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Review Article

TRPM2: a candidate therapeutic target for treating neurological diseases

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Abstract

Transient receptor potential melastatin 2 (TRPM2) is a calcium (Ca^{2+})-permeable non-selective cation channel belonging to the TRP ion channel family. Oxidative stress-induced TRPM2 activation provokes aberrant intracellular Ca^{2+} accumulation and cell death in a variety of cell types, including neurons. Aberrant TRPM2 function has been implicated in several neurological disorders including ischemia/stroke, Alzheimer's disease, neuropathic pain, Parkinson's disease and bipolar disorder. In addition to research identifying a role for TRPM2 in disease, progress has been made in the identification of physiological functions of TRPM2 in the brain, including recent evidence that TRPM2 is necessary for the induction of *N*-methyl-*D*-aspartate (NMDA) receptor-dependent long-term depression, an important form of synaptic plasticity at glutamate synapses. Here, we summarize recent evidence on the role of TRPM2 in the central nervous system (CNS) in health and disease and discuss the potential therapeutic implications of targeting TRPM2. Collectively, these studies suggest that TRPM2 represents a prospective novel therapeutic target for neurological disorders.

Keywords: transient receptor potential melastatin 2 (TRPM2); synaptic plasticity; neuropathic pain; Alzheimer's disease; Parkinson's disease; bipolar disorder; neurodegeneration

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Introduction

Transient receptor potential melastatin 2 (TRPM2) is a calcium (Ca^{2+})-permeable non-selective cation channel first cloned and described in 1998[1]. The last 20 years have led to the identification of mechanisms that regulate TRPM2 and a better understanding of factors that augment or diminish the function of the channel. However, the mechanisms by which these factors dynamically regulate TRPM2 function in health and disease remains poorly understood. In the central nervous system (CNS), TRPM2 mRNA is most abundant among transient receptor potential (TRP) channels[2]. An increasing body of evidence has revealed a number of important contributions of TRPM2 channels to CNS physiology and pathophysiology. This review will summarize this evidence, with a focus on synaptic plasticity and neurodegeneration.

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TRPM2 channel structure

The first TRP gene was originally identified in *Drosophila*, where it participates in phototransduction[3]. Mammalian TRP channels are divided into six subfamilies including the canonical (C), vanilloid (V), polycystin (P), mucolipin (ML), ankyrin (A), and melastatin (M) subfamilies[4, 5]. The gene encoding human TRPM2, previously known as TRPC7 and LTRPC2, is located on chromosome 21q22.3[1]. The gene is 90 kb in length and contains 33 exons, producing a 5876-bp full-length transcript. This transcript codes for the full-length 1503-amino acid protein in humans, with a predicted molecular mass of ~170 kDa. In addition to the full-length transcript, there are also several splice variants of TRPM2 (Figure 1). These variants include TRPM2-ΔN, with a deletion of amino acids 538–557; TRPM2-ΔC, with a deletion of amino acids 1292–1325; TRPM2-ΔNΔC, missing both amino acids 538-557 and 1292-1325; striatum short form TRPM2 (SSF-TRPM2), a variant identified in the striatum in which amino acids 1–214 are deleted; TRPM2-S, a variant that is truncated after the second transmembrane domain, producing an 845-residue splice variant; and TRPM2-TE, a splice variant identified using compu-

