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Review

Ionotropic receptors and ion channels in ischemic neuronal death and dysfunction

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Loss of energy supply to neurons during stroke induces a rapid loss of membrane potential that is called the anoxic depolarization. Anoxic depolarizations result in tremendous physiological stress on the neurons because of the dysregulation of ionic fluxes and the loss of ATP to drive ion pumps that maintain electrochemical gradients. In this review, we present an overview of some of the ionotropic receptors and ion channels that are thought to contribute to the anoxic depolarization of neurons and subsequently, to cell death. The ionotropic receptors for glutamate and ATP that function as ligand-gated cation channels are critical in the death and dysfunction of neurons. Interestingly, two of these receptors (P2X7 and NMDAR) have been shown to couple to the pannexin-1 (Panx1) ion channel. We also discuss the important roles of transient receptor potential (TRP) channels and acid-sensing ion channels (ASICs) in responses to ischemia. The central challenge that emerges from our current understanding of the anoxic depolarization is the need to elucidate the mechanistic and temporal interrelations of these ion channels to fully appreciate their impact on neurons during stroke.

Keywords: stroke; anoxic depolarization; ionotropic receptors; NMDA receptors; P2X7 receptors; ion channels; pannexin channels; TRP channels; acid-sensing ion channels (ASICs)

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Introduction

Ischemia is a consequence of the loss of blood flow to tissues or organs. The principle result is a restriction in the delivery of the energy substrates, oxygen and glucose, to cells in the affected area. It is important to note that ischemia also causes several other detrimental stimuli, including acidosis (hypercapnia) and cessation of blood flow that is likely important for regulation of vascular tone. In the laboratory *in vitro* setting, ischemia is typically modelled as its constituent components, anoxia, hypoglycemia, O_2/glucose deprivation (OGD), or acidification; the primary reason being that it makes dissecting the complex molecular mechanisms of cellular death and dysfunction more tractable.

In the brain, ischemia occurs as a consequence of stroke or cardiac arrest. One of the early, major effects of ischemia on neurons is the appearance of a large inward current that is carried by cation influx, and is responsible for the anoxic depolarization (AD). The AD can be measured *in vitro* and *in vivo*, and consists of a rapid loss of membrane potential, unregulated calcium influx, loss of the neuron's ability to produce ATP and characteristic dendritic and axonal beading[1–3]. The focus of this review is to summarize the roles of some of these cation channels and their contribution to the AD and its inward currents. Specifically, glutamate-gated ion channels, ATP-binding purinergic receptors, pannexin1 channels, and other channel mediators (TRP and ASIC) will be discussed. One central challenge of the field is to reconcile the temporal and mechanistic relationships of these various ion channels, so that effective therapeutics for neuroprotection and enhanced recovery can be developed.

Glutamate-gated ionotropic receptors and ischemia

The longstanding paradigm of ischemia-induced cell death has been that uncontrolled opening of ionotropic glutamate receptors induces excitotoxcity, and that this death of neurons by over-activation is responsible for the neurological deficits following stroke. In response to increased release of presynaptic glutamate[4] or reversal of uptake mechanisms in astrocytes[5], there is an accumulation of extracellular glutamate and subsequent over-stimulation of post-synaptic *N*-methyl-*D*-aspartate (NMDA) and 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) receptors that mediate cationic inward

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currents (Figure 1).

NMDA receptors (NMDARs) are cation permeable ligand-gated ion channels that have prominent roles in physiological synaptic functions. Activation of NMDARs contributes to the plasticity of synaptic strength, and by extension, the sub-cellular mechanisms underlying some forms of learning and memory. NMDARs are unique from other ligand-gated ion channels insofar as they are "coincidence detectors"; not only do they require glutamate and glycine (or *D*-serine) as co-agonists to induce channel opening[6–9], but they also need

