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Review Article

The role of TRPM2 channels in neurons, glial cells and the blood-brain barrier in cerebral ischemia and hypoxia

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Abstract

Stroke is one of the major causes of mortality and morbidity worldwide, yet novel therapeutic treatments for this condition are lacking. This review focuses on the roles of the transient receptor potential melastatin 2 (TRPM2) ion channels in cellular damage following hypoxia-ischemia and their potential as a future therapeutic target for stroke. Here, we highlight the complex molecular signaling that takes place in neurons, glial cells and the blood-brain barrier following ischemic insult. We also describe the evidence of TRPM2 involvement in these processes, as shown from numerous *in vitro* and *in vivo* studies that utilize genetic and pharmacological approaches. This evidence implicates TRPM2 in a broad range of pathways that take place every stage of cerebral ischemic injury, thus making TRPM2 a promising target for drug development for stroke and other neurodegenerative conditions of the central nervous system.

Keywords: ion channels; TRPM2/transient receptor potential melastatin 2; cerebral ischemia; stroke; hypoxia; oxygen-glucose deprivation; glycogen synthase kinase 3-β; neuroprotection; inhibitor

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Introduction

Stroke is a complex neurological condition that causes irreparable destruction of brain tissue. In 2013, stroke was the 5th leading cause of death in the United States, where it accounted for 1 out of every 20 deaths and killing as many as 130 000 people each year[1]. While recent advances in research have identified several potential mechanisms underlying neuronal death following stroke, the treatments for this condition remain limited. It has been estimated that by 2050, the incidence of stroke will more than double, and by 2030, total stroke-related direct medical costs will rise from $71.6 billion to $184.1 billion[1]. Therefore, there is an urgent need for novel therapeutic opportunities beyond the current stroke treatments.

The current understanding of stroke pathology revolves around the biochemical cascade that begins with ischemia and lasts long after blood flow is restored[2, 3]. Energy failure and ATP depletion due to low oxygen levels lead to a massive release of glutamate into the synaptic cleft and to excessive Ca^{2+} influx through α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-Methyl-*D*-aspartic acid (NMDA) receptors. As a result, pro-apoptotic signaling and reactive oxygen species production are activated[2, 3]. The severity of the ischemic insult dictates the extent of further neuronal cell death, activation of inflammatory cascades and prolonged apoptosis[3]. A previous and popular strategy was pharmacological targeting of the activation of NMDA receptors after the excessive glutamate release following ischemia. Several compounds, including dizocilpine maleate (MK-801)[4, 5], aptiganel hydrochloride (Cerestat)[6], dextromethorphan (DMX)[7] and CGS 19755 (Selfotel)[8, 9] showed promising results in rodent models. However, by 2001, all clinical trials using NMDA receptor antagonists as treatment for stroke were deemed unsuccessful[10-15]. Therefore, effort was re-focused on finding novel, non-glutamate therapeutic targets for hypoxic-ischemic cell death.

Non-glutamate targets for stroke therapy

Several non-glutamate ion channels have been identified as potential therapeutic targets for cerebral ischemia in rodent models, including the following examples.

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1) **Acid-sensing ion channels.** The ASIC1a channel is widely expressed in the brain, and its activation has been shown to contribute to neuronal cell death in both *in vitro* and *in vivo* models[16, 17].

2) **Volume-regulated anion channels.** Cerebral edema and swelling are some of the factors contributing to delayed neuronal death following cerebral ischemia. Volume-regulated anion channels (VRACs) regulate cell volume via efflux of ions that are followed by water[18]. In astrocytes, VRACs have been shown to conduct excitatory amino acids (EAAs), aspartate and glutamate, after ischemia, thus contributing to excitotoxicity[19-21].

3) **Hemichannels.** Hemichannels, proteins involved in the formation of gap junctions, have also been proposed as non-glutamate contributors of neuronal death in ischemia[22]. They have been implicated in contributing to anoxic depolarization, leading to cell death in the penumbra region, as well as efflux of vital nutrients from neurons, thus worsening the effects of energy failure caused by low-oxygen conditions[23-25].

