·Minireview·

Modulation of the activity of dopaminergic neurons by SK channels: a potential target for the treatment of Parkinson's disease?

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Abstract: SK channels are small conductance calcium-activated potassium channels that are widely expressed in different neurons with distinct subtypes. They play an important role in modulating synaptic plasticity, dopaminergic neurotransmission, and learning and memory. The present review was mainly focused on the recent findings on the contradictory roles of SK channels in modulating dopaminergic neurons in substantia nigra and in the pathogenesis of Parkinson's disease (PD). Besides, whether modulation of SK channels could be a potential target for PD treatment was also discussed.

Keywords: small-conductance calcium-activated potassium channel; Parkinson's disease; afterhyperpolarization; dopaminergic neuron

1   Introduction

Small conductance calcium-activated potassium channels (SK channels) play a fundamental role in all the excitable cells and act as key regulators of neuronal excitability. The SK channel family consists of 4 members: SK1, SK2, SK3 and SK4, which can be further divided into 2 subtypes: SK1-3 and SK4. The SK1-3 members have been cloned from mammalian systems. They are encoded by *KCNN1*, *KCNN2* and *KCNN3*, respectively, and are expressed differentially in excitable tissues of brain and in peripheral tissues[1]. Differently, the transcript of SK4 channel is only found in non-excitable tissues, such as placenta and lung[2].

Parkinson's disease (PD) is a progressive neurodegenerative disorder caused by loss of dopaminergic neurons in the substantia nigra. Several hypotheses have been proposed concerning the pathogenesis of PD, including mitochondrial dysfunction, oxidative stress, inflammatory reactions, and apoptosis of dopaminergic neurons and other related cells in the basal ganglia. Moreover, recent studies on the K^+ channel gene expression in the basal ganglia show that the dysfunctions of various K^+ channels, such as Kv, K_{ATP}, Kir2 and SK, may contribute to the pathogenesis of PD[3,4]. Among these K^+ channels, SK channel blockade increases dopaminergic transmission and is involved tightly in the dopaminergic system disorders[5]. The present review mainly focuses on the structures and functions of SK channels, and their roles in modulating dopaminergic neurotransmission. In addition, the novel and crucial roles of SK channels in the pathogenesis of PD and whether modulation of SK channels could provide a new therapeutic target in PD treatment are

also discussed.

2   Structure and function of SK channels

2.1 Structure of SK channels As voltage-independent po-
tassium channels, the potassium-selective SK channels are
activated by the increase in the intracellular level of calcium
following one or several action potentials in neurons. The
SK channels are symmetric tetramers formed by 4 identical
subunits. Each subunit has 6 transmembrane regions. The
N- and C-termini reside within the cell (Fig.1)[6,7]. The essen-
tial feature of SK channels is the gating of them by intercellu-
lar calcium. However, SK channels are not directly activated
by calcium ions. Maylie J and colleagues have found that
there is no obvious calcium-binding domain in SK channels.
Actually, the calcium gating is conferred by calmodulin, which
is constitutively bound to the C-terminus of the channel and
serves as a Ca^{2+} sensor in SK channels[8]. Upon binding of
calcium to calmodulin, SK channels are activated and the

membrane is hyperpolarized, causing the reduction of cell
excitability within hundreds of milliseconds. This phenom-
enon is called afterhyperpolarization (AHP), and the media-
tion of medium/slow AHP is considered as a major function
of SK channels in neurons in the central nervous system
(CNS)[2,9].

Studies using *in situ* hybridization and immunohis-
tochemistry methods have shown that in rat brain, subtypes
of SK channels are widely expressed in the CNS[5,10]. SK1 and
SK2 channels are expressed at their highest levels in the
cortex and the hippocampus, while SK3 channel is expressed
at the highest level in subcortical areas, such as substantia
nigra, dorsal raphe and locus coeruleus. SK3 channel is also
found to be highly expressed in dopaminergic neurons in
substantia nigra[5,9,10]. A similar pattern of SK channel distri-
bution has also been described in human brain[11].

