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OVERVIEW

TRPM2 Cation Channels, Oxidative Stress and Neurological Diseases: Where Are We Now?

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Abstract The Na+ and Ca^{2+}-permeable melastatin related transient receptor potential 2 (TRPM2) channels can be gated either by ADP-ribose (ADPR) in concert with Ca^{2+} or by hydrogen peroxide (H_2O_2), an experimental model for oxidative stress, binding to the channel's enzymatic Nudix domain. Since the mechanisms that lead to TRPM2 gating in response to ADPR and H_2O_2 are not understood in neuronal cells, I summarized previous findings and important recent advances in the understanding of Ca^{2+} influx via TRPM2 channels in different neuronal cell types and disease processes. Considering that TRPM2 is activated by oxidative stress, mediated cell death and inflammation, and is highly expressed in brain, the channel has been investigated in the context of central nervous system. TRPM2 plays a role in H_2O_2 and amyloid β-peptide induced striatal cell death. Genetic variants of the TRPM2 gene confer a risk of developing Western Pacific amyotropic lateral sclerosis and parkinsonism-dementia complex and bipolar disorders. TRPM2 also contributes to traumatic brain injury processes such as oxidative stress, inflammation and neuronal death. There are a limited number of TRPM2 channel blockers and they seem to be cell specific. For example, ADPR-induced Ca^{2+} influx in rat hippocampal cells was not blocked by N-(p-amylcinnomoyl)anthralic acid (ACA), the IP_3 receptor inhibitor 2-aminoethoxydiphenyl borate or PLC inhibitor flufenamic acid (FFA). However, the Ca^{2+} entry in rat primary striatal cells was blocked by ACA and FFA. In conclusion TRPM2 channels in neuronal cells can be gated by either ADPR or H_2O_2. It seems to that the exact relationship between TRPM2 channels activation and neuronal cell death still remains to be determined.

Keywords TRPM2 · Ca^{2+} · Neuronal cells · Oxidative stress · ADP-ribose · Glial cells · Alzheimer' disease

Abbreviations

2-APB	2-Aminoethoxydiphenyl borate.
ACA	N-(p-amylcinnomoyl)anthranilic acid
AD	Alzheimer's disease
ADPR	Adenosine diphosphatase ribose
CHO	Chinese hamster ovary
CNS	Central nervous system
DRG	Dorsal rood ganglion
FFA	Flufenamic acid
HEK	Human embryonic kidney
PARG	Poly (ADP-ribose) glycohydrolase
PARP	Poly(ADP-ribose) polymerase
PD	Parkinson disease
PKC	Protein kinase C
ROS	Reactive oxygen species
TNF-α	Tumor necrosis factor alpha
TRP	Transient receptor potential

Introduction

Of all the cell types in the body, neuronal cells may be among the most vulnerable to oxidative stress. These cells are continuously exposed to reactive oxygen species (ROS)

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generated via the auto-oxidation of polyunsaturated fatty acids [1]. A variety of neurodegenerative disease states have been also associated with oxidative stress [2]. Oxidative stress is thought to be mediated by excessive exposure of cells to reactive oxygen or nitrogen species, which can be generated by ischemia, radiation, seizure, trauma etc. Exposure to oxidative stress induces both apoptotic-like delayed neural death and necrosis in cultured neurons, mediated by a rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), mitochondrial dysfunction, and activation of poly[adenosine diphosphate ribose (ADPR)] polymerase (PARP) due to DNA damage [2]. In this regard, one potentially important calcium influx pathway may be activation Na^+ and Ca^{2+} permeable Transient Receptor Potential (TRP) channels. Of particular interest is a member of the melastatin subfamily (TRPM2, it is also known as LTRPC2) that is ROS and ADPR gated [3, 4]. TRPM2 channels are widely expressed in brain, and TRPM2 channels have been implicated in neuronal damage induced by oxidants, amyloid β-peptide and tumor necrosis factor alpha (TNF-α) [2, 4]. Therefore, they are regarded as a potential therapeutic target in some neurological disorders including Alzheimer's disease (AD), bipolar disorders, Parkinson disease (PD) and traumatic brain injury.

TRPM2, TRPM6, and TRPM7 share a feature unique among known channels, by having a functional enzyme moiety in the C-terminal domain. In TRPM2, this is a type of Nudix hydrolase (NUDT9-H) that can bind to and hydrolyze ADPR, although not as effectively as other known Nudix ADPR-hydrolases (Fig. 1) [5]. Binding of ADPR to NUDT9-H activates the channel, allowing the passage of cations down their electrochemical gradient. Because TRPM2 is a plasma membrane channel, Ca^{2+} and Na^+ will flow into the cell when TRPM2 opens. ADPR is the most potent physiological activator of TRPM2, but other less potent activators have been proposed [6, 7]. These include nicotinamide adenine dinucleotide (NAD^+) [4], oxidants such as H_2O_2 [4], and cyclic ADPR (cADPR) [5]. NAD^+ and H_2O_2 increase intracellular levels of ADPR and could thus gate TRPM2 either directly [5–7] or indirectly [8, 9].

Several chemicals have been reported to act, as TRPM2 agonists. However, they cannot be easily used for experimental purposes. For example, imidazole, derivates, miconazole and clotrimazole, inhibit TRPM2 in an irreversible manner. Flufenamic acid (FFA) is also a TRPM2 channel blocker. FFA is a nonsteroidal anti-inflammatory drug, is not easily dissolved in aqueous solution which makes it difficult to prepare solutions containing the high concentrations of FFA necessary to inhibit TRPM2. ADPR-induced TRPM2 currents are blocked by FFA [10]. N-(p-amylcinnomoyl)anthranilic acid (ACA) blocks TRPM2 channels blocker although it is relatively nonspecific, inhibiting both phospholipase A2 as well as TRPM2 channels [11]. 2-aminoethyldiphenyl borate (2-APB) blocks 1,4,5-trisphosphate (InsP3) receptors as well as store-operated channels, and it is also a TRPM2 channel blocker in TRPM2-expressing cell lines and rat pancreatic islet cells [12]. All of the TRPM2 channel antagonists, with the exception of 2-APB, either gradually or irreversibly block ADPR-induced currents.