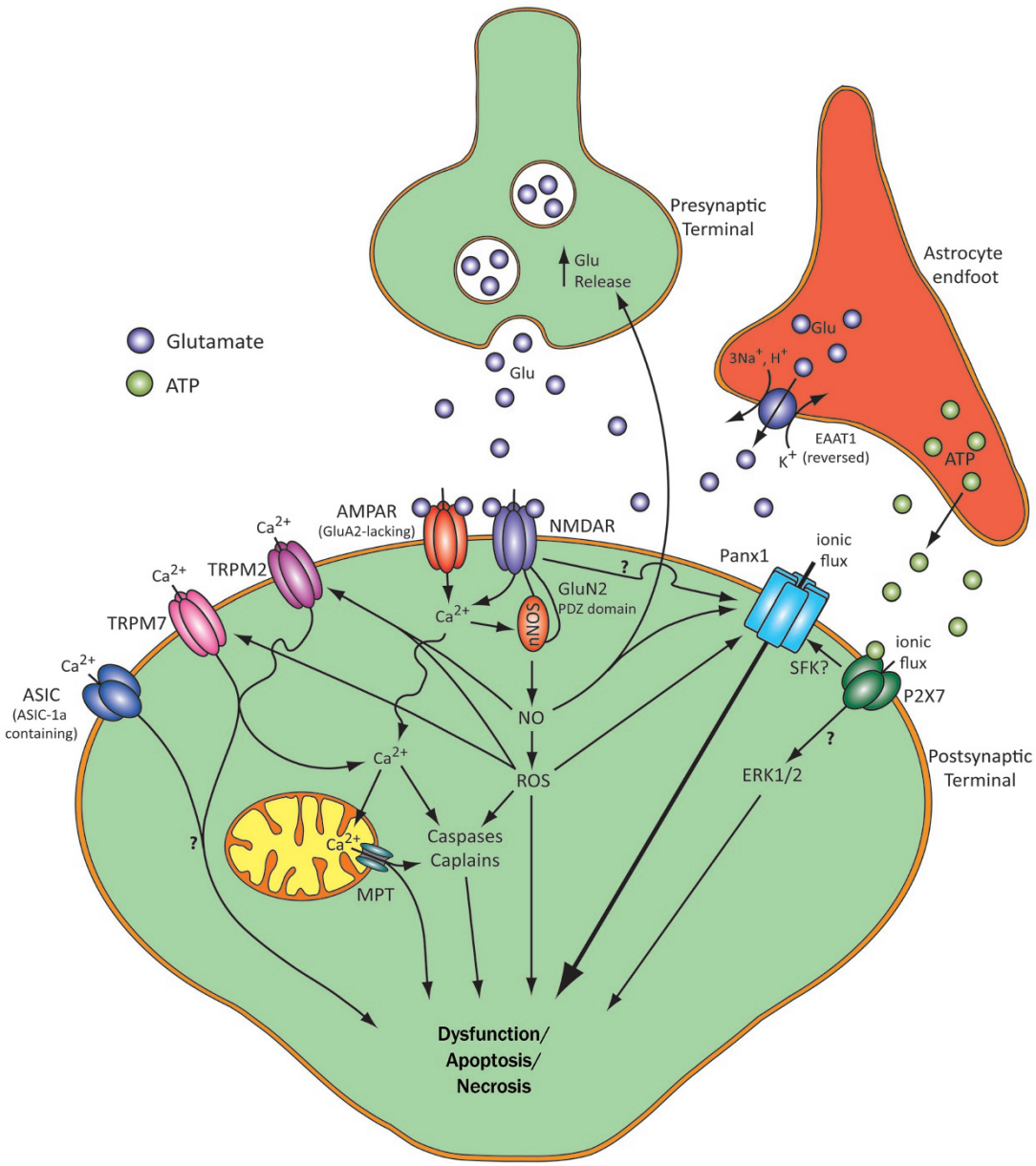

Figure 1. Key ion channels that contribute to cell death signaling cascades during ischemia. For clarity, channels in the post-synpatic membrane are highlighted, but it is important to note that both pre-synpatic and astrocytic channels are also likely critical. Ischemia triggers enhanced presynaptic glutamate release and reversal of the astrocytic glutamate reuptake transporter (EAAT1), amounting to a dramatic increase in glutamatergic signaling via postsynaptic NMDARs and AMPARs. Ca^{2+} through NMDARs and GluA2-lacking AMPARs can stimulate NO production by Ca^{2+}-dependent nNOS, which can react with reactive oxygen species (ROS) to form highly damaging intermediates. NO and NO-ROS reaction products (i.e. peroxynitrite) may activate TRPM2/7 and Panx1 channels. Retrograde diffusion of NO can enhance presynaptic glutamate release, further exacerbating postsynaptic excitotoxicity. Decreases in extracellular pH leads to ASIC opening and Ca^{2+}-influx (for ASIC-1a homomers), which may in turn contribute to cell death. Increases in extracellular ATP concentrations stimulate P2X7 opening, possibly activating Panx1 channels via Src Family Kinases (SFK), as well as stimulating ERK1/2 function to induce cell death. Activation of any/all of the ion channels mentioned here will yield an increase in intracellular Ca^{2+} levels, which can activate downstream caspases, calpains, and trigger mitochondrial permeability transition; all of which have been implicated in neuronal dysfunction/apoptosis/necrosis.

a concurrent membrane depolarization to alleviate a voltage-sensitive Mg^{2+} block in the pore[10]. NMDAR opening allows influx of Na^{+} (which will contribute to membrane depolarization), and more importantly Ca^{2+} influx, which mediates subsequent physiological as well as pathophysiological effects.

NMDARs are heterotetramers comprised of GluN1 (ubiquitously expressed), GluN2(A-D) and GluN3(A-B) subunits; the resultant receptor composition will govern Ca^{2+} permeability, and the affinity of Mg^{2+} for the pore and glutamate and glycine at their ligand-binding sites[11–14]. In that light, NMDAR contribution to glutamate excitotoxicity is thought to be directly dependent on subunit expression. For example, GluN3A-containing NMDARs are impermeable to Ca^{2+}, have decreased Mg^{2+} block[11, 15], and have been reported to be neuroprotective during ischemia[16].

