Potassium Channels in Parkinson's Disease: Potential Roles in its Pathogenesis and Innovative Molecular Targets for Treatment

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### Abbreviations

<table>
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<th>Abbreviation</th>
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<tbody>
<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
</tr>
<tr>
<td>AgTx-2</td>
<td>Agitoxin 2</td>
</tr>
<tr>
<td>AHP</td>
<td>Afterhyperpolarization</td>
</tr>
<tr>
<td>AKT</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP-activated protein kinase</td>
</tr>
<tr>
<td>BDS</td>
<td>Blood-depressing substance</td>
</tr>
<tr>
<td>BK</td>
<td>Large-conductance calcium-activated potassium (channel)</td>
</tr>
<tr>
<td>C3aR</td>
<td>Complement C3a receptor</td>
</tr>
<tr>
<td>CaM</td>
<td>Calmodulin</td>
</tr>
<tr>
<td>CaV</td>
<td>Voltage-gated calcium (channel)</td>
</tr>
<tr>
<td>ChTx</td>
<td>Charybdotoxin</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>D2R</td>
<td>Dopamine D2 receptor</td>
</tr>
<tr>
<td>DA</td>
<td>Dopaminergic</td>
</tr>
<tr>
<td>DAT</td>
<td>Dopamine transporter</td>
</tr>
<tr>
<td>E_k</td>
<td>Equilibrium potential for K⁺</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>Half maximal effective concentration</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-Aminobutyric acid</td>
</tr>
<tr>
<td>GIRK</td>
<td>G protein-coupled inwardly rectifying potassium (channel)</td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotropin-releasing hormone</td>
</tr>
<tr>
<td>GP</td>
<td>Globus pallidus</td>
</tr>
<tr>
<td>GPCR</td>
<td>G protein-coupled receptor</td>
</tr>
<tr>
<td>GxTx</td>
<td>Guangxitoxin</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>H₂S</td>
<td>Hydrogen sulfide</td>
</tr>
<tr>
<td>I₄₋</td>
<td>A-type voltage-dependent K⁺ currents</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
<td>IK</td>
<td>Intermediate-conductance calcium-activated potassium (channel)</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>K₂P</td>
<td>Two-pore-domain potassium (channel)</td>
</tr>
<tr>
<td>K₅₆P</td>
<td>ATP-sensitive potassium (channel)</td>
</tr>
<tr>
<td>K Ca⁺</td>
<td>Calcium-activated potassium (channel)</td>
</tr>
<tr>
<td>Kir</td>
<td>Inwardly rectifying potassium (channel)</td>
</tr>
<tr>
<td>K_v</td>
<td>Voltage-gated potassium (channel)</td>
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<tr>
<td>LTCC</td>
<td>L-type voltage-gated calcium channel</td>
</tr>
<tr>
<td>MgxTx</td>
<td>Margatoxin</td>
</tr>
<tr>
<td>mito-K₅₆P</td>
<td>Mitochondrial ATP-sensitive potassium (channel)</td>
</tr>
<tr>
<td>mTOR</td>
<td>Mammalian target of rapamycin</td>
</tr>
<tr>
<td>Naᵥ</td>
<td>Voltage-gated sodium (channel)</td>
</tr>
<tr>
<td>NMDAR</td>
<td>N-methyl-D-aspartate receptor</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>PGC</td>
<td>Peroxisome proliferator-activated receptor-gamma coactivator</td>
</tr>
<tr>
<td>PIP₂</td>
<td>Phosphatidylinositol 4,5-bisphosphate</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein kinase A</td>
</tr>
<tr>
<td>PM</td>
<td>Plasma membrane</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SK</td>
<td>Small-conductance calcium-activated potassium (channel)</td>
</tr>
<tr>
<td>SN</td>
<td>Substantia nigra</td>
</tr>
<tr>
<td>SNpc</td>
<td>Substantia nigra pars compacta</td>
</tr>
<tr>
<td>SNpr</td>
<td>Substantia nigra pars reticulata</td>
</tr>
<tr>
<td>SOC</td>
<td>Store-operated calcium (channel)</td>
</tr>
<tr>
<td>SSRI</td>
<td>Serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>SSTR4</td>
<td>Somatostatin receptor subtype 4</td>
</tr>
<tr>
<td>SUR</td>
<td>Sulfonylurea receptor subunit</td>
</tr>
<tr>
<td>TH-</td>
<td>Hydroxylase negative</td>
</tr>
<tr>
<td>TH+</td>
<td>Hydroxylase positive</td>
</tr>
<tr>
<td>TPN</td>
<td>Tertiapin</td>
</tr>
<tr>
<td>UCP</td>
<td>Uncoupling protein</td>
</tr>
<tr>
<td>V&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>Half-activating voltage</td>
</tr>
<tr>
<td>VMAT</td>
<td>Vesicular monoamine transporter</td>
</tr>
<tr>
<td>VSD</td>
<td>Voltage sensor domain</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
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</table>
Abstract

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) region of the midbrain. The loss of neurons results in a subsequent reduction of dopamine in the striatum, which underlies the core motor symptoms of PD. To date, there are no effective treatments to stop, slow, or reverse the pathological progression of dopaminergic neurodegeneration. This unfortunate predicament is because of the current early stages in understanding the biological targets and pathways involved in PD pathogenesis. Ion channels have become emerging targets for new therapeutic development for PD due to their essential roles in neuronal function and neuroinflammation. Potassium channels are the most prominent ion channel family and have been shown to be critically important in PD pathology because of their roles in modulating neuronal excitability, neurotransmitter release, synaptic transmission, and neuroinflammation. In this review, members of the subfamilies of voltage-gated K⁺ channels, inward rectifying K⁺ channels, and Ca²⁺-activated potassium channels are described. Evidence of the role of these channels in PD aetiology is discussed together with the latest views on related pathological mechanisms and their potential as biological targets for developing neuroprotective drugs for PD.
Significance Statement

Parkinson's disease (PD) is the second most common neurodegenerative disorder, featuring progressive degeneration of dopaminergic neurons in the midbrain. It is a multifactorial disease involving multiple risk factors and complex pathobiological mechanisms. Mounting evidence suggests that ion channels play vital roles in the pathogenesis and progression of PD by regulating neuronal excitability and immune cell function. Therefore, they have become "hot" biological targets for PD, as demonstrated by multiple clinical trials of drug candidates targeting ion channels for PD therapy.
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I. Introduction – Parkinson’s Disease & Ion Channels

Parkinson’s disease (PD) was first identified by English physician James Parkinson in 1817 (Parkinson, 2002) and is the second most common neurodegenerative disorder (Poewe et al., 2017). The disease features progressive degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) (Poewe et al., 2017). The loss of DA neurons results in the cardinal motor symptoms of PD, such as tremor, bradykinesia, postural instability, and rigidity (Jankovic, 2008), often accompanied by non-motor symptoms such as cognitive impairment, sleeping disturbance, olfaction dysfunction, and depression (Chaudhuri et al., 2006).

PD is a multifactorial disease with several well-acknowledged risk factors, such as aging, gender, genetic variables, environmental exposures, and oxidative stress (Figure 1). These factors interact and aggravate each other, affecting numerous cellular functions and increasing an individual's vulnerability. Pathologically, various mechanisms are involved in the degeneration of DA neurons, including neuroinflammation, mitochondrial and lysosomal dysfunction, calcium imbalance, ER stress, protein aggregation, and iron accumulation (Figure 1) (Blesa et al., 2015; Cali et al., 2011; Goldman, 2014; Hirsch and Hunot, 2009; Jenner, 2003; Ma et al., 2021).

Although serotonin and norepinephrine-expressing neurons within the CNS can regenerate their cranial projecting axons (Dougherty et al., 2020; Jin et al., 2016), the loss of DA neurons in PD is permanent and irreversible (Ransohoff, 2016). However, the degeneration of DA neurons can be a long and gradual process, so PD patients can live relatively normal lives until a significant number of DA neurons are lost from the SNpc (Kalia and Lang, 2015;
Poewe et al., 2017). DA neurons are the main source of dopamine in the mammalian central nervous system (CNS) (Chinta and Andersen, 2005). They are essential for voluntary movement, cognition, emotion, working memory, and reward pathways (Bromberg-Martin et al., 2010; Chinta and Andersen, 2005; Groves, 1983; McNamara et al., 2014; Salgado-Pineda et al., 2005). As a result, insufficient dopamine levels lead not only to profoundly disabling motor symptoms, but also to mood changes and memory loss (Chinta and Andersen, 2005).

The exceptional and selective vulnerability of DA neurons in the SNpc to the PD risk factors has been an attractive question in the field (Bayer et al., 1995; Chan et al., 2010). One of the compelling hypotheses is that these neurons are more vulnerable to neurodegeneration than other neuronal types because of their long and complex axons, and subsequent higher energy and calcium requirements (Bolam and Pissadaki, 2012; Pissadaki and Bolam, 2013).

DA neurons are featured by their pacemaker (spontaneous action potential firing) properties in vivo and in vitro, displaying distinct electrical activity patterns (Guzman et al., 2009; Puopolo et al., 2007). This robust, autonomous, self-generated electrical activity is critical for dopamine secretion and maintenance of normal basal ganglia activity and is generated and modulated by a variety of orchestrated ion channel activities (Albin et al., 1989; Gonon and Bloch, 1998). Voltage-gated sodium (Na\textsubscript{V}) channels control pacemaker frequency; as such, inhibition of Na\textsubscript{V} channels by tetrodotoxin abolishes spike generation and reveals slow oscillatory potentials mediated by L-type voltage-gated calcium channel (LTCC) (Chan et al., 2007; Ilin et al., 2021; Puopolo et al., 2007; Tucker et al., 2012; Yee et al., 2019).
Calcium channels, although not directly triggering the firing of action potentials, regulate the precision, frequency, and robustness of pacemaking (Benkert et al., 2019; Duda et al., 2016; Guzman et al., 2009; Guzman et al., 2010; Poetschke et al., 2015). The SN DA neurons only have a modest Ca\(^{2+}\) buffering ability, and the balance of intra- and extracellular Ca\(^{2+}\) can be easily disrupted (Foehring et al., 2009). Disruption of calcium homeostasis and mitochondrial function caused by the SN DA neuron-specific calcium oscillations have been indicated to play a key role in PD pathogenesis (Schapira, 2008; Zaichick et al., 2017). Therefore, targeting voltage-gated calcium (Ca\(v\)) channels to reduce activity-related calcium overload and mitochondrial dysfunction in SN DA neurons has become a promising therapeutic strategy for PD (Biglan et al., 2017; Boag et al., 2021; Simuni et al., 2010).

Another important cation, the potassium (K\(^{+}\)) ion, is essential in establishing resting membrane potential, maintaining cellular homeostasis, and regulating neuronal excitability (Hille, 2001). K\(^{+}\) channels have received increasing attention in PD research due to their multifaceted roles in PD pathophysiology. For example, in the SN, the A-type voltage-dependent K\(^{+}\) currents (\(I_A\)), characterized by relatively fast inactivation and mediating neuronal action potential repolarization, have been demonstrated to fine-tune the pacemaker firing rates in DA neurons (Hahn et al., 2003; Liss et al., 2001). Potassium channels have also been demonstrated to be involved in neuroinflammation in PD, resulting in the overactivity of brain immune cells, including dendritic cells, astrocytes, and microglia, triggered by various stressors, such as aggregated \(\alpha\)-synuclein (the pathological marker of PD), toxins, infection, and aging (Hirsch et al., 2012; Mosley et al., 2006; Wang et al., 2015a). In addition, it is well known that abnormal activation of potassium channels is the hallmark of over-activated microglia (Di Lucente et al., 2018; Kettenmann et al., 1993; Nguyen et al., 2017; Norenberg et al., 1994; Sarkar et al., 2020), while uncontrolled microglial activities aggravate the
neurodegeneration in PD (Badanjak et al., 2021; Luo et al., 2010). Therefore, K⁺ channels are promising targets for various aspects of PD pathology.

II. Overview of potassium channels – the largest ion channel family

Potassium channels are integral membrane proteins that highly selectively transport K⁺ across the cell membrane. Being present in all living organisms, K⁺ channels have evolved to play a variety of roles in different cell types; however, some critical features have been conserved throughout this most prominent ion channel family. The most important common feature is their high selectivity for K⁺; some K⁺ channels have ~1000 times higher selectivity for K⁺ over other small cations, such as Na⁺ and Li⁺ (Noskov and Roux, 2006; Yellen, 2002). Such a high selectivity is essential for maintaining the resting membrane potential, which is largely determined by the equilibrium potential of K⁺ ions (Noskov and Roux, 2006). During an action potential, this K⁺ selectivity is also crucial for K⁺ channels to be responsible for the repolarization phase following membrane depolarization mediated by Na⁺ and/or Ca²⁺ influx, which is required for returning the membrane to its negative resting potential to terminate the action potential signal (Bean, 2007; Lawson, 2000).

The high level of selectivity of K⁺ channels is achieved structurally. Potassium channels function as tetramers, with four pore-forming domains from the four subunits comprising a K⁺-conducting pore in the center of the tetramer (Gonzalez et al., 2012; Heginbotham et al., 1994; Ketchum et al., 1995; Miller, 2000). Within the pore, there is a selectivity filter constituted by a highly conserved “signature sequence (T-I/V-G-Y/F-G)” in the GYG motif, where four ion binding sites are coordinated by the backbone carbonyl oxygen atoms and the threonine side chains (Berneche and Roux, 2005; Cheng et al., 2011; Doyle et al., 1998; Heginbotham et al., 1994). This incredible architecture of the selectivity filter, which allows
for the discrimination between $K^+$ and other cations, also endows another property of $K^+$ channels, i.e., the extremely fast conduction at near diffusion-limited rates of $K^+$ ions, which is critical for quickly repolarizing the cell membrane during action potentials.

