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Review

The transient receptor potential melastatin 2: a new therapeutical target for Parkinson's disease?

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From the Contents

Abstract

The transient receptor potential melastatin 2 is a calcium-permeable cation channel member of the TRP family. Also known as an oxidative stress-activated channel, the transient receptor potential melastatin 2 gating mechanism is dependent on reactive oxygen species. In pathological conditions, transient receptor potential melastatin 2 is overactivated, leading to a Ca^{2+} influx that alters cell homeostasis and promotes cell death. The role of transient receptor potential melastatin 2 in neurodegenerative diseases, including Alzheimer's disease and ischemia, has already been described and reviewed. However, data on transient receptor potential melastatin 2 involvement in Parkinson's disease pathology has emerged only in recent years and the issue lacks review studies that focus specifically on this topic. The present review aims to elucidate the role of the transient receptor potential melastatin 2 channel in Parkinson's disease by reviewing, summarizing, and discussing the *in vitro*, *in vivo*, and human studies published until August 2022. Here we describe fourteen studies that evaluated the transient receptor potential melastatin 2 channel in Parkinson's disease. The Parkinson's disease model used, transient receptor potential melastatin 2 antagonist and genetic approaches, and the main outcomes reported were discussed. The studies described transient receptor potential melastatin 2 activation and enhanced expression in different Parkinson's disease models. They also evidenced protective and restorative effects when using transient receptor potential melastatin 2 antagonists, knockout, or silencing. This review provides a literature overview and suggests where there is a need for more research. As a perspective point, this review shows evidence that supports transient receptor potential melastatin 2 as a pharmacological target for Parkinson's disease in the future.

Key Words: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); 1-methyl-4-phenylpyridinium (MPP+); 6-hydroxydopamine; AG490; clotrimazole; flufenamic acid; N-(p-amylcinnamoyl) anthranilic acid; Parkinson's disease; poly-ADPR polymerase type 1 (PARP1); rotenone; paraquat; transient receptor potential melastatin 2 (TRPM2)

Introduction

Transient receptor potential (TRP) channels were first described in 1969 (Cosens and Manning, 1969). Their study found a mutation that led to a rapid decay in Drosophila's phototransduction receptor potential during bright illumination. The mutant protein, later shown to be a cation channel, was called transient receptor potential (*trp*). Later, the *trp* gene was cloned (Montell and Rubin, 1989), and other TRP-like channels were identified, including mammal homologs (Phillips et al., 1992; Petersen et al., 1995; Wes et al., 1995). The TRP family comprises a channel family with six subgroups: canonical (TRPC), vanilloid (TRPV), ankyrin (TRPA), polycystin (TRPP), mucolipin (TRPML), and melastatin (TRPM). These channels are involved in diverse physiological functions, including thermosensation, chemosensation, mechanosensation, nociception, and visual processes. They have differential sensitivities to ions, such as Ca^{2+}, K^+, and Na^+. However, different channels of the family share structural similarities, as all of them are six-transmembrane channels, with a pore between the fifth and sixth domains, and the N- and C-terminals are cytoplasmatic domains (Voets et al., 2005; Sumoza-Toledo and Penner, 2011; Zhao et al., 2021).

The melastatin family was named after its first described member, the tumor suppressor melastatin (TRPM1) channel (Duncan et al., 1998). This subfamily is divided into eight subtypes: TRPM1 to TRPM8. The second member, TRPM2, was first described in 1998 and was previously known as TRPC7 (Nagamine et al., 1998). TRPM2 has a widespread tissue distribution and represents the most expressed TRP in the brain (Nagamine et al., 1998; Fonfria et al., 2006). Its expression occurs in both neuronal and non-neuronal cells, including microglia and astrocytes (Kraft et al., 2004; Kaneko et al., 2006; Bond and Greenfield, 2007; Lee et al., 2010). TRPM2 has a characteristic protein in the C-terminal dominium, the NUDT9-homology (NUDT9-H). This protein is a homolog to a nucleoside diphosphate-linked moiety X (Nudix) hydrolase protein presented in mitochondria. NUDT9 is an adenosine diphosphate ribose (ADPR) pyrophosphatase enzyme. However, in TRMP2 the NUDT9-H appears to be a critical binding site for ADPR, which directly induces the channel gating (Huang et al., 2020).