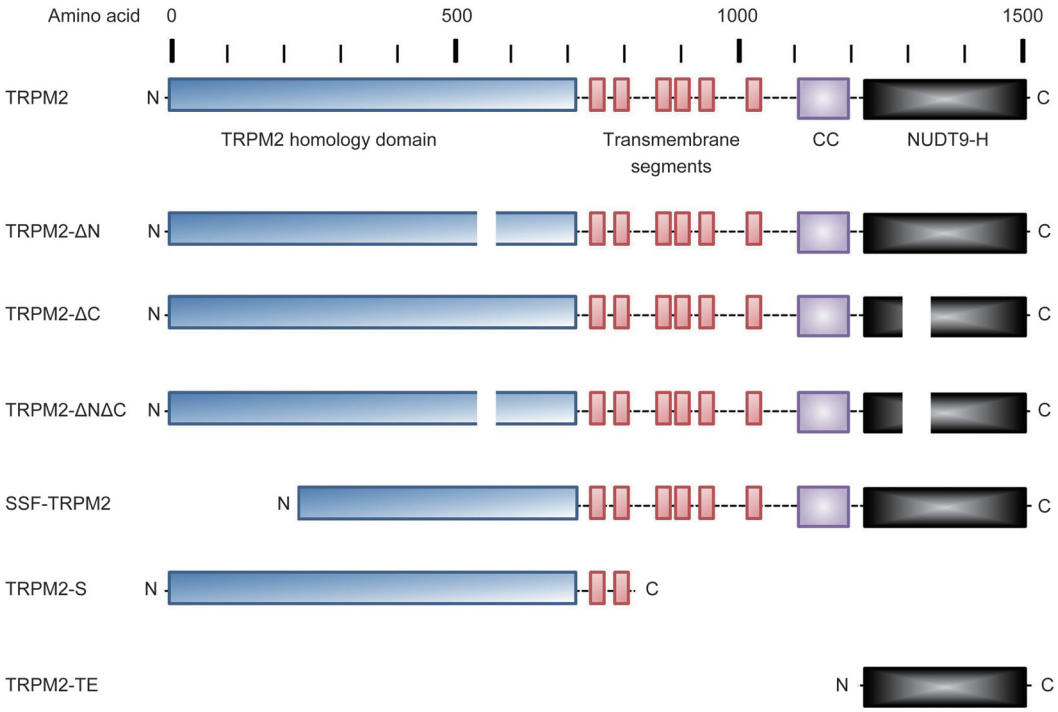

Figure 1. TRPM2 Isoforms. Schematic representation of full-length TRPM2 and TRPM2 isoforms, demonstrating the approximate locations of domains and deletions. The N-terminus of TRPM2 contains the TRPM homology domain and the IQ-like motif. This is followed by six transmembrane domains, with a pore-forming re-entry loop between domains 5 and 6. The C-terminus contains the coiled-coiled (CC) motif and the NUDT9-H motif. TRPM2 isoforms include TRPM2-ΔN (deletion of amino acids (aa) 538-557); TRPM2-ΔC (deletion of aa 1292-1325); TRPM2-ΔNΔC (deletion of aa538-557 and aa1292-1325); striatum short form TRPM2 (SSF-TRPM2, deletion of aa1-214); TRPM2-S, a variant that is truncated after the second transmembrane domain, producing an 845-residue splice variant; and TRPM2-TE, a splice variant identified with computational analysis demonstrating up-regulated expression in melanoma and other tumor types[6–11].

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The TRPM2 protein structure includes 6 transmembrane domains, with a pore-forming re-entry loop located between domains 5 and 6. Regulatory features of the channel are contained within the cytoplasmic N- and C-terminal regions of the channel (Figure 2). The N-terminus contains the TRPM homology domain, a region of approximately 700 amino acids that is largely conserved across all TRPM subfamily members[12]. Additionally, there is an IQ-like motif that is involved in Ca^{2+}-calmodulin binding to the channel and participates in TRPM2 activation[13, 14]. In the C-terminus of TRPM2, the coiled-coiled motif participates in subunit interaction and assembly of the channel into its functional tetrameric form[15]. The C-terminus of TRPM2 also includes a nucleoside diphosphate-linked moiety X-type homology motif (NUDT9-H). This motif is required for gating of the channel by adenosine diphosphate ribose (ADPR), an intracellular ligand for TRPM2. Although the sequence in this region is highly homologous to NUDT9 (50% sequence identity), a mitochondrial ADPR hydrolase that converts ADPR to adenosine monophosphate (AMP) and ribose-5-phosphate, this region demonstrates less than 1% of the enzymatic activity[16, 17]. More recently, NUDT9-H has been suggested to not possess enzymatic activity. Rather, the previously reported apparent activity is proposed to be due to the instability and spontaneous degradation of ADPR[18]. The impaired enzyme activity of NUDT9-H , when compared to the enzyme activity of NUDT9, is attributed to amino acid substitutions within the NUDT9-H motif at E1405I/F1406L[19]. Consistent with this suggestion, the introduction of the reverse substitution within NUDT9-H (*ie*, I1405E/L1406F) restores the enzyme activity to that of NUDT9 and abolishes TRPM2 activation by ADPR[14, 20, 21]. This observation suggests that NUDT9-H has adapted during evolution to function as an intracellular ligand-binding domain.

Gating mechanisms and channel properties

The TRPM2 channel has a single-channel conductance of approximately 60–80 pS, with a linear current-voltage relationship and a reversal potential of 0 mV[16, 22, 23]. These characteristics demonstrate that TRPM2 is a non-selective cation channel without voltage-dependent behavior. The permeability ratio of Ca^{2+} to Na^{+} (PCa:PNa) is approximately 0.7, indicating that the inward current is predominantly carried by Na^{+}[23, 24]. Despite this, substantial Ca^{2+} is able to flux into the cell due to the channel's long open time of several hundred milliseconds[16, 23, 24]. The permeability of the channel to divalent cations including Ca^{2+} and Mg^{2+} is regulated by glutamic acid, aspartate, and glutamine residues located between the pore helix and the selectivity filter in the TRPM2 channel pore[25].

