

**COMMENTARY**

# The action of a BK channel opener

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Agonists and antagonists of an ion channel are important tools to probe the functional role of the channel in specific cells and tissues. The effects of the agonist and antagonist on the cellular and tissue physiology and pathophysiology can be indications for the ion channel as a drug target for certain diseases. Agonists and antagonists can also be effective tools for understanding molecular mechanisms for function of the channel. Obviously, to know what a tool does will help a better use of the tool. NS11021 is a BK channel opener (Bentzen et al., 2007) that has been used as a tool to probe the functional role of BK channels in various tissues and the molecular mechanism of BK channel gating. In this issue of the *Journal of General Physiology*, Rockman et al. present a comprehensive study of NS11021 modulation of BK channels.

BK channels are K<sup>+</sup> channels activated by voltage and intracellular Ca<sup>2+</sup>, which are distributed in neurons and smooth muscle cells, as well as other cell types. During an action potential, BK channels are activated by depolarization and Ca<sup>2+</sup> entry via voltage-gated Ca<sup>2+</sup> channels, and the K<sup>+</sup> currents carried by BK channels shorten action potential duration and result in the fast after hyperpolarization (Lancaster and Nicoll, 1987; Shao et al., 1999; Petkov, 2014). BK channels activated by local Ca<sup>2+</sup> release from intracellular Ca<sup>2+</sup> stores also generate transient outward currents (Brown et al., 1983; Nelson et al., 1995; ZhuGe et al., 1998). Thus, in the cells containing BK channels in their plasma membrane, BK channel activation opposes membrane depolarization, reduces membrane excitability, and provides a negative feedback mechanism to regulate the intracellular Ca<sup>2+</sup> concentration. BK channels have also been found to regulate membrane potential and Ca<sup>2+</sup> store in intracellular organelles, including mitochondria (Siemen et al., 1999), nucleus (Mazzanti et al., 1990; Li et al., 2014), and lysosome (Cao et al., 2015; Wang et al., 2017) by sensing voltage, Ca<sup>2+</sup>, and various signaling molecules (Toro et al., 2014).

How do these cellular functions of BK channels affect the physiology and pathophysiology in specific tissues? NS11021 has been used in some of the studies that answer this question. In

studies of ischemia and reperfusion in isolated, perfused rat hearts, Bentzen et al. found that mitochondria BK channel activation by NS11021 perfusion reduced tissue damage, decreasing infarct size by more than 70% (Bentzen et al., 2009; 2010). A similar result was observed when studies were performed in mouse hearts (Soltysinska et al., 2014; Frankenreiter et al., 2018), and NS11021 reduced infarction area in comparison with the BK knockout (Slo1<sup>-/-</sup>) hearts. NS11021 also increased survival of isolated ventricular myocytes subjected to metabolic inhibition followed by reenergization (Borchert et al., 2013). The cardiac protection effects of NS11021 were suggested to derive from a beneficial effect on energetics due to enhanced K<sup>+</sup> uptake via BK channels that resulted in mitochondrial swelling with little change in mitochondrial membrane potential (Aon et al., 2010). In a study of erectile dysfunction, Kun et al. (2009) found that NS11021 activation of BK channels relaxed erectile tissue in vitro and improved erectile responses in rats. This study suggests that BK channel openers may be an alternative in the treatment of erectile dysfunction, which may be a symptom of cardiovascular diseases (Jackson et al., 2006), to phosphodiesterase 5 inhibitors such as sildenafil, which are contraindicated in men concurrently taking organic nitrates and may cause severe side effects in patients with other symptoms of cardiovascular diseases (Cheitlin et al., 1999). NS11021 has also been used to study the physiological and pathophysiological roles of BK channels in urinary bladder smooth muscle (Layne et al., 2010; Fernandes et al., 2015), myometrial smooth muscle (Wakle-Prabakaran et al., 2016), trigeminal ganglion neurons (Wulf-Johansson et al., 2010; Liu et al., 2014), and pancreatic duct epithelium (Venglovecz et al., 2011).