In the current review I summarized previous findings and important recent advances in the understanding of Ca^{2+} influx via TRPM2 channels in different neuronal cell types and in disease processes. I discussed the possible use of nonspecific TRPM2 channels blockers in neuronal cells.

TRP Superfamily

The *trp* gene was identified through genetic studies of a mutation in the *Drosophilia* visual transduction system. The term of "TRP" is derived from "transient receptor potential", because photoreceptors with the *trp* gene mutant fail to generate the Ca^{2+}-dependent "sustained" phase of receptor potential and therefore fail to show subsequent Ca^{2+}-dependent adaptation to light [13]. There are 30 mammalian TRP channels, grouped into six subfamilies. Members of the TRP channels superfamily include TRP canonical (TRPC) subfamily consisting of 7, TRP vanilloid (TRPV) subfamily consisting of 6, TRP melastatin (TRPM) subfamily consisting of 8, TRP polycystein (TRPP) subfamily consisting of 3, TRP mucolipin (ML) subfamily consisting of 3, and TRP ankyrin (TRPA) subfamily consisting of only one member. Some members of this superfamily are poorly characterized, but these channels are attracting increasing interest because of their apparent involvement in several human diseases. TRP channels have a basic structure similar to voltage-gated potassium channels, with homo- or hetero-tetrameric arrangements around a central ion conducting pore formed from a sequence between the 5th and 6th transmembrane domains [14]. The N-termini of TRPV and TRPC, but not those of TRPM channels, contain multiple ankyrin binding repeats. The C-terminal part of 6th segment in TRPC and TRPM channels includes the 'TRP C and N domains', a conserved stretch of 25 amino acids starting with the nearly invariant 'TRP box' that is missing in TRPV channels. In addition, all TRP channels have multiple regulatory protein interaction sites. Multiple protein kinase A (PKA) and C (PKC) putative phosphorylation sites have been identified and partially tested for function [15]. Phosphatidylinositide 3-kinase SH2-recognition domains have also been identified in several TRP channels [15, 16].

TRP channels contribute to changes in $[Ca^{2+}]_i$ concentrations by acting as Ca^{2+} entry channels in the plasma membrane directly or by changing membrane potential,

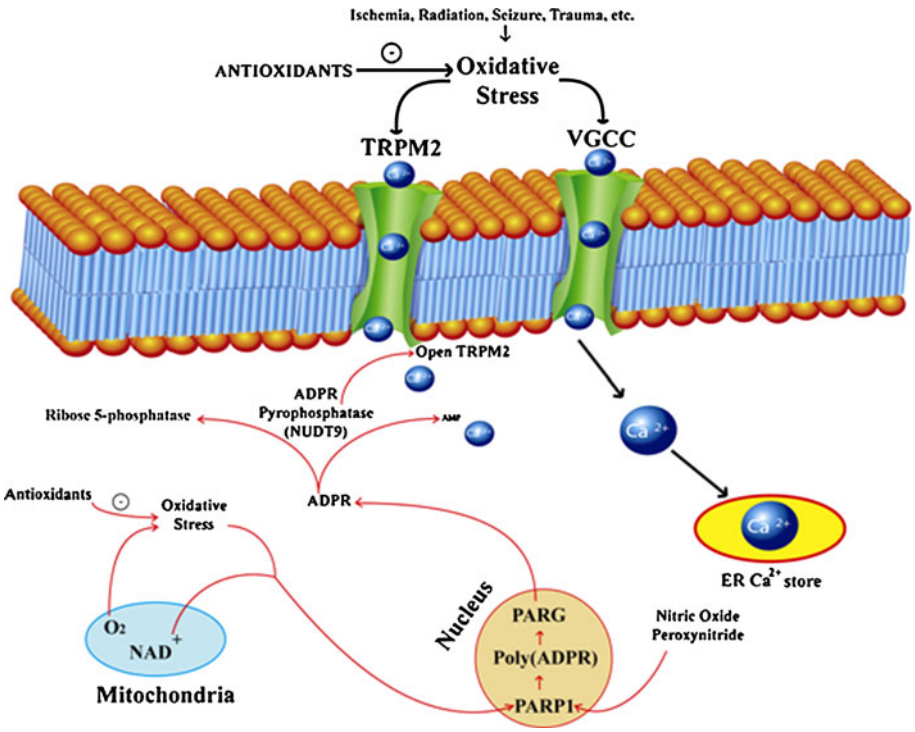

Fig. 1 Cells regulate intracellular Ca^{2+} levels lightly and excessive Ca^{2+} loads can lead to inappropriate activation of process that are normally operate at low levels, causing metabolic derangements and eventual cell death. For example, excesses elevations in intracellular Ca^{2+} due to ischemia, radiation, trauma etc. may activate enzymatic degradation, induce formation of reactive oxygen species (ROS) or disrupt normal mitochondrial function leading to oxidative stress and bioenergetic failure. Voltage gated Ca^{2+} channels (VGCC) and transient potential melastatin 2 (TRPM2)-mediated Ca^{2+} entry triggers a neurotoxic signal cascade involving the activation of neuronal nitric oxide (NO) synthase (nNOS), formation of the toxic ROS and NO and activation of the pro-apoptotic protein poly[ADP-ribose, (ADPR)] polymerase (PARP-1). The TRPM2 channels are containing C (Nudix box) and N sections. Nodix box domain has ADP-ribose pyrophosphatase activity. The TRPM2 channels can be gated by H_2O_2 and ADP-Ribose via activation ADP-ribose pyrophosphatase [1, 8]. Antioxidants regulate Ca^{2+} influx into cytosol by inhibition of ROS. Sustained depolarization of mitochondrial membranes and enhanced ROS production activates TRPM2 and VGCC and Ca^{2+} influx increases by activation of TRPM2 via ROS [67]. The molecular pathway may be a cause of neurological symptoms and represents a fruitful subject for further study