Human $K^+$ channels are composed of 79 pore-forming members ($\alpha$-subunits), constituting four subfamilies based on their structural and functional properties, i.e., voltage-gated $K^+$ ($K_v$) channels, inwardly rectifying $K^+$ ($K_{ir}$) channels, calcium-activated $K^+$ ($K_{Ca}$) channels and two-pore-domain $K^+$ ($K_{2P}$) channels (Figure 2) (Villa et al., 2020). The number of functioning $K^+$ channel proteins is actually much greater than this because of the formation of heteromeric channels, alternative splicing and alternative translation initiation of subunits, and different co-assembly with accessory proteins. These channels not only control resting membrane potential and action potentials but also regulate hormone secretion, neurotransmitter release, internal signaling pathways, and apoptosis (Fernandez-Fernandez and Lamas, 2021; Lawson, 2000; Maingret et al., 2008; Martire et al., 2004; Vervaeke et al., 2006; Yu, 2003). This wide array of functions requires that $K^+$ channels must respond rapidly to sensing changes in the local environment. This is achieved by the extraordinarily complex and efficient gating mechanisms evolved by these channels, which accurately couple the perception of external stimuli with the physical opening or closing of the channel pore to control ion flux (Noskov and Roux, 2006). Depending on the channel type, these channels are primarily gated by membrane voltage changes ($K_v$ channels), cellular mediators ($K_{ir}$ channels), intracellular calcium oscillations ($K_{Ca}$ channels), or pH and temperature ($K_{2P}$ channels).

Many $K^+$ channels function in the brain and have been reported to be involved in PD pathology. In this review, we mainly consider the $K_v$, $K_{ir}$ and $K_{Ca}$ subfamilies due to their evidence-supported roles in preventing or modulating the disease progression. For each subfamily, we focus on the key members that play significant roles in PD and discuss their
overall physical properties and physiological functions, as well as their pathological indications in PD and underlying mechanisms. We also describe existing therapeutic agents acting on these channels and outline why these channels represent an under-exploited biological target for PD with the potential for future drug development.

III. Voltage-gated potassium (Kv) channels

Voltage-gated potassium (Kv) channels, which are tightly gated by transmembrane potential changes, form the largest and most complex family of K^+ channels with 40 members classified into Kv1- Kv12 subfamilies (Gutman et al., 2005). These channels function as homotetramers or heterotetramers, with each subunit consisting of six transmembrane segments (S1-S6) and N- and C-terminal cytoplasmic tails. The K^+-conducting pore is formed by S5-S6 from each subunit, flanked by four voltage sensor domains (VSDs) composed of S1-S4 in each subunit (Figure 2) (Lopez et al., 1994; Yool and Schwarz, 1991; Yu et al., 2005). The voltage-dependent gating of Kv channels starts from the sensation of transmembrane potential changes by S4 segments, which stimulates the activation of the VSDs first (Catterall, 2010; Chanda et al., 2005; Doherty et al., 2010). VSD activation drives conformational changes, leading to the propagation of VSD movements to the ion conduction pore via a helical linker. Then, through VSD-pore coupling, rearrangement of the pore leads to pore opening and K^+ conduction (Bezanilla, 2005; Cui, 2016; Delemotte et al., 2010; Schoppa and Sigworth, 1998; Tytgat et al., 1993; Zagotta et al., 1994). The activation speed of Kv channels determines the height and width of action potentials, which in turn, establishes the time course and the amount of Ca^{2+} entry through Cav channels during the action potential, regulating neurotransmitter release (Jan and Jan, 2012; Kaczmarek and Zhang, 2017).
Following activation, many Kv channels undergo a time-dependent inactivation process that functionally shuts down the K\(^+\) conduction and prevents reactivation until the channel recovers from this non-conducting inactivated state. Different Kv channel subtypes present significant differences in the inactivation properties, such as the time course of inactivation and recovery, state dependence, voltage dependence, and sensitivity to extracellular cations (Kurata and Fedida, 2006). These inactivation properties directly affect various roles of Kv channels in regulating cellular excitability and repolarization of the action potential. Furthermore, many Kv channel inhibitors work by targeting the inactivation gating mechanism (Leung, 2012). Therefore, both the activation and inactivation gating mechanisms exhibited by Kv channels provide pivotal physiological means.

Kv channels are widely expressed in most cell types and play diverse roles depending on the cell type (Yellen, 2002). In the brain, they play essential regulatory roles in both neurons and glial cells (Del Pino et al., 2015; Menegola et al., 2008; Piccialli et al., 2020; Rhodes et al., 2004; Tian et al., 2019). Within Kv channel family, the Kv1-4 and Kv7 subfamilies are the primary members involved in PD pathology.

### A. Kv1 channels

The Kv1 (KCNA) subfamily has eight members (Kv1.1-Kv1.8) that are widely expressed in the brain (Curran et al., 1992; Grissmer et al., 1990; Gutman et al., 2005; Philipson et al., 1990). In PD, the hyper-cholinergic state is one of the pathological features, with anti-cholinergic drugs being used to treat PD before L-DOPA was used in the clinic (Kalia and Lang, 2015). The application of cholinergics was rationalized because striatal cholinergic interneurons regulate motor function and basal ganglia output and play a significant role in PD pathophysiology (Maurice et al., 2015b). It has long been known that outward K\(^+\) currents provide negative feedback to the depolarization of cholinergic interneurons, limiting their
burst firing rate and tonic activity (Figure 3) (Song et al., 1998). However, the identity of the channels mediating such $K^+$ currents was unknown. Interestingly, disruption of $K_V1$ (mainly $K_V1.3$) channel function has recently been shown to underlie the hyper-excitability of striatal cholinergic interneurons, suggesting that dysfunctional $K_V1.3$ channels may be responsible for the hyper-cholinergic state in PD (Tubert et al., 2016).

$K_V1$ channels also contribute to neuroinflammation in multiple neurodegenerative diseases (Rangaraju et al., 2015; Sarkar et al., 2020; Wang et al., 2019). The neuroinflammatory response typically starts with the activation of immune cells in the brain, such as microglia and astrocytes (DiSabato et al., 2016). One of the hallmarks of microglia activation is the up-regulation of $K^+$ channels, including $K_V$ channels (Rangaraju et al., 2015; Wang et al., 2019), of which the $K_V1.3$ channel is required for microglial cytokine production and microglia-mediated neurodegeneration (Di Lucente et al., 2018; Fordyce et al., 2005; Sarkar et al., 2020).

$K_V1.3$ is a classical Shaker-type $K^+$ channel with an activation threshold between -50 mV and -60 mV (Cahalan et al., 1985; Pahapill and Schlichter, 1992). It has also been demonstrated to be transcriptionally up-regulated by aggregated $\alpha$-synuclein in both PD animal models and post-mortem human brains (Sarkar et al., 2020). Furthermore, inhibition of $K_V1.3$ channel function can significantly ameliorate neuroinflammation and neurodegeneration in multiple PD animal models (Sarkar et al., 2020). Therefore, $K_V1.3$ is a multifunctional player in PD pathology, making it a highly promising biological target for PD therapy. Not surprisingly, identifying potent and specific $K_V1.3$ inhibitors has been a hot topic pursued by researchers and the pharmaceutical industry.
Many \( K_{V1.3} \) inhibitors are currently available, including diphenoxylate (Nguyen et al., 2012), benzyl piperidines (UK 78,282) (Hanson et al., 1999), verapamil (Rauer and Grissmer, 1996), phenoxyalkoxypsoralens (Schmitz et al., 2005; Vennekamp et al., 2004), dihydroquinolines (CP-339818, CP393223 and CP-394322) (Nguyen et al., 1996), benzamides (Miao et al., 2003), clofazimine (Ren et al., 2008), and furoquinoline (Butenschon et al., 2001) (Table 1).

To date, the most successful small molecule inhibitor is PAP-1 (5-(4-phenoxybutoxy)-psoralen), a phenoxyalkoxypsoralen developed based on the non-selective \( K_{V1.3} \) inhibitor, Psora-4 (5-(4-phenylbutoxy)-psoralen) (Schmitz et al., 2005; Vennekamp et al., 2004).

PAP-1 binds preferentially to the C-type inactivated state of \( K_{V1.3} \) channels and exhibits a use-dependent inhibition with high potency (IC\(_{50} = 2 \) nM) (Schmitz et al., 2005). The selectivity of PAP-1, although not ideal, is better than other small molecule inhibitors, demonstrating 23-fold greater selectivity over \( K_{V1.5} \), and 33- to 125-fold greater selectivity over other \( K_{V1} \)-family channels (Schmitz et al., 2005). As an orally available and brain penetrant compound, PAP-1 has become the inhibitor of choice for animal studies of neurodegenerative diseases. Indeed, it has been shown to be able to reduce neuroinflammation and neurodegeneration in both PD and AD animal models and improve cognition or neurological deficits in AD (Maezawa et al., 2018; Sarkar et al., 2020).

Another category of \( K_{V1.3} \) inhibitors has been developed from peptide toxins, such as scorpion toxins (e.g., Charybdotoxin (ChTx), Margatoxin (MgTx), Agitoxin 2 (AgTx-2), \( \alpha \)-KTx3.7 (OsK-1), and snake toxins (e.g., BF9) (Bartok et al., 2014; Chen et al., 2001; Mouhat et al., 2005; Pimentel et al., 2008). These peptidic \( K_{V1.3} \) inhibitors were used in both in vitro
and in vivo studies to demonstrate the potential therapeutic effects of blocking Kv1.3-mediated K⁺ currents in different disease models, including PD (Koo et al., 1997; Tubert et al., 2016; Yang et al., 2014a). One prominent example is ShK, a 35-residue disulfide-rich peptide toxin purified from the sea anemone, Stichodactyla helianthus (Norton et al., 2004; Tudor et al., 1996). ShK has been a sought-after drug candidate because it potently blocks Kv1.3 channels with an IC₅₀ of ~10 pM (Kalman et al., 1998). However, ShK is also an efficient inhibitor for Kv1.1, Kv1.6, Kv1.2, Kv3.2 and KCa3.1 channels with picomolar to nanomolar potency (Chi et al., 2012). The Kv1.1 channel is a particularly dangerous off-target molecule of candidate drugs, as it plays an essential role in the control of neuronal excitability in CNS (Robbins and Tempel, 2012). In humans, Kv1.1 dysfunction can cause epilepsy, episodic ataxia, and involuntary muscular contractions (Adelman et al., 1995; Liguori et al., 2001; Zuberi et al., 1999).

To improve the selectivity for Kv1.3 over the other channels, over 300 ShK analogs have been generated. The most successful analog, ShK-186 (Dalazatide, the FDA-approved name), has >100-fold selectivity for Kv1.3 over Kv1.1 and shows efficacy in various animal models of chronic inflammatory diseases (Chang et al., 2015; Pennington et al., 2015; Tarcha et al., 2012). While ShK-186 has progressed through human clinical trials, additional analogs have been developed to further increase the stability and selectivity of the peptide (Pennington et al., 2015; Wang et al., 2019). Therefore, further studies examining these peptidic Kv1.3 inhibitors in PD animal models are warranted.
B. \( \text{K}_\text{v}2 \) channels

\( \text{K}_\text{v}2 \) (or KCNB) channels (\( \text{K}_\text{v}2.1 \) and \( \text{K}_\text{v}2.2 \)) are widely expressed in the human body. In the brain, they suppress neuronal hyperexcitability by sensing metabolic stress and the intracellular \( \text{Ca}^{2+} \) level, thus playing a critical role in maintaining neuronal activity (Misonou et al., 2005). One of the unique hallmarks that distinguish \( \text{K}_\text{v}2.1 \) channels from their homologs is their clustered expression on the plasmid membrane of neurons (Figure 3) (Fox et al., 2013; Lim et al., 2000). \( \text{K}_\text{v}2.1 \) surface expression is regulated by AMP-activated protein kinase (AMPK), which phosphorylates \( \text{K}_\text{v}2.1 \) at Ser 440 and Ser 537 in its cytoplasmic tail (Figure 3) (Chao et al., 2018; Ikematsu et al., 2011; Wu et al., 2015).

Increased clustering of \( \text{K}_\text{v}2.1 \) on the plasma membrane can lead to apoptosis and neurodegeneration by increasing \( \text{K}^+ \) efflux, resulting in more hyperpolarized cells and altered cellular volume (Chao et al., 2018). Specifically, \( \text{K}_\text{v}2.1 \) clusters at the endoplasmic reticulum (ER)-plasma membrane (PM) junctions in neurons, promoting the functional coupling of PM L-type \( \text{Ca}^{2+} \) channels (LTCC) to ER ryanodine receptor and enhancing the opening of the LTCC (Johnson et al., 2018; Vierra et al., 2019). In DA neurons, the clustering of \( \text{K}_\text{v}2.1 \) decreases the lateral mobility of dopamine transporters, which results in dysregulation of dopamine transmission (Lebowitz et al., 2019). The impairment caused by increased \( \text{K}_\text{v}2.1 \) surface expression can be abolished in two ways, first, by inhibition of protein kinase A (PKA, an activator of AMPK), which prevents the activation of AMPK and its phosphorylation of \( \text{K}_\text{v}2.1 \); and second, by a \( \text{K}_\text{v}2.1 \) inhibitor such as Guangxitoxin (GxTx) (Chao et al., 2018). GxTx is a peptide toxin isolated from the venom of the tarantula, \textit{Plesiophictus guangxiensis} (Tilley et al., 2019). This peptide slows \( \text{K}_\text{v}2.1 \) activation and
accelerates its deactivation by binding to the S3b-S4 paddle motif in its voltage sensor domain, limiting its movement (Tilley et al., 2019).

