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Cytotoxic edema: mechanisms of pathological cell swelling

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Abstract

Cerebral edema is caused by a variety of pathological conditions that affect the brain. It is associated with two separate pathophysiological processes with distinct molecular and physiological antecedents: those related to cytotoxic (cellular) edema of neurons and astrocytes, and those related to transcapillary flux of Na^+ and other ions, water, and serum macromolecules. In this review, the authors focus exclusively on the first of these two processes. Cytotoxic edema results from unchecked or uncompensated influx of cations, mainly Na^+, through cation channels. The authors review the different cation channels that have been implicated in the formation of cytotoxic edema of astrocytes and neurons in different pathological states. A better understanding of these molecular mechanisms holds the promise of improved treatments of cerebral edema and of the secondary injury produced by this pathological process.

Keywords

cation channel; cytotoxic edema; hypoxia; stroke; sulfonylurea receptor 1; traumatic brain injury

CYTOTOXIC EDEMA IN THE CNS is typically accompanied by brain swelling. Edema can result from almost any insult to the brain, including trauma, infarction, neoplasm, abscess, or conditions such as hypoxia or toxic or metabolic perturbation.10,93,94 Stroke and traumatic brain injury are especially prevalent causes of morbidity and mortality. In the US, stroke is the third most common cause of death, with more than 730,000 first-time incidents each year.47,126 Traumatic brain injury afflicts 1.4 million people yearly, resulting in 50,000 deaths and 235,000 hospitalizations.82,83,131,151

Cytotoxic edema is defined as the premorbid cellular process, otherwise known as cellular edema, oncotic cell swelling, or oncosis, whereby extracellular Na^+ and other cations enter into neurons and astrocytes and accumulate intracellularly, in part due to failure of energy-dependent mechanisms of extrusion. Unchecked influx of cations occurs largely through cation channels. Cation influx, in turn, drives influx of anions, which maintains electrical neutrality, and in combination these phenomena drive influx of water, resulting in osmotic expansion of the cell, that is, cytotoxic edema. Cytotoxic edema by itself does not result in brain swelling, but formation of cytotoxic edema depletes the extracellular space of Na^+, Cl^-, and water, thereby creating a new gradient for these molecules across the capillary of the blood–brain barrier. With appropriate changes in capillary permeability,138 the new gradient created by cytotoxic edema results in driving transcapillary formation of ionic

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edema. Thus, cytotoxic edema is important in its own right, because it signals a premorbid cellular process that almost inevitably leads to oncotic or necrotic cell death. But equally important, cytotoxic edema supplies the driving force for the formation of ionic edema, which is the process that introduces new mass (Na^+, Cl^-, H_2O) that is ultimately responsible for brain swelling.138

Excellent reviews that summarize current knowledge on this topic have been published.10,21,37,46,71-73,112,125,166 In our recent review,138 we examined molecular mechanisms involved in transcapillary flux of Na^+, water, plasma ultrafiltrate, and blood that lead to brain swelling. In this paper, we review molecular mechanisms involved in cytotoxic edema.

Pathophysiology of Cytotoxic Edema

When an insult to the brain results in ischemia or hypoxia, very little new ATP can be produced due to abrogation of oxidative phosphorylation.56 Cells quickly use up their reserves of ATP and, unless normoxia is restored, the deranged cellular machinery loses its ability to sustain homeostasis. Primary active transport, mainly ATP-dependent Na^+/K^+ ATPase that requires continuous expenditure of ATP, is necessary to maintain homeostais.146,162 The balance between survival and death is determined by the struggle between electrogenic pump activity and channels that enable Na^+ to enter into cells.137,161 Cellular survival requires that Na^+ be continuously extruded from the intracellular compartment, because this is critical to maintaining normal cell volume.

Depletion of ATP is accompanied by unchecked influx of extracellular ions, primarily Na^+, down their electrochemical gradients. This influx is driven by energy stored in preexisting ionic gradients across the cell membrane.84 Sodium ion influx in turn drives Cl^- influx via chloride channels, and the resultant increase in intracellular osmolarity drives inflow of water via AQP channels (among others).8,11,12,46,71-73,75 Extracellular water flows into the cell interior, resulting in an increase in intracellular fluid volume, at the expense of the extracellular space. Morphologically, this process results in alterations in membrane surface architecture with prominent bleb formation. In the initial stages of cytotoxic edema, the blood–brain barrier is intact and largely impermeable to ions and fluids, so extracellular ions and water loss are not replenished. Thus, fluid movement involved in formation of cytotoxic edema does not lead to any change in total brain volume, despite the observable increase in cell size.