ADPR is generated under oxidative stress through diverse intracellular pathways. One of them takes place in the nucleus, where poly-ADPR polymerase type 1 (PARP1) and poly-ADPR-glycohydrolase (PARG) enzymes catalyze the cleavage of nicotinamide adenine dinucleotide (NAD^+) into nicotinamide and ADPR. Another source of ADPR is the hydrolysis of NAD^+ by mitochondrial NADases. A third source is represented by the glycohydrolases, such as CD38 and CD157 (**Figure 1**). Besides ADPR, other molecules coactivate or indirectly activate TRPM2, including hydrogen peroxide (H_2O_2), cyclic ADPR, Ca^{2+}, nicotinic acid adenine dinucleotide phosphate (NAADP), and 2′-O-acetyl-ADP-ribose (OAADPr) (Belrose and Jackson, 2018; Szollosi, 2021). In 2019, scientists were able to describe the TRPM2 3D structure using a cryo-electron microscopy technique, which enabled them to discover a new binding region for ADPR in the N-terminal dominium (Huang et al., 2019). This reveals that, despite the advances in the knowledge of TRPM2 structure, there are still many open questions regarding TRMP2 gating mechanisms and functions.

Also known as an oxidative stress-activated channel, TRPM2 has several physiological and pathophysiological roles. For example, TRPM2 is involved in pancreatic β-cell insulin secretion (Togashi et al., 2006), immune cell chemokine production (Yamamoto et al., 2008), and endothelium permeability (Hecquet et al., 2010). However, the most distinguishing role of TRPM2 is related to cell death. In one of the first studies on this topic, susceptibly to cell death was shown to be mediated by TRPM2 activation in response to reactive oxygen species (ROS) (Hara et al., 2002). Thereafter, increasing evidence suggests the involvement of TRPM2 in neurodegenerative diseases. For instance, amyloid beta-peptide, a hallmark of Alzheimer's disease, was able to induce cell death through TRPM2 activation in cultured rat striatal neurons (Fonfria et al., 2005). Studies over the past few years explored this relationship and made significant progress in elucidating TRPM2 contribution to neurodegenerative diseases, especially Alzheimer's disease (Jiang et al., 2018), ischemic stroke (Turlova et al., 2018), and bipolar disorder (Belrose and Jackson, 2018).

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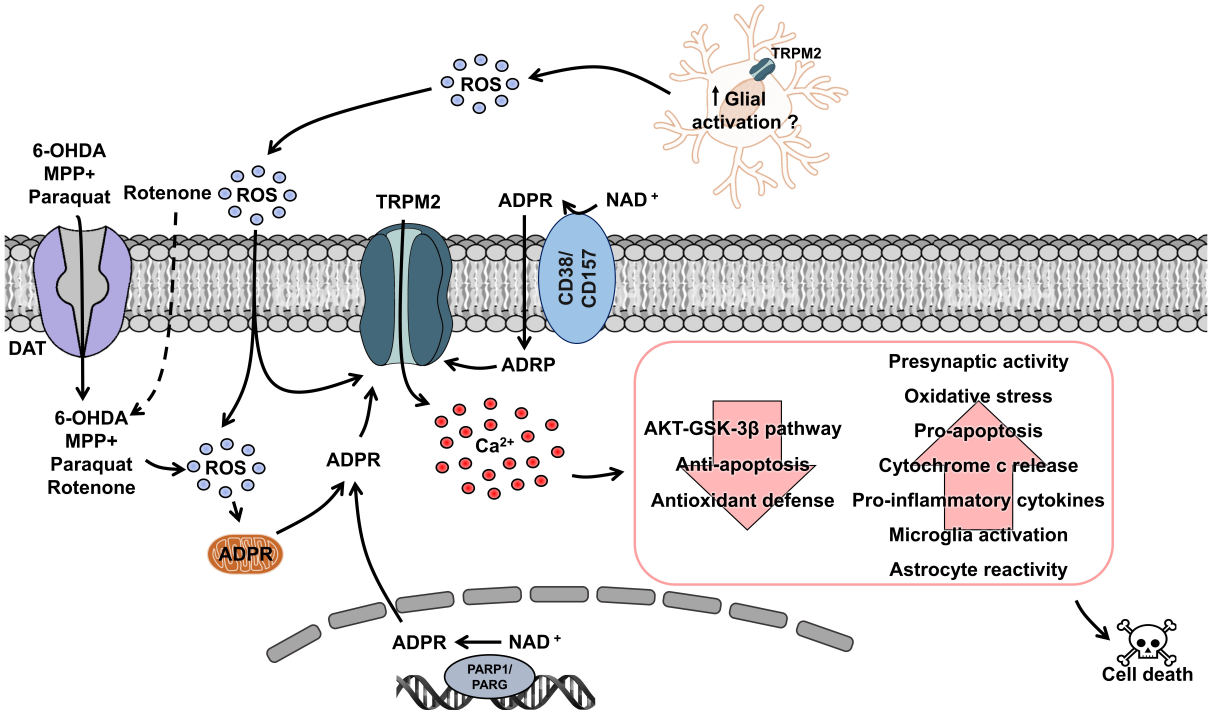