C. \( \text{KV3 channels} \)

\( \text{KV3 (KCNC) channels (KV3.1 and KV3.2) are mainly expressed in neurons in the globus pallidus (GP), one of the primary targets of the striatum that regulates spike repolarization and after potentials that determine refractory periods (Hernandez-Pineda et al., 1999; Wilson, 2015). After the degeneration of DA neurons innervating the basal ganglia, many GP neurons begin to discharge more frequently in bursts and fire at higher rates within bursts, which inhibits the motor commands from the cortex (Hutchinson et al., 1997; Hutchison et al., 1994).} \)

Abnormal GP electrical function is one of the key reasons for tremors and rigidity in PD symptoms (Taha et al., 1996; Wichmann and DeLong, 1996). \( \text{KV3 channels were found to be responsible for this unusual high-frequency discharge or “fast-spiking” excitation pattern in GP neurons (Figure 3) (Baranauskas et al., 1999; Weiser et al., 1994). The fast deactivation of KV3 channels allows efficient removal of its impediment to neuronal depolarization, therefore facilitating the rapid generation of “fast-spiking” action potentials (Baranauskas et al., 2003). So a strategy for PD treatment is to decrease the deactivation efficiency of KV3 channels, particularly KV3.4 channels, thus selectively reducing the abnormally high frequency bursting in GP neurons (Baranauskas et al., 2003).} \)


KV3 channels can be inhibited by blood-depressing substance-I and -II (BDS-I and BDS-II) extracted from the sea anemone, *Anemonia sulcate* (Diochot et al., 1998; Yeung et al., 2005). BDS slows KV3 activation by binding to the S3b-S4 paddle motif in the voltage-sensing domain (Yeung et al., 2005). This inhibition mechanism is similar to how HaTx/GaTx inhibits KV2 (Tilley et al., 2019), which appears to be the most effective pharmacological strategy to inhibit KV channels.

As an oxidation-sensitive channel, KV3.4 also plays an essential role in oxidative stress-induced damage to neurons in PD (Song et al., 2017). The inhibition of KV3.4 by BDS-II blocked mitochondrial membrane depolarization and cytochrome c release induced by MPP⁺. Furthermore, BDS-II protected SH-SY5Y cells against MPP⁺-induced cell death, suggesting that KV3.4 may be a potential new therapeutic paradigm for PD treatment (Song et al., 2017).

D. KV4 channels

KV4 (KCND) channels (KV4.1, KV4.2 and KV4.3) are widely expressed in the striatum, particularly in medium spiny neurons, and are involved in both motor and non-motor deficits in the early stages of PD (Day et al., 2008; Takagi et al., 2005). Blockage of KV4-mediated K⁺ currents reduced motor symptoms, such as resting tremors and rigidity, as well as non-motor symptoms, such as anxiety (Aidi-Knani et al., 2015; Day et al., 2008). These channels also play an important role in emotion and memory functions mediated by basal ganglia (Aidi-Knani et al., 2015).

Quality of life in PD patients is significantly affected by gastrointestinal diseases, the second most common non-motor manifestation of PD (Park et al., 2015). The gastrointestinal
disorders in PD may result from increased intestinal permeability by enteric α-synuclein (Forsyth et al., 2011). The leakage of α-synuclein and other inflammatory mediators may spread to the brain by the gut-brain axis through the vagus nerve, which links the dorsal motor nucleus in the medulla with the viscera, especially the gastrointestinal tract (Holmqvist et al., 2014; Perez-Pardo et al., 2017; Pomfrett et al., 2007). The α-synuclein in the medullary dorsal motor nucleus of the vagus nerve selectively increases the Kv4 surface density, which slows the action potential depolarization, thereby reducing the pacemaker frequency of these neurons (Figure 3) (Chiu et al., 2021). Lower neuronal excitability further decreases gastrointestinal motility, causing constipation in PD (Chiu et al., 2021; Park et al., 2015; Travagli and Anselmi, 2016). Thus, the inhibition of Kv4 could be a potential strategy for treating gastrointestinal disorders caused by PD, improving the quality of life of PD patients.

E. Kv7 channels

Kv7 (KCNQ) channels (Kv7.1-Kv7.5) are widely expressed in the peripheral and central nervous systems (Chen et al., 2018; Hansen, 2006). Both Kv7.2 and Kv7.3 are the predominant subtypes expressed in DA neurons in the midbrain (Chen et al., 2018; Martire et al., 2007). The primary function of Kv7 in neurons is regulating the firing frequency by controlling M-current (Figure 3) (Hansen, 2006; Peretz et al., 2007). The M-current is named after its classical inhibitory pathway, i.e., muscarinic acetylcholine receptor activation that closes the channel (Bordas et al., 2015; Brown and Adams, 1980; Delmas and Brown, 2005; Marrion, 1997). Of note, the M-current is a type of slowly activating, non-inactivating K+ current that raises the threshold of action potential firing (Brown and Adams, 1980; Marrion, 1997). Physiologically, M-currents shape the action potential firing properties, contribute to
spike frequency adaptation, and regulate presynaptic functions (Huang and Trussell, 2011; Madison and Nicoll, 1984; Nigro et al., 2014).

In the midbrain, DA neurons can switch between three distinct firing modes, i.e., tonic, irregular and burst firing, under physiological conditions (Brazhnik et al., 2008; Grace and Bunney, 1984). M-currents specifically modulate the burst firing, which controls synaptic concentrations of dopamine (Chergui et al., 1994; Drion et al., 2010). Inhibiting Kv7 has been shown to promote the bursting of DA neurons and protect these neurons from degeneration in PD animal models (Drion et al., 2010; Liu et al., 2018). The most commonly used Kv7 selective blocker is 4-pyridinylmethyl-9(10H)-anthracenone (XE991), which has been demonstrated in multiple studies to increase dopamine synthesis in the striatal region (Bian et al., 2020; Drion et al., 2010; Hansen et al., 2006; Liu et al., 2018; Shi et al., 2013). Interestingly, Kv7 opener retigabine was shown to reduce the severity of L-DOPA-induced dyskinesias in a 6-OHDA rat PD model without affecting the antiparkinsonian efficacy of L-DOPA (Sander et al., 2012). Furthermore, 6-OHDA-induced SNpc lesions were recently demonstrated to decrease the expression of Kv7.2 in the lateral habenula neurons, increase these neurons’ firing rate, and impair working memory. Lateral habenula injection of M-channel activator retigabine could enhance working memory, while M-channel blocker XE-991 exhibited the opposite effect (Bian et al., 2020). Therefore, fine regulation of Kv7 channels in specific brain regions may present a new approach for treating PD.

In summary, Kv1.3, Kv2.1, Kv3.4 and Kv7.2 are the most prominent members of the Kv channel family involved in PD. Kv channels contribute to PD pathology primarily by regulating the hyper-excitability of striatal cholinergic interneurons, microglial cytokine production, LTCC activation-triggered dopamine transmission, electrical function of GP neurons, GI motility, and bursting of DA neurons. Importantly, the role played by a Kv channel needs to be examined in specific cell types and brain regions.
IV. Inwardly rectifying potassium (Kir) channels

Inwardly rectifying potassium (Kir) channels are known for their greater tendency to conduct K⁺ ions in the inward direction (into the cell) than in the outward direction (out of the cell) at any driving force of the opposite direction (Hibino et al., 2010). This feature differs from other K⁺ channels, providing Kir channels with their original name as "anomalous rectifiers" (Hibino et al., 2010). The inward rectification of Kir channels is caused by the voltage-dependent block of the channel pore by intracellular polyamines and Ma²⁺, not an intrinsic gating property of the channels (Lopatin et al., 1994; Matsuda et al., 1987; Vandenberg, 1987). Under physiological conditions, voltage-independent Kir channels play critical roles in maintaining the resting membrane potential, regulating action potential duration, and controlling K⁺ homeostasis (Baronas and Kurata, 2014; Hibino et al., 2010).

The Kir channels can be categorized into four major groups based on their structural and functional differences, namely G protein-coupled inwardly rectifying potassium channels (GIRK, Kir 3.x); ATP-sensitive potassium channels (K_ATP, Kir6.x); classic Kir channels (Kir2.x); and K⁺ transport channels (Kir1.x; Kir4.x; Kir5.x and Kir7.x) (Hibino et al., 2010). Some Kir channels are strong rectifiers (Kir2.x and Kir3.x), others are weak rectifiers (Kir1.1 and Kir6.x), while none of them are perfect rectifiers (i.e., they all conduct outward K⁺ currents to some extent (Gonzalez et al., 2012; Hibino et al., 2010). The inward rectification is not an intrinsic gating property of Kir channels. Instead, it results from high-affinity blockade of the channel pore from the intracellular side of the channel by endogenous polyamines (e.g., spermine) and Mg²⁺, which significantly decreases the outward currents at depolarized membrane potentials (relative to potassium's reversal potential (Hibino et al., 2010). Kir channels are expressed in numerous types of cells, including, but not limited to
astrocytes, dopaminergic neurons, microglia, and oligodendrocytes (Boucsein et al., 2000; Brasko et al., 2017; Eulitz et al., 2007a; Steinhauser et al., 2012). Amongst all of these Kir channel subfamilies, the GIRKs are the best-studied Kir channels in PD pathology.

A. G protein-coupled inwardly rectifying potassium channels (GIRKs)

The GIRKs (Kir3 channels) are composed of four members GIRK1-4 (Kir3.1-3.4) encoded by the \( KCNJ3, \ KCNJ6, \ KCNJ9 \), and \( KCNJ5 \) genes, respectively. They regulate cellular excitabilities in the brain and heart (Hibino et al., 2010; Kano et al., 2019). The functional GIRK channels are tetramers formed by homomeric or heteromeric Kir3.x subtypes (Corey and Clapham, 1998; Hibino et al., 2010; Inanobe et al., 1999a; Inanobe et al., 1999b; Ishihara et al., 2009; Jelacic et al., 2000). In the brain, the majority of functional GIRK channels are Kir3.1/3.2, Kir3.1/3.3, Kir3.2/3.3, and homotetramers of Kir2.2 (Inanobe et al., 1999b; Lesage et al., 1995; Luscher and Slesinger, 2010). These channels contribute to neuronal plasticity and memory control (Marron Fernandez de Velasco et al., 2015).

As their name suggests, GIRK channels are regulated by G protein-coupled receptors (GPCRs), which are transmembrane receptors that can be activated by extracellular ligands (such as neurotransmitters and hormones) and regulate multiple intracellular signaling cascades (Hilger et al., 2018). As shown in Figure 4, when a GPCR is activated by its ligand, it binds with the GDP-bound heterotrimer G-protein complex (\( G_{\alpha\beta\gamma} \)), leading to the replacement of GDP by GTP and dissociation of \( G_{\alpha\beta\gamma} \) into \( G_{\alpha}\text{GTP} \) and \( G_{\beta\gamma} \) (Oldham and Hamm, 2008). The \( G_{\beta\gamma} \) complex can interact with GIRK channels by binding to the TM2 helix and activating the channel (Kofuji et al., 1995; Logothetis et al., 1987).
A variety of GPCRs activate GIRKs, such as GABA<sub>B</sub>, M2-muscarinic, A1-adenosine, α2-adrenergic, dopamine D2, 5-HT<sub>1A</sub> serotonin, somatostatin, and galanin receptors (Figure 4) (Constantin and Wray, 2016; Gorham et al., 2014; Kim and Johnston, 2015; Luscher et al., 1997; McCall et al., 2019; Raymond et al., 1999; Wellner-Kienitz et al., 2001).

In cortical neurons, GABA<sub>B</sub>-stimulated GIRK activation inhibits glutamate release (Fernandez-Alacid et al., 2009). In addition, coupling A1-adenosine receptors and GIRK contributes to intrinsic membrane properties and excitation-inhibitory balance in dorsal CA1 neurons (Kim and Johnston, 2015). In gonadotropin-releasing hormone (GnRH)-expressing neurons, GIRKs activated by galanin receptor 1 lead to Ca<sup>2+</sup> oscillation and decreased neuronal activities (Constantin and Wray, 2016). The somatostatin-activated somatostatin receptor subtype 4 (SSTR4) also induces GIRK currents, which inhibits voltage-stimulated Ca<sup>2+</sup> influx in the pain processing pathway (Gorham et al., 2014). Therefore, through their coupling to GPCRs, GIRKs mediate the effects of many neurotransmitters, neuromodulators, and hormones (Figure 4). This effect of GIRKs contributes to the general homeostasis and particular synaptic plasticity processes of neurons and modulating memory and pain signaling pathways (Abney et al., 2019; Cooper et al., 2012; Djebari et al., 2021; Lippiello et al., 2020). Accordingly, GIRKs are involved in various neurological diseases, including Alzheimer's disease (Mondragon-Rodriguez et al., 2020; Sanchez-Rodriguez et al., 2019), alcohol abuse (Clarke et al., 2011; Kobayashi et al., 1999), nicotine addiction (Saccone et al., 2007), and epilepsy (Huang et al., 2018).
1. The involvement of GIRKs in PD

In DA neurons, dopamine receptor D$_2$ can be activated by the binding of dopamine, which then activates GIRKs, leading to cell hyperpolarization and inhibition of dopamine release (Liss et al., 1999; Lujan et al., 2009; Marcott et al., 2014). GIRK channels are critical in PD, as evidenced by the GIRK2 (Kir3.2) channel and its pathological role in the well-studied PD animal model, the *weaver* mouse.