Cells in both gray and white matter are affected by cytotoxic edema.52 Cellular swelling begins within 30 minutes of MCA occlusion, particularly around capillaries, persists for up to 24 hours after reperfusion,43,44 and results in an average reduction of extracellular space from the normal 20% down to 4 to 10%.86,147 Astrocytic swelling is much more prominent than neuronal swelling. Astrocytes are more prone to pathological swelling than neurons, because they are involved in clearance of K^+ and glutamate, which cause osmotic overload that in turn promotes water inflow. Astrocytic but not neuronal NKCC is upregulated by elevated extracellular K^+ and cell swelling.100,142,143 Expression of high levels of the water channel AQP4 is also important.90,91

When compensatory mechanisms such as ionic pumps in the plasma membrane are exceeded or fail altogether, the swollen cell dies.148 This pathway to cell death was called oncosis (derived from the Greek word “onkos,” which means swelling) by von Recklinghausen, specifically to describe cell death by swelling. This term is a more specific way of classifying cellular demise than the less precise terms “accidental cell death” or “necrosis.” Oncotic cell death also differs in important ways from apoptotic cell death. At the electron-microscopic level, the difference between the two pathways leading to cell

death becomes apparent: oncosis leads to cells that show a noticeable increase in volume and presents with membrane damage to plasmalemma and other organelle membranes, along with loss of membrane phospholipids and disappearance of stainable nuclei at the late stage;14,15 in contrast, apoptosis presents with cell involution and shrinkage89,111 before death ensues.

Cation Channels Involved in Cytotoxic Edema

Experimental evidence shows a clearly delineated sequence of metabolic responses of brain tissue to a decrease in blood flow.60-62,66,69 The brain area where blood flow is either absent or measures less than 10 ml/100 g (brain tissue)/min is rapidly and irreversibly damaged in less than 6 minutes, forming an “ischemic core.” This infarcted tissue is surrounded by the “penumbra” of hypoxic but living tissue with blood flow greater than 20 ml/100 g (brain tissue)/min. Cells in the penumbra undergo cytotoxic edema and other changes that are potentially reversible if perfusion is restored within the first few hours after injury. If hypoxic conditions persist, however, penumbral cells with cytotoxic edema eventually die, extending the course of cell death deeper into the parenchyma than the originally involved core. The penumbra, therefore, is the main therapeutic target in the prevention of ischemic stroke and injury.

A number of studies have now shown that pharmacological inhibition of ion channels, including nonselective cation channels, reduces focal ischemic injury in rodent models of ischemic stroke.59,98,118,172 Nonselective cation channels are distinguished from selective cation channels by their permeability properties; ion selective channels are typically permeable to a single cation, such as Na^+, K^+, or Ca^{2+}, whereas a nonselective cation channel may allow flux of any monovalent cation, or even a mixture of monovalent and divalent cations. It is likely that these channels play an important role in secondary injury in the penumbra,139 and thus targeting these channels offers the possibility of reducing secondary injury. In the sections that follow, we review several of the nonselective cation channels that have been implicated in cytotoxic edema and secondary injury in the penumbra.

The ASIC Channel

Acid-sensing ion channels are members of the recently discovered epithelial sodium channel/degenerin gene family of ion channels. Acid-sensing ion channel genes encode proton-gated cation channels in both the central and peripheral nervous system.153,154 Six different ASIC subunits have been cloned to date, which are encoded by four genes, *ASIC1–ASIC4*.16

Acid-sensing ion channels are hydrogen ion–gated cation channels that are activated as pH falls but are generally inactive at physiological pH (7.4). All ASICs are permeable to Na^+ and, to a lesser degree, to Ca^{2+} and are blocked by amiloride. Activation of these channels leads to an increase in cell excitability.