modulating the driving force for other Ca^{2+} entry channels. All functionally characterized TRP channels are permeable to Ca^{2+} with the exceptions of TRPM4 and TRPM5, which are only permeable to monovalent cations. Two mammalian TRPs, TRPV5 and TRPV6, are highly Ca^{2+} permeable. TRPM6 channels are highly selective to Mg^{2+} [16]. TRPM7 is also a cation selective ion channel that is highly permeable to both Ca^{2+} and Mg^{2+}, but it can also conduct essential or toxic metals. At least three TRP channels, TRPV1, TRPML1, and TRPP3, are highly permeable to H^+ ions [17].

TRPM Subfamily

The TRPM2 subfamily consist of eight members, which are sub-divided into three subgroups on the basis of sequence homology; TRPM1/TRPM3, TRPM4/TRPM5 and TRPM6/7, with TRPM2/TRPM8 being distinct proteins from the other groups (Fig. 2) [1].

Three members of this subfamily carry a functional enzyme in their COOH-termini: TRPM2 contains a functional NUDT9 homology domain, exhibiting ADPR pyrophophatase activity (Fig. 1), whereas both TRPM6 and TRPM7 contain a functional COOH-terminal serine/threonine kinase. Except for TRPM1 which is not as yet functionally characterized, all TRPM channels are cation channels, although the Ca^{2+} permeability is diverse, ranging from highly permeable (TRPM6/TRPM7 and splice variants of TRPM3) to Ca^{2+} impermeable (TRPM4 and TRPM5). TRPM4 and TRPM5 are heat-sensitive, Ca^{2+} activated channels [17]. TRPM2 is activated by ADPR, H_2O_2 and heat. TRPM2 and TRPM7 are involved in oxidative stress-induced cell death [4]. Interestingly, TRPM2 and TRPM7 have been reported to play pathological roles in the brain [18]. TRPM8 is the infamous cold receptor [19]. TRPM3 channels are, much like TRPM6 and TRPM7, regulated by intracellular Mg^{2+} levels. TRPM3 channels show considerable constitutive activity, and TRPM3 activation was also reported after cell swelling and by

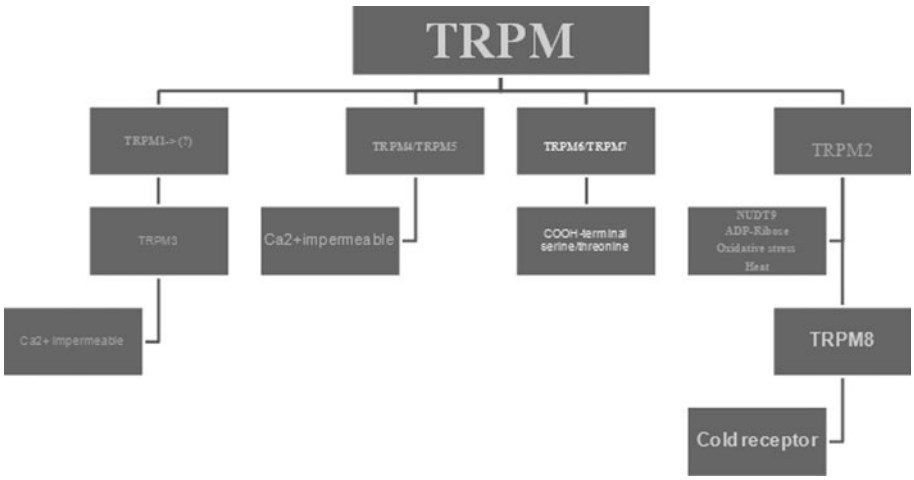

Fig. 2 The TRPM2 subfamily consist of 8 members, which are sub-divided into four subgroups on the basis of sequence homology; TRPM1/TRPM3, TRPM4/TRPM5, TRPM6/7 and TRPM2/TRPM8

sphingosine [20]. TRPM7 channels are also activated by oxidative stress, ADPR and both.

TRPM2 Channels

The founding member of the TRPM family is melastatin (TRPM1), so named because it was first described in connection with metastatic melanomas. TRPM2 was formerly known as TRPC2 and LTRPC2. TRPM2 channels were first identified in 1998 [21] and subsequently recognized as a member of the TRPM family [14]. All eight TRPM family members have been linked to disease, either by functional studies in mouse models or by genetic evidence [17]. TRPM2 channels are nonselective cation channels. They are highly expressed in brain [4], where they are preferentially localized in microglia cells, the host macrophages of the central nervous system. A cation current with TRPM2-like properties has been described in rat striatal neurons [22].

Molecular Mechanisms on the Activation of TRPM2 in Neurons

Three extracellular signals are known to active TRPM2; oxidative stress, ADPR/NAD^+ metabolism and TNF-α [4]. The channel may also be temperature sensitive with body temperature acting as 'an endogenous co-activator' for TRPM2 [14].

Oxidative Stress

Oxidative stress is defined as an imbalance between higher cellular levels and ROS, e.g., superoxide and hydroxyl radicals and cellular antioxidant defense. Generation of ROS is ubiquitous since ROS are generated during aerobic metabolism, i.e., mitochondrial oxidation and phagocytosis. In order to scavenge ROS, various defense systems exist in the brain [2].