Figure 1 | **Schematic figure of proposed transient receptor potential melastatin 2 (TRPM2) involvement in Parkinson's disease (PD) models.**
TRPM2 is activated by adenosine diphosphate ribose (ADPR) that is generated via where poly-ADPR polymerase type 1 (PARP1) and poly-ADPR-glycohydrolase (PARG) in the nucleus, NADases in mitochondria, or glycohydrolases (CD38 and CD157). Reactive oxygen species can also coactivate TRPM2. ADPR generation is enhanced in an oxidative stress environment, such as the one evoked by different neurotoxins (6-hydroxidopamine [6-OHDA], 1-methyl-4-phenylpyridinium [MPP^+], paraquat, and rotenone) in PD models. In this scenario, TRPM2 is overactivated, and a Ca^{2+} overload results in the decrease of protein kinase B/glycogen synthase kinase type 3β (AKT/GSK-3β) signaling, anti-apoptosis (B-cell lymphoma 2 [Bcl-2]), and antioxidant defense (superoxide dismutase activity and glutathione levels). In addition, presynaptic activity (extracellular glutamate release), oxidative stress, pro-apoptosis (caspase-3, caspase-9, calpain, Bcl-2 homologous antagonist/killer [Bak], BH3 interacting-domain death agonist [Bid], and Bcl-2-like protein 4 [Bad]), cytochrome c release, pro-inflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin 1β [IL-1β], and interleukin-6 [IL-6], microglia activation (ionized calcium-binding adaptor molecule 1 [Iba-1] and inducible nitric oxide synthase [iNOS]), and astrocyte reactivity (glial fibrillary acidic protein [GFAP] and astroglial S100 calcium-binding protein β [S100β]) are all enhanced. This leads to cell death and neurodegeneration in PD models. It is not clear if glial cells have a different role in this mechanism, as astrocytes and microglia also express TRPM2 channels. These molecular pathways may be involved in PD pathomechanism and TRPM2 represents a potential target for pharmacological treatment approaches. Created with BioRender.com and PowerPoint.

Parkinson's disease (PD) is the second most common neurodegenerative disease. It is estimated that the global burden of PD could increase up to 17 million patients by 2040, which will become an enormous public health issue (Dorsey et al., 2018). The main characteristics of PD are the neurodegeneration of dopaminergic neurons of the substantia nigra pars compacta (SNc), the reduction of dopamine release in the striatum (caudate/putamen, CPu), the presence of abnormal α-synuclein aggregates (Lewy bodies), and the resulting appearance of motor symptoms, including bradykinesia, resting tremor, incoordination, and gating alterations (Poewe et al., 2017). The gold treatment currently used in clinical practice, levodopa, has several limitations, such as the dyskinesia that appears after prolonged use (Fabbri et al., 2018). In this way, new therapeutical approaches are in urge, and novel, potential pharmacological targets have been extensively studied.

Considering the potential role of TRPM2 in different neurological conditions, it is logical to hypothesize a contribution of this channel to the pathophysiology of Parkinson´s disease. And even further, the understanding of this relationship can lead to new pharmacological targets and possible new treatments in the future. In this way, studies that evaluate that hypothesis are necessary and fundamental. The crucial information related to TRPM2 and its involvement in PD has emerged only in recent years. Reviews about TRPM2 in neurological disorders have been published before (See Steinman et al. (2022) for more details). However, until now, no work has appeared with a specific focus on TRMP2 and Parkinson´s disease. In this review, we summarized and discussed studies that evaluated the role of TRPM2 in PD, including *in vitro* studies, animal models, and human research.

Search Strategy and Selection Criteria

A literature systematic review was performed on PubMed, EMBASE, and Web of Science databases between March and August of 2022. The following keywords were used: "Parkinson" and "TRPM2". Articles were screened based on Title and Abstract. Further articles were retrieved through citation tracking. Fourteen articles were selected. They include cell culture, animals, and human studies from 2008 to 2022.