First noted in sensory neurons,77-79 and later implicated in acid-induced nociception in mammals,152 ASICs have most recently been shown to be involved in the detection of ischemic pain.105,170 The ASIC subunits ASIC1a and ASIC2a have attracted scientific attention in the context of neuroprotection. Ischemia and hypoxia result in a marked reduction in tissue pH due to uncontrolled generation of lactic acid, and acidosis is an important determinant of neurological injury.106,124 The ASIC1a subunit may be responsible for acidosis-mediated, glutamate receptor-independent neuronal injury.167,168 The probability of opening of ASIC1a increases as the pH decreases below 7.0, and activation is half of maximum at a pH of 6.2, which is in the range of pH that is thought to

occur within the penumbra and core of an infarct, especially in the context of hyperglycemia.106 Activation of ASIC1a is promoted by stretching of the membrane, release of arachidonic acid, production of lactate,6,64 or a drop in extracellular Ca^{2+} concentration,65 conditions that occur within an infarct as cells swell, Ca^{2+}-dependent phospholipases are activated, and Ca^{2+} influx occurs.19

Activation of ASIC1a in vitro results in an increase in intracellular Ca^{2+} and induces time-dependent neuronal injury that occurs in the presence of the blockers of voltage-gated Ca^{2+} channels and glutamate receptors. In rodent in vivo models of ischemic stroke, intracerebroventricular administration of the ASIC1a blockers amiloride and tarantula toxin (psalmotoxin 1) prior to onset of ischemia, as well as knockout of the ASIC1a gene, reportedly prevents ischemic injury.168

The channel ASIC2a has garnered particular interest because transient global ischemia induces its expression in the rat brain, including in neurons of the hippocampus and cortex. 68

The SUR1-Regulated $NC_{Ca\text{-}ATP}$ Channel

The $NC_{Ca\text{-}ATP}$ channel is a novel cation channel that conducts all inorganic monovalent cations, but is impermeable to Ca^{2+} and Mg^{2+}.26 Opening of this channel requires nanomolar Ca^{2+} on the cytoplasmic side. Physiological levels of ATP intracellularly block $NC_{Ca\text{-}ATP}$ channel opening, whereas depletion of ATP triggers channel opening.

The $NC_{Ca\text{-}ATP}$ channel is believed to be composed of poreforming and regulatory subunits. The regulatory subunit is SUR1, the same as that for K_{ATP} channels in pancreatic β cells.25 Knockdown of SUR1 using antisense oligodeoxy-nucleotide reduces SUR1 expression137 and prevents expression of functional $NC_{Ca\text{-}ATP}$ channels (Simard and Chen, unpublished data). Because SUR1 is involved in channel regulation, pharmacological agents that affect the SUR1-regulated K_{ATP} channel also affect the $NC_{Ca\text{-}ATP}$ channel. Thus, $NC_{Ca\text{-}ATP}$ channel opening is blocked by sulfonylurea compounds such as tolbutamide and glibenclamide, and channel activity is increased by diazoxide.

The $NC_{Ca\text{-}ATP}$ channel is not constitutively expressed, but is expressed in the CNS following hypoxia or injury. The channel was first discovered in freshly isolated reactive astrocytes obtained from the hypoxic inner zone of the gliotic capsule.25,26 Since then, it has also been identified in neurons from the core of an ischemic stroke.137 In rat models of ischemic stroke, the SUR1 regulatory subunit is transcriptionally upregulated in neurons, astrocytes, and capillary endothelial cells.

The consequences of channel opening have been studied in isolated cells that express the channel, by depleting ATP using Na^+ azide or Na^+ cyanide in addition to 2-deoxyglucose, or by using diazoxide to open the channel without ATP depletion. These treatments induce a strong inward current that depolarizes the cell completely to 0 mV and induces cytotoxic edema and cell blebbing. These effects are reproduced without ATP depletion by diazoxide. 26 After these treatments, cells die predominantly by oncosis, not by apoptosis.137

The effect of channel blocking using glibenclamide has been studied in vitro in reactive astrocytes that express the channel.25,137 In cells exposed to Na^+ azide to intentionally deplete ATP, glibenclamide blocks membrane depolarization, significantly reduces blebbing associated with cytotoxic edema, and significantly reduces oncotic cell death.

The effect of channel blocking using glibenclamide has also been studied in vivo in two rat models of ischemic stroke.137 In a model of massive ischemic stroke with malignant

cerebral edema associated with high mortality (68%), glibenclamide reduced mortality and cerebral edema (excess water) by half. In a model of stroke induced by thromboemboli with delayed spontaneous reperfusion, glibenclamide reduced lesion volume by half, and its use is associated with cortical sparing attributed to improved leptomeningeal collateral blood flow due to reduced mass effect from edema.