Ca^{2+} conductance through NMDARs can promote both apoptotic and necrotic pathways. One means through which NMDARs achieve this is via direct activation of neuronal nitric oxide synthase (nNOS; Figure 1), which is anchored in a complex with NMDARs via a PSD-95 binding domain on the C-terminus of GluN2 subunits[17]. Ca^{2+} currents through NMDARs transiently activate nNOS to produce nitric oxide (NO), which subsequently activates a plethora of cascades. NO production directly induces ATP depletion by inhibiting mitochondrial cytochrome oxidase, by generating reactive oxygen species (ROS), and enhancing presynaptic glutamate release through retrograde signalling[18–20]. Concurrent increases in intracellular Ca^{2+} and ROS may also lead to mitochondrial permeability transition (and thus cytochrome *c* release), as well as activating caspases and calpains, which trigger apoptosis and necrosis[21, 22] (Figure 1).

It is by no means a stretch to conclude that activation of NMDARs plays a crucial role in perpetuating cell death pathways, and yet clinical development of NMDAR-targeting pharmacological interventions was ineffective in treating or minimizing stroke damage in patients. In spite of the considerable promise of neuroprotection of NMDAR block from *in vitro* and *in vivo* animal studies, clinical trials on all NMDAR antagonists were halted due to lack of efficacy[23, 24]. NMDARs are not, however, the sole conduit for Ca^{2+} entry during ischemia (see below), and therefore targeting Ca^{2+}-signalling cascades may be a more strategic approach to blocking neuronal death. Emerging evidence suggests key differences between neuronal responses to activation of synaptic or extrasynaptic NMDARs. The more-abundant, extrasynaptic NMDARs promote cell death[25], while synaptic NMDARs might in fact be neuroprotective through Ca^{2+} dependent activation of CREB (for recent review, see[26]).

In addition to NMDARs, AMPA receptors are also proposed to mediate cell death during ischemia[27]. AMPARs are tetrameric ligand gated ion channels, composed of a combination of GluA1-4 subunits and, unlike NMDARs, are activated solely by glutamate binding. Though historically not considered to be as critical as the NMDAR in perpetuating excitotoxic cell death, AMPARs may also mediate (or initiate) pathological cationic influx. Indeed, early studies on rodent models have shown that administration of AMPAR antagonists can be neuroprotective during ischemia[28, 29]. One key feature that differentiates some AMPARs from NMDARs is the *inability* of GluA2 containing AMPARs to conduct Ca^{2+}, reducing the possibility of activating Ca^{2+}-mediated neurotoxic cascades directly. However, AMPARs may contribute indirectly to neurotoxic cascades through membrane depolarizations that are sufficient to remove the Mg^{2+} block of NMDAR and facilitate opening or by recruitment of other Ca^{2+} influx pathways.