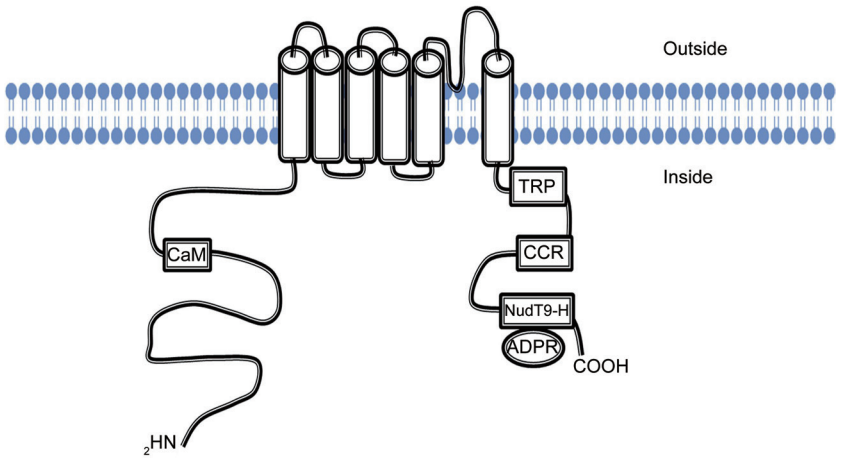

Figure 2. TRPM2 Structure. TRPM2 is comprised of intracellular amino and carboxy-terminal regions and six transmembrane segments with a re-entry loop that forms a pore between segments 5 and 6. The amino terminus contains the TRPM2 homology domain, which is largely conserved across all TRPM family members. The amino terminus also contains a Ca^{2+}-calmodulin (CaM) binding motif that participates in channel activation. The carboxy terminus contains a highly conserved TRP box (TRP), a coiled-coiled region (CCR) that may participate in tetrameric assembly, and the nucleoside diphosphate-linked moiety X-type homology motif (NUDT9-H), which binds adenosine diphosphate ribose (ADPR), the intracellular agonist for TRPM2.

TRPM2 represents a particularly unique channel in that it is activated in response to reactive oxygen and nitrogen species (ROS/RNS)[26]. Exposure to ROS/RNS is generally accepted to promote channel activation through the generation of ADPR, an agonist for TRPM2[16]. The precise interaction between the ligand ADPR and the NUDT9-H domain continues to be investigated, with recent evidence further characterizing the precise residues critical for binding and activation[27, 28]. Oxidative stress may generate ADPR through two pathways. In the first pathway, ROS/RNS leads to DNA damage and activation of poly-ADPR polymerase (PARP) and poly-ADPR glycohydrolase (PARG). PARP and PARG then act in concert to convert nicotinamide adenine dinucleotide (NAD^+) into polymers of ADPR, which are subsequently degraded into ADPR monomers. Monomeric ADPR then acts as an intracellular ligand to activate TRPM2 channels[29, 30]. The second proposed mechanism involves the breakdown of NAD^+ into ADPR in the mitochondria by NUDT9 ADPRase. To demonstrate this mechanism, Perraud and colleagues used an elegant approach in which they targeted NUDT9, which is able to degrade ADPR, to the mitochondria. When mitochondria-targeted NUDT9 was expressed in human embryonic kidney-293 (HEK-293) cells stably expressing human TRPM2 (HEK293-TRPM2), they demonstrated that TRPM2 channel activation in response to H_2O_2 exposure was largely abrogated[21]. These findings suggest that the mitochondrial production of ADPR is critical for the activation of TRPM2 channels in response to oxidative stress.

In addition to ADPR, several studies have suggested that NAD^+ is capable of activating TRPM2 channels[23, 31, 32]; however, this finding has not been consistently reproduced in the literature[8, 16]. In comparison to the latency of TRPM2 activation by ADPR, the latency to TRPM2 activation after NAD^+ application is significantly longer, raising the possibility that NAD^+ activates TRPM2 indirectly following its conversion to ADPR[23]. TRPM2 has also been shown to be activated by structural analogues of ADPR, including cyclic ADPR (cADPR), nicotinic acid adenine dinucleotide phosphate (NAADP) and 2′-O-acetyl-ADP-ribose (OAADPr), a product of the sirtuin family of protein deacetylases[33]. Whether cADPR and NAADP act as direct agonists, contributing to TRPM2 activation through their conversion to ADPR, or modify the concentration-response curve of ADPR required for channel activation is still unclear[34–38]. Intriguingly, a recent study seeking to identify the structural requirements for TRPM2 channel activation assessed the agonist activity of a series of ADPR analogues harboring modifications to the purine base, the pyrophosphate group or the terminal ribose[39]. This investigation led to the identification of 2′-deoxy-ADPR, a TRPM2 superagonist capable of eliciting whole-cell currents >10-fold larger than those elicited by ADPR. Using HPLC and mass spectrometry, 2′-deoxy-ADPR was identified as an endogenous metabolite generated through the concerted activity of cytosolic nicotinamide mononucleotide adenylyltransferase 2 (NMNAT-2) and the nicotinamide adenine dinucleotide glycohydrolase CD38. Progress in understanding the relative influence of these candidate intracellular ligands in regulating TRPM2 activity will require knowledge of the signaling context and concentrations achieved under various physiological and pathological conditions.