The TRP Channels

The TRP channel superfamily derives its name from its role in *Drosophila* phototransduction. This family contains more than 50 members, 28 of which are known to be expressed in mammals. These channels vary in their modes of activation. Some TRP channels are constitutively open, and others react to diverse stimuli such as pH, redox state, osmolarity, stretching, voltage, and intracellular Ca^{2+}.109,164 Some of these channels are selective for Ca^{2+}, and others are nonselective and permeable to monovalent and/or divalent cations. The TRPs are subdivided into six subfamilies based on homology. The TRP proteins tend to form heteromultimers and can exhibit interdependent expression.1,122

Analysis of promoter regions of TRPC and TRPM subfamily members TRPC1−7 and TRPM1−8 shows these members to possess multiple consensus binding sites for one or more of the transcription factors linked to ischemic stroke, suggesting possible involvement in hypoxic injury to CNS.139

Recent in vitro work unmasked a Ca^{2+}-mediated cell death mechanism associated with a Ca^{2+}-permeable non-selective cation conductance carried by TRPM7 in cultures of mixed cortical neurons subjected to oxygen/glucose deprivation, followed by a return to normoxic conditions with an antiexcitotoxic combination.1 Suppressing TRPM7 expression blocked TRPM7 currents, which are known to be potentiated by acidosis, anoxic Ca^{2+} uptake, production of reactive oxygen species, and anoxic death.67 Most important, channel blocking eliminated the need for the antiexcitotoxic mixture and permitted the survival of neurons previously destined to die from prolonged anoxia.1 Both TRPM7 and TRPM2 are now believed to be important contributors to the paradoxical increase in intracellular Ca^{2+} levels that can lead to cell death following restoration of extracellular Ca^{2+} and/or postischemia.2,3,88,95,107 The subfamily member TRPM2 is abundantly expressed in the brain, where it functions as a cell death–mediating Ca^{2+}-permeable cation channel. It possesses both ion channel and ADP-ribose hydrolase functions. Stress-related accumulation of cytosolic ADP-ribose released from mitochondria is required for gating of TRPM2 channels.120 Inhibition of TRPM2 function by poly-ADP-ribose-polymerase-1 inhibitors protects cells from oxidative stress-induced death.53,98,99

Studies of TRPC channels in vivo suggest that they serve as redox sensors49 in regulating endothelial barrier function,5 which is crucial in the formation of edema in ischemic stroke. 138 The TRPC4 protein is also found to be significantly elevated in the rat striatum and hippocampus in rat models of MCA occlusion, in which immunoreactivity is localized to neuronal membranes.42 Numerous other TRP proteins have been shown to be localized in the mammalian vasculature.13,39,85,157,174

The TRPV1 subunit, widely expressed in the brain, has been suggested to be involved in neurodegenerative mechanisms occurring in ischemia and hypoxia.92,97 In a model of global cerebral ischemia in gerbils, both capsaicin118,119 and rimonabant20,55 exert neuroprotective effects that are at least partially attributable to TRPV1.

The NKCC Channel

The electroneutral cotransporter NKCC is encoded by a gene from the cation–chloride cotransporter family. This channel mediates the coupled movement of Na^+ and/or K^+ with

Cl^-, with a stoichiometry of $1Na^+:2K^+:2Cl^-$. Activity of this transporter is involved in regulatory ion responses of glia, neurons, endothelium, and choroid plexus epithelial cells.144,156,165,173 Although two isoforms are found, only NKCC1, the "housekeeping" isoform of the NKCC channel, plays a role in sodium secretion and absorption, cell volume regulation, and maintenance of intracellular Cl^- concentration in the CNS.32,50,130,169 Loop diuretics, such as bumetanide, can inhibit the channel.38

The NKCC1 isoform is involved in secondary transport of inorganic ions. The driving force for ion flux originates in the Na^+ gradient created by Na^+/K^+-ATPase, with an important contribution of the Cl^- gradient in epithelial cells. The NKCC cotransporter requires that all three ions (Na^+, K^+ , and Cl^-) be simultaneously present on the same side of the membrane.130 A decrease in intracellular Cl^-, hypertonic stress, increased intracellular Ca^{2+}, and β-adrenergic receptor stimulation31,34,81,135 result in phosphorylation of NKCC1, which increases channel activity. Kinases and phosphatases contribute to NKCC1 regulation through their opposing effects.31,35,51,87,121