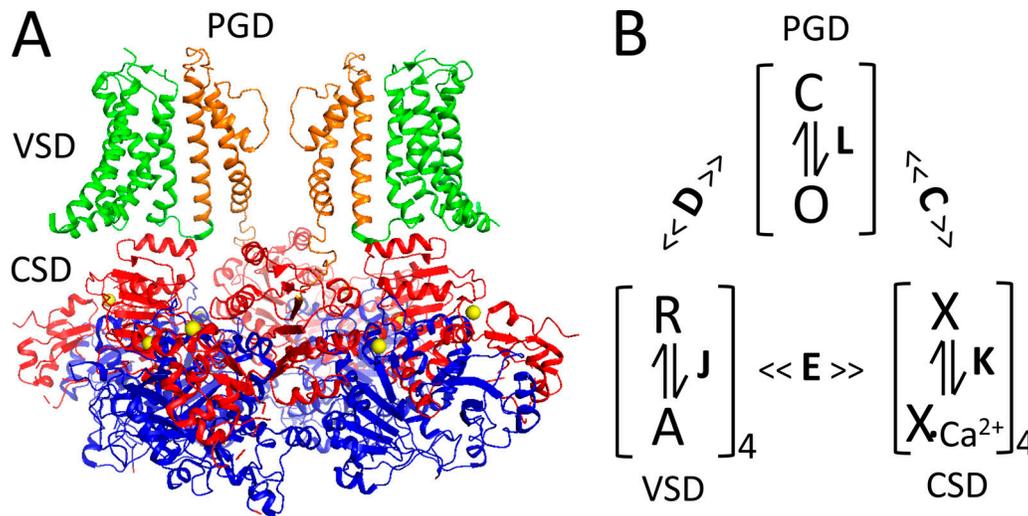
BK channels are formed by four Slo1 subunits (Fig. 1 A). Each Slo1 subunit contains seven transmembrane segments (S0–S6), in which S0 to S4 form the voltage sensor domain (VSD) and S5–S6 form the pore-gate domain (PGD). The membrane spanning domain of the channel is thus comprised of four VSDs surrounding a central pore formed by PGDs from all four subunits. The cytosolic domain of the four subunits form a ring-like

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This work is part of the special collection entitled "Electrical Signaling in the Heart and Nervous System: A Joint Meeting of the Society of General Physiologists and Latin American Society of Biophysicists."

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**Figure 1. BK channel structure and gating mechanisms. (A)** Structure of the human BK channel (Protein Database accession no. 6V38; [Tao and MacKinnon, 2019](#)). The VSD (green) and PGD (orange) of only two subunits are shown for clarity.  $\text{Ca}^{2+}$  ions bound to the CSD (red and blue) are shown as yellow dots. **(B)** An allosteric gating mechanism ([Horrigan and Aldrich, 2002](#)). L, J, and K are equilibrium constants, which are influenced by conformational changes in the PGD, VSD, and CSD domains and interactions among the three domains. D, C, and E are allosteric constants for these interactions.

structure (gating ring) and each Slo1 subunit harbors two  $\text{Ca}^{2+}$  binding sites so that a BK channel contains a total of eight  $\text{Ca}^{2+}$  binding sites, and the cytosolic domain is thus called the  $\text{Ca}^{2+}$ -sensing domain (CSD; [Fig. 1 A](#)). BK channels have a selectivity filter in the pore that is highly conserved among  $\text{K}^+$  channels, but unlike most other  $\text{K}^+$  channels, the pore of BK channels can be accessed by bulky quaternary ammonium blockers, which bind to the site just beneath the selectivity filter, when the channel is either open or closed to  $\text{K}^+$  flux ([Li and Aldrich, 2004](#); [Wilkins and Aldrich, 2006](#); [Tang et al., 2009](#)). Methanethiosulfonate reagents can also access and modify cysteines in the pore when BK channels are closed to  $\text{K}^+$  flux ([Zhou et al., 2011](#)). These results lead to the hypothesis that the activation gate that restricts  $\text{K}^+$  ion flux is not located at the cytosolic side but resides in the selectivity filter. Consistent with this hypothesis, when the cryo-EM structures of BK channels were solved with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  bound (presumably at the open state) and metal free (at the closed state), the pore below the selectivity filter does not show any physical barrier for  $\text{K}^+$  ions or QA blockers ([Hite et al., 2017](#); [Tao et al., 2017](#); [Tao and MacKinnon, 2019](#)).