2.2 Function of SK channels As one of the 3 types (fast,
medium and slow) of AHP, medium AHP (m-AHP) can also
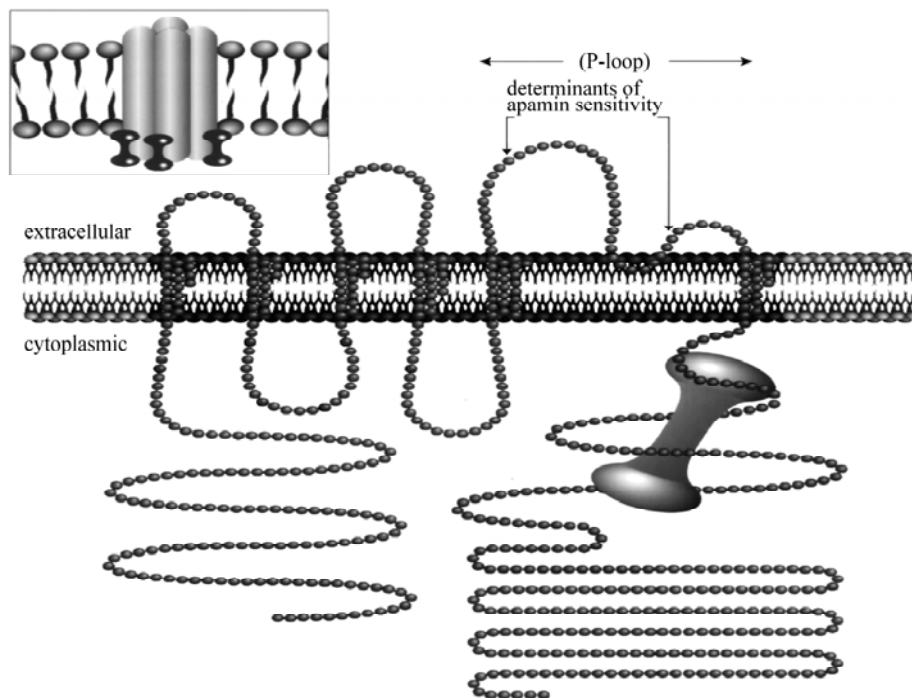
**Fig. 1 Structure of SK subunits (adopted from Bond CT *et al.*, 1999)[7]. The main panel shows the putative subunit topology of a single subunit of SK2,
with 6 transmembrane domains. The N- and C-termini reside within the cell. Calmodulin is constitutively bound to the proximal portion of the
intracellular C-terminus, and within this domain, undergoes calcium-dependent interactions with the α-subunit. The pore region (P-loop) contains
two amino acids that locate on each side of the deep pore, and can be blocked by apamin. The top left insert shows a possible model of SK channels,
the symmetrical tetramers with 4 α-subunits, each binding to the calmodulin.**

be activated rapidly and lasts 200–400 ms in midbrain dopaminergic neurons, hippocampal pyramidal cells and locus coeruleus neurons, mainly mediated by SK channels[2,9,12]. SK channels play a functional role in controlling the firing rate (the number of action potentials generated per unit of time) and the firing pattern (the way of action potentials distributed over time) by producing m-AHP, thus being critical for understanding the functions of many transmitter systems. For instance, midbrain dopaminergic neurons release much more dopamine when they fire in bursts than at a same mean firing rate[9, 13]. SK channel blockers will increase the firing rate and cell sensitivity to excitatory inputs, whereas SK channel openers will produce the opposite effects[9,14]. Apamin, an alkaline 18-amino acid peptide from the venom of the honey bee, *Apis mellifera*, has been reported to be a selective blocker of SK channels and can bind to the residues both inside and outside of the pore region of the SK channels[15,16]. Apamin can alter the duration and frequency of membrane potential oscillations and induce bursting activity *in vitro*, which is qualitatively similar to the spontaneous activity observed *in vivo*[17]. In the contrast, 1-ethyl-2-benzimidazolinone (EBIO) can enhance the activation of SK channels and shift the apparent calcium sensitivity of SK channels. Thus, EBIO application increases both medium and slow AHP, and strongly reduces electrical activity[18].

Similar with other cation channels that contain consensus phosphorylation sites for protein kinases, mutagenesis and mass spectrometry studies have identified 3 protein kinase A (PKA) phosphorylation sites within the distal C-terminus of the SK2 cytoplasmic residues: Ser568, Ser569 and Ser570, which are responsible for the trafficking effect of PKA activation on SK2 surface expression[19]. In addition, there has been evidence that modulation of SK2 surface expression plays an important role in regulating NMDA receptor-mediated synaptic potentials and Ca^{2+} influx, and is tightly associated with cellular plasticity, learning and memory[20,21].

3   Roles of SK channels in PD

3.1 Roles of SK channels in dopaminergic neurons As mentioned above, SK3 channels mediate m-AHP and are involved in generating and modulating the cellular firing pattern.