The *weaver* mouse is a naturally occurring mutant strain characterized by tonic-clonic seizures, male infertility, and PD-like phenotypes including ataxia and tremor (Liss et al., 1999). Neurologically, *weaver* mice show progressive depletion of cerebellar granule cells and DA neurons in the substantia nigra (Bayer et al., 1996; Rakic and Sidman, 1973; Rezai and Yoon, 1972; Schmidt et al., 1982). These phenotypes are caused by a naturally developed homozygous point mutation p.G156S in *GIRK2*, which disrupts the selectivity filter of the channel pore and converts GIRK2 from a highly selective K$^+$ channel into a non-selective cation channel (*Figure 5*) (Heginbotham et al., 1992; Soman et al., 1995).

As a result, the Na$^+$ to K$^+$ permeability increases from ~0.03 for the native Kir3.1/3.2 heteromeric tetramers to ~0.74 for the Kir3.1/3.2$^{G156S}$ (Navarro et al., 1996). The loss of K$^+$ selectivity results in excessive inward leakage of Na$^+$ currents, which stimulate voltage-gated Ca$^{2+}$ channels and NMDA-glutamate receptors, leading to Ca$^{2+}$ overload and cell death (*Figure 5*) (Chen et al., 2014a; Hess, 1996; Surmeier et al., 1996). Consistent with these findings, a local anesthetic, *N*-ethyl lidocaine, that inhibits Na$_V$ channels (Ragsdale et al., 1994), has an inhibitory effect on mKir3.2$^{G156S}$ channels (Kofuji et al., 1996; Slesinger, 2001). Other cation channel blockers, such as the NMDA receptor inhibitor MK-801 (Dizocilpine,
INN), and the non-specific Cav channel blocker, Verapamil, can rescue the phenotype of *weaver* mice, potentially by inhibiting bulk cation influx and the resulting neuronal depolarization (Huettner and Bean, 1988; Kofuji et al., 1996; Lashgari et al., 2007; Liesi and Wright, 1996).

The effects of the p.G156S mutation on GIRK2 channel function vary depending on the expression level and relative abundance of GIRK2 compared to other GIRK channels (Slesinger et al., 1996). A higher abundance of GIRK2 in the substantial nigra may contribute to the selective neurodegeneration in this region (Karschin et al., 1996). In *weaver* mice, DA neuron death in the substantial nigra does not occur by apoptosis, but is primarily mediated via microglia-associated neuroinflammation (Figure 5) (Peng et al., 2006). In fact, minocycline, a microglia activation inhibitor, was found to attenuate nigrostriatal dopaminergic neurodegeneration and locomotor dysfunction in *weaver* mice (Peng et al., 2006).

Interestingly, despite the significant difference between rodent brain and human brain, similar de novo GIRK2 mutations have also been identified in patients with Keppen-Lubinsky syndrome, a rare disease characterized by a hyperkinetic movement disorder (characterized by abnormal involuntary movement), intellectual disability, and developmental delay (Horvath et al., 2018; Masotti et al., 2015). One patient was found to carry a missense mutation p.G154S, which is equivalent to the *weaver* mutation p.G156S in mice (Masotti et al., 2015). Another patient carries a p.L171R mutation, leading to the loss of K^+^ selectivity and membrane depolarisation, similar to the *weaver* mutation (Horvath et al., 2018). Furthermore, the abnormal inward currents mediated by hKir3.2^L171R^ can also be blocked by
QX-314 (Horvath et al., 2018). Although no loss of DA neurons has been reported in these Keppen-Lubinsky syndrome patients, their hyperkinetic dyskinesia symptoms raise the question of whether this syndrome and PD share some pathobiological mechanisms.

2. GIRK channels and the differential vulnerability of SNpc dopaminergic neurons

As mentioned above, one of the conundrums of most neurodegenerative diseases is the difference in the vulnerability of different brain regions, even though they have similar neuron types. In addition to the well-known differential vulnerability of DA neurons in the SNpc and the ventral tegmental area (Brichta et al., 2013; Dauer and Przedborski, 2003), post-mortem studies have also shown that DA neuron degeneration mainly affects the ventral tier of SNpc (losing 70%~90% neurons) compared to the dorsal tier of the SNpc (losing 25%~70% neurons), even though they have a similar neuron composition (Double et al., 2010; Fearnley and Lees, 1991; Hirsch et al., 1997). This difference in neurons within the same defined cytoarchitectural area suggests more complex causes than just positional differences.

This differential vulnerability of DA neurons might result from multiple factors contributing to the pathogenesis and progression of the disease in an accumulated or coordinated way. For example, compared to the dorsal tier of SNpc, the higher vulnerability of DA neurons in the ventral tier of the SNpc may be attributed to: (i) a more complex neuronal projection received from both the caudate nucleus and putamen (versus projection from caudate nucleus only for the dorsal tier) (Haber et al., 2000; Joel and Weiner, 2000); (ii) a lower expression of neuroprotective factors, such as trophin proteins (e.g., glial-derived neurotrophic factors, neurotrophins and epidermal growth factor receptors) (Barroso-Chinea et al., 2005; Nishio et al., 1998; Seroogy et al., 1994), proteins involved in synaptic and intracellular vesicle
trafficking (e.g., Ras-related protein RAB3B, RAB13, SNX8, and ANXA), and proteins involved in metal-binding and redox processes, including copper metabolism, e.g., metallothioneins, etc. (Monzon-Sandoval et al., 2020); (iii) a deficiency in antioxidant systems (Bjorklund et al., 2021); (iv) increased iron deposition (He et al., 2020); (v) a higher ratio of dopamine transporter (DAT) to vesicular monoamine transporter (VMAT), leading to neurotoxic accumulation of cytosolic dopamine (Double et al., 2010; Gonzalez-Hernandez et al., 2004; Kanaan et al., 2010); (vi) a different expression level of receptors, transporters and ion channels, which regulate the dopamine signaling and neuron excitability (Brichta and Greengard, 2014; Chan et al., 2010; Chan et al., 2007; Hurd et al., 2001).

Among these potential factors, the GIRK2 channels are an interesting, but controversial contributor. Initial in-situ hybridization and immunohistochemistry studies showed that GIRK2 expression is overall enriched in SNpc compared to the VTA DA neurons (Schein et al., 1998). Subsequent studies in humans, mice, and rats further suggested that GIRK2 is mainly expressed in the ventral tier of SNpc (Bjorklund and Dunnett, 2007; Chung et al., 2005; Mendez et al., 2005; Thompson et al., 2005). Therefore it was speculated that GIRK2 might contribute to the high vulnerability of DA neurons in the ventral tier of SNpc (Double et al., 2010).

However, a study of the distribution of all four GIRK channels in rat DA neurons demonstrated that GIRK2 was broadly expressed in SNpc, but also the lateral VTA (Eulitz et al., 2007b). More recently, Reyes et al. studied GIRK2 expression in both human and mouse midbrains and revealed that a vast majority of DA neurons in both dorsal and ventral tiers of SNpc have strong GIRK2 expression, and there is no significant difference between them.
Moreover, most VTA TH-positive neurons also express GIRK2, although the percentage of high GIRK2-expressing neurons in VTA was significantly lower than in the SNpc (Reyes et al., 2012). Another immunohistochemistry study in mice confirmed these findings by showing that GIRK2 is expressed in almost all TH-positive neurons in both tiers of the SNpc, and the majority of VTA TH-positive neurons (Fu et al., 2012).

Taken together, the overall expression of GIRK2 is significantly lower in VTA DA neurons compared to SNpc neurons. However, some VTA DA neurons have as strong GIRK2 expression as SNpc neurons. These findings are intriguing and warrant further studies to elucidate the exact role of GIRK2 in the highly complex vulnerability of DA neurons.

3. GIRK modifiers and their application in PD

The GIRK channels are biological targets for drug development in several diseases, such as depression (Llamosas et al., 2015), Down syndrome (Kleschevnikov et al., 2017), Keppen-Lubinsky syndrome (Masotti et al., 2015), and schizophrenia (Yamada et al., 2012). GIRK activators, such as ML297 (VU0456810), the first selective GIRK activator (Kaufmann et al., 2013; Wydeven et al., 2014); naringin, a natural flavonoid compound found in grapefruit (Yow et al., 2011); and flupirtine, a non-opioid analgesic agent with muscle relaxant properties (Osborne et al., 1998), have been used to treat conditions such as chronic pain, epilepsy and Alzheimer’s disease (Huang et al., 2018; Kimura et al., 2020; Sanchez-Rodriguez et al., 2017).
Due to the significant pathobiological roles of over-activated GIRK channels in PD, GIRK inhibitors are of interest (Table 1). Currently, available GIRK inhibitors include anti-psychotic drugs, selective serotonin reuptake inhibitors (SSRI), and Schering-Plough (SCH) compounds (SCH28080) (Zhao et al., 2021a). The anti-psychotic drug clozapine inhibits the brain-type GIRK1/2 heteromeric channels and cardiac-type GIRK1/4 heteromeric channels (Kobayashi et al., 1998). The SSRI drug, fluoxetine, can inhibit GIRK1/2 channel activities from the extracellular side at low micromolar concentrations (Kobayashi et al., 2003). The dopamine D1 antagonist SCH28080 inhibits serotonergic neurons in the dorsal raphe nucleus (Montalbano et al., 2015).

In addition to these compounds, chemicals such as benzopyrene derivatives NTC-801, honeybee venom peptide tertiapin (TPN) and its derivatives Tertiapin-Q, and antitussives have all shown the ability to inhibit GIRK channel activity (Drain et al., 1998; Hamasaki et al., 2013; Machida et al., 2011; Soeda et al., 2016; Walsh, 2011). Also, as a sub-family of the Kir channels, GIRKs can be inhibited by the Kir selective inhibitor Ba^{2+} (Kobayashi et al., 1998; 2000; Walsh, 2011).

Some of the above-listed GIRK inhibitors have been tested in clinical trials as PD treatments. For example, in PD psychosis clinical trials, clozapine was effective for PD psychotic symptoms, including hallucination, delusion, and sleep disturbance, but showed no side effects of increasing motor symptoms (Klein et al., 2003; Miyasaki et al., 2006). Fluoxetine passed a double-blind, randomized clinical trial as a treatment for multiple system atrophy, a disease that displays many PD-like symptoms (Rascol et al., 2021). These clinical trials strongly support the potential benefit of GIRK inhibitors for PD treatment.
Another important subfamily of Kir channels, the ATP-sensitive potassium (K\textsubscript{ATP}) channels, were first identified in cardiac muscle, but are predominantly expressed in the nervous system (Noma, 1983; Thomzig et al., 2005; Thomzig et al., 2001). Expression of different types of K\textsubscript{ATP} channels was found in various brain regions, including, but not limited to the hippocampus, hypothalamus, and SN (Fujimura et al., 1997; Schiemann et al., 2012; Spanswick et al., 1997).

K\textsubscript{ATP} channels are composed of four identical pore-forming Kir subunits (Kir6.1 or Kir6.2, encoded by \textit{KCNJ8} and \textit{KCNJ11}, respectively), and four identical regulatory sulfonylurea receptor subunits (SUR1, SUR2A, or SUR2B), forming an octameric complex (Figure 6) (Lee et al., 2017; Shyng and Nichols, 1997). Each Kir subunit contains an ATP-binding site on the cytoplasmic domain, and the binding of ATP leads to pore closure (Antcliff et al., 2005; Craig et al., 2008; Drain et al., 1998; Nichols, 2006; Nichols et al., 1996; Trapp et al., 2003; Zingman et al., 2001), which has been clearly demonstrated by the recently solved three-dimensional structures of K\textsubscript{ATP} channels (Lee et al., 2017; Martin et al., 2017; Puljung, 2018; Wu et al., 2018).

Each SUR subunit, which belongs to the ABC transporter family, contains two adenosine nucleotide-binding sites. Occupancy of the nucleotide-binding sites on SUR1 by ADP-Mg\textsuperscript{2+} activates the K\textsubscript{ATP} channel, a condition triggered either by direct binding of ADP-Mg\textsuperscript{2+} or by binding and subsequent hydrolysis of ADP-Mg\textsuperscript{2+} (Craig et al., 2008; Vedovato et al., 2015;
Zingman et al., 2001). Consequently, $K_{ATP}$ acts as a cellular sensor and modulates membrane excitability based on the intracellular metabolic state: a high ATP/ADP ratio closes the $K_{ATP}$ channel pore, increasing the excitability, while a low ATP/ADP ratio reduces excitability by opening it (Craig et al., 2008; Gribble et al., 2000; Zingman et al., 2001).

Furthermore, $K_{ATP}$ channel activity is also regulated by a variety of other metabolic signals, such as insulin, leptin, ghrelin, long-chain fatty acids, phosphatidylinositol 4,5-bisphosphate (PIP$_2$), pH, hydrogen peroxide (H$_2$O$_2$), reactive oxygen/nitrogen species (ROS/RNS), and nitric oxide (NO), all of which contribute to the essential properties of $K_{ATP}$ channels as metabolic sensors (Dragicevic et al., 2015; Fan and Makielski, 1997; Yang et al., 2020). Such a multifactorial regulatory mechanism is particularly important for neurons, where $K_{ATP}$ channels function as an energy control valve that integrates the ATP/ADP ratio with electroactivity to enable sophisticated neuronal functions (Lawson, 2000; Liss et al., 2005; Liss and Roeper, 2001; Nichols, 2006).