The majority of AMPARs expressed in neocortical and hippocampal pyramidal neurons are GluA2-containing channels[30–32], a subunit that contains a positively charged arginine (R) in the pore forming domain of the channel, rendering the AMPAR impermeable to Ca^{2+} ions[33]. Transgenic expression of a glutamine (Q) in lieu of arginine (R) on GluA2 is permissive of Ca^{2+} conduction[34]; prolonged opening of GluA2(Q)-containing AMPARs (and not GluA2(R) receptors), are proposed to play a pivotal role during ischemic cell death[34]. On the other hand, GluA2-lacking receptors (consisting of GluA1, GluA3, or GluA4) are permeable to divalent Ca^{2+} and Zn^{2+} [35, 36], and are strongly implicated in global ischemia/glutamate excitotoxicity *in vivo*[37].

AMPAR trafficking and subunit assembly are dynamic processes under both physiological (for example, in long-term potentiation and depression) and pathophysiological conditions (for review, see[38]). Regulation of AMPARs has been extensively studied during neurodegenerative conditions, such epilepsy, brain trauma, and ischemia. Notably, surface expression of GluA2-containing AMPARs is downregulated following ischemic insult, with a subsequent increase in AMPAR-mediated Ca^{2+} influx (by GluA2-lacking AMPARs) following global ischemia[39, 40]. Similar to NMDARs, activation of AMPARs have also been shown to induce NO production via nNOS[41], as well as Ca^{2+}-dependent calpain activity in culture[42]. Contrary to this, AMPA stimulation might promote neuroprotection through CREB and BDNF activity[43]. Thus, it is clear that GluA2-lacking AMPARs confer a significant role in mediating cell death during glutamate excitotoxicity, which is likely occurring through Ca^{2+}-dependent pathways analogous to those seen in NMDAR overstimulation.

Purinergic receptors

There are two major groups of purinergic receptors, adenosine-gated P1 and P2 that are opened by uridine tri and di-phosphates as well as adenosine[44, 45]. The P1 receptors are metabotropic and include the A_1 and A_3 subtypes, which enhance phospholipase C activity and inhibit adenylyl cyclase, and the A_{2A} and A_{2B} subtypes that enhance production of cAMP. The P2 receptors comprise both the ionotropic P2X, and metabotropic G protein-coupled P2Y receptors. The P2 family are widely expressed in tissues and in diverse cell types, including neurons, astrocytes, microglia and oligodendrocytes in the central nervous system. Both adenosine and adenosine-5′-triphosphate (ATP) can signal in physiological and pathological conditions and whether this is neuroprotective or neurodegenerative depends upon the receptor subtypes

involved[44, 45].

Following the onset of ischemia, the intracellular concentration of ATP decreases[46, 47], resulting in a dramatic ionic imbalance and subsequent anoxic depolarization-like events[48]. This is associated with enhanced glutamate (see above) and ATP release into the extracellular milieu[49], resulting in activation of P2 receptors. After prolonged activation, P2X7 receptor function can transition from a small, cation permeable channel to one with characteristics of a significantly larger, non-specific pore[50, 51]. Several models for how this occurs have been proposed (reviewed in[52]), the most recent involving the recruitment of pannexin-1 (Panx1) channels[53, 54] (reviewed in[55]; see below).

Disruption of the membrane, as well as apparent activation of Panx1 permits rapid efflux of ATP from the cytosol to the extracellular space. The physiological concentration of ATP is typically high in neurons (millimolar range) — ischemia can induce a rapid increase in extracellular ATP to cytotoxic levels, such that cells (which are uninjured initially) can rapidly succumb to cell death[56, 57]. In addition to release of ATP through Panx1, the purine may also leave neurons and astrocytes by several other mechanisms, including permeation through connexin hemichannel's exocytosis[58–61], and via the ABC transporters/osmolytic transporters that are linked to anion channels[62, 63]. Regardless of the exact mechanism of release, it is clear that excessive ATP can contribute to cell death due to recruitment of a 'death complex' that includes P2X7 receptors and Panx1[64, 65].