In midbrain dopaminergic neurons, they are expressed at high levels. Midbrain dopaminergic neurons mainly exhibit 2 firing patterns *in vivo*: single spike firing and burst firing, latter of which codes for reward-related events. However, these neurons usually exhibit a pacemaker-like, very regular, single-spike firing pattern *in vitro*, associated with loss of synaptic afferents[2,22]. SK channels are activated selectively via T-type calcium channels in dopaminergic neurons, and both SK and T-type channels are essential for the stability of spontaneous pacemaker activity[23,24]. Furthermore, Ji H and co-workers have found that pharmacological modulation of the apparent Ca^{2+} affinity of SK channels by different modulators can alter the excitability of substantia nigra dopaminergic neurons. As a positive modulator of SK channels, 6,7-dichloro-1H-indole-2,3-dione 3-oxime (NS309) can inhibit spontaneous firing, enhance m-AHP in substantia nigra dopaminergic neurons, and decrease neuronal excitability. The negative SK channel modulator R-N-(benzimidazol-2-y1)-1, 2, 3, 4-tetrohydro-1-naphtylamine (NS8593) exhibits strong Ca^{2+}-dependence, and potently shifts the SK channel Ca^{2+} response curve, albeit in the opposite (rightward) direction. NS8593 application results in elimination of m-AHP in dopaminergic neurons, which is associated with the disruption in pacemaker firing and the emergence of bursting activity driven by a plateau oscillation in membrane potential[25]. Meanwhile, Foehring RC and collegues have revealed that changes in intracellular calcium ion concentration play a very important role in several events, including AHPs and subthreshold oscillations underlying autonomous firing in dopamine cells. Continuous Ca^{2+} load due to Ca^{2+}-dependent rhythmicity has been proposed to be responsible for the death of dopamine cells in PD and during normal aging. A low endogenous calcium buffering capacity may make dopamine cells vulnerable to Ca^{2+}-dependent pathology. Therefore, SK channels, associated with calcium ions, play an important role in the excitability of neuronal circuitry in substantia nigra[26].

Previous studies have identified that SK3 protein is expressed differentially in dopaminergic neurons in substantia nigra and ventral tegmental area (VTA)[7]. Compared with those in the VTA, dopaminergic neurons in the substantia nigra exhibit a significantly higher SK3 protein level and larger

calcium-sensitive IAHPs. Results of electrophysiological recordings with cell filling and immunohistochemistry demonstrate that SK3 expression is related to the degree of pacemaker precision, while SK channels in VTA dopaminergic neurons are not involved in the control of pacemaker frequency or precision. These findings show that the differential expression of SK3 channels is critical for neuronal activity control in dopaminergic neurons[27].

3.2 Contradictory roles of SK channels in the pathogenesis of PD Current pharmacologic treatment of PD involves not only neuroprotection, but also restoration, such as providing new neurons or stimulating the growth and the function of remaining cells. Interestingly, SK channels exert contradictory effects in the regulation of dopamine release and in the alteration of dopamine phenotype[28]. Firstly, SK modulation results in alterations of dopamine release[29]. Blockade of SK channels *in vivo* induces a shift of substantia nigra cells from tonic to burst firing, which dramatically increases the amount of dopamine release from synaptic terminals of substantia nigra neurons. Therefore, increasing or prolonging dopamine re-uptake into cytosol through the dopamine transporter (DAT) can induce an increase in cytosolic dopamine level or its synthesis. Initially, this increase in dopamine level will compensate the loss of tyrosine hydroxylase-positive (TH+) nigral neurons. However, when dopamine level is excessively increased, the opposite effects will occur, since dopamine has an unstable catechol ring that will spontaneously oxidize to produce H_2O_2, superoxide and dopamine-o-quinone, when exposed to molecular oxygen. Dopamine can also be deaminated by monoamine oxidase (MAO) to yield dihydroxy-phenyl acetic acid (DOPAC) and H_2O_2. H_2O_2 then converts to hydroxyl radicals in the presence of iron. Besides, dopamine can cause damage in mitochondria[30,31]. Therefore, when dopamine is free in the cytosol and accumulates to a higher level than usual, cell damage will be induced[32]. Secondly, SK modulation is involved in the alterations of dopamine phenotype of neurons[28,33]. Following loss of TH+ substantia nigra compacta (SNc) neurons in a rat model of 6-hydroxy-DA (6-OHDA)-induced hemi-parkinsonism, the number of TH+ neurons partially recovers, which has been reported to occur via cell phenotype "shift", from TH- to TH+.

Infusion of one SK agonist directly into substantia nigra in normal mice for 2 weeks increases the number of TH+ neurons whereas infusion of one SK antagonist decreases its number. Thus, the number of TH+ neurons can be bidirectionally altered by SK channel modulation. The effect of SK channels on TH expression may be partially attributed to neuroprotection against increased dopamine toxicity that would lead to cell damage. However, the relationship between SK and TH still needs further investigation.