4) **Transient Receptor Potential Melastatin (TRPM) subfamily.** Transient Receptor Potential melastatin (TRPM) channels are calcium-permeable, ubiquitously expressed cation channels[26, 27]. TRPM7 has recently emerged as one of the major contributors to non-glutamate-induced cell death following ischemia[28-30]. *In vitro* pharmacological inhibition[31] and *in vivo* siRNA suppression of TRPM7 in rodents[32] were shown to significantly reduce neuronal death. Moreover, conditions such as low pH and reactive oxygen species were found to enhance TRPM7 activity[33, 34]. The contribution of a closely related TRPM family member, TRPM2, to hypoxic-ischemic brain injury has also been investigated.

In this review, we focus on the role of TRPM2 in neuronal and non-neuronal mechanisms that contribute to the devastating effects of cerebral ischemia.

TRPM2: structure and biophysical properties

TRPM2, the second member of the melastatin subfamily of the transient receptor potential (TRP) channel superfamily, is a calcium-permeable, non-selective cation channel[35]. It is broadly expressed within the CNS, heart, lung, liver and pancreas[36]. At the cellular level, TRPM2 has been identified in multiple cell types, including neurons[37-42], microglia[43-49], astrocytes[50], macrophages[51, 52], neutrophils[53-55], dendritic cells[56], megakaryocytes[57], endothelial vascular cells[58-62], cardiomyocytes[63] and pancreatic β-cells[64, 65]. The ubiquitous distribution of TRPM2 indicates that it may play roles in a wide range of physiological processes. In addition to its role as a plasma membrane channel, TRPM2 has been shown to also be localized to the lysosomal compartment, where it regulates calcium mobilization from intracellular compartments and contributes to H_2O_2-induced apoptosis of β cells[66].

The human *TRPM2* gene is located on chromosome 21q22.3, spanning approximately 90 kb and encoding 1503 amino acid residues[67]. TRPM2 has also been cloned from mouse and rat tissues, encoding 1507 amino acid residues, with a predicted molecular weight of 172 kDa and 83%–85% similarity to human TRPM2 at the nucleotide and protein levels, repectively[68, 69]. Molecularly, the TRPM2 channel is composed of four identical subunits, each consisting of a 730-amino acid N terminus with an IQ-like calmodulin-binding motif (amino acids 406-416)[70], 6 transmembrane domains (S1-S6) with a pore-forming loop located between S5 and S6, and a C terminus containing a highly conserved TRP box, a coiled-coil domain and a unique adenosine diphosphate ribose (ADPR) pyrophosphatase NUDT9-H domain (amino acids 1197-1503) (Figure 1)[71]. The NUDT9-H domain contains an 11-residue ADPR binding pocket[72]; TRPM2 has been shown to be gated by free ADPR[71]. A site-directed mutagenesis study identified that hydrogen bonding of Arg1433 and Tyr1349 is necessary for TRPM2 activation by ADPR[73]. The enzymatic activity of NUDT9-H is not required for channel gating[74], however it plays a role in TRPM2 surface expression[75]. When expressed on its own, the NUDT9-H domain also has measurable enzymatic activity, thus making TRPM2 a "chanzyme". However, the specific role of that activity remains to be defined[71]. In addition to the NUDT9-H domain, the C-terminus contains a coiled-coil domain that was shown to be critical in mediating the tetrameric assembly of the channel[76]. The N-terminal IQ-like motif is important in activating TRPM2 current by intracellular Ca^{2+} in an ADPR-free manner[70, 77-79]. In addition to full-length TRPM2 (TRPM2-L), several splice isoforms with varying degrees of activity have been identified: TRPM2-ΔN[80], TRPM2-ΔC[80], TRPM2-S[59], TRPM2-SSF[81] and TRPM2-TE[82].