ROS act as subcellular messengers when they are generated excessively or when enzymatic and non-enzymatic defense systems are impaired [1], and can regulate such complex processes as mitogenic signal transduction, gene expression, and cell proliferation. While many intra- and extracellular molecules may participate in neuronal injury and cell apoptosis, accumulation of oxidative stress due to excessive generation of ROS appears to be a potential factor for cell damage and death [2].

Role of Oxidative Stress on Activation of TRPM2

Oxidative stress, for which application of H_2O_2 is an experimental paradigm, induces TRPM2 currents and an increase in $[Ca^{2+}]_i$ concentrations in various cell types transfected with TRPM2 [6, 7], as well as in pancreatic β-cells [9], neutrophil granulocytes [5], and U937 monocytes [19, 23]. However, the exact mediator for H_2O_2-induced TRPM2 channel activation remains to be identified. The ROS sensitivity of TRPM2 could be mediated by NAD^+ [4] or ADPR [4, 24] released from mitochondria or through protein oxidation [25]. H_2O_2 and cADPR have been proposed to potentiate the effect of ADPR at lower concentrations and to gate the TRPM2 channel directly at higher concentrations [26].

It was reported that oxidative stress-induced TRPM2 activation is triggered via the production of ADPR from mitochondria [1]. N domain of TRPM2 has ADPR pyrophosphates enzyme activity and the enzyme activated by oxidative stress and ADPR. ADPR is synthesized from NAD of mitochondria and nucleolus via different three ways [1, 8]. In the nucleus, ADPR generation is attributed to a pathway involving poly(ADPR) polymerase-1 (PARP-1), and may be initiated by DNA damage because of different factors such as oxidative stress and radiation. Free ADPR is

generated following the degradation of poly(ADPR) by poly(ADPR) glycohydrolase (PARG) and ADP-ribosyl protein lyase activities [27]. Thus, it may be hypothesized that free-ADPR, which activates TRPM2, is produced by the activation of PARP-1 and PARG. However, electrophysiological studies have shown that PARP inhibitors don't interfere with activation of TRPM2 by ADPR [8, 28].

Role of ADPR/NAD^+ Products on Activation of TRPM2

Ca^{2+} influx via TRPM2 is thought to occur via production of ADPR. ADPR may arise from a mitochondrial source or alternatively via activation of poly(ADPR). ADPR may also arise from a mitochondrial source [25].

NAD^+ has been reported to stimulate TRPM2 [4, 6]. TRPM2 contains a characteristic structural feature known as a Nudix domain in its C-terminal cytosolic tail [23]. A Nudix domain is a consensus region that is known to be present in a class of pyrophosphates that degrade nucleoside diphosphates (Fig. 1) [1]. Indeed, the nudix domain of TRPM2 cleaves ADPR, a breakdown product of NAD^+ and cyclic ADPR, the latter being an intracellular second messenger that stimulates Ca^{2+} release by ryanodine receptors [4]. While ADPR is hydrolyzed by TRPM2, it also activates TRPM2 and induces TRPM2 currents when infused by patch pipettes [4, 23]. In two recent studies, we observed activation of TRPM2 by NAD^+, which supports the idea that this substance activates TRPM2 directly [9, 10]. A more recent study [29] reported that extracellular cyclic-ADP-ribose (cADPR) induced temperature-sensitive $[Ca^{2+}]_i$ concentration increases by Ca^{2+} influx through TRPM2 channels in rodent NG108-15 neuronal cells.

Previously published data indicated that TRPM2 can be gated by H_2O_2 and it has been suggested that this occurs independently of ADPR [1, 6, 7, 24]. Perraud et al. [23] demonstrated that reducing the ADPR concentration within mitochondria largely suppresses H_2O_2-mediated activation of TRPM2, suggesting that the gating mechanism of H_2O_2 is primarily based on its ability to release ADPR from mitochondria, which then proceeds to active TRPM2. However, Kolisek et al. [30] recently demonstrated that H_2O_2 may act as a direct stimulus for TRPM2 activation, as it can initiate the release of ADPR from mitochondria and at the same time function as a potentiating cofactor of ADPR. In our recent study, we observed that the H_2O_2-mediated activation of TRPM2 appears to result from a direct gating mechanism, because TRPM2 activation by H_2O_2 was relatively rapid in whole cell recordings, and the compound triggered single-channel activity in excised membrane patches [6]. After adding H_2O_2 to the bath after recording in the inside out configuration, the patches were washed by intracellular buffer and then H_2O_2 was given again. The channel was activated again by a second administration of H_2O_2 although no intracellular components should be present. From this, we concluded that H_2O_2-induced, single channel activity observed in excised membrane patches is likely caused by a direct gating mechanism.

Methodological problems are likely responsible for differing reports on the activation of TRPM2 by NAD^+, ADPR and H_2O_2. For examples, in a previous study [24] using HEK293 cells as an expression system, we demonstrated characteristic currents through the splice variant of TRPM-ΔC induced by H_2O_2. In later experiments using a CHO expression system, we could stimulate wild-type TRPM2 channels with NAD^+ but we could not stimulate them with H_2O_2 with sufficient consistency [30].

Second problem leading to differing results arises from the use of different cell types to study TRPM channel activation by H_2O_2, ADPR and its metabolites. On the other hand, channel activation by H_2O_2 appears to represent a cell-specific process in cells with endogenous expression of TRPM2. For example, the TRPM2 channel is activated by H_2O_2 in HEK293 cells [4, 24], CHO cells [31], CRI-GI rat insulinoma cell lines [9], rat primary striatal cultures [32] but not in human neutrophil granulocytes [5]. Kolisek et al. [30] in experiments with HEK293 cells found that cADPR stimulated TRPM2 channels. Later, this report was supported by the results of Gasser et al. [33] who reported that TRPM2 channels in Jurkat cells were gated by cADPR. However, a study from Aachen did not support the results for neutrophils granulocytes [5] where TRPM2 channels were not activated by cADPR.