Cell Culture Studies

Ten studies reported TRPM2 involvement in toxin-induced cellular models of PD (**Table 1**). Cellular models are low-cost, fast, and reliable. These models are very useful to understand specific contributions in a disease or to test molecular targets. To mimic PD, studies often use neurotoxins that promote neuronal death. The selected studies used: 1-methyl-4-phenylpyridinium (MPP^+) (n = 6), rotenone (n = 2), paraquat (n = 1), and 6-hydroxydopamine (6-OHDA) (n = 1). MPP+ is a metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that entry cells by the dopamine transporter, inhibits mitochondrial complex I, and increases the generation of ROS. 6-OHDA, a dopamine analog, is transported by dopamine transporter into the cell, blocks mitochondria complex I, and can be auto-oxidated. Rotenone and paraquat are pesticides and herbicides. Rotenone diffuses into the cell and also inhibits mitochondria complex I. Paraquat is transported by dopamine transporter and generates ROS. All neurotoxins lead to oxidative stress and cell death, representing reliable models for the study of PD (Pingale and Gupta, 2020; Ferreira et al., 2021).

The selected studies used different approaches to evaluate the TRPM2 role, including the use of antagonists (n = 8), agonists (n = 1), and genetic tools (n = 4). The antagonists used included: 2-aminoethoxydiphenyl borate (2-APB), N-(p-amylcinnamoyl) anthranilic acid (ACA), clotrimazole, flufenamic acid (FFA), 3,4-Dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone (DPQ), and N-(6-oxo-5,6-dihydrophenanthridin-2-yl)-(N,N-dimethylamino) acetamide hydrochloride (PJ34). FFA, ACA, 2-APB, and clotrimazole were used as TRPM2 inhibitors. FFA, a non-steroidal anti-inflammatory compound; clotrimazole, an antifungal agent; ACA, a phospholipase A2 inhibitor; and 2-APB, first reported as an inositol 1,4,5-trisphosphate receptor antagonist, all can inhibit TRPM2 and prevent currents activated by intracellular ADPR (Hill et al., 2004; Kraft et al., 2006; Togashi et al., 2008). However, all are unspecific and broad-spectrum blockers, as they can also act on different ion channels, including other TRP members (Xu et al., 2005; Harteneck et al., 2007; Meseguer et al., 2008; Guinamard et al., 2013). The TRPM2 blockers diverge in inhibitory efficiency, reversibility, and specificity. Thus, there are several limitations when the objective is to investigate a TRPM2-specific contribution to a disease, as experimental results can be ambiguous. In addition, these inhibitors can be unsuitable for *in vivo* studies (See (Zhang et al., 2020) for a detailed review on TRPM2 inhibitors).

DPQ and PJ34 are PARP1 inhibitors. As PARP1 is a source of ADPR, it mediates TRPM2 activation. Thus, the use of PARP1 inhibitors is useful to inhibit TRPM2 channels. This was observed before in TRPM2-HEK293 cells (Fonfria et al., 2004). However, this is not the only TRPM2 activation mechanism, as PARP1 enzymes are not the only ADPR source. In addition, PARP1 participates in other physiological pathways, for instance in DNA repair (Ko and Ren, 2012), which can be a problem when using a PARP1 inhibitor in animal studies. The selected studies for this review also used TRPM2 overexpression and silencing