1. **$K_{ATP}$ is involved in neuronal protection and selective vulnerability**

Under physiological conditions, $K_{ATP}$ channels in the brain are closed, while under abnormal conditions such as ischemia or metabolic stress, the intracellular ATP concentration drops sharply, thereby opening $K_{ATP}$ channels and leading to K$^+$ efflux (Katsura et al., 1992; Yamada and Inagaki, 2005). These actions would lead to neuron membrane hyperpolarization, which reduces neuronal excitability. On the positive side, in the early phase of brain metabolic deficiency, silencing of electrical activity in the substantia nigra pars reticulata (SNpr) by the opening of $K_{ATP}$ can stop the progression of a seizure (Yamada and Inagaki, 2005). Under these pathological conditions, the transient opening of $K_{ATP}$ will counteract the
excitotoxic Na\(^+\)/Ca\(^{2+}\) overload and reduce abnormal neuronal excitability (Soundarapandian et al., 2007). Also, activation of \(K_{\text{ATP}}\) channels has a protective effect in ischemic post-conditioning, not only in the cardiac system, but also in the central nervous system. Indeed, in \textit{vivo} animal studies demonstrated that knocking out Kir6.2 exacerbates ischemic infarction, while overexpressing Kir6.2 ameliorates neuronal injury from ischemic insults (Morisaki et al., 2022; Pertiwi et al., 2019; Zhao et al., 2021b; Zheng et al., 2022).

However, in dopaminergic neurons, the hyperpolarization caused by over-activated \(K_{\text{ATP}}\) channels abolishes the electrical activity and reduces neurotransmitter release (Liss and Roeper, 2001; Yamada and Inagaki, 2005). Indeed, \(K_{\text{ATP}}\) channels have been shown to modulate the release of multiple neurotransmitters such as glutamate (Soundarapandian et al., 2007), GABA (Maneuf et al., 1996b), or dopamine (Avshalumov and Rice, 2003) (Figure 6).

As discussed above, the DA neurons in the substantia nigra (SN) are more vulnerable in PD than DA neurons in the ventral tegmental area (VTA). DA neurons at both sites have functional \(K_{\text{ATP}}\) channels (Kir6.2/SUR) expressed (Schiemann et al., 2012). However, \(K_{\text{ATP}}\) channels are selectively activated in response to mitochondrial respiratory chain complex I inhibition in SN DA neurons, but not in VTA DA neurons (Liss et al., 2005). This may be due to a lower expression level of mitochondrial uncoupling protein (UCP-2) in SN, which makes SN DA neurons more sensitive to metabolic stresses and more active in silencing the neurons electrically (Liss et al., 2005). Accordingly, genetic inactivation of Kir6.2 resulted in a selective rescue of SN, but not VTA DA neurons in two mechanistically distinct PD mouse models, \textit{i.e.}, the MPTP-induced model and the \textit{weaver} mouse model (Liss et al., 2005). This was confirmed in a recent Kir6.2 knockout study demonstrating that deletion of \(K_{\text{ATP}}\)
channels selectively restored the reduction of both DA neuron number and dopamine transmitter level and suppressed the excessive iron accumulation in the nigrostriatum of MPTP-treated PD mice (Zhang et al., 2018). Nevertheless, selective vulnerability in PD is a complex mechanism involving multiple factors. It remains largely elusive how exactly $K_{\text{ATP}}$ channels are involved and how much they contribute to the selective DA neuron vulnerability.

In contrast to Kir6.2, Kir6.1 is prominently expressed in astrocytes and regulates their cellular functions. Knockout of Kir6.1 in astrocytes leads to dysfunctional mitophagy, resulting in accumulation of damaged mitochondria, production of ROS and neuroinflammation (Hu et al., 2019). Consequently, astrocytic Kir6.1 KO mice demonstrate more significant DA neurodegeneration in the SNpc, lower dopamine levels in the striatum, and more significant motor dysfunction than controls (Hu et al., 2019).

The Kir6.1 channel may also protect neurons from neurodegeneration by cross-talking with the NF-κB/complement C3/complement C3a receptor (C3aR) pathway in astrocytes (Chen et al., 2021). Chen and colleagues demonstrated more dopaminergic neuron death and astrocytic complement reactivity in the SNpc area in astrocytic Kir6.1 knockout mice than in controls (Chen et al., 2021). Astrocytic Kir6.1 KO may promote astroglial NF-κB activation to stimulate more extracellular release of complement C3, which then interacts with neuronal C3aR to induce neurodegeneration (Chen et al., 2021). Another recent study has demonstrated that the small signaling molecule, hydrogen sulfide (H$_2$S), can alleviate 6-OHDA-induced Parkinsonism and increase dopaminergic neuron survival by activating $K_{\text{ATP}}$ (Minaei et al., 2021). These significant protective effects of $K_{\text{ATP}}$ channels in PD settings suggest that the defects in $K_{\text{ATP}}$ function may play an essential role in PD pathogenesis.
2. K\textsubscript{ATP} is involved in mitochondrial dysfunction-induced neuronal death

Mitochondrial damage and dysfunction are key pathological mechanisms in multiple neurodegenerative diseases, including PD (Bose and Beal, 2016; Guo et al., 2013). Mitochondrial activities, especially electron transport chain complex I activities, were shown to be significantly reduced in post-mortem studies of the SN in sporadic PD patients (Janetzky et al., 1994; Schapira et al., 1990). Complex I (also known as NADH: ubiquinone oxidoreductase or Type I NADH dehydrogenase) is a large enzyme catalyzing the first step of the mitochondrial electron transport chain and drives the generation of the mitochondrial electrochemical membrane potential (Sharma et al., 2009). Neurotoxins such as rotenone and MPP\textsuperscript{+}, and chemicals such as pyridaben, trichloroethylene, and fenpyroximate, all directly inhibit complex I, increase oxidative stress, and ultimately induce DA neuron degeneration (Gash et al., 2008; Sherer et al., 2003; Sherer et al., 2007; Smeyne and Jackson-Lewis, 2005).

K\textsubscript{ATP} channels are also expressed on the inner mitochondrial membrane (mito-K\textsubscript{ATP}) (Inoue et al., 1991; Paucek et al., 1992). The mitochondria are the dynamic structure whose matrix volume would change under different conditions (Anastacio et al., 2013). Earlier studies suggested that mito-K\textsubscript{ATP} may regulate the matrix volume of mitochondria (Lim et al., 2002; Szewczyk et al., 1993). Under stressful conditions, the mitochondrial uptake of K\textsuperscript{+} would decrease, leading to an imbalance between K\textsuperscript{+} influx and efflux and mitochondrial matrix contraction (Figure 6) (Al-Dadah et al., 2007; Garlid et al., 2003). The contraction of the mitochondrial matrix increases the distance among intermembrane enzymes essential for mitochondrial functions, compromising energy transfer efficiency (Anastacio et al., 2013). When activating mito-K\textsubscript{ATP}, the K\textsuperscript{+} uptake is increased, which is accompanied with an
increased uptake of inorganic phosphates, anions, and water (Ardehali and O’Rourke, 2005). Subsequently, the mitochondrial matrix contraction can be reversed back to normal (Figure 6) (Dos Santos et al., 2002).

The mitoK\textsubscript{ATP} channels regulate the oxygen-free radical formation and the mitochondrial inner membrane potential, thereby coupling energy metabolism with bioelectrical activities of mitochondria (Peng et al., 2018). They are also key players in mitochondrial preconditioning in the brain, a protective mechanism primarily regulated by mitochondria. In this process, preconditioning using a harmful insult at low doses can significantly inhibit the metabolic stress and the expression of pro-apoptotic proteins, thus protecting the brain from future severe damage (Ardehali and O’Rourke, 2005; Xu et al., 2001). In this regard, the mitoK\textsubscript{ATP} opener, diazoxide, exhibits neuroprotective effects against oxidative stress-induced damage and cellular dysfunction (Nakagawa et al., 2013; Zarch et al., 2009). Interestingly, under conditions denoted by ATP-deficiency, the affinity of mito-K\textsubscript{ATP} to diazoxide increases significantly (Akopova et al., 2020).

MitoK\textsubscript{ATP} activators have also been demonstrated to protect against DA neurodegeneration induced by chemicals targeting mitochondrial complexes I (Tai and Truong, 2002; Yang and Perry, 2009; Yang et al., 2004; Yang et al., 2005a; Yang et al., 2005b). However, there have been controversies regarding the role of mito-K\textsubscript{ATP} activators in Parkinson’s disease, and the effects seem to vary depending on the model used. For example, Peng et al. showed that, in the rotenone-induced chronic PD model, diazoxide aggravated DA neurodegeneration both in vitro and in vivo, while the mito-K\textsubscript{ATP} inhibitor 5-hydroxydecanoate showed the opposite effects (Peng et al., 2018). However, diazoxide showed protective effects in a rotenone-induced acute PD model (Peng et al., 2018). Modulation of mito-K\textsubscript{ATP} activity may influence
the expression levels of key proteins regulating mitochondrial biogenesis, fission/fusion, and homeostasis, such as peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α (Peng et al., 2018). Further studies are required to elucidate the detailed molecular mechanism of mito-\(K_{\text{ATP}}\) function in PD.

3. \(K_{\text{ATP}}\) modulates GABA level and \(\alpha\)-synuclein secretion in PD

GABA (\(\gamma\)-Aminobutyric acid) is a widely expressed neurotransmitter and GABA-dependent control is a common mechanism in most types of neurons (Blaszczyk, 2016). GABAergic terminals derived from the striatum, especially the pallidum, form the primary synaptic input to DA neurones in the SN (Bolam and Smith, 1990). The GABAergic neurons or GABA-dependent control may not be directly related to PD pathogenesis, but could have an essential role in controlling motor symptoms in PD (Blaszczyk, 2016). A post-mortem study showed a ~36% decrease in GABA concentration in the thalamus of PD patients compared to the controls (Gerlach et al., 1996). Moreover, PD patients with tremor-dominant symptoms had even lower GABA concentrations compared to patients with non-tremor-dominant symptoms (Gong et al., 2018). A recent study also confirmed that the GABA levels in the motor cortex are inversely related to the severity of motor symptoms (van Nuland et al., 2020). As such, understanding the potential protective effect of GABAergic neurons may be beneficial to the development of new treatments for PD.

\(K_{\text{ATP}}\) has been reported to be involved in GABA modulation in the SN (Amalric et al., 1992; Maneuf et al., 1996a). Inhibition of \(K_{\text{ATP}}\) in the absence of glucose increased GABA release in the hippocampus (Margaill et al., 1992), whereas \(K_{\text{ATP}}\) activation by diazoxide decreased GABA levels in the ventromedial hypothalamus (Li et al., 2017). In PD, Maneuf et al.
demonstrated that \( K_{ATP} \) openers such as diazoxide and cromaklim reduced the release of GABA in the SN (Maneuf et al., 1996a). Furthermore, an intra-globus pallidus injection of diazoxide alleviates akinesia in a dose-dependent manner in a reserpine-induced rat PD model (Maneuf et al., 1996a).

Interestingly, the synergistic effects of GABA, GABA receptors, and \( K_{ATP} \) channels were demonstrated to be one of the potential factors influencing the secretion of \( \alpha \)-synuclein, a key player in PD pathology (Emmanouilidou et al., 2016). Activation of the SUR1-\( K_{ATP} \) channels on the membrane of GABAergic neurons leads to membrane hyperpolarization and reduces GABA release (Amoroso et al., 1990). Decreased GABA levels leads to decreased GABA\(_B\) receptor activity on adjacent presynaptic glutamatergic nerve endings (Kerr and Ong, 1995). Under physiological conditions, activation of presynaptic GABA\(_B\) receptors suppresses neurotransmitter release by inhibiting Ca\(^{2+}\) channels (Alten et al., 2022). When GABA\(_B\) receptors are less active, surrounding Ca\(^{2+}\) channels are less inhibited, leading to an increased intracellular Ca\(^{2+}\) concentration, which triggers calcium-dependent \( \alpha \)-synuclein secretion (Emmanouilidou et al., 2016).

4. **\( K_{ATP} \) modifiers and their potential applications in PD**

Decades of research on \( K_{ATP} \) channels have identified numerous drugs or chemicals as \( K_{ATP} \) activators or inhibitors. Some have been applied extensively in both *in vitro* and *in vivo* studies examining PD (Table 1).
Diazoxide (7-chloro-3-methyl-4H-1,2,4-benzothiadiazine 1,1-dioxide) is the first well-studied \( K_{\text{ATP}} \) opener, which was initially approved to treat low blood sugar and acute hypertension (Koch-Weser, 1974; van Hamersvelt et al., 1996). As a specific mito-\( K_{\text{ATP}} \) channel opener and a blood-brain barrier permeable medication, diazoxide has been studied for its potential use in the treatment of PD. Some research has suggested that diazoxide suppresses neurotoxin-induced microglial activation and subsequent neuroinflammation, reduces ROS generation in primary midbrain neurons, and ameliorates excessive GABA release (Liu et al., 2006; Maneuf et al., 1996a; Tai et al., 2003; Xie et al., 2010; Yang et al., 2006; Zhou et al., 2008). These effects of diazoxide protect neurons from degeneration in various PD cell and animal models (Liu et al., 2006; Maneuf et al., 1996a; Tai et al., 2003; Xie et al., 2010; Yang et al., 2006; Zhou et al., 2008).