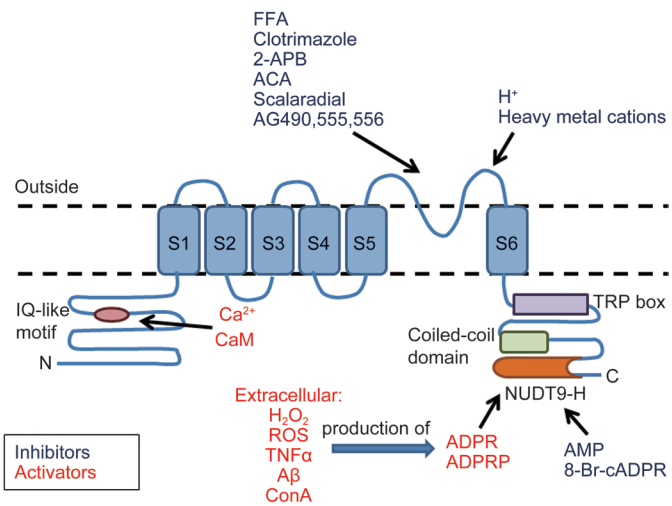

Figure 1. TRPM2 protein structure and modulators of TRPM2 activity. Extracellular agents such as H_2O_2, ROS, TNFα, Aβ and concanavalin A enhanced TRPM2 activity via production of intracellular ADP that gates TRPM2 via binding to the NUDT9-H domain. AMP and 8-Br-cADPR reduce TRPM2 activity through interaction with the NUDT9-H domain. Protons and divalent heavy metal cations reduce TRPM2 activity through interaction with the pore region. Ca^{2+} gates TRPM2 via CaM interaction with N-terminal IQ-like motif. Structurally unrelated compounds, such as FFA, clotrimazole, 2-APB, ACA, scalaradial and AG490, 555 and 556, are also able to inhibit TRPM2, although their mechanisms of action remain to be elucidated.

Upon activation, TRPM2 displays a linear current-voltage (I-V) relationship, with a single-channel conductance range of 52-60 pS at negative potentials and ~72 pS at positive potentials[69, 71, 79, 83, 84]. TRPM2 is permeable to Na^+, K^+, Ca^{2+} and Mg^{2+}. The relative permeabilities of TRPM2 to these ions are reported as P_K/P_{Na} – 1.1, P_{Ca}/P_{Na} – 0.9 and P_{Mg}/P_{Na} – 0.5[85]. Several key residues identified within the pore-forming region dictate channel function as well as conductance of certain ions. The two conserved cysteine residues, C996 and C1008, were shown to be critical for TRPM2 gating by ADPR and voltage[86]. The point mutation P1018L[87], present in Guamanian amyotrophic lateral sclerosis and parkinsonism-dementia patients, produces a fast-desensitizing channel. The permeability of TRPM2 to Ca^{2+} and Mg^{2+} was shown to be controlled by four residues within the pore-forming loop: E960, Q981, D987 and E1022[85].

Several extracellular stimuli, including reactive oxygen species[88], H_2O_2[80], amyloid β-peptide[89], concanavalin A[90], tumor necrosis factor-α[91] and zinc ions[45], have been shown to induce TRPM2 activation via metabolic production of intracellular ADPR. While ADPR is considered to be the most potent TRPM2 activator (EC_{50} of 10–80 μmol/L)[71, 92], there has been much controversy around other proposed TRPM2 activators. Several studies suggested that other nucleotides, such as 2′-*O*-acetyl-ADPr (OAADPr), cyclic ADP (cAPDr), nicotinamide-adenine dinucleotide (NAD), nicotinic acid-adenine dinucleotide (NAAD), and NAAD-phosphate (NAADP), can also enhance TRPM2 activity[93, 94]. However, a recent study showed, using affinity-purified-specific ADPR hydrolase to purify commercially available pyridine dinucleotides, that NAD, NAAD and NAADP were incapable of stimulating TRPM2 activity, even at concentrations substantially higher than cytosolic. Instead, they identified ADPR-2′-phosphate (ADPRP) as a direct TRPM2 agonist[95]. Full activation of TRPM2 is highly dependent on the presence of intracellular and/or extracellular Ca^{2+}; ADPR-induced TRPM2 current was shown to be significantly reduced in the absence of Ca^{2+}. It has been proposed that intracellular calcium sensitizes TRPM2 to ADPR via calcium-dependent interaction of calmodulin (CaM) with the N-terminal IQ-like motif. Calcium can also gate the channel in the absence of ADPR, with an EC_{50} of 17 μmol/L[70, 77-79].