Table 1 | **Cell culture/tissue studies with PD models and TRPM2**

		PD model				
	Cell culture/Tissue	Toxin	Dose	TRPM2 antagonist/ agonist/ approach	Dose	Main outcomes
Freestone et al., 2009	Wistar rat brain slices (SNc)	Rotenone	200 nM	ACA and FFA	ACA: 20 μM FFA: 50 μM	ACA: ↓ rotenone-induced intracellular Ca^{2+} rise FFA: ↓ rotenone-induced intracellular Ca^{2+} rise (tendency)
Yee et al., 2017	Wistar rat brain slices (Locus coeruleus)	Rotenone	1 μM	ACA, FFA, and clotrimazole	ACA: 20 μM FFA: 50 μM clotrimazole: 20 μM	ACA: ↓ rotenone-induced intracellular Ca^{2+} rise ACA: abolished the neuronal firing rate increase evoked by rotenone FFA: ↓ rotenone-induced intracellular Ca^{2+} rise Clotrimazole: ↓ rotenone-induced intracellular Ca^{2+} rise (tendency) TRPM2 expression colocalizes with TH
Zhou and Han, 2017	SH-SY5Y PC12	6-OHDA	100 μM	TRPM2 overexpression	NA	GATA3 silenced: ↑ TRPM2 mRNA level GATA3 overexpression: ↓ TRPM2 mRNA level GATA3 interacted with the promoter region of TRPM2 TRPM2 overexpression + GATA3 overexpression: ↓ cell proliferation and survival, and ↑ ROS production and caspase-3 activity after 6-OHDA
Sun et al., 2018	SH-SY5Y	MPP^+	500 μM	TRPM2 siRNA, TRPM2 overexpression, ACA and FFA	ACA: 10 μM FFA: 50 μM	MPP^+ ↑ TRPM2 levels ACA and FFA: ↓ MPP^+-induced intracellular Ca^{2+} rise FFA: ↓ calpain activation, ↑ mitochondrial cytochrome c levels, ↓ caspase-3, Bak, Bid, and Bad protein levels, and partially rescued cell death upon MPP^+ TRPM2 siRNA: abolished the MPP+-induced Ca^{2+} influx, ↓ TRPM2 levels, ↓ caspase-3, Bak, Bid, and Bad protein levels, rescued cell death, and ↓ caspase-3 activity upon MPP^+ TRPM2 overexpression: ↑ H_2O_2-induced currents, ↓ cell survival, ↑ cell death upon MPP^+
Ding et al., 2019	SH-SY5Y	MPP^+	2 mM	TRPM2 siRNA	NA	MPP^+ ↑ TRPM2 mRNA and protein levels TRPM2 is a direct target of miR-625 miR-625 overexpression: ↓ TRPM2 mRNA and protein levels miR-625 overexpression + lnc-p21 overexpression: ↑ TRPM2 mRNA and protein levels TRPM2siRNA: ↑ MPP^+-induced cell viability, ↓ cell cytotoxicity, ↓ apoptosis, ↓ caspase-3 activity and Bax levels, ↑Bcl-2 level, ↓ ROS, ↑ SOD activity, and ↓ TNF-α, IL1β and IL-6 levels
Tamano et al., 2019	Wistar rat brain slices (SNc)	Paraquat	40 μM	ACA	40 μM	ACA: ↓ exocytosis, ↓ glutamate, ↑ extracellular Zn^{2+}, and ↓ intracellular Zn^{2+}
Yıldızhan and Nazıroğlu, 2020	TRPM2-WT and TRPM2-KO primary microglia	MPP^+	0.5 mM	TRPM2 KO ACA, 2-APB DPQ, PJ34 (PARP inhibitors)	ACA: 25 μM 2-APB: 100 μM DPQ: 30 μM PJ34: 1 μM	TRPM2-WT: MPP^+ ↑ PARP1 and TRPM2 levels ACA and 2-APB in TRPM2-WT: ↓ cell death, ↓mitochondrial membrane depolarization, ↓ ROS production, ↓caspase-3, ↓ caspase-9, ↓ PARP1, and TRPM2 protein levels TRPM2-KO: no effects of MPP+, no increase in intracellular Ca^{2+} DPQ and PJ34: ↓ MPP-induced increases in intracellular Ca^{2+}
Yıldızhan and Nazıroğlu, 2022	SH-SY5Y	MPP^+	0.5 mM	ACA, 2-APB cumene-hydrogen peroxide (CPx)	ACA: 25 μM 2-APB: 100 μM CPx: 1mM	MPP^+ ↑ TRPM2 and PARP1 protein levels CPx: ↑ MPP^+-induced intracellular Ca^{2+} rise 2-APB: ↓ MPP+-induced intracellular Ca^{2+} rise 2-APB and ACA: ↓ cell death Selenium treatment: ↓ TRMP2 increase and MPP^+-induced intracellular Ca^{2+} rise
Vaidya et al., 2022	SH-SY5Y	MPP^+	1 mM	2-APB	3 and 10 μM	2-APB: ↓ MPP- induced intracellular Ca^{2+} rise
Yildizhan et al., 2022	C57BL/6 primary hippocampal neurons	MPP^+	0.5 nM	ACA	25 μM	MPP^+ ↑ PARP1 and TRPM2 levels Resveratrol: ↓ PARP1 and TRPM2 levels ACA: ↓ MPP^+-induced intracellular Ca^{2+} rise

2-APB: 2-Aminoethoxydiphenyl borate; 6-OHDA: 6-hydroxidopamine; ACA: N-(p-Amylcinnamoyl) anthranilic acid; Bad: Bcl-2 associated agonist of cell death; Bak: Bcl-2 homologous antagonist/killer; Bax: Bcl-2-like protein 4; Bcl-2: B-cell lymphoma 2; Bid: BH3 interacting-domain death agonist; Ca^{2+}: calcium; DPQ: 3,4-Dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone; FFA: flufenamic acid; GATA3: GATA binding protein 3; IL-1β: interleukin-1β; IL-6: interleukin-6; lnc-p21: long non-coding RNA-p21; miR-625: microRNA 625; MPP^+: 1-methyl-4-phenylpyridinium; NA: not applicable; PARP1: poly[ADP-ribose] polymerase 1; PD: Parkinson's disease; PJ34: N-(6-Oxo-5,6-dihydrophenanthridin-2-yl)-(N,N-dimethylamino) acetamide hydrochloride; ROS: reactive oxygen species; SNc: substantia nigra pars compacta; SOD: superoxide dismutase; TH: tyrosine hydroxylase; TNF-α: tumor necrosis factor-α; TRPM2: Transient receptor potential melastatin 2; TRPM2-KO: TRPM2 knockout; TRPM2siRNA: TRPM2 small interfering RNA; TRPM2-WT: TRPM2 wild type; Zn^{2+}: zinc.