By studying NS11021 activation of BK channels [Schewe et al. \(2019\)](#) proposed a mechanism for how the selectivity filter gates BK channels. NS11021 was found to belong to a class of negatively charged activators (NCAs) that open  $\text{K}^+$  channels with a selectivity filter (SF) gate with equal efficiency. These  $\text{K}^+$  channels include the mechanosensitive two pore domain ( $\text{K}_{2\text{P}}$ ) channels TREK-1 and TREK-2, the voltage gated hERG, and BK channels. NS11021 was suggested to bind to BK channels in the pore below the SF based on the comparison to the structural and functional interactions of another NCA, BL-1249, with  $\text{K}_{2\text{P}}$  channels, molecular dynamic simulations, and the experimental result that QA blocking of BK channels was antagonized by NS11021. The similar actions of NS11021 on BK channels in comparing to that of NCAs on  $\text{K}_{2\text{P}}$  channels led the authors to propose that, as in  $\text{K}_{2\text{P}}$  channels, the negative charge of NS11021 increases  $\text{K}^+$  occupancy in the pore and SF of BK channels; thus,

BK channels adopt a low-activity state (closed) of their SF at rest, and voltage and  $\text{Ca}^{2+}$  induce structural changes that drive the SF into an active (open) state. Here, it is worth mentioning a molecular dynamic simulations study of BK channels by [Jia et al. \(2018\)](#). This study suggested that in the metal-free structure (closed) of BK channels the pore shape and surface hydrophobicity allow the channel to undergo transitions of hydrophobic dewetting, resulting in a large free energy barrier for  $\text{K}^+$  to enter the pore, thereby forming a hydrophobic gate to stop  $\text{K}^+$  flux. The dry pore remains physically open and QA blockers can readily access to the binding site inside the pore. This hydrophobic gate is an alternative hypothesis to that of the SF gate. In the dry pore, a negative charge from NS11021 may attract  $\text{K}^+$  and water molecules to enhance BK channel activity.

While NS11021 has been used in the above-mentioned studies, what NS11021 does to alter BK channel function is still not clear, although the original report of NS11021 characterized the enhancement of BK channel activation by the compound ([Bentzen et al., 2007](#)). The mechanism of drug action is a complex problem due to the properties of BK channel gating. The three structural domains, PGD, VSD, and CSD ([Fig. 1 A](#)), all undergo conformational changes in BK channel gating, with the PGD going through the transitions between the closed and open state (C–O), the VSD between the resting and activated states (R–A), and the CSD between the  $\text{Ca}^{2+}$ -unbound and  $\text{Ca}^{2+}$ -bound state (X –  $\text{X}_{\text{Ca}^{2+}}$ ). These domains interact one another to control channel opening, with the interactions dependent on the conformation of each domain ([Fig. 1 B](#)). These conformational changes in the PGD, VSD, and CSD domains and the interactions among them were characterized ([Cui et al., 2009](#)) and quantitatively described by a gating scheme ([Horrigan and Aldrich, 2002](#)). To understand what NS11021 does to the BK channel, we need to know if the compound affects the conformational change in any of the domains or any of the interactions among the domains.

[Rockman et al. \(2020\)](#) investigated the mechanism of NS11021 modulation of BK channels by first recording BK channel currents

at various voltages (−240–120 mV),  $\text{Ca}^{2+}$  concentrations (0–100  $\mu\text{M}$ , which saturates  $\text{Ca}^{2+}$  binding), and NS11021 concentrations (0–30  $\mu\text{M}$ ). These results confirm that NS11021 activates BK channels by shifting the voltage dependence of channel opening (the conductance–voltage relation,  $G$ – $V$ ) to more negative voltages. Thus, at a given voltage the channel is more likely to open in the presence of NS11021. These results also show that NS11021 slows down the rate of deactivation but has little effect on the rate of activation, and that NS11021 shifts  $G$ – $V$  relations similarly at all  $\text{Ca}^{2+}$  concentrations. The authors then measured the effects of NS11021 on BK channel gating in some extreme experimental conditions, in which the conformational changes of PGD, VSD, and CSD domains and their influence on channel gating can be isolated and examined individually. These experiments reached the following conclusions. First, NS11021 does not affect  $\text{Ca}^{2+}$  binding and does not act primarily through CSD activation. This is first suggested by the results that the compound shifts  $G$ – $V$  relations similarly at all  $\text{Ca}^{2+}$  concentrations and is further demonstrated by the effects of the compound on a truncated BK channel, Slo1c-Kv-MinT. In Slo1c-Kv-MinT the CSD is removed and replaced with a short amino acid sequence, such that the channel can be activated by voltage but no longer by  $\text{Ca}^{2+}$  due to the lack of CSD (Budelli et al., 2013). NS11021 shifted the  $G$ – $V$  of Slo1c-Kv-MinT to negative voltages similarly as for the intact WT BK channels, supporting the conclusion that the CSD is not involved in the action of NS11021. Second, NS11021 activates BK channels independently from conformational changes of the VSD. This is first suggested by the result that the compound decreased deactivation rate of the channel when the voltage was returned to negative voltages (less than −180 mV) where the VSD returned to the resting state. To more directly demonstrate this mechanism, the authors recorded BK currents elicited by these negative voltages, where the VSD stays in the resting state but the GPD spontaneously opens with a small probability ( $P_o \sim 10^{-7}$ – $10^{-6}$ ; Horrigan et al., 1999; Cui and Aldrich, 2000). Although in their patch clamp study the membrane patch contained hundreds of BK channels, the authors could record single channel openings since the  $P_o$  is very low at these voltages and channel opening is a rare event. NS11021 increased BK channel opening by driving the channel to gate from single, brief openings toward bursts of two or more openings with longer lifetime durations. These results led the authors to conclude that NS11021 activation may occur primarily through the PGD.

