Canadian Science Publishing | Canadian Journal of Physiology and Pharmacology

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Journal:	*Canadian Journal of Physiology and Pharmacology*
Manuscript ID	cjpp-2018-0516.R1
Manuscript Type:	Review
Date Submitted by the Author:	n/a
Complete List of Authors:	Almassy, Janos; University of Debrecen, Department of Physiology; Nánási, Péter; University of Debrecen, Department of Physiology
Is the invited manuscript for consideration in a Special Issue:	2018 IACS Cuba
Keyword:	BK channel, MaxiK, Horrigan-Aldrich model, structure, review

Brief structural insight into the allosteric gating mechanism of BK (Slo) channel

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Abstract

The big conductance Ca^{2+} dependent K^+ channel also known as BK, MaxiK, Slo1 or KCa1.1 is a ligand- and voltage gated K^+ channel. Although, structure-function studies of the past decades, involving mutagenesis and electrophysiological measurements revealed fine details of the mechanism of BK channel gating, the exact molecular details remained unknown until the quaternary structure of the protein has been solved at a resolution of 3.5 Å using cryo-electron microscopy. In this short review, we are going to summarize these results and interpret the gating model of the BK channel in the light of the recent structural results.

Keywords: BK channel, MaxiK, Slo1, KCa1.1, structure, Horrigan-Aldrich model, review

Introduction

The big conductance Ca^{2+} dependent K^+ channel (BK channel) has a unitary conductance of ~200 pS. The channel is gated by both voltage and Ca^{2+}, which enables it to functionally couple intracellular Ca^{2+} signaling to the electrical activity of cells, such as neurons, auditory hair cells, smooth muscle cells, skeletal muscle fibers, and adrenal chromaffin cells. In these cells, membrane depolarization results in Ca^{2+} entry through voltage-gated Ca^{2+} channels, which activates cellular functions and BK channels simultaneously. Subsequent K^+ efflux causes membrane hyperpolarization, which terminates further Ca^{2+}-entry and the biological process too. Therefore, BK channels are essential negative feedback regulators of Ca^{2+} mediated physiological processes such as neurotransmitter- and hormone release, neuronal excitability, vascular tone and bronchial diameter. Consequently, pharmacological modulation of BK channel function is considered to be a good therapeutic strategy for instance in epilepsy, hypertension and asthma. However, in order to design better medications, we need to understand the structure-function relationship of the channel. Fortunately, a recent work mainly performed by Mackinnon`s group provided detailed structural insights into BK channel gating. The aim of the current paper is to briefly summarize these new structural results of the pore forming α subunit and correlate them with the gating model created by Horrigan and Aldrich (HA model) earlier (Horrigan and Aldrich, 2002; Horrigan and Aldrich, 1999; Horrigan et al. 1999; Wu et al. 2010; Yuan et al. 2010; Yuan et al. 2011; Tao et al, 2017; Hite et al. 2017).

The Horrigan-Aldrich gating model

Allosteric voltage gating

Similar to other voltage-dependent ion channels, BK channel`s open probability increases with depolarized membrane potentials. The fact that BK current can be elicited using suprathreshold voltage steps even in the virtual absence of Ca^{2+} and that the macroscopic current is preceded by gating current, supports the idea that BK channel contains intrinsic voltage sensors.

The K^+ conductance-voltage relationship follows a Boltzmann (sigmoidal) function, whose midpoint is shifted toward more hyperpolarized voltages by increasing intracellular [Ca^{2+}]. This dual influence of voltage and Ca^{2+} on channel gating was described in details in the past decades, which resulted in the widely accepted Horrigan-Aldrich (HA) model. The model (figure 1.) describes the three events of BK channel gating, including voltage sensor activation, Ca^{2+} binding, channel opening and the interaction between these processes. Horrigan and Aldrich systematically determined the equilibrium constants J, L and K for the transitions between the resting (R) and activated (A) states of the voltage sensor, the open (O) and closed (C) states of the gate and the Ca^{2+}-free (X) and Ca^{2+}-bound (XCa^{2+}) states, respectively, by isolating the subset of transitions using extreme conditions and measuring of ionic and gating currents. The kinetic and steady-state parameters of the gating current provide information about voltage sensor movements, whereas the properties of the ionic current describe the activation gate.