In addition to agonists, several non-specific inhibitors of TRPM2 have been described. Adenosine monophosphate (AMP) inhibits the channel activity, potentially via binding to the NUDT9-H domain with IC_{50} values of 10 μmol/L and 70 μmol/L for endogenous and recombinant channels, respectively[53, 92, 96]. 8-Bromo-cyclic inosine diphosphoribose (8-Br-cADPR, IC_{50} 100 μmol/L) was shown to inhibit TRPM2 gating by cADPR and H_2O_2[92]. Protons[97-99] and divalent heavy metal cations[100-102] also caused TRPM2 inhibition by targeting the extracellular pore region. Several structurally unrelated pharmacological agents have been identified as TRPM2 inhibitors. Those include flufenamic acid (FFA, IC_{50} 50–1000 μmol/L)[103], the anti-fungal agents clotrimazole and econazole (IC_{50} 3–30 μmol/L)[104], 2-aminoethoxydiphenyl borate (2-APB, IC_{50} 1.2 μmol/L)[105], *N*-(*p*-amylcinnamoyl) anthranilic acid (ACA, IC_{50} 1.7 μmol/L)[106], tyrphostin AG-related compounds (AG490, AG555 and AG556)[107, 108] and the marine-derived compounds scalaradial and 12-deacetylscalaradial (IC_{50} 210 nmol/L)[109]. It is important to note that these compounds affect a wide variety of ion channels and proteins, and none of them are selective for TRPM2. Therefore, efforts should be made to develop TRPM2-specific inhibitors in order to further elucidate the physiological functions of this channel.

In addition to nucleotides and pharmacological agents, TRPM2 activity was also shown to be modulated via interactions with other proteins. As mentioned above, CaM-TRPM2 interaction at the N-terminal IQ-like motif facilitates TRPM2 activation by Ca^{2+}. Mutation of the IQ-like motif or expression of a CaM mutant that is unable to bind Ca^{2+} significantly inhibits the rate of development of H_2O_2-induced TRPM2 Ca^{2+} conductance[70, 79]. The non-receptor protein tyrosine phosphatase PTPL1 directly interacts with TRPM2 and reduces TRPM2 phosphorylation, Ca^{2+} influx and cell death induced by H_2O_2 and TNF-α in the human monocytic cell line U937[110].

Neuronal TRPM2 in cerebral ischemia

Neuronal cell death is the hallmark of ischemic insult resulting in life-long, debilitating and irreversible consequences for survivors. TRPM2 is broadly expressed in neurons. Primary rat cortical cultures exposed to H_2O_2 undergo rapid apoptotic cell death; treating these neurons with TRPM2 siRNA significantly inhibits H_2O_2-induced intracellular Ca^{2+} influx and neuronal cell death[41]. This indicates that neuron-specific TRPM2 may contribute to the pathology of cerebral ischemia. CA1 hippocampal neurons are highly vulnerable to oxidative stress, and ischemic injury often causes irreparable damage to the hippocampus. CA1 neurons from rat hippocampal slices showed H_2O_2-induced inward current that was inhibited by the TRPM2 antagonist clotrimazole[111]. Moreover, activation of TRPM2-like currents in these neurons required concomitant activation and Ca^{2+} influx via voltage-gated Ca^{2+} channels and NMDARs, the two events that take place following hypoxia-ischemia[111]. Another study demonstrated that TRPM2-deficient CA1 pyramidal neurons were resistant to increases in cytosolic Zn^{2+} concentrations, thus implicating TRPM2 in delayed neuronal cell death post-ischemia[112].

The contribution of TRPM2 to ischemic cell death has also been addressed in several animal models of hypoxia-ischemia. Compared to wild-type mice, TRPM2-null mice subjected to transient middle cerebral artery occlusion (tMCAO) exhibited a reduction of approximately 40% in infarct volumes[39]. However, when TRPM2-null mice were subjected to permanent MCAO (pMCAO), the infarct severity was comparable to that of wild-type mice. This was hypothesized to be due to the lack of reperfusion following the procedure, thus eliminating the production of H_2O_2, which is a major activator of TRPM2-like currents. Thus, it is possible that TRPM2-null mice are only resistant to ischemia-reperfusion injury due to reduced vulnerability to H_2O_2, while under pMCAO conditions, the insult becomes so severe that it outweighs the neuroprotective

effects of TRPM2 deletion[39]. Similarly, hippocampal neurons cultured from these mice showed reduced cell death following one hour of oxygen-glucose deprivation (OGD)[39]. At the molecular level, the study observed the activation of the pro-survival Akt pathway and the inhibition of downstream glycogen synthase kinase 3β (GSK-3β), thus tipping the scale towards cell survival in TRPM2-null mice[39].