Studies of TRPM2 Knockout Mice

The first TRPM2 knockout (TRPM2-KO) mouse study was published in 2008 [19], and additional studies using this model have continued. For example, an important advance in understanding the biological role of TRPM2 is provided by a study on TRPM2-KO mice [19]. The H_2O_2-activated signaling cascade involved in the production of inflammatory protein-2 cytokine is impaired in monocytes from TRPM2-KO mice. Recently, Lange et al. [34] reported that in TRPM2-KO mice, TRPM2 functions as a Ca^{2+}-release channel activated by intracellular ADPR in a lysosomal compartment in addition to its role as a plasma membrane channel. They showed that both functions of TRPM2 are critically linked to hydrogen peroxide-induced beta cell death. Additionally, extracellular ADPR production by the ectoenzyme CD38 from its substrates NAD^+ or cADPR causes IP_3-dependent Ca^{2+} release via P2Y and adenosine receptors. Lange et al. [34] concluded that ADPR and

TRPM2 represent multimodal signaling elements regulating Ca^{2+} mobilization in β-cells through membrane depolarization, Ca^{2+} influx, and release of Ca^{2+} from intracellular stores.

Role of TRPM2 Channels in Neuronal Cells

Northern blotting and quantitative PCR techniques indicted that TRPM2 is broadly expressed in the central nervous system (CNS). However, as this evidence was derived from homogenized tissue samples, it does not allow expression in neurons to be distinguished from that in glial cells. The importance of making such a distinction is highlighted by a recent study that failed to identify TRPM2 transcripts or functional channels in cerebellar granule cells and astrocytes [35]. Rather, TRPM2 was detected in microglial cells leading to the suggestion that the CNS distribution of TRPM2 corresponds to its expression in non-neuronal cells (Table 1) [36, 37].

Role of Oxidative Stress on TRPM2 Channels in Microglia and Astroglia Cells

The NADPH-oxidase in phagocytic cells such as microglia is an electron transport system that catalyzes the reduction of oxygen to superoxide radical. Under physiological conditions, the system contributes to the elimination of pathogens but under chronic inflammatory conditions such as scleroderma and liver fibrosis it is through to induce neurodegeneration by the massive accumulation of superoxide radical [2]. Monocyte superoxide, in high glucose media, is released by the NADPH-oxidase but not by the mitochondrial respiratory chain, and antioxidant such as α-tocopherol inhibits superoxide release via inhibition of PKC-α. The assembly of a functional NADPH-oxidase complex at the plasma membrane depends on the phosphorylation and subsequent translocation of several cytosolic subunits ($p40^{phox}$, $p47^{phox}$, $p67^{phox}$, and Rac1/2) [38]. In microglia cells, antioxidants inactivates PKC via phosphatase-mediated pathway (PP1 or PP2A) and, as a consequence, block the phosphorylation-dependent translocation of $p67^{phox}$ to the plasma membrane. As a result, the production of superoxide radical by the microglial NADPH-oxidase system is substantially inhibited, offering a partial explanation for the beneficial effect of antioxidants such as α-tocopherol on a variety of neurodegenerative diseases [2].

Like other activated macrophages, microglia remove bacteria and cellular debris and produce a diverse range mediators of the inflammatory cascade including arachidonic acid derivatives and H_2O_2. Thus microglia cells are a key factor in the immune defense and tissue repair in the central nervous system. Kraft et al. [35] described a novel calcium influx pathway in microglial cells coupled to hydrogen peroxide and ADPR and provided evidence that this pathway involved TRPM2 although they failed to detect TRPM2 in cultured cerebellar granule neurons. Recently, Ohana et al. [39] investigated expression of putative Ca^{2+}-permeable TRPM2 channels in rat cultured microglial cells by quantitative real-time RT-PCR. They detected transcripts in the rat cultured microglial cells for TRPM2 genes.

Although the role of astroglia in the progression of neurodegenerative disease is still relatively unknown, their importance in regulating the normal and abnormal neuronal

Table 1 Effects of oxidative stress, adenosine diphosphoribose (ADPR) and cyclicADPR (cADPR) on TRPM channels in neuronal cells

Activator	Cells	Effects	Reference
ADPR	Rat microglia	Activator	Kraft et al. [35]
ADPR	Pyramidal neurons and CA1	Unknown	Ohana et al. [39]
ADPR	Hippocampus interneurons	Insufficient	Olah et al. [37]
ADPR	Dorsal root ganglion of mouse	Activator	Nazıroğlu et al. [submitted]
cADPR	NG108-15 neuroblastoma cells	Activator	Amina et al. [29]
Both ADPR and oxidative stress	Cortical neurons	Activator	Kaneko et al. [45]
	Rat microglia	No effects	Kraft et al. [35]
Oxidative stress	Striatal neurons	Activator	Smith et al. [65]
Oxidative stress	Rat primary striatal neurons	Activator	Fonfria et al. [32]
Oxidative stress	Hippocampal neurons	Activator	Lipski et al. [42]
Oxidative stress	C13 microglia cells	Activator	Hill et al. [22]
Oxidative stress	C13 microglia cells	Activator	Fonfria et al. [66]
Oxidative stress	Astroglia	Activator	Bond and Greenfield [41]
Oxidative stress	Substantia nigra	Activator	Freestone et al. [53]
Oxidative stress	Rat primary striatal neurons	Activator	Olah et al. [37]
Oxidative stress	Hippocampal cells	Activator	Bai and Lipski [43]

environment is attracting increasing attention. Through Ca^{2+} signaling cascades, astroglia control gene expression, neuronal differentiation, and programmed cell death, which are all integral to developmental and degenerative processes. Under conditions of oxidative stress, glial cells provide energy support for neurons, exert a protective function by scavenging and detoxifying ROS, and direct neuronal resistance or vulnerability to degeneration through Ca^{2+}-dependent secretion of trophic or inflammatory factors [40]. There are few reports on TRPM2 in astroglia. Bond and Greenfield [41] reported that the additive effects of L-VGCC blockade and TRPM2 inhibition during oxidative stress significantly enhanced recovery from protein synthesis suppression and repressed subsequent compensatory protein over-expression. These results indicated that Ca^{2+} signaling is integral to astroglial transcriptional and translational responses to oxidative stress.