There are several studies that demonstrate upregulation of purinergic receptors and neuroprotective effects of antagonizing ATP signalling during ischemia and brain injury (extensively reviewed in[66]) — for the purpose of this review, we will only mention the most recent studies. Ischemia reportedly elevates expression of several P2 receptors (P2X1, 2, 4, 7, and P2Y4) in dissociated neuronal or organotypic cultures, suggesting that these subunits may be important for pathological responses to ATP during insults[67–69]. In support of this notion, block of P2X7 during focal ischemia reduced infarct size[70] and, analogous to neuroprotection, protected optic nerve oligedendrocytes from ischemic damage[71].

The non-selective P2 receptor antagonists, PPADS (pyridoxalphosphate-6-azophenyl-2′,4′-disulfonate) and suramin decreased infarct size and facilitated functional recovery following animal stroke models[72–74]. Similar effects have been reported *in vitro*, where inhibition of P2Y1, P2X3, and P2X7 receptors in rat hippocampal slices exposed to OGD significantly attenuated depression of field excitatory postsynaptic potentials (fEPSPs) and anoxic depolarizations[75]. The same study also demonstrated that downstream activation of the kinases ERK1/2 was involved in synaptic failure and neuronal damage. Interestingly, activation of purinergic receptors by ATP may also be neuroprotective. ATP released during cortical spreading depression was reported to activate P2Y receptors, followed by synthesis of new proteins, which in turn exerted neuroprotective effects, possibly through preconditioning[60].

Under normal conditions, adenosine typically inhibits neuronal excitability by attenuating evoked release of glutamate from the presynaptic neuron[76–78]. The effects of adenosine receptor activation, similar to P2X/P2Y receptors, are reported to be both protective and detrimental. For example, inhibition of A_{2A} signalling in a model of focal ischemia is thought to be neuroprotective[79], while ischemic brain injury was reduced by activation of A_3 receptors[80].

Ischemic elevation of extracellular adenosine may be due to increased activity/expression of exonucleotidase, which participates in hydrolysis of ATP to adenosine[81–83]. It is likely therefore, that the release of ATP and adenosine under ischemic conditions is mechanistically and temporally separated[49], suggesting that the initial elevations in ATP can activate neurodegenerative mechanisms that may be followed, after conversion of ATP to adenosine by neuroprotective and neurodegenerative roles for the purines. However, this appears to be strongly dependent upon CNS region, animal model and the type of insult, and requires more investigation to elucidate these complex mechanisms[80, 84–96].

Pannexin channels

The pannexin family of proteins was first identified by Panchin *et al*[97] over a decade ago by using a degenerate PCR strategy in a search for vertebrate homologs of invertebrate gap junctions (innexins). It is now known that there are three family members (Pannexin-1, -2, and -3) with differential tissue distributions. Northern blot analysis shows that Pannexin-1 (Panx1) mRNA is found in wide variety of tissues, and has high expression levels in the brain and immune cells[98]. Expression of Panx2 mRNA on the other hand, is restricted to the brain and appears to have an intracellular distribution when modified by *S*-palmitoylation in neural progenitor cells[98, 99]. Alignment of mRNA and amino acid sequences demonstrate significant similarity between Panx3 and Panx1 with Panx3 being more closely related to Panx1 than Panx2, but the tissue distribution of Panx3 appears to be limited to synovial fibroblasts and osteoblasts[98].

The first indications that Panx1 channels were involved in anoxic depolarizations came from our work[100] where we showed using acutely isolated hippocampal neurons that OGD activated Panx1 channels. The main implications of this work were that Panx1 channels could be directly activated by ischemia, and the mechanism by which this activation occurred was independent of ligand-gated receptors because blocking NMDA, AMPA and P2X7 receptors failed to alter the large anoxic depolarization's inward currents activated by OGD[100]. It was later suggested that Panx1 opening in isolated neurons was mediated by the production of NO, and the authors' predicted that this could involve a nitrosylation reaction[101]. However, the mechanism by which NO acts on Panx1 to mediate channel opening during ischemia has not been identified. One intriguing possibility is that cysteine residues in the C-terminal are involved in activation of Panx1 by NO because mutation of C346 produced constitutively active channels and cell death[102].