Recent studies propose that the functional state of SK channels plays a role in determining TH expression and dopamine synthesis in substantia nigra neurons. In the short-term, an increase in TH expression can meet the normal demand, while in the long-term, a decrease in TH expression can protect against the neurotoxicity of cytosolic dopamine at a high level. Therefore, in the long-term, attenuating SK channel function would lead to a decrease in dopamine synthesis, while enhancing SK channel function would lead to an increase in dopamine synthesis. Thus, increasing the function of SK channels may increase or maintain dopamine synthesis in the substantia nigra neurons in PD patients, which can help relieve long-term motor symptoms. Meanwhile, since blockade of SK3 channels would lead to burst firing and consequently an increase in dopamine release from dopaminergic neurons, it is thought that inhibition of SK3 channels can either slow down the degeneration of dopaminergic neurons in the early phase of PD by increasing excitability and activity of dopaminergic neurons, or alleviate PD symptoms by enhancing dopamine release from the residual dopaminergic neurons. It is proposed that SK could play different roles according to the duration (short-term or long-term) and environment condition (physiological or pathophysiological) (Fig. 2). However, further studies are needed to clarify the contradictory roles of SK channels.

4   Conclusion

The predominant roles of SK channels in neurons include regulating dendritic excitability, excitatory synaptic transmission, synaptic plasticity, and learning and memory. Accumulating evidence has indicated that SK channels, especially the SK3 channels, play an important role in dopam-
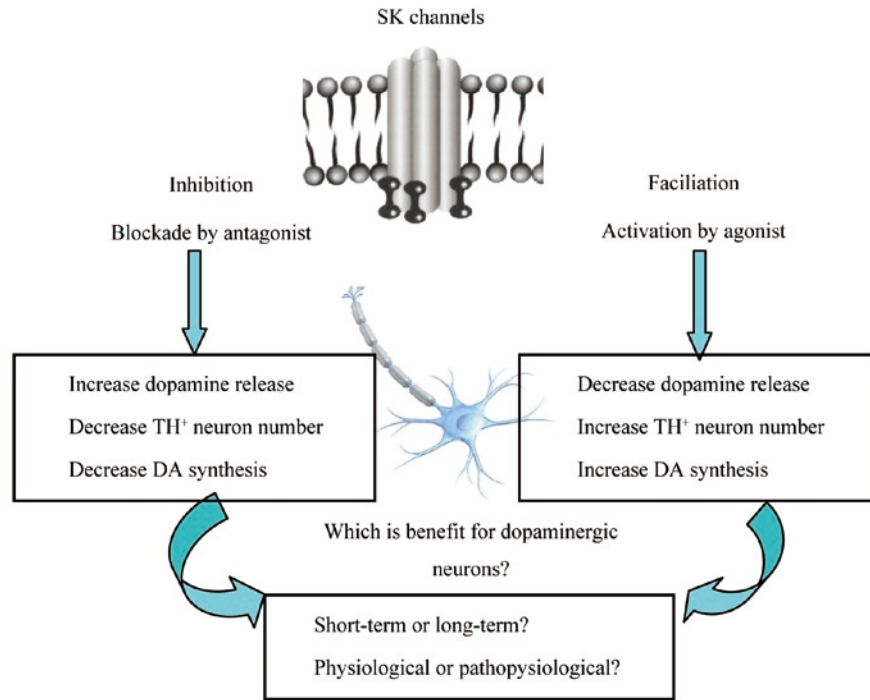
Fig. 2 Contradictory roles of SK channels in the pathogenesis of PD.

inergic neurons in the substantia nigra. On one hand, blockade of SK3 channels can result in burst firing in dopaminergic neurons and promote the release of dopamine, which gives a hint that application of SK3 channel blockers in PD patients may alleviate some symptoms. On the other hand, although the classical excitotoxic hypothesis suggests that increases in neuronal activity and dopamine level will accelerate neurodegeneration, studies reveal that this may be not true for all the neurodegenerative diseases. Besides, enhancing the function of SK channels may increase or maintain dopamine synthesis in the substantia nigra neurons in PD patients, which should be helpful to alleviate the long-term motor symptoms of PD. Thus, concerning the 2 reversed viewpoints, further studies are needed to reveal whether enhancement or inhibition of SK channel function is more important and more beneficial to the improvement of PD symptoms. However, it is at least clear that SK channel function is involved in the pathogenesis of PD, and SK channels may be a new therapeutic target for PD treatment.

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小电导钙激活钾通道对多巴胺能神经元活性的调节：可能成为一种治疗帕金森病的新策略

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摘要： 小电导钙激活钾通道也即 SK 通道，其广泛表达于不同亚型的神经元中，在调节突触可塑性、学习与记忆和多巴胺能神经传递过程中发挥重要作用。本文重点讨论了 SK 通道调节黑质多巴胺能神经元的作用及其在帕金森病发病机制中的争议性角色，并对调控 SK 通道是否可成为一种新的治疗帕金森病的方法进行了探讨。
关键词： 小电导钙激活钾通道；帕金森病；后超级化；多巴胺能神经元