In order to investigate the coupling between voltage sensors and the activation gate (i.e. the mechanism of voltage-dependent gating), the Ca^{2+}- and voltage dependent states should be distinguished. Therefore, the voltage dependent

behavior of the channel was determined in 0 Ca^{2+} to make sure the Ca^{2+}-dependent mechanisms do not influence gating. Compared to other voltage-dependent K^+ channels (Kv), BK channel opening shows weaker voltage dependence. Also, in response to a voltage step, K^+ current develops with an exponential time course after a short voltage dependent delay. Interestingly, at very negative membrane potentials, when the voltage sensors are in resting position, thus the probability of openings triggered by voltage sensor movement is very small, considerably high open probability was observed. It means that BK channels can open without voltage and Ca^{2+}. In addition, the time constant of the current activation was less voltage sensitive at very negative voltages (-360–-40 mV) compared to a higher voltage range (+30–+110 mV). These properties of the current kinetics are inconsistent with the classic, sequential gating model, which is usually used to describe other voltage-dependent (Kv) channel function. In the sequential model, the voltage sensors move independently, but all four sensors must move to active position in order to open the gate. The gate opening is obligatory, after all four voltage sensors are active (obligatory coupling). In contrast, the electrophysiology data of BK channels suggest that the voltage sensors and the gate are coupled through an allosteric mechanism (figure 1.). In the HA-model, the voltage sensors of the four subunits move independently too, but open transition does not require all four (or any) subunits to be activated and also, the voltage sensors can activate whether the channels are closed or open. However, there is an allosteric interaction between the two processes, meaning that the active state of the voltage sensor (J^{0-4}) facilitates channel opening (with an allosteric factor D), but they are not coupled obligatorily. Furthermore, the amplitude of the slow component of the gating current (which

is related to the transmembrane movement of gating charges) correlates with the duration of step depolarization, (i.e. with the number of open channels) suggesting that the allosteric activity is mutual: the voltage sensor activation is easier in the open state. (Horrigan and Aldrich, 1999; Horrigan et al. 1999; Shelley et al, 2010; Zhang et al, 2017).

Ca^{2+} dependent allosteric activation

The main question of Horrigan and Aldrich`s research was whether $Ca^{2+}$ shifts the conductance-voltage curve toward less depolarized voltages by affecting the coupling between the voltage sensors and the channel activation gate (D) or directly increasing the channel gate`s equilibrium constant (L). The question was answered by comparing the kinetic and steady-state characteristics of gating- and ionic currents recorded in 0 or 70 μM Ca^{2+}. In 70 μM Ca^{2+}, both the conductance-voltage and charge-voltage relationship midpoint shifted to more hyperpolarized potentials, but the charge-voltage relationship became steeper, suggesting that Ca^{2+} affects the voltage sensor and the channel gate in different ways. Furthermore, Ca^{2+} had minor effect on the fast component of the ON gating current, indicating that Ca^{2+} has only a small effect on voltage sensor activation when the channels are closed. In contrast, at very negative voltages, when the voltage sensors are forced at resting state, Ca^{2+} increased the open probability significantly (1000 fold). These results suggest that Ca^{2+} increases the equilibrium constant of the closed-open state transition of the gate.

The [Ca^{2+}]-conductance curve follows a Hill function with a coefficient higher than 1, which indicates that the channel contains at least 2 Ca^{2+} binding sites and Ca^{2+} binding is cooperative. Even though the influence of Ca^{2+}on the voltage

sensor is weak, the voltage dependence of K^+ conductance is steeper in high [Ca^{2+}]. This indicates that the positive cooperativity is enhanced by voltage (Horrigan and Aldrich, 2002; Qian et al, 2006, Zhang et al, 2017).

In summary, Ca^{2+} dependent activation of BK channel is an allosteric mechanism. Ca^{2+} primarily enhances the equilibrium constant between the open-closed transition and slightly the allosteric factor E.