Is Panx1 activation important for neuronal death during stroke? This question has taken some time to answer considering that the first description of Panx1 activation by ischemia was in 2006. Recently, two groups from Heidelberg, the Monyer and Schwaninger labs collaborated to show that genetic deletion of both Panx1 and Panx2 decreased stroke lesion volumes in mice subjected to permanent middle cerebral artery occlusion[103]. These results were both exciting and intriguing. The exciting part is, of course, that knockout of pannexin channels contributed to neuroprotection in stroke. The intriguing part lies in the observations that Panx1/2 knockouts appear phenotypically normal (i.e. breed normally and don't have any obvious behavioural abnormalities), and that both channels had to be knocked out to detect significant neuroprotection. Given the wide distribution in Panx1 tissue expression, one might expect a more deleterious effect when not present. This suggests that there may be some developmental compensation for deletion of pannexins. Another possibility, is that there is no normal physiological role for pannexins. This however, seems highly unlikely because data are emerging that Panx1 is involved in ATP release from cells[104, 105] and is important in regulating proliferation of neuronal stem cells[106].

How are pannexins being activated during stroke? Clearly a stroke in the brain is much more complicated than just OGD of isolated neurons. As described above, one of the central consequences of ischemic exposure is the uncontrolled release of the neurotransmitters, glutamate and ATP. In 2008, we described a role for the NMDA receptor in activating Panx1, which contributed to interictal (*ie* aberrant bursting) in hippocampal pyramidal neurons in acute brain slices[107]. This work demonstrated that Panx1 can be involved in neuronal plasticity, but also that over-stimulation of NMDA receptors can recruit Panx1, implicating Panx1 channels in excitotoxic neuronal death (Figure 1). It is important to note that direct demonstration of an NMDAR-Panx1 role in excitotoxicity has not yet been shown, but is certainly suggested by the work of Bargiotas *et al*[103] in Panx1/2 knockout mice. Furthermore, the intermediary mechanism(s) that couple NMDARs to Panx1 have not yet been characterized.

Interestingly, the purinergic receptor, P2X7 can directly couple to Panx1, although the mechanistic details, like NMDAR-Panx1, remain unknown. The nature of the role of Panx1 in functioning as the large pore of the P2X receptors is still controversial and has been reviewed elsewhere[55] so it will not be the focus here. Regardless of whether or not Panx1 is the "large pore mode" of the P2X7 channel, it is clear from work from several labs that P2X7 receptors can induce opening of Panx1[108–112]. In J774 cells, this appears to involve the Src family of protein tyronsine kinases (SFKs[110]). It has also been reported that SFK activity is increased during ischemia, suggesting that in neurons expressing both P2X7 and Panx1, SFKs could play a role in neuronal death[113, 114]. Other studies, however, also suggest that SFKs may have a neuroprotective role following ischemic insult by a mechanism that may involve regulation of ERK and stimulating proliferation of dentage gyrus neuronal cells[115, 116]. The relationship between pannexin channels and purinergic receptors was further demonstrated in the study by Kawamura Jr *et al*[117], who uncovered a significant contribution of Panx1 to neuronal excitability through ATP release.

Mechanisms governing Panx1 activation, other than recruitment by ligand-gated ion channels, have also been proposed. These include truncation of the Panx1 C-terminal by caspases. Chekeni *et al*[64] suggested Panx1 was responsible in part for the release of ATP and UTP during apoptosis, which recruits monocytes and macrophages. In their model, Chekeni *et al*, propose that Panx1 is targeted for cleavage at the C-terminus by caspase-3 and -7, resulting in channel opening, and consequential purine release. Thus, Panx1 can release "find-me" signals to immune cells during apoptosis. Interestingly, Panx1 was not required for the apoptotic process, but rather seems to act as the pathway for release of death signals[118]. Panx1 may also be opened by rises in extracellular K^+, independently of membrane depolarization and contribute to seizure phenotypes[119]. Similarly, in a more recent study by Gulbransen *et al*[109], we show that Panx1 is involved in death of neurons in the enteric nervous system (gut) following inflammation models of Crohn's and colitis. The mechanism of neuronal death is however, not clear but appears to involve activation of the inflammasome[53].