A recent study using both the PC12 cell model and Sprague-Dawley rat model demonstrated that, opposite to the expected protective effects, diazoxide aggravated rotenone-induced dopamine neurodegeneration (Peng et al., 2018). In contrast, the mito\( K_{\text{ATP}} \) channel inhibitor 5-hydroxydecanoate (5-HD) improved rotenone-induced dopamine neurodegeneration (Peng et al., 2018). The underlying mechanism for such disparity remains elusive, but it may be due to different subtypes of mito\( K_{\text{ATP}} \) channels involved in target cells/tissues in various studies or differences between acute versus chronic rotenone-induced PD models (Peng et al., 2018). Diazoxide may be more effective at protecting against the initial toxic effects of rotenone in acute models, but may not be as effective at reversing the ongoing degeneration of dopaminergic neurons in chronic models.

Notably, it has been recently established that SUR1, the predominant SUR isoform in neuronal cells, also modulates non-selective cation channel TRPM4, forming \( Ca^{2+} \)-activated,
voltage-dependent, ATP-suppressive TRMP4/SUR1 channels in the CNS (Hansen, 2006; Woo et al., 2013). Diazoxide can directly activate SUR1-TRPM4 channels that mediate the influx of monovalent cations, leading to membrane depolarization, cytotoxic edema, and oncotic cell death (Alquisiras-Burgos et al., 2022; Jha et al., 2021; Simard et al., 2006). No substantial evidence is available demonstrating a direct role of TRPM4 in PD yet, but TRPM4 is an essential co-activator of the NMDA receptors, and regulates Ca\(^{2+}\) homeostasis and microglial functions (Kurland et al., 2016; Menigoz et al., 2016). Therefore, the involvement of other diazoxide-regulated ion channels may also contribute to the observed discrepancy in the effectiveness of diazoxide in different PD models.

Another K\(_{ATP}\) channel activator, Iptakalim (2, 3-dimethyl-N-(1-methylethyl)-2-butanamine hydrochloride, Ipt), is also permeable to the blood-brain barrier and has been used to treat ischemic stroke (Sikka et al., 2012; Wang et al., 2004). Ipt exhibits potent neuroprotection against rotenone-induced neurotoxicity by reducing inducible nitric oxide synthase (iNOS) expression and activity in PD studies (Yang et al., 2004; Yang et al., 2006). Furthermore, activation of mito-K\(_{ATP}\) by Ipt can inhibit the activation of microglia and reduce the damage caused by microglia-mediated neuroinflammation or cell death caused by neurotoxins such as MPP\(^{+}\) and rotenone (Hu et al., 2005; Yang et al., 2004; Zhou et al., 2007).

The FDA-approved anti-hypertensive drugs, pinacidil and cromakalim, are also neuroprotective as K\(_{ATP}\) activators (Deng et al., 2009; Lauritzen et al., 1997; Shukry et al., 2015; Wang et al., 2011). Even brief application of K\(_{ATP}\) openers, including diazoxide, Ipt, pinacidil, and cromakalim, can be neuroprotective against rotenone and/or MPP\(^{+}\)-induced cell death in PD models (Tai and Truong, 2002; Yang et al., 2004; Yang et al., 2005a; Yang et al., 2005b).
Furthermore, these effects can be abolished by the \( K_{ATP} \) blockers (Findlay, 1992; Tai and Truong, 2002; Zhou et al., 2009).

Paradoxically, glibenclamide, a specific suppressor of the SUR subunit, has been shown to significantly improve motor performance, slow the decline of striatal DA neurons density and dopamine level, and reduce neuronal apoptosis in a rotenone-induced PD mouse model (Abdelkader et al., 2020). Therefore, as noted above, mito-\( K_{ATP} \) may have a binary effect upon activation: mito-KATP activation is neuroprotective under conditions such as transient hypoxia or ischemia; while if an acute condition turns into a chronic condition, the activation of mito-\( K_{ATP} \) is tuned to have the opposite effect, accelerating neurodegeneration (Peng et al., 2018).

C. Classic Kir channels and PD

The classic Kir channels, Kir2 channels (Kir2.1-2.4 or IRK1-4), function as homomeric or heteromeric tetramers and play key roles in regulating basal ganglia dopaminergic neurotransmission (Hibino et al., 2010; Ishihara et al., 2009; Pruss et al., 2003; Schram et al., 2002; Tinker et al., 1996). In PD settings, the depletion of dopamine leads to a variety of structural and biochemical changes, either compensatory or maladaptive (Shen et al., 2007b). One of the consequences is the relative up-regulation of Kir2.3 expression, which stimulates enhanced regulation of Kir2.3 by the M1 muscarinic receptor (Shen et al., 2007a).

Activated M1 muscarinic receptors potently down-regulate Kir2.3 channel currents in striatopallidal medium spiny neurons, but not in the same type of neurons in the neighbouring
striatonigral region, suggesting another mechanism contributing to the differential vulnerability in PD (Shen et al., 2007a). Interestingly, the mRNA levels of all four Kir2 channels were down-regulated in peripheral blood lymphocytes from PD patients compared with age-matched controls (Gui et al., 2011). It would be useful to examine further whether reduced Kir2 expression correlates with stages of PD progression and whether it can be used as a peripheral biomarker for early diagnosis.

In summary, the Kir channel family is an exciting and distinguished group of K+ channels for PD. Not only because the first naturally occurring mouse model of PD was due to a single mutation in Kir channels (GIRK2/Kir3.2), but also because these channels mediate the physiological effects of many neurotransmitters, neuromodulators and hormones; they are sensitive to cellular energy levels and control the selective vulnerability of SNpc DA neurons. These channels also help restore mitochondrial function and modulate GABA levels and α-synuclein secretion. Therefore, it is critical to consider how to precisely regulate Kir channels in a highly coordinated manner.

V. Ca2+-activated potassium channels (KCa)

Another important K+ channel family, Ca2+-activated potassium (KCa) channels, are activated by elevated intracellular Ca2+ concentration (Stocker, 2004). The KCa family consists of eight members, including three main subtypes, i.e., the large-conductance KCa (BK) channels (KCa1.1 or KCNMA1 or Slo1), the small-conductance KCa (SK) channels (KCa2.1-2.3 or KCNN1-3), and the intermediate-conductance KCa (IK) channels (KCa3.1 or KCNN4), and three other members KCa4.1, KCa4.2 and KCa5.1 (Ishii et al., 1997; Joiner et al., 1997; Kohler et al., 1996).
BK channels are voltage-dependent and can be synergistically activated through the direct binding of calcium and magnesium ions (Contet et al., 2016; Cui et al., 2009; Shi and Cui, 2001; Zhang et al., 2001). In contrast, SK and IK channels are voltage insensitive and do not directly bind Ca\(^{2+}\), but are instead regulated by Ca\(^{2+}\) through calmodulin (CaM) that binds to their C-terminus, with channel opening occurring in response to Ca\(^{2+}\) binding to CaM (Fanger et al., 1999; Xia et al., 1998). Functional SK channels are heteromeric complexes with constitutively bound CaM (Maylie et al., 2004; Xia et al., 1998).

These K\(_{Ca}\) channels link the elevation of intracellular calcium ions to membrane potential, exerting a hyperpolarizing influence by inducing, upon activation, a massive efflux of K\(^+\) (Contet et al., 2016; Daniel et al., 2021). Numerous sources of Ca\(^{2+}\), including the activation of Ca\(_V\) channels, the release of intracellular Ca\(^{2+}\) stores, and the activation of Ca\(^{2+}\)-permeable ionotropic neurotransmitter receptors, can activate K\(_{Ca}\) channels (Gueguinou et al., 2014). The kinetics and consequences of K\(_{Ca}\) channel activation are determined by the interactions among the Ca\(^{2+}\) source, K\(_{Ca}\) channels, and various modulators. Remarkably, K\(_{Ca}\) channels in turn control the activity of multiple Ca\(^{2+}\) channels and, therefore, they are also critically involved in coordinating diverse Ca\(^{2+}\) signaling pathways and controlling Ca\(^{2+}\) signal amplitude and duration (Adelman et al., 2012; Sun et al., 2020).

A. **BK channels**

BK channels (K\(_{Ca1.1}\) or KCNMA1 or Slo1) are characterized by a large single-channel conductance (~200 pS), high K\(^+\) selectivity, and the ability to be exquisitely regulated by both
membrane potential and local $[\text{Ca}^{2+}]_{\text{cyto}}$ and $[\text{Mg}^{2+}]_{\text{cyto}}$ (Latorre et al., 1982; Marty, 1981). They are broadly expressed in both excitable and non-excitable cells and regulate diverse physiological processes, such as neuronal excitability, neurotransmitter release, endothelial functions, and muscle contraction (Latorre et al., 2017; Trimmer, 2015). Notably, the open probability of BK channels increases with membrane depolarization and elevated $[\text{Ca}^{2+}]_{\text{cyto}}$ (Cui et al., 1997). The kinetic behavior of these channels is critical for their fundamental roles in modulating membrane excitability and intracellular $\text{Ca}^{2+}$ homeostasis (Sancho and Kyle, 2021).

In neurons, BK channels regulate the timing and duration of $\text{K}^+$ conductance; therefore, they influence the propagation, shape and frequency of action potentials depending on the cellular context (Figure 7) (Contet et al., 2016; Lara et al., 1999; Lin et al., 2014; Lovell and McCobb, 2001). Specifically, BK channels contribute to AP repolarization and fast afterhyperpolarization (fAHP); inhibition of BK channels reduces the spontaneous firing activity, which is one of the mechanisms by which methamphetamine exerts pharmacological effects on regulating the firing activities of DA neurons (Lin et al., 2016). Interestingly, BK channels are neither strictly excitatory nor inhibitory. Pharmacological or genetic inhibition of BK channels in the CNS can increase or decrease the neuronal firing activity, depending on the cellular context and relative magnitude of other ion channels (Du et al., 2019; Gu et al., 2007; Matthews et al., 2008; Pitts et al., 2006). This capacity to neutralize the neuronal excitability, i.e., tuning down the over-activated neurons and tuning up the firing of over-quiet ones, makes BK channels an attractive target for multiple neurological disorders (Contet et al., 2016; Gu et al., 2007; Matthews et al., 2008; Meredith et al., 2006; Nelson et al., 2003; Pitts et al., 2006; Zhu et al., 2018).
In addition, as regulators of presynaptic neurotransmitter release, BK channels have been reported to be down-regulated by excessive ROS, a significant contributor to DA neuron loss in PD (Figure 7) (Robitaille and Charlton, 1992; Robitaille et al., 1993; Tang et al., 2004). Excessive ROS could result from various factors, such as low glutathione levels, abnormal iron accumulation, calcium imbalances and dysregulated dopamine metabolism (Ma et al., 2021). ROS impairs BK channel function by reducing its sensitivity to intracellular [Ca^{2+}], so activation of BK channels has been used as a therapeutic strategy for treating PD (Tang et al., 2004).

The BK channel agonist, zonisamide (Zonegran, a sulfonamide drug), has been approved since 2009 as an adjunct to Levodopa in treating motor symptoms in PD (Matsunaga et al., 2017; Yang and Perry, 2009). At doses of 25-50 mg/day, zonisamide significantly reduces UPDRS III scores and daily "off" time in PD patients without increasing disabling dyskinesia (Murata et al., 2015). The mode of action of zonisamide is quite complex, primarily involving: (1) inhibition of monoamine oxidase-B (MAO-B), preventing ROS generation from the metabolism of dopamine, and generation of MPP\(^+\) from MPTP by MAO-B (Sonsalla et al., 2010; Uemura et al., 2017); (2) blocking T-type Ca\(_V\) channels to restore the Ca\(^{2+}\) balance in DA neurons (Kunisawa et al., 2018; Yang et al., 2014b); (3) protection of neurons from degeneration by inducing neurotrophic factors (Choudhury et al., 2011; Sano et al., 2015), inhibiting oxidative stress and apoptosis (Condello et al., 2013; Yurekli et al., 2013), and repressing neuroinflammation (Hossain et al., 2018); (4) modulating levodopa-dopamine metabolism in the striatum and enhancing the therapeutic effect of levodopa (Murata, 2004; Nishijima et al., 2018); and (5) ameliorating levodopa-induced dyskinesia by downregulation of endocannabinoid CB1 and adenosine A2A receptors (Oki et al., 2017). As an oral drug with 100% bioavailability and a long half-life (49.7-62.5 h), zonisamide has been tested in multiple randomized controlled clinical trials and has proven safe and efficacious as
adjunctive therapy for PD patients (Fox et al., 2018; Murata et al., 2015; Murata et al., 2007). Nevertheless, it is still to be elucidated how the activation of BK channels contributes to the above mechanisms and the alleviation of PD symptoms.