Role of Oxidative Stress on TRPM2 Channel in Hippocampal Neurons

As it was mentioned in previous section, TRPM2 is expressed in diverse cell types and despite convincing evidence for high expression in the mammalian brain, much of this signal was attributed to strong expression in non-neuronal cells. Thus, the existence of functional TRPM2 channels in neurons is controversial at best. Recently, Lipski et al. [42] reported that TRP or TRP-like channels including TRPM2 in hippocampal CA1 neurons are activated by cellular stress and contribute to ischemia-induced membrane depolarization, intracellular calcium accumulation and cell swelling. More recently, Olah et al. [37] reported that functional TRPM2 channels are highly expressed in pyramidal neurons of hippocampus, including those of CA1 interneurons in hippocampal slices. They also reported that ADPR alone is insufficient to gate TRPM2 in hippocampal neurons. They concluded that concomitant influx of Ca^{2+} through voltage dependent Ca^{2+} channels and or NMDAR is necessary to fully active TRPM2 channels. Recently, Bai and Lipski [43] investigated expression of TRPM2 and TRPV4 channels and their potential role in oxidative stress-induced cell damage in organotypic hippocampal slice cultures. In this study oxidative stress induced by H_2O_2 (600 μM) caused preferential damage of pyramidal neurons although oxidative stress induced with mercaptosuccinate (400 μM) or buthionine sulfoximine (4 μM) damaged astrocytes. Antioxidants (Trolox 500 μM, MitoE 2 μM) reduced both neuronal and astrocytic cell damage.

TRPM2 Channels and Dorsal Root Ganglion Neurons

There are several types of sensory neurons in the DRG, with responsiveness to different of external and internal stimuli. These stimuli, i.e. nociceptive, thermal or mechanical, activate different receptors and ion channels that are present in the nerve terminals at the sensory receptive fields and their expression in selective subsets of DRG neurons determines the response profile of non-selective cation channels that play important functions in sensory neurons. TRPM8 is the only TRPM channel with a clearly assigned function in DRG neurons. It is activated by innocuous cool stimuli and responds to menthol and icilin with intracellular Ca^{2+} elevations [16, 17]. Recently, TRPM2 channels were for the first time detected in DRG cells of mouse [44]. These investigators observed also that levels of TRPM2 channels in DRG cells were significantly higher in lumbar tissue than in thoracic tissue in the adult mouse although they did not detect the channels in the DRG cells of embryonic day (12) to 12 weeks age. Recently, my group was performed in whole cell patch clamp experiments in DRG cells of mouse and we observed that the TRPM2 channels in DRG cells of mouse were activated by either ADPR (1 mM) or rotenone (1 μM) (Submitted data).

Role of TRPM2 Channels in Oxidative Stress-Induced Neurological Diseases

TRPM2 is highly expressed in the brain, in both microglia and neuronal cells, but its biological role in these cells still needs to be understood. There are few studies on the role of TRPM2 cations channels in neurological diseases those few suggest that Ca^{2+} influx via TRPM2 is necessary for microglia and other phagocytes to mount effective inflammatory and clearance responses [35].

Alzheimer's Disease, Oxidative Stress and TRPM2 Channels

Alzheimer's disease (AD) is a form of dementia in which patients show neurodegeneration, complete loss of cognitive abilities and prematurely die. Central to the neurodegenerative process is the inability of neurons to properly regulate $[Ca^{2+}]_i$ concentration. In AD, correlations between the pathological hallmarks of AD (amyloid plaques and neurofibrillary tangles) and perturbed cellular Ca^{2+} homeostasis have been established in studies of patients, as well as in animal and cell culture models of AD. Specifically, increased levels of amyloid β-peptide (Aβ) induce neurotoxic factors including ROS and cytokines, which impair cellular Ca^{2+} homeostasis and render neurons vulnerable to apoptosis and excitotoxicity [18].

Ca^{2+} influx via H_2O_2-activated TRPM2 induces cell death in rat insulinoma RIN-5F cells expressing TRPM2; TRPM2-specific antisense oligonucleotide significantly suppressed Ca^{2+} influx and cell death induced by H_2O_2 [4].

TRPM2-specific antisense almost completely abolished TNF-α-evoked $[Ca^{2+}]_i$ concentration oscillation in RIN-5F cells, similar to the effect of omitting extracellular Ca^{2+}, and also significantly suppressed TNF-α-induced death in RIN-5F cells [4]. These results suggest an important role of TRPM2 in TNF-α-activated Ca^{2+} channels that mediate cell death. Therefore, it is possible that TNF-α released from Aβ-activated microglia triggers neuronal cell death via TRPM2 activation in AD. In fact, TRPM2 expressed in rat cortical neurons is critically involved in H_2O_2-induced Ca^{2+} influx that causes neuronal cell death [45]. Importantly, Fonfria et al. [32] have suggested that activation of TRPM2, functionally expressed in primary cultures of rat striatum, contributes to Aβ- and oxidative stress-induced striatal cell death, thereby providing evidence that TRPM2 activity may contribute to neuronal cell death in pathophysiological circumstances in which ROS are generated abundantly.