In addition to NUDT9-H agonist binding, TRPM2 activation has a strict intracellular Ca^{2+} requirement[16, 22, 40-42]. Ca^{2+} dependence is imparted via the Ca^{2+}-sensitive association of calmodulin (CaM) to an IQ-like domain localized within the intracellular N-terminus of TRPM2 (amino acids 406–416)[13, 14]. Mutation of the IQ-like CaM binding motif abrogates channel activation by hydrogen peroxide[13] and ADPR[14]. Similarly, ADPR-evoked TRPM2 currents are inhibited by calmidazolium, an inhibitor of CaM[41]. Interestingly, TRPM2 splice isoforms (TRPM2-ΔN, TRPM2-ΔC, TRPM2-ΔNΔC, and SSF-

TRPM2) and mutants that do not respond to ADPR can be activated by Ca^{2+}, albeit at very high concentrations (EC_{50}=~17 μmol/L in the absence of exogenously supplied ADPR)[14]. This Ca^{2+}-dependent activation suggests that elevated Ca^{2+} alone may be sufficient to activate TRPM2. In this regard, the rise in the intracellular Ca^{2+} concentration within micro- and nano-domains surrounding open Ca^{2+}-permeable channels is estimated to reach as high as 1–100 μmol/L[43]. Whether Ca^{2+} reaches a concentration sufficient to directly promote Ca^{2+}/CaM-dependent TRPM2 channel openings, for example, in response to Ca^{2+} influx via voltage- or ligand-operated channels localized in close proximity to TRPM2, remains to be determined. More realistically, intracellular Ca^{2+} and ADPR likely act co-operatively to effect TRPM2 channel activation. Such co-operativity between ADPR and Ca^{2+} is likely to have important functional implications. In neutrophil granulocytes, endogenous levels of ADPR, estimated at ~5 μmol/L, are sufficient to allow TRPM2 to respond to varying intracellular levels of Ca^{2+}, for example, in response to the release of Ca^{2+} from intracellular stores[22]. Analogously, in hippocampal pyramidal neurons, intracellular application of ADPR alone is insufficient to initiate robust TRPM2 activation. However, supplemental Ca^{2+} entry via voltage- or NMDA receptor-gated Ca^{2+}-permeable channels is necessary[42]. Collectively, these results suggest that TRPM2 integrates intracellular signaling events that provoke changes in the intracellular concentrations of ADPR (and possibly other endogenous agonists) and Ca^{2+}.

Additional gating modifiers

In addition to ADPR and Ca^{2+}, several other factors may influence TRPM2 gating. For example, extracellular pH has been shown to inhibit TRPM2 channels with a half-maximal inhibitory concentration (IC_{50}) ranging from pH 4.7 to 6.5, depending on the concentration of Ca^{2+} used in the solution. Acidic pH is proposed to reduce TRPM2 channel conductance by binding to external residues and/or through permeation of H^+ and subsequent competition for intracellular Ca^{2+} binding sites on the channel[44–46]. Similarly, an intracellular acidic environment also inhibits TRPM2 channels. This inhibition appears to involve Asp933, since substitution of this residue changes the IC_{50} from pH 6.7 to pH 5.5. Furthermore, increasing the concentration of intracellular calcium shifts the IC_{50} from pH 6.7 to pH 6.3, suggesting that intracellular protons may compete with calcium for a binding site[44]. In addition to pH, glutathione (GSH), a naturally occurring antioxidant, can act as an inhibitor of TRPM2 channels[47]. GSH has been shown to inhibit TRPM2-mediated cell death induced by H_2O_2 and TNF-α in insulinoma and monocyte cell lines[31]. Conversely, chemical depletion of intracellular GSH induces an increase in intracellular Ca^{2+} through TRPM2 channels expressed in glia or dorsal root ganglion sensory neurons[48, 49]. Our own results have demonstrated that TRPM2 activity is enhanced in cultured hippocampal neurons over time *in vitro* due to the loss of tonic inhibition by GSH[47]. Although the precise mechanism has yet to be defined, we have demonstrated that GSH causes a rightward shift in the ADPR concentration-response curve, increasing the EC_{50} for channel activation by ADPR from 77 μmol/L to 269 μmol/L. This observation suggest that GSH may compete for the ADPR binding site[47].