Genetic deletion of TRPM2 was also shown to be neuroprotective in a developmental model of hypoxic-ischemic brain damage[113]. Compared to wild-type littermates, $TRPM2^{+/-}$ and $TRPM2^{-/-}$ neonatal mice had reduced brain infarct volumes, improved sensorimotor outcomes, reduced expression of inflammatory markers and reduced loss of brain mass following hypoxia-ischemia[113]. At the molecular level, $TRPM2^{+/-}$ and $TRPM2^{-/-}$ neonatal mice showed increased pro-survival signaling, suggesting that genetic knockout of TRPM2 exerts its neuroprotective effects via the Akt/GSK-3β pathway[113]. These results confirmed the findings from the adult mouse model of tMCAO. Another study used a novel inhibitor of GSK-3β, TDZD-8, to show that deactivation of GSK-3β via phosphorylation on Ser9 is neuroprotective in a neonatal mouse model of hypoxia-ischemia. These findings confirmed the involvement of the Akt/GSK-3β signaling pathway in neuronal survival following ischemic insult[114].

The role of neuronal TRPM2 in cerebral ischemia was shown to be sexually dimorphic. Pharmacological inhibition of TRPM2 with ACA[106], 2-APB[105], clotrimazole (CTZ)[104], flufenamic acid[103], and TRPM2 shRNA treatment significantly reduced cell death following OGD in neurons from male but not female animals[115]. Additionally, intrastriatal lentiviral infection with TRPM2 shRNA following middle cerebral artery occlusion (MCAO) resulted in markedly reduced striatal infarct volumes in male but not female mice[115]. Another study has shown that TRPM2 channels in male, but not female, hippocampal neurons were activated during reperfusion following OGD[116]. Similarly, inhibition of TRPM2 activity with clotrimazole 30 min after transient global cerebral ischemia due to cardiac arrest reduced CA1 hippocampal neuronal death only in male mice[117]. Pre-treatment of adult and aged male mice with the TRPM2 inhibitor tat-M2NX resulted in reduced infarct volumes, while no effect was observed in female mice[118]. These sex differences have been postulated to be due male-specific androgen signaling, and to preferentially enhanced activity of the enzyme poly(ADP-ribose) polymerase-1 (PARP-1) in the male brain following ischemia[119]. In female mice, androgens were not sufficient to produce TRPM2 activation[120]. Collectively, these studies indicate that TRPM2 is expressed in neurons, becomes activated under ischemic brain conditions and contributes to cell death in a sexually dimorphic manner.

Non-neuronal TRPM2 in cerebral ischemia

Pathological post-ischemic changes also require the involvement of non-neuronal cells, such as microglia, astrocytes and other immune cells. Microglia, the macrophages of the central nervous system, were previously implicated in pathology following hypoxic-ischemic injury due to their role in generating a range of inflammatory mediators, such as ROS, cytokines, free radicals, glutamate, proteases, nitric oxide (NO) and H_2O_2[121]. Lipopolysaccharide (LPS)-activated primary rat microglia had detectable levels of TRPM2 mRNA and exhibited a robust TRPM2-like Ca^{2+} conductance following the application of H_2O_2[47]. Similarly, activation of microglia has also been detected following tMCAO injury. In tMCAO rodent model, cortical mRNA levels of TRPM2 increased in a time-dependent manner, peaking at 7 d post-injury, suggesting a contribution to brain damage following ischemia[43]. Patch-clamp experiments in human C13 microglia and primary rat microglia in a model of H_2O_2-induced oxidative stress revealed an upregulation of a TRPM2-like conductance, which was reversibly blocked by flufenamic acid[43]. A recent study using bone marrow chimeric mice demonstrated that *Trpm2* deficiency is protective due to TRPM2-mediated regulation of the migratory ability of peripheral immune cells (neutrophils and macrophages) that infiltrate the injury site and exacerbate post-ischemic inflammation[48]. At the molecular level, microglial activation was shown to be caused by an increase in TRPM2 activity due to generation of ROS and activation of PARP-1[49]. Moreover, this increase in TRPM2 activity was suppressed by inhibition of protein kinase C (PKC) and NADPH oxidase (NOX), as well as proline-rich tyrosine kinase 2β (PYK2) and downstream MEK/ERK signaling[49]. Therefore, it has been suggested that PKC/NOX-mediated generation of ROS and subsequent activation of PARP-1 lead to activation of microglial TRPM2. Additionally, activation of the PYK2/MEK/ERK pathway downstream of TRPM2 acts as a positive feedback mechanism for further activation of TRPM2[49]. Another study demonstrated that the release of the pro-inflammatory cytokine interleukin-1β from microglia and U937 monocytes occurs due to TRPM2-dependent activation of NLRP3 inflammasomes[122, 123]. These mechanistic findings provide insight into the role of TRPM2 in microglial activation and neuroinflammation.