Transient receptor potential (Melastatin) channels

Transient receptor potential (TRP) channels are a family of tetrameric cation-permeable channels that employ unique mechanisms of activation, spanning mechanosensation (membrane stretch), temperature, and naturally occurring exogenous agonists[120, 121]. These channels are expressed throughout the nervous system, and have been noted to play a critical role in delayed cell death after ischemic insult in the CNS[122]. The TRP channel family consists of six main subfamilies, and are involved in a whole host of processes (downstream of Ca^{2+} influx) such as sensation, cell proliferation and fertility[123]. Two species of TRP channels from the TRPM (Melastatin) super family including TRPM2 and TRPM7, have been reported to contribute to neuronal cell death in the brain[124].

Cell death due to TRPM2 and TRPM7 appear to be a consequence of delayed calcium dysregulation following ischemia[124] (Figure 1). TRMP2 can be activated by arachidonic acid, reactive oxygen species (ROS), nitric oxide (NO) and adenine 5′-diphosphoribose (ADPR), while TRPM7 is activated by various components involved in stroke such as peroxynitirite, free radicals and change in extracellular pH[125]. TRPM2 and TRPM7 are permeable to Ca^{2+} while TRPM2 is also permeable to Na^+ and K^+ [126]. TRPM7 is also thought to mediate the influx of other divalent metals such as Zn^{2+} and is also permeable to Mg^{2+} [127]. Conductance of Zn^{2+} and Mg^{2+} by TRPM7 occlude monovalent ions from permeating through the pore[122]. Unlike TRPM2, which produce a linear current-voltage curve, TRPM7 currents exhibit a large outward rectification[124]. However, this outward rectification will linearize when divalent cations, such as calcium, are absent from

the extracellular space[124], which may allow for opening of the channel at resting potentials or under pathophysiological conditions such as ischemia.

TRPM7 was shown by Aarts *et al* (2003) to mediate a cation current (I_{OGD}) that the authors reported to be lethal to neurons[122]. This current arises under conditions of OGD and is activated by ROS, which increases Ca^{2+} uptake by the neuron[122] (Figure 1). Furthermore, the use of antiexcitotoxic therapy (AET), with drugs such as MK-801, CNQX and nimodipine, could protect neurons from death if given one hour prior to stroke[128]. When TRPM7 was knocked down in culture by siRNA, cell death due to anoxia decreased significantly even without the use of AET[122]. This demonstrates the importance of TRPM7 in *in vitro* models of toxicity, such that when TRPM7 is inhibited or silenced there is increased neuronal survival during ischemia[122]. The importance of TRPM7 channels in neuronal death using *in vivo* stroke rodent models has been shown by either silencing TRPM7 directly or when its activation (among other pathways) was disrupted with use of a PSD-95 interfering peptide[129, 130]. A recent and exciting report shows that disruption of TRPM7/PSD95/neuronal nitric oxide synthase with the NR2B C-terminal mimetic peptide dramatically reduced focal stroke damage in primates[129]. Taken together, these studies have demonstrated a clear role for TRPM7 in neuronal death caused by OGD.

TRPM2 channels are also strongly implicated in neuronal death through a mechanism that involves a large calcium influx induced by oxidative stress, exogenous hydrogen peroxide (H_2O_2), or tumor necrosis factor α (TNF-α)[131]. Zhang *et al* (2003) activated an isoform of TRPM2 with H_2O_2 that caused cell death in expression systems[132]. Consistent with this, when TRPM2 was either pharmacologically inhibited or knocked down by shRNA strategies, there was reduced calcium influx and greater cell survival. An increase in cortical levels of TRPM2 mRNA has also been reported following ischemia, but whether or not this is detrimental to neuronal survival *in vivo* is unclear[133], and it appears that TRPM2 is important for oxidative stress induced cell death. A role for TRPM2 in neuronal death *in vivo* is clearly the logical next step to confirm the importance of these channels.