**B. SK channels**

SK channels ($K_{Ca.1-2.3}$ or KCNN1-3) are named after their small single-channel conductance of ~10 pS, and are expressed in the plasma membrane, the endoplasmic reticulum (ER), and the inner mitochondrial membrane (Adelman et al., 2012). In the plasma membrane of neurons, SK channels are in close proximity to $N$-methyl-$D$-aspartate receptors (NMDAR) and are activated by NMDAR-dependent Ca$^{2+}$ influx (Ngo-Anh et al., 2005). Once activated, the SK channel-induced K$^+$ efflux leads to membrane hyperpolarization, thereby attenuating excessive Ca$^{2+}$ influx in response to excitotoxic activation of NMDAR by glutamate (Dolga et al., 2011). In mitochondria, activation of mito-SK channels can restore the mitochondrial membrane potential, prevents mitochondrial fission, and reduce mitochondrial Ca$^{2+}$ overload and the generation of mitochondrial ROS (Figure 7) (Honrath et al., 2017). Therefore, SK channels play a fundamental role in regulating synaptic plasticity and [Ca$^{2+}$], homeostasis in many types of neurons (Adelman et al., 2012; Bond et al., 2005; Desai et al., 2000; Xia et al., 1998). Accordingly, they have become an emerging target for their neuroprotective effects under conditions such as ER stress, oxidative cell death, excitotoxicity and ischemia (Allen et al., 2011; Richter et al., 2015; Richter et al., 2016).

During an action potential, SK channels are activated by the transient increase of intracellular Ca$^{2+}$ concentration, and the K$^+$ efflux due to their activation mediates the medium afterhyperpolarization (mAHP) phase, which regulates neuronal firing frequency (Figure 7) (Adelman et al., 2012; Dwivedi and Bhalla, 2021). In DA neurons, SK currents are
crucial in regulating the spontaneous firing patterns by decreasing spike-to-spike variability and supporting the precision of tonic firing as both an intrinsic cellular property and in response to synaptic inputs (Canavier and Landry, 2006; Komendantov et al., 2004; Waroux et al., 2005). This is critical for PD, as dopamine release at DA neuron projection sites depends on the rate and pattern of DA neuron firing (Paladini et al., 2003). Biophysical and pharmacological studies have demonstrated that \( K_{Ca.2.3} \) channels are the predominant mediators of SK effects in SN DA neurons, but are not involved in the pacemaker control in VTA DA neurons (Wolfart et al., 2001). Accordingly, SN DA neurons have highly precise firing activities, while VTA DA neurons discharge more irregularly (Wolfart et al., 2001).

More specifically, the medium afterhyperpolarization phase regulated by SK channels controls the timing and stability of DA neuron pacemaker activity (Adelman et al., 2012; Bond et al., 2005; Wolfart et al., 2001). This regulatory effect has been directly supported by the observation that the firing regularity in midbrain DA neurons can be compromised by blocking SK channels (Iyer et al., 2017). Furthermore, in a 6-OHDA-induced rodent PD model, SK channels were found to precisely control the phenotypes of SN DA neurons, where a phenotype "shift" from tyrosine hydroxylase negative (TH\(^{-}\)) cells to hydroxylase positive (TH\(^{+}\)) cells was observed during the convalescent phase after 6-OHDA treatment (Stanic et al., 2003). Aumann et al. further reported that such a shift could be manipulated by modulating SK channels. Infusion of an SK agonist into the SNC area in the mouse brain increased the number of TH\(^{+}\) neurons, while an SK antagonist showed the opposite effects (Aumann et al., 2008). Along this line, activation of SK channels was shown to attenuate spontaneous irregular firing by enhancing mAHP, and preserve mitochondrial function, ATP levels and the dendritic network, thereby reducing neurotoxicity and spontaneous loss of DA.
neurons, whereas inhibition of SK channels reduced the number of DA neurons (Benitez et al., 2011; Dolga et al., 2014; Wang et al., 2015b). In contrast, the SK channel antagonist, apamin, a globular peptide neurotoxin from bee venom, exacerbated the toxic effects of 6-OHDA and decreased DA neuron numbers (Wang et al., 2015b). These results suggest that increasing SK channel activity could potentially preserve DA neurons and be beneficial to PD patients.

Nevertheless, apamin has also been reported to increase basal dopamine levels in the striatum, thereby ameliorating motor deficits and improving both motor activities and non-motor symptoms in PD models (Alvarez and Sabatini, 2007; Chen et al., 2014b; Maurice et al., 2015a). However, in a clinical trial with monthly bee venom injections, PD patients did not show improvement after 11 months of treatment. The authors suggested that administering higher doses of bee venom at an increased frequency may improve symptoms (Hartmann et al., 2016). Possibly, the controversy is a result of the different models used. Given that SK channels affect both excitability and spiking patterns, they are expected to have complex effects on disease pathophysiology, which would lead to different outcomes in different PD settings. More detailed studies are required to understand the contribution of SK channels to PD and how to alleviate PD symptoms by appropriately modifying SK channel activity.

C. IK channels

The IK channels (KCa3.1 or KCNN4), which have an intermediate conductance of ~40 pS, play an essential role in cellular proliferation, differentiation, migration, and cytokine production in innate and adaptive immune systems (Cruse et al., 2006; Di et al., 2010; Du et
al., 2019; Millership et al., 2011). The membrane hyperpolarization caused by K⁺ efflux through activated KCa3.1 channels facilitates Ca²⁺ influx; accordingly, activation of KCa3.1 was found to promote Ca²⁺ signaling and microglia activation and migration (Figure 7) (Daniel et al., 2021; Ferreira and Schlichter, 2013; Kaushal et al., 2007; Schilling et al., 2004).

A recent study using an MPTP-induced PD mouse model showed that genetic knockout or pharmacological blockade of the KCa3.1 channels could attenuate microgliosis and neuroinflammation in the SNpc area, rescue the tyrosine hydroxylase (TH)-positive neuron number, and improve the locomotor ability of these mice (Lu et al., 2019). These effects can be partially attributed to the involvement of KCa3.1 in store-operated calcium (SOC) channel-mediated Ca²⁺ overload and ER stress via the protein kinase B (AKT) signaling pathway (Yu et al., 2018). Interestingly, KCa3.1 deletion could redeem the AKT/mammalian target of rapamycin (mTOR) signaling both in vivo and in vitro (Lu et al., 2019). Currently, there are no available drugs targeting IK channels for PD treatment. However, the pivotal role of KCa3.1 in microgliosis-induced neurotoxicity suggests that the IK channel could also be a fascinating biological target for PD study and therapy.

Given the diverse functions of KCa channels in CNS, including regulation of neuronal firing frequency and patterns, synaptic transmission, rhythmic activity, mitochondrial function and intracellular Ca²⁺ homeostasis, our current understanding of the involvement of KCa channels in PD may only scratch the surface. Further studies using robust research models (such as KCa knockout animal models and optimized PD models) and more specific KCa channel modulators, as well as comprehensive investigation from cellular to whole animal levels, are needed to elucidate the link between KCa channels and PD.
VI. Conclusion and perspectives

PD is a multifactorial neurodegenerative disease involving a variety of risk factors and complex pathobiological mechanisms (Figure 1) (Goldman, 2014; Nguyen et al., 2019; Winklhofer and Haass, 2010). These factors exacerbate each other, resulting in highly diverse pathogenesis and symptoms observed in PD patients. This neurodegenerative disorder is therefore regarded as multiple pathological states under the same “PD” umbrella. Accordingly, more scientific effort is needed to understand the various molecular mechanisms that stratify PD patients into different disease contexts.

Mounting evidence suggests that ion channels play a key role in the pathogenesis and progression of PD by regulating neuronal excitability and immune cell function (Hahn et al., 2003; Kettenmann et al., 1993; Liss and Roeper, 2001; Sarkar et al., 2020). These unique membrane proteins have therefore become promising biological targets for PD treatment. For example, due to the vital role of voltage-gated calcium ($\text{Ca}_V$) channels in the pathogenesis of PD, the dihydropyridine class anti-hypertensive drug and L-type $\text{Ca}_V$ channel blocker, isradipine (trade name DynaCirc or Prescal), has been through multiple clinical trials (Investigators, 2006; McFarthing and Simuni, 2019; Parkinson Study Group, 2020; Venuto et al., 2021). New clinical trials of the T-type $\text{Ca}_V$ channel blocker CX-8998 and zonisamide are also underway (https://clinicaltrials.gov).

However, the development of $\text{K}^+$ channel targeting drugs for PD is still a “blue sky” endeavor. One of the major challenges is that $\text{K}^+$ channels are broadly expressed throughout the body. It is therefore critical to avoid side effects caused by both on-target and off-target mechanisms.
Another major difficulty is achieving subtype selectivity when several homologous members are within the same subfamily. Biologics, such as antibodies and peptides, can often provide better potency and subtype selectivity due to their much larger size and consequently more extensive interactions with the target channel. The poor blood-brain barrier permeability is, unfortunately, often a barrier to using biologics for neurological disorders. Remarkably, recent preclinical trials of nanoparticle-mediated entry of dopamine, nerve growth factor or glial cell line-derived neurotrophic factor into the brain have revealed exciting opportunities (Saraiva et al., 2016). As such, combining $K^+$ channel-specific biologics with targeted delivery might be a promising strategy for treating PD.

Overall, despite being the most abundant channel type in the CNS, the study of $K^+$ channels is still in its infancy in terms of understanding their roles in the pathobiology and treatment of PD. In spite of the growing body of evidence discussed in this review, more systematic studies, especially in vivo studies, are needed to move forward.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Chen, Feng, Quinn, Pountney, Richardson, Mellick, Ma
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Footnotes

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Figure and Table legends

Figure 1 The key risk factors and pathological features of PD.

Figure 2 The representative 3D structures of the four potassium channel subfamilies. The 3D structure model was built by SWISS-Model. The grey shades represent the plasma membranes. The model channels: Kv1.3 (7EJ1); Kir2.2 (3SPG); Slo1 K⁺ channel (BK) (6V22); K₂P3.1 (6RV2) (Liu et al., 2021; Miller and Long, 2012; Rödström et al., 2020; Waterhouse et al., 2018)

Figure 3 Featured Kv channels in CNS. (1) The activation of Kv1 channels mediates K⁺ efflux to counterbalance the influx of Ca²⁺ ions, regulating the excitability of striatal cholinergic interneurons. (2) The PKA-AMPK pathway regulates Kv2 surface clustering. The clustered Kv2 channels boost K⁺ efflux and can lead to cell death. (3) Kv3 channels are predominantly expressed in the globus pallidus (GP) and generate “fast-spiking” currents. (4) Kv4 channels regulate pacemaker frequency and are related to PD motor symptoms. The spread of α-synuclein increases the plasma membrane expression of Kv4 and leads to a decrease in gastrointestinal motility. (5) Kv7 channels control the generation of M-current, a type of slowly activating, non-inactivating K⁺ current that raises the threshold of action potential firing.

Figure 4 Illustration of the activation of GIRK channels by GPCRs and related neurological effects.
Figure 5 Schematic diagram of *weaver* mouse pathogenesis. The mutation, p.G156S, changes GIRK2 (Kir3.2) from a highly selective K⁺ channel into a non-selective cation channel. The excessive cation influx through mutated Kir3.2 channels increases the excitability of DA neurons in the substantia nigra, which leads to neurodegeneration and Parkinsonism symptoms.

Figure 6 The involvement of K<sub>ATP</sub> channels in PD pathogenesis. The activation of K<sub>ATP</sub> channels on the plasma membrane hyperpolarizes the membrane, further inhibiting dopamine release. The mitoK<sub>ATP</sub> channels are activated when mitochondrial contraction occurs and reverse the mitochondrial contraction by increased K⁺ uptake.

Figure 7 The schematic diagram of K<sub>Ca</sub> channel functions in the CNS. The K<sub>Ca</sub> channel family comprises BK, SK, and IK channels. BK channels regulate the propagation, shape, and frequency of the action potential (AP). Reactive oxygen species (ROS) inhibit BK channel functions by decreasing its sensitivity to intracellular [Ca²⁺]. SK channels expressed on the plasma membrane mediate the medium afterhyperpolarization phase of AP, while those on the mitochondria have protective functions by restoring mitochondrial membrane potential, preventing mitochondrial fission, and reducing mitochondrial Ca²⁺ overload and ROS generation. Membrane hyperpolarization caused by IK channel activation promotes Ca²⁺ influx, leading to the activation of Ca²⁺ signaling pathways, and activation/migration of mitochondria.
Table 1 Overview of key $K^+$ channel modulators in PD
### TABLE 1