Ca^{2+} influx through TRPM2 then causes a positive feedback loop of ROS production, which kills the neuronal cells. In primary cortical neurons, knockdown of TRPM7 also results in knockdown of TRPM2, suggesting that expression of these two proteins in cortical neurons is co-ordinated in some manner. Yoshida et al. [46] reported that H_2O_2-induced Ca^{2+} responses mediated by TRPM7 were very small compared to those from TRPM2. Considering that TRPM2 and TRPM7 are activated by ROS and plays a critical role for the progression of anoxic cell death in a model of in vitro ischemic neuronal injury, it is then possible that Ca^{2+} overload via oxidative stress-activated TRPM7 may participate in the development of AD [18].

TRPM2 Channels, Western Pacific Amyotrophic Lateral Sclerosis and Parkinson Disease

Among neurodegenerative diseases, ALS and PD are ideal for studying the relative contributions of genes and environment in disease etiology because they occur in geographically separate foci among three genetically different, homogenous groups of people [47]. Intensive research conducted over the years led to the identification of two candidate environmental factors: (1) severely low levels of Ca^{2+} and Mg^{2+} in the soil and drinking water [48]; and (2) the putative neurotoxin-methylamino-L-alanine derived from the cycad plant, a traditional food source in Guam [49]. These findings led to the hypothesis that prolonged exposure to such an environment could be involved in the pathogenesis of Western Pacific ALS and PD [49]. Supporting this hypothesis are observations that afflicted individuals exhibit altered Ca^{2+} metabolism, and experimental reports of neuronal damage in experimental models of animals fed diets that mimic the mineral composition in the disease foci environment [50].

TRPM2 has been implicated in cell death induced by oxidants [4, 7, 24]. The presence of low Mg^{2+} and high transition metals in the Western Pacific ALS and PD foci create conditions of increased oxidative stress [47]. As a channel with high expression profile in microglia and neuronal cells that could sense and respond to oxidative stress, TRPM2 is an attractive candidate for a susceptibility gene for these disorders. Hermosura et al. [47] reported the presence of heterozygous TRPM2P1018L, a heterozygous variant of TRPM2 in the pathogenesis of Western Pacific ALS and PD. They observed also that P1018L forms functional channels that activate in response to H_2O_2 and ADPR. However, in the presence of physiological concentrations of extracellular Ca^{2+}, P1018L channels inactivate quickly and are thus unable to allow sustained ion influx. In intact cells, this is manifested as an attenuated Ca^{2+} rise in response to H_2O_2. Defective Ca^{2+} handling is implicated in many diseases, including neurodegeneration. Of particular interest is a recent report that described another Pro-to-Leu substitution, this time in CalHM1, a putative Ca^{2+}-permeable channel [51]. CalHM1P86L increases risk for Alzheimer's disease. In functional studies, cells expressing CalHM1P86L proteins exhibited attenuated Ca^{2+} permeability, reduced cytosolic Ca^{2+} levels, and increased amyloid deposition. The similarity in the effects of CalHM1P86L and TRPM2P1018L suggests that attenuation of intracellular Ca^{2+} rises and its effects on downstream signaling pathways may contribute to the pathophysiological mechanism in neurodegenerative diseases [47].

One frequent studied animal model of PD, relevant to the 'environmental toxin' hypothesis of PD [52], is based on the use of rotenone, a naturally occurring isoflavonoid from the tropical plants *Lonchocarpus* and *Derris* [53]. Rotenone inhibits complex I of the mitochondrial electron chain reaction. It also decreases intracellular ATP levels and increases the production of ROS [54]. It also releases glutamate from presynaptic terminals' leading to an additional increase in ROS production [55]. ROS gates TRPM2 channels [5]. Recently, Freestone et al. [54] indicated that TRPM2 or TRPM2-like channels are activated by ROS during exposure of substantia nigra pars compacta (SNc) neurons to rotenone. Their evidences were based on the following observations: rotenone induced a fast rise in $[Ca^{2+}]_i$ concentration although the rise in $[Ca^{2+}]_i$ concentration was reduced by N-(p-amylcinnamoyl) anthranilic acid (ACA); scavenging ROS by Trolox also decreased the rotenone-induced $[Ca^{2+}]_i$ concentration increase.

TRPM2 Channels and Bipolar Disorders

While the C-terminal cytosolic tail mediates the interaction with ADPR, the role of the N-terminal tail that is also

located within the cytosol has not been defined on a molecular level. A number of N-terminal truncated isoforms of TRPM2 have been identified and in some cases they regulate the function of the full-length channel [36]. In general, the N terminus is indispensable. Deletion of a stretch of 20 amino acid residues (Δ537–556), as has been found in the TRPM2-ΔN splice variant in neutrophils, abolishes any channel function [24]. This ΔN stretch comprises several structural elements which may explain why TRPM2-ΔN is dysfunctional. First, it contains an IQ-like sequence motif that represents a CaM binding domain [56]. Interestingly, a further IQ-like motif is found immediately downstream of the ΔN stretch. Second, the ΔN stretch contains two PxxP motifs that are characteristic for sites enabling interaction with other proteins [57]. For TRP channels the significance of endogenous PxxP motifs until now has only been investigated in the TRPC channel subfamily [58]. Although so far no proteins are known that form functional protein complexes with TRPM2, TRPM2 is unusually rich in PxxP motifs in comparison to other channels of the TRP superfamily. As a further remarkable property of the ΔN stretch, the conservative substitution of a single amino acid residue (D543E) was correlated with the presence of bipolar disorder in members of a family with a high incidence of this disease [59, 60]. Recently, it was reported that no functional role can be attributed to any of the structural motifs within the ΔN stretch, and that this sequence may act as a spacer segment for other functional sites in the N terminus [61].