as genetic approaches. This tool has an important advantage, as it allows the investigator to specifically assess the TRPM2 channel. Nevertheless, despite being very useful to understand physiological mechanisms in experimental approaches, gene therapies are a long way from the reality of human studies. Therefore, pharmacological approaches are still necessary.

All selected studies provided evidence that TRPM2 is activated in PD cellular models, and that TRPM2 inhibition was able to prevent or rescue the cellular damage evoked by the neurotoxin used. One study demonstrated that TRPM2 colocalizes with dopaminergic neurons (Yee et al., 2017), whereas others showed that MPP^+ treatment increased TRPM2 (Sun et al., 2018; Ding et al., 2019; Yıldızhan and Nazıroğlu, 2020, 2022; Yildizhan et al., 2022) and PARP1 (Yıldızhan and Nazıroğlu, 2020, 2022; Yildizhan et al., 2022) levels, indicating a TRPM2 activation in PD models. In addition, the treatments revealed neuroprotective effects. ACA was able to attenuate toxin-induced Ca^{2+} rise (Freestone et al., 2009; Yee et al., 2017; Sun et al., 2018; Yildizhan et al., 2022), abolish the neuronal firing rate increase evoked by rotenone (Yee et al., 2017), inhibit the enhancement of presynaptic activity (reducing exocytosis and glutamate concentration), and revert the effects of paraquat by decreasing intracellular Zn^{2+} and increasing extracellular Zn^{2+} (Tamano et al., 2019). Other MPP^+-induced deficits, including cell death, mitochondrial membrane depolarization, ROS production, and increased caspase-3, caspase-9, PARP1, and TRPM2 protein levels were also prevented by ACA (Yıldızhan and Nazıroğlu, 2020, 2022). FFA reduced toxin-induced Ca^{2+} rise (Freestone et al., 2009; Yee et al., 2017; Sun et al., 2018) and cell death (decreased calpain activation, caspase-3, Bak, Bid, and Bad protein levels, and increased mitochondrial cytochrome c levels) (Sun et al., 2018). The use of clotrimazole revealed an attenuated toxin-induced Ca^{2+} rise (Yee et al., 2017). Also, 2-APB attenuated toxin-induced Ca^{2+} rise (Yıldızhan and Nazıroğlu, 2022; Vaidya et al., 2022) and reduced cell death (Yıldızhan and Nazıroğlu, 2022), as well other MPP^+-induced deficits. The latter effects were revealed by the rescued mitochondrial membrane potential and decreased ROS production, caspase-3, caspase-9, PARP1, and TRPM2 protein levels (Yıldızhan and Nazıroğlu, 2020). Finally, DPQ and PJ34 also attenuated toxin-induced Ca^{2+} rise (Yıldızhan and Nazıroğlu, 2020).

Genetic approaches corroborate the hypothesis that TRPM2 has an important role in Parkinson´s disease, as TRPM2 silencing abolished the MPP^+-induced Ca^{2+} influx, reduced TRPM2 levels, increased cell viability, decreased cell cytotoxicity, reduced apoptosis (reduced caspase-3, Bak, Bid, and Bad protein levels, and caspase-3 activity, and increased Bcl-2 levels), reduced ROS and cytokine levels (tumor necrosis factor-α, interleukin-1β, and interleukin-6), and increased superoxide dismutase activity (an antioxidant enzyme) (Sun et al., 2018; Ding et al., 2019). Moreover, when cells from TRPM2 knockout mice were submitted to MPP^+ treatment, no effect was observed and there was no increase in intracellular Ca^{2+} levels (Yıldızhan and Nazıroğlu, 2020). Finally, overexpressing TRPM2 generated an increase in intracellular Ca^{2+} currents, a decrease in cell survival, an increase in cell death, and an increase in ROS levels and caspase-3 activity (Zhou and Han, 2017; Sun et al., 2018). These various observations support the idea that TRPM2 activation may be involved in PD pathology and thus inhibition of this channel might be a promising pharmacological target.