Physiological roles of TRPM2

TRPM2 mRNA expression assessed by quantitative real-time polymerase chain reaction (qRT-PCR) is nearly ubiquitous in all tissues examined, except for bone and cartilage. As such, TRPM2 has been implicated in physiological processes in a host of tissues and organ systems. First, TRPM2 participates in insulin secretion from pancreatic β-cells[32, 50, 51]. A role for TRPM2 in inflammation has also been established whereby TRPM2 activation has been shown to promote inflammation and immune responses through the production of cytokines CXCL8 (previously known as interleukin-8 [IL-8]), IL-6, IL-10, and TNF-α[52, 53]. More recently, TRPM2 has been implicated in phagosome maturation, which has been shown to increase bacterial clearance and reduce mortality in a mouse model of *E coli* sepsis[54]. Interestingly, TRPM2 dampens the inflammatory response through cellular depolarization and subsequent reduction of ROS production in phagocytes, thereby minimizing excess inflammation[55]. Additionally, the channel has been implicated in lung inflammation and associated diseases[56]. Lastly, oxidative stress-induced TRPM2 activation mediates Ca^{2+} entry into endothelial cells, leading to vascular barrier dysfunction via opening of interendothelial junctions, although the precise mechanism(s) involved requires further investigation[57, 58].

TRPM2 in the CNS

TRPM2 mRNA is most abundant in the brain[2, 24, 59]. Within the CNS, TRPM2 expression has been demonstrated in microglia, astrocytes, and neuronal populations in the hippocampus, substantia nigra, striatum, and cortex, as well as dorsal root ganglion (DRG) sensory neurons in the spinal cord[2, 24, 42, 59–63]. Within the hippocampus, TRPM2 mRNA is localized to the pyramidal cell layer, and preliminary evidence has demonstrated a predominantly extrasynaptic distribution in cultured hippocampal neurons[42]. TRPM2 currents in these cells can be activated by ROS/RNS exposure or by intracellularly applied ADPR contingent on supplemental Ca^{2+} influx through voltage-gated calcium channels (VGCCs) or N-methyl-D-aspartate (NMDA) receptors[42].

The requirement for Ca^{2+} entry via VGCCs and/or NMDA receptors (NMDARs) is in keeping with the recognized Ca^{2+}/CaM co-dependence of TRPM2 activation by NUDT9-H ligands. The strict requirement for co-stimulation of VGCCs or NMDARs to activate TRPM2 in hippocampal neurons is intriguing given the importance of each of these channels in neuronal function. In light of the essential contribution of NMDARs to the induction of multiple forms of synaptic plasticity[64, 65], the functional link between NMDARs and TRPM2 is of particular interest to us. In a recent study, we demonstrated that TRPM2 channels are necessary for the establishment of long-term depression (LTD), a specific form of NMDAR-dependent synaptic plasticity[66]. Impaired

NMDAR-dependent LTD in TRPM2 knock-out (KO) hippocampal slices was associated with a reduced expression of postsynaptic density protein 95 (PSD-95) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPARs). Moreover, the loss of NMDAR-dependent LTD was recapitulated with the TRPM2 antagonist clotrimazole, demonstrating that the observed changes in synaptic plasticity were not due to developmental changes induced by genetic deletion of TRPM2. Mechanistically, the resulting changes could be attributed to inactivation of glycogen synthase kinase 3β (GSK-3β) based on two key findings: 1) loss of TRPM2 was associated with increased inactivation of GSK-3β through its phosphorylation at Ser9, and 2) LTD deficits were rescued by stimulating GSK-3β activity downstream of dopamine D2 receptors 66. The signaling pathway through which TRPM2 regulates GSK-3β activity is likely to involve Ca^{2+}. Calcineurin (also known as PP2B), a phosphatase regulated by Ca^{2+}, dephosphorylates GSK-3β at Ser9 and may mediate the change in GSK-3β activation downstream of TRPM2. Evidence supporting such a mechanism was recently provided[67].

Importantly, we have demonstrated that the loss of TRPM2 expression does not affect the expression of NMDAR subunits GluN1, GluN2A and GluN2B 66, the predominant NMDAR subunits expressed in the hippocampus. In contrast, a subsequent study reported that knockout of TRPM2 impacted the expression of both GluN2A and GluN2B but not GluN1[68]. In this study, the reported changes in expression were evident by western blot analysis of whole hippocampal lysates but not when examined by immunofluorescence[68]. The reason for these discordant western blot findings is not clear. Importantly, the lack of altered expression was confirmed in our study by the assessment of NMDAR function in hippocampal pyramidal neurons. In this way, we demonstrated that NMDA-evoked current amplitude, desensitization (steady state/peak) and sensitivity to Ro25-6981, a highly specific GluN2B antagonist, are identical in hippocampal neurons derived from TRPM2 WT and KO mice[66]. Similarly, the amplitude of synaptically evoked NMDAR-mediated responses was not impacted by genetic deletion of TRPM2[66]. These findings strongly suggest that genetic deletion of TRPM2 does not impact NMDAR expression, at least among the complement of functioning receptors expressed at the cell surface.