Rockman et al. (2020) explored the mechanism of NS11021 modulation further. A fit of the relation of BK channel activation versus NS11021 concentrations by the Hill equation produced a Hill coefficient of  $\sim 1$ . This result indicates that either one NS11021 molecule binds to the channel to activate the channel or multiple NS11021 molecules bind but activate the channel independently. Since the four VSDs and CSDs in BK channels affect PGD opening allosterically with a positive cooperativity (Horrigan and Aldrich, 2002), this result is not compatible with NS11021 binding to VSDs or CSDs, but is more consistent with the mechanism that the compound binds to the confluent PGD to modulate channel opening. The authors also fitted the steady-state channel activation by NS11021 at the entire voltage and  $\text{Ca}^{2+}$  concentration ranges with the HA allosteric gating model (Fig. 1 B; Horrigan and Aldrich, 2002). Corresponding to each change of the NS11021

concentration, a change solely in  $L_o$  allowed the model best fit with all experimental data, where  $L_o$  is the equilibrium constant for the C–O transition of the GPD (Fig. 1 B) in the absence of VSD or CSD activations. This result supports the conclusion that NS11021 makes the PGD more likely to open by altering the intrinsic equilibrium between the C–O transition. Furthermore, using a reduced model, in which the CSD effect is removed and the channel is activated by voltage, the authors also fitted the activation and deactivation kinetics of the channel recorded at 0  $\text{Ca}^{2+}$ . An increase of the forward rate (C–O) and decrease of the backward rate (O–C) was observed due to NS11021 stimulation. This independent fitting showed that the NS11021 effect can be reproduced by a similar change in  $L_o$  as in the fittings by the HA model. Taken together, the study suggests that NS11021 activates BK channels by stabilizing the PGD in the open state and destabilizing it in the closed state.

This new understanding of NS11021 modulation of BK channels provides a broader basis for analyzing the results that NS11021 helps to obtain. For instance, this mechanism is consistent with the study of Schewe et al. (2019) that NS11021 may bind to the BK channel in the pore to affect the open probability of PGD regardless the specific mechanisms of voltage and  $\text{Ca}^{2+}$  dependent gating. On the other hand, unlike TREK-2 channels, in which its negatively charged activator (NCA) enhances single channel conductance as an indication of affecting the SF gate, NS11021 does not seem to affect single channel conductance of BK channels (Bentzen et al., 2007; Rockman et al., 2020). In another case, BK channel openers have been suggested for therapy of BK channel associated diseases. However, different BK channel openers act with different mechanisms. For instance, the structurally different BK channel opener Cym04 was proposed to affect the coupling between VSD and PGD (Gessner et al., 2012). Unlike NS11021, which activates BK channels even at extremely negative voltages (Rockman et al., 2020), Cym04 activates BK channels less in negative voltages than in positive voltages (Gessner et al., 2012). Does such a difference in voltage dependence affect the therapeutic outcome or side effects? Such questions can be addressed in reference to the mechanisms of drug action.

## Acknowledgments

Richard W. Aldrich served as guest editor.

This work was supported by National Institutes of Health grant R01 HL126774 (to J. Cui).

J. Cui is a cofounder of a startup company VivoCor LLC, which is targeting  $I_{K_s}$  channels for the treatment of cardiac arrhythmia.

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