Millimolar concentration of Mg^{2+} was also shown to shift the conductance-voltage curve to more hyperpolarized membrane potentials. However, the effect of Mg^{2+} was not different in 0 or 300 μM (saturating) Ca^{2+}, indicating that this binding site is independent from the above mentioned Ca^{2+} binding sites (hence the name Mg^{2+} binding sites). This result is supported by the finding, that mutations significantly reducing Ca^{2+} sensitivity, have only little effect on Mg^{2+} sensitivity. Mg^{2+} affected the OFF-gating charge current (when the channel is open) significantly, but had little effect on the ON gating current (when the channel is closed), which suggests that Mg^{2+} stabilizes the open state of the channel by enhancing the allosteric factor D (rather than the equilibrium constant J) (Shi and Cui, 2001; Zhang et al, 2001; Horrigan and Ma, 2008; Yang et al, 2007).

Although, the HA model described the BK channel gating mechanism, the most important questions about the BK channel, whether these functionally interacting voltage- Ca^{2+}- and Mg^{2+}-sensor domains are physically coupled in the quaternary structure and how they regulate the pore, was only answered recently by crystal- and cryo-electron microscope structure studies. Next, we are going to summarize these new results.

The structure of BK channel

In accordance with the HA-model, BK channel consists of three structural components: a pore domain, a voltage sensing domain (VSD) and a cytosolic domain (C-terminal domain, CTD). The VSD and the pore domain contain 7 transmembrane helices together (S0-4 and S5-6). The CTD consists of two RCK (regulator of K^+ conductance) subdomains. In the quaternary structure of the homotetrameric channel, these domains assemble such that they form three distinct layers: the transmembrane layer, the RCK1- and the RCK2 layer.

The pore domain

The selectivity filter is the same as in other K^+ channels. It is formed by the loop (P-loop) connecting S5 (outer helix) and S6 (inner helix). The pore dilates as a funnel toward the cytosol and lined with negative side chains, which is compatible with the high conductance of the channel. The N-terminal of S5 is connected to the VSD, whereas the C-terminal of S6 is connected to CTD through a long linker (figure 2) (Tao et al, 2017; Hite et al. 2017).

The voltage-sensing domain

The structure reveals the structural reasons of the weak voltage dependence of BK channels compared to Kv channels. In voltage dependent K^+ channels, the S4 helix of the VSD contains positively charged amino acids (gating charges) that move across the membrane when the voltage sensor switches from resting to active position. One reason of the weaker voltage dependence is that the S4 in BK channel contains less gating charges compared to Kv channels (3 vs. 6/subunit). The secondary structure of S4 is also different, and as a consequence, the S4 helix

is located closer to the intracellular side of the membrane, therefore, during membrane depolarization, the length of movement toward the extracellular space is limited compared to Kv channels, which also accounts for the smaller gating current and the weaker voltage dependence.

The positively charged side chains of S4 are guided through the low dielectric environment of the membrane by negatively charged residues located in S2. This structure, called „gating charge transfer center" is incomplete in the BK channel.

The weak voltage dependence can be also attributed to the shorter length of the linker between the S4 and S5 in the VSD. The shorter linker do not keep S5 and S6 away from each other, therefore there is a much tighter association between the helices, which restricts the movements of S4. Probably another consequence of this short linker is that the BK channel`s VSD is not domain-swapped (unlike in Kv chnannels), which means that the VSD interacts with the pore domain of the same monomer (the linker is not long enough to reach the neighboring subunit) (Tao et al, 2017; Hite et al. 2017).

The unique S0 helix is located at the periphery of the VSD.

The cytosolic domain (CTD)

The CTD comprises the 2/3 of the primary sequence (~800 amino acids). In contrast to VSD, CTD is domain-swapped, which means that a CTD interacts with a VSD of the adjacent subunit (figure 2C). The CTD can be divided to two RCK subdomains. They consist of ordered topology of secondary structures, which form an N-terminal and a C-terminal lobe in each RCK domains. The corresponding lobes of the RCK1 and RCK2 of the same subunit interlock at the junction called „flexible interface" to form a tandem RCK domain. These tandem