Animal Model Studies

Five studies evaluated TRPM2 in animal models of PD (**Table 2**). Animal models allow researchers to understand a more complex scenario, as interactions between different cell types and systems can be assessed. The selected studies used: 6-OHDA (n = 2), MPTP (n = 2), and paraquat (n = 1) to generate PD models. As cited above, these toxins induce oxidative stress and cause dopaminergic degeneration. Despite the different administration routes (intraperitoneal or intracerebral injections) and doses used, all were able to promote dopaminergic neuronal death, providing good models to mimic PD neuropathology.

The expression and function of TRPM2 channels in SNc were described in 2011, and the authors used Western Blot and immunofluorescence to attest to TRPM2 expression and colocalization in SNc dopaminergic neurons. They also described that H_2O_2 evoked inward currents and intracellular calcium rise in these neurons, whereas TRPM2 blockers (clotrimazole, FFA, and ACA) inhibited this effect (Chung et al., 2011). Later, TRPM2 expression was also described in SN pars reticulata GABAergic neurons, where neuronal firing rates were proved to be modulated via TRPM2 (Lee et al., 2013). These results provided evidence for the functional involvement of TRPM2 in brain areas related to PD. Two other selected studies for this review corroborate the above results by reporting TRPM2 expression in the substantia nigra. Despite not using TRPM2 antagonists or agonists, they demonstrated an increase in TRPM2 immunostaining, mRNA, and protein levels in the substantia nigra of animals that received a neurotoxin (Yu, 2014; Sun et al., 2018).

Three of the studies used pharmacological approaches that inhibit TRPM2 or PARP1. ACA was used in one study, where the investigations injected the drug simultaneously with a neurotoxin administration. They showed that ACA reduced extracellular glutamate levels and increased tyrosine hydroxylase (TH) levels, a key enzyme in dopamine production (Tamura et al., 2022). 2-APB and PJ34 were both used in another study. The treatment started 7 days after PD induction and lasted for 2 weeks. A decrease in TRPM2 levels, an increase in TH levels, a decrease in oxidative stress levels (estimated by measuring malondialdehyde levels), and an increase in the antioxidant glutathione levels were all reported in PD animals treated with DQP and 2-APB. This study also performed behavior analyses that revealed improvements in motor behavior (assessed by rotarod and open field test), and improvements in memory (assessed by Y-maze and passive avoidance test) (Vaidya et al., 2022). AG490, an inhibitor of TRPM2, was used in the 3rd study. AG490 is a tyrphostin compound reported to also inhibit JAK2. It can block TRPM2 channels as shown before and has a relevant advantage, as it does not act on other TRP channels beyond TRPM2 (Shimizu et al., 2014; Li et al., 2019). In the selected study, AG490 was administrated 20 minutes before PD induction. AG490 was able to reduce TRPM2 levels, to prevent TH decrease, microglia activation (as ionized calcium-binding adaptor molecule 1 and inducible nitric oxide synthase staining were both decreased), and astrocyte reactivity (as glial fibrillary acidic protein and S100β staining were both decreased), and also to restore AKT/GSK-3β/caspase-3 signaling pathway that was altered in the PD model used. In behavioral tests (apomorphine, cylinder test, and rotarod), motor improvement was observed (Ferreira et al., 2022).

Human Studies

Two studies analyzed human brain samples. One of them showed that TRPM2 protein levels were upregulated in post-mortem SNc of eight PD patients (Sun et al., 2018). The other study sequenced genomic DNA from nine Guamanian PD patients and found a heterozygous variant of TRPM2, the TRPM2P1018L. In this mutation, the proline in the sixth domain is substituted by a leucine. The leucine introduces local variations in the structure that renders the channel incapable of mediating Ca^{2+} influx to the same extent as a wild-type channel (Hermosura et al., 2008). Both human studies provide evidence for possible TRPM2 involvement in PD pathology.

Future Perspectives

This review presented evidence, supported by data derived from *in vitro*, animal models, and human studies, that the TRPM2 channel might represent a new pharmacological target for PD. Increased TRPM2 levels and activity were corroborated by all studies, demonstrating the contribution of this channel to PD pathomechanisms. **Figure 1** describes TRPM2 activation after PD model induction and the main triggered alterations reported by the selected studies. Moreover, the use of TRPM2 antagonists provided exciting and promising results, as all studies showed protection or restoration when using those compounds. However, the antagonists used can either inhibit other TRP channels or other molecules, thus providing partially biased results. More specific antagonists are essential to clarify the specific involvement of TRPM2 in PD and to the possible, future development of neuroprotective drugs for PD.