Acid sensing ion channels

A critical consequence of ischemia during stroke is acidosis, resulting primarily from lactate production when oxidative phosphorylation fails and neurons switch to glycolysis[134]. This decrease in pH to values below 6 can be an important cause of cell death[135]. Although the effects of acidification have been less intensly investigated compared to OGD, recent reports suggest that decreases in tissue pH during ischemia may trigger the opening of acid sensing ion channels (ASICs). The ASICs can facilitate sodium and calcium influx and thereby contribute to ionic dysregulation[136, 137].

ASICs are ligand-gated ion channels that are part of the epithelial sodium channel family (ENaC) and are activated by low extracellular pH[138, 139]. The recently resolved structure of ASIC1 shows a trimeric channel with each ASIC subunit having two transmembrane domains and a large extracellular loop comprising 350–370 amino acids[140–142]. Expression of ASICs appears limited to the peripheral and central nervous systems of chordates; non-neural cells and other phyla fail to express the channels[143]. To date, four genes (ASIC1-ASIC4) are known to code for the six different ASIC subunits (ASIC1a, -1b, -2a, -2b, -3, -4)[138, 139]. Four of the ASIC subunits (ASIC1a, 1b, 2a, and 3) can form functional homomeric channels, each having different biophysical properties[138, 139]. Although all ASIC channels conduct Na^+ upon activation and exhibit differential sensitivities for pH, only ASIC1a homomeric channels are Ca^{2+} permeable[134, 136, 137].

Block of ASIC1a contributes to neuroprotection in animal models of stroke[136, 137, 144, 145]. Cultured cortical neurons subjected to OGD elicit inward currents that could be inhibited by ASIC1a antagonists, amiloride or PcTx venom[136]. Additionally, exposure of cortical neurons to pH 6.0 in the presence of glutamatergic and voltage-sensitive calcium channel blockers leads to an increase in intracellular Ca^{2+} that is sensitive to ASIC1a blockers, amiloride or psalmotoxin[136]. *In vivo*, occlusion of the middle cerebral artery in knockout mice lacking ASIC1a had smaller infarct sizes compared to control animals[136]. In addition, it appears that ASICs are not only involved in mediating neurotoxicity during the ischemic event (Figure 1), but that blocking ASIC1a activity up to 5 h post reperfusion significantly reduced infarct volumes[144], suggesting an ongoing neurodegeneration due to ASIC1a activation. Thus, ASIC1a opening during ischemia may be a crucial mediator of acidosis-induced neuronal death.

Gao *et al* demonstrated that there is an ischemia-induced enhancement of ASIC1a activity due to phosphorylation of Ser478 and Ser479, which enhances permeability to cations[145]. It is well known NMDARs are activated during ischemia (see above)[134] and it appears that activation of NMDARs containing the NR2B subunit causes a cascade that recruits Ca^{2+}/calmodulin dependent protein II (CaMKII), which phosphorylates ASIC1a[145]. Moreover, preventing the increased ischemia-induced permeability of ASIC1a by applying NR2B or CaMKII antagonists leads to decreased intracellular calcium and decreased neuronal death[145]. In addition to the NMDAR-CaMKII cascade, extracellular spermine and dynorphin appear to mediate ASIC1a channel opening either during, or immediately following ischemia[146, 147]. Taken together, it appears that ischemia-mediated acidosis triggers ASIC1a channel opening that may mediate an alternative, glutamate receptor independent (but NR2b-modulated) calcium influx pathway. Block of ASIC-1a channels during ischemia could, therefore, lead to better functional outcomes in individuals who have suffered stroke.

Concluding remarks

Here we have discussed several key channels thought to be involved in ischemia mediated neuronal damage. One of the interesting themes emerging from the past several decades of work is that the ligand gated cation channels, NMDARs, P2XRs (and others) may couple directly to pannexin-1 chan-

nels, which would be important for potentiating the excitotoxic effects of receptor overstimulation. It is important however to remember that several other ion channels are critically involved, including members of the TRP and ASIC families and that both of these can be regulated or modulated by the NMDAR. It remains a challenge of the field to determine the mechanisms of activation of many of these channels and to quantify their temporal relationships during stroke. In particular, separation of the contribution of direct activation of channels by ischemia-induced intermediates versus activation by coupling to ligand-gated channels is critical.

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