In addition to regulating the induction of NMDAR-dependent synaptic plasticity, TRPM2 has been shown to have a physiological role in temperature sensation and thermoregulation. These roles are consistent with previous evidence demonstrating that TRPM2 can be activated by exposure to warm temperatures[50, 69]. Indeed, TRPM2 was recently shown to be expressed in a subpopulation of neurons in the preoptic area (POA) of the hypothalamus where it is involved in the sensing of hyperthermia and plays an important role in temperature homeostasis[69]. In this study, the authors demonstrated that TRPM2, expressed within POA neurons, becomes active under conditions of elevated core body temperatures (*ie*, >37 °C) and drives a compensatory hypothermic response, suggested to limit the upper fever range during heat stress. Interestingly, TRPM2 has also been shown to play a role in heat sensation by somatosensory and autonomic neurons[70]. In this report, the authors exposed mice to temperatures ranging from 23 °C to 38 °C and demonstrated that TRPM2 knockout mice showed a preference for non-noxious warmer temperatures, whereas wild-type littermates preferred cooler temperatures. Both groups tended to avoid the noxious temperature of 43 °C[70]. Collectively, these studies suggest that TRPM2 may guide both an autonomic and behavioral response that seeks to maintain thermal homeostasis.

Contribution of TRPM2 to CNS pathology

The influx of Ca^{2+} resulting from TRPM2 channel activation is key to its contribution to physiological as well as pathological processes. The intracellular concentration of Ca^{2+} is normally buffered to very low resting levels by numerous homeostatic mechanisms. Calcium signals, generated when the concentration of this divalent cation rises above this resting level, are sensed by a number of Ca^{2+}-dependent signaling molecules and serve a critical role in regulating cellular functions. Paradoxically, prolonged or excessive alterations in Ca^{2+} concentrations can lead to cytotoxicity[71–73]. Cell death as a result of cytotoxic Ca^{2+} entry via TRPM2 activated in response to oxidative stress is among the best and earliest characterized role for TRPM2. Initial evidence in HEK cells expressing TRPM2 heterologously, as well as in insulinoma and monocyte cell lines expressing TRPM2 endogenously, revealed that TRPM2 conferred susceptibility to cell death in response to H_2O_2 treatment[31]. A role for TRPM2 in cell death has since been confirmed in a variety of cell types including neuronal cell populations in response to H_2O_2, TNF-α, and Aβ peptide[74–77]. Aberrant TRPM2 function has been implicated in various neurological disorders, which is not surprising when one considers that TRPM2 expression levels are highest in the CNS, channel activation occurs in response to oxidative and nitrosative stress, and channel-mediated Ca^{2+} entry has been linked to cell death. In the sections to follow, we summarize recent progress in the understanding of the cause and consequence of pathological TRPM2 activation in the CNS.

Aging

Aging represents the leading risk factor for neurodegenerative diseases including Alzheimer's and Parkinson disease[78]. A major theory of aging as a risk factor for CNS disease suggests that neuronal Ca^{2+} dysregulation contributed by reduced antioxidant defense, increased oxidative stress and perturbed energy metabolism is a likely cause of aging-associated CNS disease[79]. TRPM2 is thought to participate in the normal aging processes within the brain. For example, normal aging is associated with a decreased concentration of glutathione (GSH) *in vitro* and *in vivo*[80–84]. The depletion of GSH with age not only increases the oxidative stress status of the cell but may also be associated with increased intracellular calcium and subsequent toxicity[85–89]. This process may involve

TRPM2, as recent work has demonstrated that TRPM2 activity is enhanced in cultured hippocampal neurons over time *in vitro* due to the loss of tonic inhibition by glutathione[47]. Relief of constitutive TRPM2 inhibition due to reduced intracellular GSH levels with aging could contribute to elevations in intracellular Ca^{2+}, consequently disrupting synaptic plasticity and reducing cell viability. Interestingly, although NMDAR-dependent long-term potentiation (LTP) is impaired with age, the total magnitude of LTP is largely preserved during normal aging, potentially through a compensatory upregulation in voltage gated calcium channel dependent LTP[90]. Given that TRPM2 is necessary for the induction of NMDAR-dependent LTD[66], we might expect that increased TRPM2 activity, in part due to reduced GSH levels with age, is associated with increased LTD. In fact, LTD has been shown to be enhanced with normal aging[91]. Combined, these changes in synaptic plasticity produce an age-dependent decrease in synaptic strength that may underlie the memory impairment associated with normal aging. Additional studies will be required to assess whether aberrant TRPM2 activity contributes to age-related alterations in plasticity and whether moderating TRPM2 activity in the aged brain could serve as a novel therapeutic intervention.