The NKCC1 isoform plays an important role in maintaining physiological intracellular Na^+ concentration levels. However, in pathological situations, such as ischemia and hypoxia, it has been shown to contribute to excessive Na^+ inflow, which results in cytotoxic edema. In vitro data in neurons show that loss of Cl^- is a sufficient and necessary stimulus for activation of NKCC1.135 Similarly, work in astrocytes shows elevated levels of extracellular K^+ to be sufficient and necessary to activate astrocytic NKCC1. Genetic ablation of NKCC1, as well as its block by bumetanide,17 causes a decrease in intracellular Cl^- in hypoxic neurons145 and blocks Na^+- and Cl^--mediated cell swelling in astrocytes.101,142,143

In vivo studies have shown that intracerebral bumetanide administered via a microdialysis probe, either prior to or during ischemia/hypoxia insult caused by temporary MCA occlusion, is neuroprotective, ameliorates brain damage, and reduces brain edema in rat focal ischemia,141,172,173 reinforcing the in vitro data. Focal cerebral ischemia in rats results in elevated NKCC1 protein levels in the ipsilateral cortex and striatum.173 Together, these data show that NKCC1 merits further study to clarify its role in the early stages of ischemic cytotoxic damage.

The NMDA Receptor Channel

The ionotropic glutamate receptor channels are designated by three main classes, based on their preferential affinity to agonists. One class, the NMDA receptor channels, is unique because it is ligand-gated by a concurrent binding of glutamic acid and glycine and is voltage-dependent. At resting membrane potential, this receptor channel is blocked by an "Mg^{2+} plug," even if both the agonists are occupying their respective binding sites. Depolarization of the cell membrane removes this Mg^{2+} block and allows the channel to conduct Na^+, K^+, and Ca^{2+}.102,134 This duplex regulation is an integral mechanism in cellular control of Ca^{2+} homeostasis in neurons. The calcium ion participates in multiple molecular mechanisms involved in various cellular processes, but also causes cell death via activation of Ca^{2+}-dependent proteases, formation of reactive oxygen species, phospholipase A2, and mitochondrial damage.80

Glutamate is the principal neurotransmitter in the CNS. The NMDA receptor channels are found on most neurons,158,159 where they are involved in multiple crucial aspects of physiological and pathological brain activity.30 Under resting conditions, the glutamate concentration in the synaptic cleft consists of approximately 0.6 μM.23,29 During hypoxic or ischemic insult, values as high as 320 μM are reached and sustained for a period of minutes to hours,149 resulting in cell depolarization, removal of the Mg^{2+} block, and an

unregulated influx of Na^+ and Ca^{2+} into the cell, with consequent cell death by excitotoxicity.128,129 It is not solely the amount of released glutamate at the synapses,29 but also the duration of exposure to this excitatory neurotransmitter, that is believed to cause injury.160

Neuronal death in stroke, trauma, and other neurological disorders has been directly linked to the activation of NMDA receptor channels and consequent Ca^{2+}-mediated excitotoxicity.57,104 It is generally agreed that acute excitotoxic neurodegeneration after glutamate receptor activation could be Ca^{2+}-independent, but dependent on Na^+ and Cl^- entry.28,127 Activation of NMDA receptors triggers a significant increase in intracellular Na^+ and Cl^- content in cortical neurons.155 Blockage of Na^+ and Cl^- entry by removal of extracellular Na^+ and Cl^- abolishes NMDA-mediated neurodegeneration.27,54

The NMDA receptor channel remains one of the most studied pertaining to CNS pathology. Several NMDA antagonists are currently undergoing clinical trials. Memantine, a channel blocker24 used as part of a combination therapy, has shown great promise in improving dementia symptoms. Other diseases, such as Parkinson and Huntington diseases, multiple sclerosis, amyotrophic lateral sclerosis, and epilepsy might benefit from treatment with NMDA antagonists.18,45,96,117,133 To date, however, NMDA receptor strategies have not been successful at a clinical level in treating ischemia- or hypoxia-related injury, despite in vitro and in vivo data that strongly support this approach.123

The AQP Channels

Transport of water across biological membranes occurs passively. Water molecules can dissolve in lipid bilayers and move across cell membranes at a low but finite rate by simple diffusion. Yet because this process is inefficient, plasma membranes of many types of cells have developed specialized water channels (the AQPs) that serve as passive conduits for water transport, greatly increasing membrane water permeability.4,76 The AQP channels are small transmembrane proteins, initially described in water transport in nephrons, that selectively transport water and in some cases glycerol, urea, and even hydrogen peroxide.22 Thirteen different AQPs have been identified in mammals. Seven members of the AQP family have been described in the CNS to date.