Traumatic Brain Injury, Oxidative Stress and TRPM2 Channel

Traumatic brain injury is a leading cause of mortality and disability among the young population. Motor vehicle accidents accounts for the majority severe traumatic brain injury cases, while falls, sporting accidents and assault are responsible for most mild to moderate injuries. Traumatic brain injury contributes to fuelling a chronic central nervous system inflammation with increased formation of proinflammatory cytokines, enzymes and ROS [62]. ROS promote oxidative stress, which leads to neurodegeneration and ultimately results in programmed cell death. TRPM2 is implicated in inflammatory pathways, specifically as a key participant in monocyte chemokine production induced by H_2O_2 [19]. More recently, Cook et al. [63] investigated TRPM2 transcript and protein levels following experimental traumatic brain injury. They reported increases in TRPM2 mRNA and protein expression in cortical and hippocampal neurons of injured animals, and suggested a role for TRPM2 in traumatic brain injury pathophysiology.

Neuronal Cells and TRPM2 Channel Blockers

Phospholipase A(2) enzymes display a superfamily of structurally different enzymes classified in at least nine subfamilies by biochemical and structural properties. N-(p-amylcinnamoyl)anthranilic acid (ACA) is often used as a broad-spectrum inhibitor for the characterization of phospholipase A(2)-mediated pathways. Compounds like ACA and ACA-like structures have been described to block the receptor-induced release of arachidonic acid and subsequent signaling cascades in the pancreas and the cardiovascular system. Harteneck et al. [11] reported that ACA at 20 μM directly blocks several transient receptor potential (TRP) channels (TRPC6, TRPM2 and TRPM8).

Several TRPM2 blockers are shown in Table 2. Fenamates like flufenamic acid (FFA) are anti-inflammatory drugs known to alter ion fluxes through the plasma membrane. They are for instance potent blockers of cation and anion channels, and FFA at ≥50 μM is now commonly used to block currents through TRP channels and receptor-operated channels [31]. Two other substances, clotrimazole and econazole, have been described to inhibit TRPM2 currents at 3–30 μM concentrations [64]. Recently, Olah et al. [37] reported that the voltage-ramp-activated ADPR-primed currents in cultured neurons were strongly depressed by recombinant TRPM2 channel blockers including clotrimazole, FFA and ACA.

Ca^{2+} influx observed during rotetone application is due to activation of TRPM2 channels. Freestone et al. [54] indicated that TRPM2 or TRPM2-like channels are activated by ROS during exposure of SNc neurons to rotenone. They observed also that ACA and FFA did not change resting $[Ca^{2+}]_i$ concentration or any other measured membrane property. ACA, however, caused a decrease in rotenone-induced $[Ca^{2+}]_i$ concentration increase while FFA induced a tendency to decrease the $[Ca^{2+}]_i$ concentration increase. Pretreatment with the antioxidant Trolox (500 μM for 10 min) also decreased the rotenone (200 nM)-induced $[Ca^{2+}]_i$ concentration rise, consistent with the crucial role of a ROS-sensitive Ca^{2+} entry mechanisms.

Bai and Lipski [43] investigated the effects of three putative blockers of TRPM2 channels. Clotrimazole (20 μM), ACA (25 μM) and FFA (200 μM), in organotypic hippocampal slice cultures. They concluded that the three chemicals failed to protect pyramidal neurons from damage by exogenous H_2O_2 (600 μM) and increased damage of these neurons and astrocytes caused by oxidative stress induced with mercaptosuccinate (400 μM) or buthionine sulfoximine (4 μM). Antioxidants (Trolox 500 μ; MitoE 2 μM) reduced both neuronal and astrocytic cell damage. Recently, my group investigated antagonist effects of 2-APB and FFA in DRG cells of mouse by whole cell patch clamp experiments. Recently, we observed that

Table 2 Effects of TRPM2 channels blockers on neuronal cells

Blockers	Cells	Effects	Reference
FFA	Rat microglia	Blocker	Kraft et al. [35]
ACA,	Rat primary striatal cells	Blocker	Olah et al. [37]
Clotrimazole,	Rat primary striatal cells	Blocker	Olah et al. [37]
FFA	Rat primary striatal cells	Blocker	Olah et al. [37]
FFA	Substantia nigra	No effect	Freestone et al. [54]
ACA	Substantia nigra	Blocker	Freestone et al. [54]
FFA	Hippocampal cells	No effect	Bai and Lipski [43]
ACA	Hippocampal cells	No effect	Bai and Lipski [43]
FFA	Dorsal root ganglion of mouse	Blocker	Nazıroğlu et al. [submitted]
2-APB	Dorsal root ganglion of mouse	Blocker	Nazıroğlu et al. [submitted]

ACA, N-(p-amylcinnomoyl)anthranilic acid; FFA, flufenamic acid; 2-APB, 2-aminoethoxydiphenyl borate

ADPR (1 mM)-induced TRPM2 channel currents in the cells were inhibited by 2-APB (50 μM) and FFA (100 μM) (Submitted data).

Conclusions

The evidence for association of TRPM2 variants with neurological diseases such as Bipolar disorders suggested that genetic variations in TRPM2 may influence susceptibility to the diseases. In addition, the identification of TRPM2 as a key component of the neurological Ca^{2+} entry pathway in response to reactive oxygen species sheds new light on the physiology and pathophysiology of neuronal cells and other cell types in the brain. Because there is substantial evidence for the deteriorating role of oxidative stress in neurological and brain dysfunction, manipulating TRPM2 function in neuronal cells may be highly useful in the future for experimental therapies of brain and neurological dysfunctions.

Endogenous ADP-ribose, NAD^+ and exogenous H_2O_2 in neuronal cells have been shown to gate TRPM2 channels although their effects seem to be cell type specific. Similarly, based on studies in cell lines, there appear to be limited indirect TRPM2 channel blockers such as ACA and FFA, and their channel blocking effects seem also to cell type specific. The lack of highly specific and protective TRPM2 blockers unfortunately prevents delineation of the exact relationship between TRPM2 channel activation and neuronal cell death. Hopefully continued research in this important area will produce new and more useful agents leading to a better understanding of the role of TRPM2 channels in physiological and pathological pathways.

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