Overview of key K⁺ channel modulators with potential in PD therapy

<table>
<thead>
<tr>
<th>Channel</th>
<th>Selected inhibitors (I) or activators (A)</th>
<th>Structure</th>
<th>Potency (IC₅₀/EC₅₀)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv1.3</td>
<td>ChTx (Charybdotoxin (a toxin from scorpion <em>Leiurus quinquestriatus hebraeus</em>)) (Bontems et al., 1992)</td>
<td><img src="image1" alt="Structure" /></td>
<td>IC₅₀ = 2.6 nM (Grissmer et al., 1994; Judge and Bever, 2006)</td>
<td>ChTx blocks Shaker-type K⁺ channels in a voltage-dependent manner by directly binding to the outer entrance of the channel pore in both the open and closed states (Goldstein and Miller, 1993; Naranjo and Miller, 1996; Thompson and Begenisich, 2000).</td>
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<td></td>
<td>(I) Clofazimine</td>
<td><img src="image2" alt="Structure" /></td>
<td>IC₅₀ = 1 μM (Faouzi et al., 2015)</td>
<td>Clofazimine blocks activated Kv1.3 use-dependently during prolonged depolarizations, resulting in accelerated channel inactivation; it also blocks deactivated channels</td>
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<tr>
<td>Compound</td>
<td>IC$_{50}$ Value</td>
<td>Description</td>
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<tr>
<td>Diphenoxylate</td>
<td>IC$<em>{50} = 300$ nM (Ren et al., 2008); IC$</em>{50} = 5 \mu$M (Nguyen et al., 2010)</td>
<td>Diphenoxylate is an opioid agonist and anti-diarrheal agent with therapeutic effects on psoriasis and other inflammatory skin conditions as a KV1.3 blocker.</td>
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<tr>
<td>Margatoxin (MgTx) (Johnson et al., 1994)</td>
<td>IC$_{50} = 50$ nM (Garcia-Calvo et al., 1993; Judge and Bever, 2006)</td>
<td>The Lys35 from MgTx occludes the channel filter to block the pore (Chen and Chung, 2014). MgTx is a non-selective for KV1.3 with a similar binding affinity to KV1.2 (Bartok et al., 2014).</td>
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<tr>
<td>OSK1 (α-KTx3.7, a toxin from the scorpion, Ort hochirus)</td>
<td>IC$_{50} = 14$ pM (Mouhat et al., 2005)</td>
<td>OsK-1 is positively charged and binds with KV1.3 at the turret region. It also interacts with KV1.1 and KV1.2, with IC$_{50}$ values of</td>
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<tr>
<td>Compound</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Blocking Mechanism</td>
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<td>scrobiculosus)</td>
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<td>(Jaravine et al., 1997)</td>
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<tr>
<td>(I) PAP-1 (5-(4-Phenoxybutoxy)psoralen)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 2 nM</td>
<td>(Schmitz et al., 2005) PAP-1 blocks Kv1.3 with a voltage dependence and preferential affinity to the C-type inactivated state of the channel. It may block the channel by coordinating a K&lt;sup&gt;+&lt;/sup&gt; ion with two PAP-1 molecules at the inner part of the selective filter (Zimin et al., 2010).</td>
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<tr>
<td>(I) Psora-4 (5-(4-Phenylbutoxy)psoralen)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 3 nM</td>
<td>(Admasu et al., 2022; Vennekamp et al., 2004) Psora-4 blocks C-type inactivated Kv1.3 in a dose-dependent manner and has 17- to 70-fold selectivity for Kv1.3 over closely related Kv1-family channels (Kv1.1, Kv1.2, Kv1.4, and Kv1.7) except for Kv1.5 (IC&lt;sub&gt;50&lt;/sub&gt; = 7.7 nM) (Vennekamp et al., 2004).</td>
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<tr>
<td>(I) ShK (a toxin from sea anemone <em>Stichodactyla helianthus</em>)</td>
<td>IC$_{50}$ = $\sim$10 pM (Kalman et al., 1998)</td>
<td>ShK binds to a shallow vestibule at the outer “mouth” of the Kv1.3 channel pore. The key residues in ShK are Lys22 and Tyr23, with Lys22 occluding the pore lumen like a cork in a bottle (Lanigan et al., 2002)</td>
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<tr>
<td>(I) ShK-186</td>
<td>N/A</td>
<td>IC$_{50}$ = 69 pM (Tarcha et al., 2012)</td>
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<tr>
<td>(I) ShK-192</td>
<td>N/A</td>
<td>IC$_{50}$ = 140 pM (Chi et al., 2012)</td>
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</table>

ShK-186 has 100-fold selectivity for Kv1.3 over Kv1.1, but its N-terminal phosphotyrosine can be rapidly dephosphorylated in serum (Tarcha et al., 2012).

ShK-192 contains a non-hydrolyzable phosphotyrosine surrogate, a methionine isostere, and a C-terminal amide. It keeps the selectivity of ShK-186 with significantly improved *in vivo* stability, but its *in vivo* effectiveness was moderately reduced.
(I) UK-78,282  

IC$_{50}$ = 200 nM (Hanson et al., 1999; Nguyen et al., 2012)  

UK-78,282 is a use-dependent blocker for $K_v1.3$ with preferential binding to the C-type inactivated state of the channel. However, it has similar potency on the $K_v1.4$ channel (Hanson et al., 1999).

<table>
<thead>
<tr>
<th>$K_v2.1$</th>
<th>GxTx</th>
<th>IC$_{50}$ = 1-2 nM (Herrington et al., 2006)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) GxTx</td>
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</table>
(Guangxitoxin 1E, a toxin from Chinese fawn tarantula)  
(Hilobrachys guangxiensis)  
(Lee et al., 2010) | GxTx inhibits $K_v2.1$ in a voltage-dependent manner via binding with conserved helix-run-helix motif in each voltage-sensing domain (VSD) independently. It inhibits the channel function by decreasing the activation rate ($\alpha$) and increasing the deactivation rate ($\beta$) of the VSD, without modifying the rates of the final pore opening transition (Gupta et al., 2015; Milescu et al., 2016). |
<table>
<thead>
<tr>
<th>Channel</th>
<th>Inhibitor</th>
<th>IC₅₀/IC₅₀ (μM)</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>KV3</td>
<td>BDS-I</td>
<td>IC₅₀ = 47 nM</td>
<td>BDS inactivates KV3 channels by binding to the S3b-S4 paddle motif in the voltage-sensing domain, slowing the activation and inactivation kinetics and shifting the activation V₁/₂ to more positive voltages (Yeung et al., 2005).</td>
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<tr>
<td>KV7</td>
<td>XE991</td>
<td>IC₅₀ = 0.98 μM</td>
<td>The mechanism of action of XE991 is unknown, but can increase the striatal pacemaker frequency and dopamine synthesis. XE991 augments hippocampal ACh release and is a cognitive enhancer following oral administration in vivo (Zaczek et al., 1998).</td>
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<td></td>
<td>ML252</td>
<td>IC₅₀ = 69 nM</td>
<td>ML252 is brain penetrant and has &gt;40-fold selectivity for KV7.2 over KV7.1 channels (IC₅₀ = 2.9 μM for</td>
</tr>
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</table>

KV3 (I) Blood depressing substance (BDS-I and BDS-II, toxins from the sea anemone, *Anemonia sulcate*) (Driscoll et al., 1989) IC₅₀ = 47 nM (BDS-I) (Diochot et al., 1998) BDS inactivates KV3 channels by binding to the S3b-S4 paddle motif in the voltage-sensing domain, slowing the activation and inactivation kinetics and shifting the activation V₁/₂ to more positive voltages (Yeung et al., 2005).

KV7 (I) XE991 (10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone) IC₅₀ = 0.98 μM for KV7.2+7.3/M-currents (Wang et al., 1998) The mechanism of action of XE991 is unknown, but can increase the striatal pacemaker frequency and dopamine synthesis. XE991 augments hippocampal ACh release and is a cognitive enhancer following oral administration in vivo (Zaczek et al., 1998).

KV7 (I) ML252 ((S)-2-phenyl-N-(2-(pyrrolidin-1-yl)phenyl)butanamide) IC₅₀ = 69 nM for KV7.2 ML252 is brain penetrant and has >40-fold selectivity for KV7.2 over KV7.1 channels (IC₅₀ = 2.9 μM for
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<th>mide)</th>
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<th>Kᵥ7.1) (Cheung et al., 2012).</th>
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<tr>
<td>Kir3</td>
<td>(GIRK)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 0.7 nM for GIRK1/4, IC&lt;sub&gt;50&lt;/sub&gt; = 24 nM for GIRK1/2 (Machida et al., 2011)</td>
<td>NTC801 significantly decreases atrial fibrillation inducibility with a prolonged atrial effective refractory period that was frequency-independent, but the mechanism of action is unknown (Machida et al., 2011).</td>
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<tr>
<td>NTC801 (aralkylamino)-2,2-dimethyl-3,4-dihydro-2H-benzopyran-3-ol</td>
<td>N/A</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; = 160 nM (GIRK1/2), 887 nM (GIRK2/3), 914 nM (GIRK1/3) (Kaufmann et al., 2013; Wen et al., 2013)</td>
<td>ML297 is brain penetrant and prevents PTZ-induced epileptic seizures in mice (Kaufmann et al., 2013).</td>
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<tr>
<td>(A) ML297 (VU0456810)</td>
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<tr>
<td>(I) Tertiapin-Q (a peptide from the venom of the European honeybee, <em>Apis mellifera</em>) (Xu and Nelson, 1993)</td>
<td>IC₅₀ = 22 nM (GIRK1/2); 8 nM (GIRK1/4) (Patel et al., 2020)</td>
<td>The K21 of Tertiapin-Q inhibits Kir3.2 mainly by occupying the outermost K⁺ binding site of the selectivity filter (Patel et al., 2020).</td>
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<tr>
<td>Kir6.X (K&lt;sub&gt;ATP&lt;/sub&gt;) (A) Cromakalim</td>
<td>EC₅₀ = 30 µM (Kir6.2/SUR 2A) (Inagaki et al., 1996)</td>
<td>Cromakalim binds to the TMS17 of SUR2 subunit (D'Hahan et al., 1999; Moreau et al., 2000).</td>
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<tr>
<td>(A) Diazoxide (7-chloro-3-methyl-4H-1,2,4-benzothiadiazine 1,1-dioxide)</td>
<td>EC₅₀ = 77.4 µM (Lebrun et al., 2000)</td>
<td>Diazoxide targets the nucleotide-binding domain-2 in SUR1/2 to stabilize the channel in a desensitized state to enhance activation (Matsuoka et al., 2000).</td>
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<tr>
<td>(I) Glibenclamide</td>
<td>IC₅₀ = 24.5 - 37.9 µM (Tominaga et al., 1995)</td>
<td>Glibenclamide specifically inhibits SUR1 subunit containing K&lt;sub&gt;ATP&lt;/sub&gt; channels (Hambrock et al., 2002; Zhou et al., 2009).</td>
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<tr>
<td>Substance</td>
<td>EC$_{50}$ Value</td>
<td>Description</td>
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<tr>
<td>Pinacidil</td>
<td>13 µM (SUR2B)</td>
<td>Activates SUR2A-containing K$_{ATP}$ channels in a nucleotide-independent manner. The K707 in the nucleotide-binding domain in SUR2A is critical for Pinacidil activity (Gribble et al., 2000).</td>
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<tr>
<td>Apamin</td>
<td>704 pM (SK1); 27 pM (SK2); 4 nM (SK3)</td>
<td>Potently and selectively inhibits SK channels by utilizing histidine residues in the channel outer pore (Lamy et al., 2010). Apamin blocks after-hyperpolarization in vitro and is brain penetrant and convulsive in vivo (Stocker et al., 2004).</td>
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<tr>
<td>Zonisamide</td>
<td>16.4 µM</td>
<td>Activates BK channels primarily by decreasing mean closed time and causing a left shift in the activation curve (Huang et al., 2007).</td>
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</tbody>
</table>
Figure 2

Voltage-gated $K^+$ ($K_V$) channels

Inwardly Rectifying $K^+$ ($Kir$) Channels

$Ca^{2+}$-activated $K^+$ channel ($K_{Ca}$)

Two-pore-domain $K^+$ channel ($K_{2P}$)
Figure 3

- **Kv3**: High-frequency Discharge in GP
- **Kv4**: Plasma Membrane Expression Increase
- **Globus Pallidus**: Reduce Pacemaker Frequency
- **Substantia Nigra**: α-Synuclein Accumulation
- **Gut-Brain Axis**: Decrease Gastrointestinal Motility
- **Calcium Overload**: Microglia Activation
- **Hyperpolarization**: Dopaminergic Neuron
- **Kv1** and **Kv2**: Cluster
- **Kv7**: M-Current Generation

**Frontal Cortex**

This image illustrates the impact of Kv3 and Kv4 on various brain regions and functions.
Figure 4

GABA
Adenosine
Dopamine
Serotonin,
Somatostatin
Galanin
......

Hyperpolarization

Postsynaptic inhibition → Hippocampal excitability and plasticity

GABA<sub>2</sub>R induced GIRK activation

A<sub>1</sub>-Adenosine R-GIRK coupling → Glutamate release inhibition

Galanin R1 induced GIRK activation → Excitatory-inhibitory equilibrium in CA1 neurons

Ca<sup>2+</sup> oscillation and reduced activity in GnRH neurons

Reduced Ca<sup>2+</sup> influx in the pain processing pathways

Somatostatin activates GIRK via SS<sub>1</sub>R4

Dopamine D<sub>2</sub>R induced GIRK activation → Drug and alcohol sensitivity and addiction

GABA<sub>B</sub> Receptor
M2-Muscarinic Receptor
A<sub>1</sub>-Adenosine Receptor
α2-Adrenergic Receptor
Dopamine D<sub>2</sub> Receptor
5-HT<sub>1A</sub> serotonin Receptor
Somatostatin Receptor
Galanin Receptor
......
Figure 5

Neurodegeneration triggered by microglia-mediated neuroinflammation

Parkinsonism Symptoms
Figure 6

Dopaminergic Neurons

Inhibit Dopamine Release

Hyperpolarization

K<sub>ATP</sub> Channel

ADP

ATP

Cytoplasm

Outer Membrane

Intermembrane Space

Inner Membrane

Mitochondrial Matrix

Reverse Contraction

Imbalanced K<sup>+</sup> Flux

Contraction
Figure 7

SK/IK Channel

Calmodulin

$\text{Ca}^{2+}$ $\text{Ca}^{2+}$

 BK Channel

RCK1 RCK2

ROS

$\text{Ca}^{2+}$ $\text{Ca}^{2+}$

SK Channel $\text{PM}$

Membrane hyperpolarization

IK Channel

Ca$^{2+}$ influx

Medium afterhyperpolarization

Restore mito membrane potential;↓ Mito diffusion;↓ Mito Ca$^{2+}$ overload & ROS

Ca$^{2+}$ signaling; Microglial activation; Microglial migration......