The AQP1 type is found in the apical domain of the choroid plexus epithelial cells, in vasculature,58,140 dorsal root ganglia cell bodies, and in both the peripheral and central branches of primary afferent neurons.136 It may be involved in human brain tumor edema formation113 and nitric-oxide–dependent vasorelaxation,58 as well as in nociceptive processing. For three AQP channel types—AQP3, AQP5, and AQP8—mRNA is found in astrocytes, with levels altered by hypoxia.171 The AQP4 type, the most abundant and well-studied member of the AQP family found in the brain, is predominantly expressed in astrocytic foot processes surrounding blood vessels and ependymocytes facing capillaries and cerebrospinal fluid.40,109 It is implicated in the formation of brain edema.70,74,108,109 The proximity of the AQP4 channel to synapses of neurons allows for efficient clearance of K^+ and water from synaptic junctions after excitation of neurons.103

Studies of AQP4-null mice demonstrated no obvious neurological abnormalities. These mice, however, show significantly lower levels of brain swelling after cellular edema produced by either acute water intoxication or ischemic stroke.91 Interestingly, transient MCA occlusion results in temporary loss of perivascular AQP4 in the neocortex,41 possibly providing an explanation for the pathophysiology involved in salutary effects of hypoxic preconditioning. In pneumococcus-induced meningitis as well, brain edema, as well as mortality, were significantly reduced116 in the absence of AQP4. None of the benefits reported in AQP4-knockout models with MCA occlusion, however, is observed in vasogenic

edema, cortical freeze injury, and brain tumor models; in contrast, brain swelling, intracranial pressure, and general outcome are all worse than in controls.114,115 Also, AQP4 deficiency impairs astroglial cell migration in brain injury.9,132

Two different isoforms of AQP9 are localized to mitochondria in tanycyte and astrocyte cell bodies11,12 and midbrain dopaminergic neurons.7 It is believed that the flux of lactate and other metabolites through AQP9 enables mitochondria to adjust to extramitochondrial cytoplasm. The AQP11 isoform is found in the endoplasmic reticulum in Purkinje cells, hippocampal neurons of CA1 and CA2, and cerebral cortical neurons.48

Given the functional synergy between ion channels and AQPs, it is only logical to expect that attempts would be made to target AQP in the brain therapeutically using either agonists or antagonists. Several carbonic anhydrase inhibitors,63 as well as quaternary ammonium compounds,33 mercurial sulfhydryl compounds, lithium, silver, and gold,110 have been shown to inhibit AQPs, but these compounds are too nonspecific and/or toxic for in vivo use.

Conclusions

Excessive accumulation of brain water in cerebral edema is of central importance in neurosurgery.36,37 Brain edema leads to worsening of ischemia, and often to herniation and death. Despite large-scale multinational interest in neuroprotection, hyperosmolar agents and supportive surgical procedures such as cerebrospinal fluid diversion and surgical decompression remain the only current treatments for brain swelling.10,150,163

Advances in translational research now clearly identify cytotoxic edema as the initial, and most important, reversible first step in the sequence that leads to ionic edema, vasogenic edema, and complete hemorrhagic conversion. Cytotoxic edema is important in all brain cells, but is most conspicuous in astrocytes. A number of non-selective cation channel blockers show promising salutary effects in cerebral ischemia or ischemic stroke models, consistent with possible involvement of their targets, including ASIC, SUR1-regulated $NC_{Ca\text{-}ATP}$ channels, and TRP channels in hypoxic insults. Although much work remains before appropriately targeted therapies can be implemented in the clinical setting, recent developments in molecular medicine hold great promise for new avenues in which CNS injury can be ameliorated.

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Abbreviations used in this paper

ADP	adenosine diphosphate
AQP	aquaporin
ASIC	acid-sensing ion channel
ATP	adenosine triphosphate
CNS	central nervous system

CSF	cerebrospinal fluid
MCA	middle cerebral artery
$NC_{Ca\text{-}ATP}$	calcium-activated, ATP-sensitive nonselective cation
NKCC	$Na^+/K^+/2Cl^-$ cotransporter
NMDA	*N*-methyl-D-aspartate
SUR1	sulfonylurea receptor 1
TRP	transient receptor potential
TRPC	TRP canonical
TRPM	TRP melastatin
TRPV	TRP vanilloid

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