Ischemic stroke

Excitotoxicity by glutamate acting upon NMDARs is long established as the dominant conceptual model underlying neuronal cell death associated with ischemic stroke. However, numerous studies have demonstrated an important additional contribution of Ca^{2+}-permeable non-selective cation channels including large-pore pannexin channels[92, 93] and TRPM7, which have been suggested to be recruited by conditions of elevated oxidative stress associated with ischemia[94]. Similarly, a number of studies have linked TRPM2 activation to conditions of elevated oxidative stress and dysregulated Ca^{2+} associated with ischemic neuronal death. Among the earliest suggestive evidence in this regard came the demonstration that cell death of cultured neurons in response to H_2O_2 treatment (50 μmol/L for 6 h or 1 mmol/L for 20 min) was greatly attenuated by knockdown of TRPM2[76]. More definitive evidence that TRPM2 contributes to ischemia-induced neuronal cell death was provided with the demonstration that pharmacological inhibitors and RNA interference using shRNA targeting TRPM2 reduced infarct volume *in vivo* and decreased neuronal cell death *in vitro* following oxygen glucose deprivation (OGD)[75]. Interestingly, this protection appeared to be specific to males, suggesting a potential sex difference in the contribution of TRPM2 to ischemic cell death[95, 96]. Other groups have confirmed the ability of TRPM2 knockout to protect against ischemic cell death (notably, only male mice were used in these studies)[68, 97, 98]. In addition to its direct contribution to neuronal death in response to ischemia, TRPM2 may contribute to the sequelae of stroke by modulating stress-induced activation of microglia, as suggested in a previous study. Here, in the transient middle cerebral artery occlusion (tMCAO) rat model of ischemia, expression of TRPM2 mRNA was demonstrated to be elevated from 1–4 weeks following stroke induction. This increase in TRPM2 expression was attributed to transcriptional upregulation of TRPM2 in microglia in response to oxidative stress and the cytokine IL-1β[59]. Lastly, TRPM2 may also participate in neonatal ischemic brain injury. One recent study looked at the role for TRPM2 in hypoxic-ischemic injury in postnatal day 7 mouse pups by ligating the right common carotid artery and subsequently exposing animals to a hypoxic environment for 2 h. This group demonstrated that TRPM2 knockout was neuroprotective, with reduced brain infarct size, improved sensorimotor function, and reduced expression of inflammatory markers[99].

Alzheimer's disease

In addition to a potential role in stroke and ischemic brain injury, TRPM2 has also been implicated in Alzheimer's disease (AD). Primary striatal cultures exposed to 20 μmol/L monomeric β-amyloid demonstrated an increase in intracellular calcium and cell death, which was partially blocked when cultures were transfected with a dominant-negative splice variant of TRPM2[74]. More definitive evidence that TRPM2 contributes to the pathology and cognitive decline was recently provided in an AD mouse model[77]. Elimination of TRPM2 was shown to reduce expression of endoplasmic reticulum stress response markers and microglia activation in the APP/PS1 Alzheimer's disease mouse model[77]. Importantly, this study also demonstrated that deletion of TRPM2 rescued the spatial memory deficits in aged APP/PS1 mice. As TRPM2 is broadly expressed in a variety of cell types in the brain, and TRPM2 was deleted globally in this study, the extent to which the beneficial effects noted were due to loss of TRPM2 function in neurons, microglia or other cell types remains to be determined. Along these lines, subsequent research has demonstrated that TRPM2 participates in Aβ-induced neuroinflammation through microglia activation and generation of TNF-α in a pathway involving ROS activation of PARP-1[100]. Moreover, Aβ has been shown to provoke TRPM2 activation in vascular endothelial cells, where TRPM2 has been proposed to contribute to cerebrovascular dysfunction in AD[101].

Neuropathic pain

Recent evidence also suggests that TRPM2 may play a role in spinal cord injury and neuropathic pain. In a rat model of spinal cord injury, intraperitoneal (IP) injection of clotrimazole conjugated to polyethylene glycol significantly reduced lipid peroxidation, an indicator of oxidative stress, when administered 5 min after spinal cord compression[102]. Whether inhibition or knockdown of TRPM2 results in higher functional performance compared to untreated controls in a spinal cord injury model is unclear. Additionally, a recent study demonstrated that TRPM2 mRNA is elevated following sciatic nerve injury. When compared with WT littermate controls, TRPM2 knockout mice show a reduced immune response and attenuation of the heightened mechanical and thermal pain responses elicited by sciatic nerve injury, suggesting that TRPM2 plays a

role in neuropathic pain[103].

Bipolar disorder and other disorders associated with TRPM2 mutations

Genetic studies have associated single nucleotide polymorphisms (SNPs) in TRPM2 to an increased susceptibility for bipolar disorder[104–107]. Interestingly, a recent study demonstrated that a TRPM2 mutation identified in patients with bipolar disorder (D543E) is associated with a loss of TRPM2 function[66]. This finding led the authors to posit that the loss of TRPM2 function may be associated with the behavioral manifestations of bipolar disorder, including mood disorders and impaired social interaction. Consistent with this assumption, TRPM2 knockout mice were found to exhibit increased anxiety and impaired social cognition. In agreement with previous reports demonstrating an important role of TRPM2 in regulating GSK-3 activity[66, 68], the authors provided further evidence suggesting that bipolar disorder-related behavior associated with loss of TRPM2 function may be due to dysregulated GSK-3 activity.