Astrocytes are another type of glia that undergo molecular and morphological changes in response to CNS insults, such as hypoxia-ischemia[124]. While the function of activated or reactive astrocytes in stroke remains controversial, it has been demonstrated that reactive astrocytes express the inducible form of nitric oxide synthase (iNOS) following ischemic injury[125]. This implicates astrocytes in NO production, which contributes to delayed neuronal cell death[125]. It has also been shown that reactive astrocytes that form a glial scar following brain injury may inhibit the growth of regenerating axons, thus reducing the recovery following injury[126]. Human astrocytes treated with the TRPM2 inhibitor clotrimazole or transfected with TRPM2 siRNA were reported to show reduced release of inflammatory and neurotoxic factors and downregulated neuroinflammatory signaling, such as the JNK, p38, ERK42/44 and NFκB pathways, in response to glutathione depletion[50]. Therefore, it is possible that TRPM2 activity in these cells could contribute at least in part to their deleterious role in brain injury. However, the role of astrocyte-expressed

TRPM2 in cerebral ischemic injury is still unclear.

Studies have also linked glial TRPM2 activation, oxidative stress and inflammatory mechanisms to neurodegenerative conditions such as Alzheimer's disease (AD)[127]. TRPM2 channel activation and subsequent Ca^{2+} influx due to oxidative stress and depletion of glutathione levels resulted in inflammatory responses in microglia and astrocytes, which may promote and exacerbate neuronal degeneration[50]. Additionally, it has been shown that in aging cultured hippocampal neurons, TRPM2 currents were enhanced with time, suggesting that TRPM2 may also contribute to neurodegeneration during neuronal senescence[40].

Therefore, the current body of literature indicates that non-neuronal TRPM2 may contribute to inflammatory responses in the CNS following ischemic insult and during other neurodegenerative conditions, potentially exacerbating the extent of brain damage.

Role of TRPM2 in the blood-brain barrier in cerebral ischemia

The blood-brain barrier (BBB) is an intricate network of cells that form a functional barrier that separates the CNS from systemic circulation. It is composed of and maintained by a variety of cell types, including pericytes, astrocytes and endothelial cells. Ischemic conditions lead to dysregulation and breakdown of the molecular integrity of the BBB, leading to vasogenic edema and increased permeability to immune cells into the damaged area[128]. TRP channels have been previously implicated in BBB permeability, and TRPM2 RNA has been detected in primary rat cultures of brain microvessel endothelial cells[129]. A recent study demonstrated that TRPM2-mediated pericyte autophagy, secondary to stress-induced Y1485 tyrosine nitration of TRPM2, played a critical role in pericyte injury and apoptosis[130]. Another study confirmed expression of TRPM2 in human pulmonary artery endothelial cell monolayers and demonstrated that H_2O_2 exposure elicited calcium influx and increased endothelial cell permeability[59]. This was attenuated by TRPM2 siRNA silencing and overexpression of the isoform TRPM2-S, which interacts with the isoform TRPM2-L and inhibits H_2O_2-induced calcium influx[59]. At the molecular level, it has been demonstrated that PARP-1 is strongly activated in endothelial cells, leading to apoptosis. PARP-1 activation has been linked to post-ischemic disruption of BBB, and administration of PARP-1 inhibitors, 3-aminobenzamide and 4-amino-1,8-naphthalamide, in rodents with transient focal ischemia resulted in decreased edema, immune cell infiltration and preservation of endothelial tight junctions[131]. PARP-1 activation has been previously shown to be required for oxidative stress-induced activation of TRPM2 in DT40 B cells, and PARP-deficient lymphocytes showed no oxidant-induced TRPM2 activation[132]. A recent study described the role of endothelial cell-expressed TRPM2 in transendothelial migration of polymorphonuclear neutrophils (PMNs)[62]. It was shown that siRNA-mediated depletion of TRPM2 in endothelial cells led to a reduction in phosphorylated VE-cadherin[62], an adhesion molecule that regulates the opening of adherens junctions and facilitates the migration of PMNs across the blood-brain barrier[133]. Infiltration of PMNs into the ischemic penumbra is one of the hallmarks of post-ischemic inflammation[134]; therefore, endothelial TRPM2 activation facilitates the secondary brain injury following neutrophil invasion. Moreover, ROS-induced activation of TRPM2 has been implicated in endothelial cell apoptosis[61]. Application of H_2O_2 or TNFα has been shown to induce TRPM2-S phosphorylation at Ser39 by PKCα, leading to supra-normal Ca^{2+} influx, activation of caspase-3 and endothelial cell death[61], which can exacerbate the breakdown of endothelial barrier. Therefore, it is possible that TRPM2 channels also contribute to the increased permeability and eventual breakdown of the BBB following ischemia, thus contributing to edema formation, inflammation and cell death, although their role needs to be confirmed by further studies.