Until now, only five studies evaluated TRPM2 contribution in animal models. Among them, only three used TRPM2 antagonists. This shows us that there is a long way for research to reach a pharmaceutical agent that could be used by PD patients. Despite that, the studies have shown encouraging results. More studies with animals will allow a better understanding of TRPM2 channel physiological function in PD. Some points need to be carefully considered, for instance, the understanding of the different roles of TRPM2 in neuronal and glial cells, which have not yet been evaluated. Considering genetic approaches, inhibition of the channel in one specific cell type, or in one brain area, instead of the whole body, is also desirable, since TRPM2 is ubiquitously expressed in the brain. Another relevant gap is the lack of studies with female animals, since sex hormones might differentially affect the channel function. Finally, studies that assess animal behavior are also important to better understand the effects of TRPM2 ablation or inhibition in PD models.

Conclusion

The first description of TRPM2 dates from 1998. Since then, scientists made considerable effort to better understand the structure and function of this channel. The description of the TRPM2 gating mechanism using cryo-electron microscopy technique in 2019 was essential to clarify questions that were open since the TRPM2 discovery. With the detailed TRPM2 structure, it is now possible to develop new drugs that can interact more specifically with the channel. This will allow for more assertive studies, a better understanding of TRPM2 involvement in Parkinson's disease, and the development of therapeutical agents that might be useful in the treatment of that devastating disease.

Table 2 | **Animals studies with PD models and TRPM2**

			PD model		TRPM2 antagonist/ approach				Treatment started period (considering PD induction)		
	Animal	Age/ weight	Toxin	Dose/injection site		Dose	Injection site	Duration		Analyzed areas	Main results
Yu et al. 2014	Wistar	210–240 g	6-OHDA	18 µg into MFB	NA	NA	NA	NA	NA	SN	6-OHDA ↑ TRPM2 staining, mRNA, and protein levels
Sun et al., 2018	C57BL/6	8–10 mon old	MPTP	5 i.p. injections of 25 mg/kg	NA	NA	NA	NA	NA	SN	MPTP ↑ TRPM2 levels
Ferreira et al., 2022	C57BL/6	3 mon old	6-OHDA	10 µg into right CPu	AG490	30 mg/kg	i.p.	Single injection	20 min before treatment	CPu and SNc	↑TH ↓ TRPM2 ↓ Iba-1, iNOS, GFAP, and S100β ↑ p-AKT/AKT and p-GSK-3β/GSK-3β protein levels ↓ Caspase-3 Improve motor behavior in apomorphine, cylinder and rotarod tests
Vaidya et al., 2022	Sprague-Dawley	8–10 wk old	MPTP	100 µg bilateral into SNc	2-APB, PJ34 (PARP inhibitor)	2-APB: 3 and 10 mg/kg PJ34: 3 and 10 mg/kg	i.p.	2 wk	7 d after treatment	CPu, midbrain, hippocampus, and cortex	↑TH ↓ TRPM2 ↓ MDA ↑ GSH Improve motor behavior in rotarod and open field test Improve memory in Y-maze and passive avoidance test
Tamura et al., 2022	Wistar	10–15 wk old	Paraquat	40 µM into right SNc	ACA	50 µM	SNc	Single injection	Simultaneously	SNc	↓ extracellular glutamate ↑TH

2-APB: 2-Aminoethoxydiphenyl borate; 6-OHDA: 6-hydroxidopamine; ACA: N-(p-Amylcinnamoyl) anthranilic acid; AG490: tyrphostin tyrosine kinase inhibitor; AKT: protein kinase B; CPu: caudate/putamen; GFAP: glial fibrillary acidic protein; GSH: glutathione; GSK-3β: glycogen synthase kinase type 3β; i.p.: intraperitoneal injection; Iba-1: ionized calcium-binding adaptor molecule 1; iNOS: inducible nitric oxide synthase; MDA: malondialdehyde; MFB: middle forebrain bungle; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NA: not applicable; p-AKT: phosphorylated protein kinase B; PARP: poly[ADP-ribose] polymerase; PD: Parkinson's disease; p-GSK-3β: phosphorylated glycogen synthase kinase type 3; PJ34: N-(6-Oxo-5,6-dihydrophenanthridin-2-yl)-(N,N-dimethylamino) acetamide hydrochloride; S100β: astroglial S100 calcium-binding protein β; SN: substantia nigra; SNc: substantia nigra pars compacta; TH: tyrosine hydroxylase; TRPM2: Transient receptor potential melastatin 2.

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