A single SNP (P1018L) in TRPM2 has also been identified in tissue from Guamanian amyotrophic lateral sclerosis (ALS) and Parkinson's disease subjects[108]. In addition to the potential genetic link with Parkinson's disease, a recent study also demonstrated that TRPM2 channels are necessary for NMDA-induced burst firing in substantia nigra pars reticulate GABAergic neurons. These authors noted that previous studies have demonstrated an increase in burst firing observed in Parkinson's disease, which may implicate TRPM2 in Parkinson's disease pathology[62]. Whether other neurological disorders are associated with TRPM2 mutations requires further investigation.

Pharmacology and therapeutic potential of drugs targeting TRPM2

Initial research into TRPM2 and its role in normal physiology and disease involved a combination of non-selective pharmacological agents and genetic knockout models. The channel is inhibited by agents such as N-(p-amycinnamoyl) anthranilic acid (N-ACA), econazole, clotrimazole, and flufenamic acid[42, 109]. Notably, many previously identified TRPM2 inhibitors are non-selective and thus have additional effects unrelated to TRPM2. For example, N-ACA inhibits calcium-activated chloride channels[110] as well as several TRP family members, including TRPC6 and TRPM8[111]. Clotrimazole, an antifungal agent, inhibits Ca^{2+}-activated potassium channels (KCa3.1)[112], cytochrome P-450[113] and NMDARs[114]. Lastly, flufenamic acid, a non-steroidal anti-inflammatory, inhibits chloride channels, calcium-activated chloride channels, and voltage-gated Ca^{2+} channels[115]. Experimentally, the limited specificity of existing inhibitors can be addressed in part through the utilization of a panel of inhibitors that have the ability to block TRPM2 in common but differ with respect to their additional off-target effects (*eg*, N-ACA and clotrimazole). Additionally, potential confounds related to the use of non-specific antagonists can be circumvented through judicious use of established TRPM2 knockout mice[53]. Therapeutically, the lack of specificity of existing TRPM2 inhibitors is a major limitation. In this regard, some recent developments have provided hope that TRPM2 is, in fact, a druggable target.

Research in the last 5 years has led to the identification of several novel inhibitors of TRPM2. These inhibitors certainly have a role in experimental application, and some may also show promise as compounds that may be developed into novel therapeutics. The use of a modified ADPR analogue, 8Br-ADPR, was initially shown to inhibit ADPR-activated calcium influx in mouse neutrophils and dendritic cells[116]. Following this paper, several other modified compounds were designed, and their ability to antagonize TRPM2 was evaluated, including the antagonist 8-Ph-2′-deoxy-ADPR, which displays an IC_{50} of 3 μmol/L[117]. Luo and colleagues also recently synthesized novel ADPR analogues capable of selectively inhibiting TRPM2 channel currents *in vitro* at low concentrations without affecting TRPM7, TRPM8, TRPV1, or TRPV3[118]. Additionally, a TRPM2 peptide inhibitor tat-M2NX, a cell-permeable peptide, was recently designed to correspond to the C-terminal NUDT9-H domain. This peptide has been shown to decrease calcium influx *in vitro* and decrease infarct volume following transient middle cerebral artery occlusion when provided either prior to the infarct or 3 hours following the insult[119].

Summary

As highlighted in this review, numerous studies have now established the important contribution of TRPM2 to a wide variety of CNS diseases, including ischemia/stroke, Alzheimer's disease, neuropathic pain, bipolar disorder, and Parkinson's disease. Collectively, these studies suggest that therapeutic interventions able to moderate aberrant TRPM2 activation may be effective in treating these debilitating neurological disorders. This therapeutic potential is counterbalanced by reports demonstrating that TRPM2 also contributes to many physiological processes. The involvement of TRPM2 in these numerous physiological processes raises some concerns with regards to the potential side effects of drugs able to block TRPM2 function. These concerns predominantly relate to the roles of the channel in immune function, insulin release, and temperature sensation and regulation. Of note, genetic deletion of TRPM2 is well tolerated and does not appear to alter behavior, including locomotor activity assessed on the open field test, anxiety behaviors assessed by the elevated plus maze, or spatial memory deficits assessed by the Barnes maze[77]. Ultimately, assessment of the risk-benefit profile of TRPM2 as a therapeutic target will require the development of specific TRPM2 inhibitors with favorable pharmacokinetic and pharmacodynamic properties. Further research will be needed to identify which specific patient populations would derive the most benefit and to assess whether side effects of TRPM2 inhibition are clinically significant and thus preclude the consideration of drugs targeting this channel.

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