport pathways like the NKCC1, the SUR1-regulated NCCa-ATP channel, and aquaporin water channels (9, 42, 32). Because \( K_{H} \) remains close to zero, \( J_{v} \) is positive, and transendothelial fluid transfer occurs, resulting in the accumulation of ionic edema fluid. With vasogenic edema, both \( K_{H} \) and \( K_{O} \) are increased, with the increase in \( K_{H} \) due to the degradation of endothelial cell tight junctions (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 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-independent 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 SGLT1-regulated NCCa-ATP channels, non-voltage- or ligand-gated sodium channels on the luminal and ad luminal 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 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 metalloproteins; matrix metalloproteins 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, across plasma membranes of cells, are intrinsic membrane proteins that transport chloride ions, together with sodium and/or potassium ions, across plasma membranes of cells. The stoichiometrically similar chloride transporters in the brain's extracellular space. Chloride and water follow via chloride channels and aquaporins (e.g., aquaporin-1), respectively.

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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, astrocyste-derived, interleukin-6 (IL-6) activates the 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 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, 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 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 ~50% 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 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 astrocytes in ischemia-induced brain edema. NKCC1 activation elicits excessive solute uptake (9, 37).

During early phases of ischemia, partial failure of the Na+/K+ ATPase and neural 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 radioabeled 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 unlesioned 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. Acute excitotoxic neurodegeneration after stroke is associated with neuronal death after OGD or by application of NMDA in cultured neurons [3, 8]. In hippocampal slices, bumetanide prevents OGD or by application of NMDA in cultured neurons [3, 8]. In hippocampal slices, bumetanide prevents 

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 neuronal seizures (FDA IND no. 101690; see http://www.cureepilepsy.org/research/current.asp), and adults with medically intractable and surgically unresectable temporal lobe epilepsy [25, 24, 20]. The use of bumetanide specifically target NKCC1, and thus do not have the side effects of NKCC2 inhibitors, which are used at doses that provide specific inhibitory action of NKCC1 in the CNS, without a profound diuretic effect [24]. Thus the use of bumetanide might be beneficial for the treatment of ischemic cerebral edema.

References

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