Conclusions

While considerable progress has been made in recent years towards elucidating the cellular and molecular pathogenesis of ischemic brain injury, effective and potent treatments for stroke patients are still lacking. There is increasing evidence that TRPM2 regulates a broad range of pathways in neurons, glia and the cells of the BBB, thus contributing to every stage of brain injury development after ischemia (summarized in

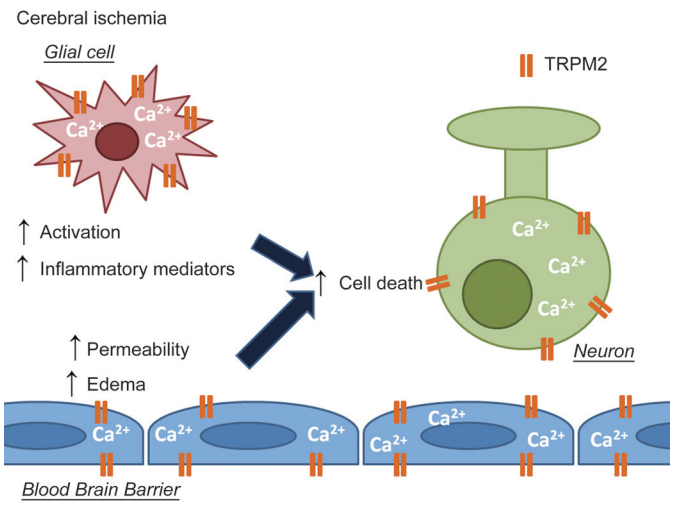

Figure 2. The effects of TRPM2 activation on neurons, glia and the blood-brain barrier under hypoxic/ischemic conditions. TRPM2 activation secondary to oxidative stress and hydrogen peroxide production under ischemic conditions leads to a variety of responses in neurons, glial cells and the cells composing the blood-brain barrier. It has been shown that in neurons, TRPM2 is activated under ischemic conditions and contributes to neuronal cell death, potentially in a sexually dimorphic manner. Current literature indicates that TRPM2 channels may contribute to increased permeability and breakdown of the blood-brain barrier under ischemic conditions. In glial cells, TRPM2 has been shown to mediate the release of neuroinflammatory factors, thus exacerbating the brain damage under ischemic conditions. Together, the evidence indicates that TRPM2 regulates a wide range of pathological events occurring during ischemia, thus making this channel a major target for drug development.

Figure 2). This evidence makes TRPM2 a promising target for further research and therapeutic development for several reasons. First, broad expression of TRPM2 in CNS and vasculature suggests that TRPM2 inhibition could be more effective at treating ischemic brain injury, compared to conventional therapies. Second, contributions of TRPM2 to different stages of brain injury suggest that therapeutic agents that target TRPM2 activity may have a longer therapeutic window than conventional therapies. Finally, gaining in-depth insight into TRPM2 downstream signaling may lead to development of therapies that specifically target TRPM2 signaling in specific cell types, leading to specialized treatments for different neurodegenerative conditions.

Abbreviations

TRPM2 channel, Transient Receptor Potential Melastatin 2 channel; OGD, oxygen-glucose deprivation; GSK-3β, glycogen synthase kinase 3 beta.

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