RCK pairs (4x2) assemble together with neighboring tandem RCKs such that the N-lobes surround a central pore and the C-lobes face outside. This inter-subunit interaction is called „assembly interface" whereas the whole structure of the tetrameric tandem RCKs is called „gating ring" (figure 2A). The gating ring was named after its essential role in channel gating. Mutagenesis studies identified a series of conserved aspartic acid residues in RCK2 which are responsible for the Ca^{2+} activation of the channel, referred to as „Ca^{2+} bowl". The Ca^{2+} bowl is a helix-loop helix motif similar to EF-hands. Its first helix also participates in the interaction with the adjacent RCK1. Ca^{2+} is coordinated by the main-chain carbonyl oxygen atoms and oxygen atoms from the side-chain carboxylate groups from the RCK2 domain and made complete by a side-chain carboxylate from RCK1 of the neighboring subunit. Therefore, in the quaternary structure, the Ca^{2+} bowl is located at the assembly interface.

When the Ca^{2+} bowl is deleted, BK channel is still activated by Ca^{2+} with lower affinity, suggesting that the channel is regulated by another Ca^{2+} sensor too. This Ca^{2+} binding site is located in the groove between the N- and C-terminal lobes of RCK1 and interacts with residues in the loop of the Ca^{2+}-bowl. This relationship provides a possible explanation for the mechanism of the positive cooperative interaction between the two Ca^{2+} binding sites, which is discussed in details in the next section (Wu et al. 2010; Yuan et al. 2010; Yuan et al. 2011; Tao et al, 2017; Hite et al. 2017).

The second Ca^{2+} binding site in RCK1 actually functions as a Mg^{2+} binding site under physiological conditions. Mutational analysis revealed that is possibly located between VSD and CTD in RCK1. Accordingly, the EM-structure demonstrated a density-peak (corresponding to Mg^{2+}) in the interface between

VSD and RCK1. The cryo-EM structure shows that this binding site is formed by glutamic acids of two β-strands running parallel with the membrane on the top of the gating ring, close to the S0-S1 linker and the linker connecting the gating ring and the channel pore. This linker contributes to the binding site by a hydroxyl group of a serine residue. Apparently, Mg^{2+} functions as glue between these three structures and therefore stabilizes the relative orientation of the pore domain, the VSD and CTD and therefore, definitely modifies this protein-protein (intersubunit) interaction. The ion in the structure looks important for the coupling between the transmembrane domain and CTD (Tao et al, 2017; Hite et al. 2017; Yang et al. 2007; Yang et al. 2008). This idea is also supported by experimental data showing that mutations in the C-terminal of S4 and in the S4-S5 linker abolish the Mg^{2+} sensitivity of the channel (Hu et al, 2003).

Structural background of allosteric gating

In this section, we discuss how Ca^{2+} binding changes the shape of the gating ring and how this change is converted to pore opening and how the gating ring communicates with the voltage sensor. A cartoon illustration of the gating mechanism is shown in Figure 2B.

After Ca^{2+} binds to the Ca^{2+}-bowl, an asparagine in the adjacent RCK1 approaches the ion to make the coordination complete, which induces the whole RCK to move to open conformation. Similarly, Ca^{2+} binding to the RCK1 binding site is made complete by an asparagine, which bends toward Ca^{2+}. This movement is also coupled to the transition between the closed and open conformation of RCK1. The whole RCK1 layer of the gating ring that faces the transmembrane domains is subject to substantial conformational changes when moves to open

conformation. The N-terminal lobe tilts away from the pore axis and changes its angle according to RCK2 and VSD. As a result, -while the conformation of RCK2 does not change much- the gating ring opens up like a „petals of a flower" and expands its diameter. There are probably two pathways which transmit these movements toward the pore domain. First, upon Ca^{2+} binding, CTD is compressed against the voltage sensors and the S4-S5 linker (of the adjacent subunit), which causes a lateral shift of S4 away the pore. As the interaction between S4 and S5 is strong, their relative position do not change, thus S5 follows S4 and sways outward away the pore. Consequently, the displacement of S5 modifies the position and shape of S6 (the pore lining inner helix), such that it bends below the selectivity filter, which pulls the pore open. The other mechanism for pore opening is based on the connection between CTD and S6 (inner pore helix) through a linker, which directly mediates the opening force to the pore when RCK1 tilts. Note that the inter-subunit organization of the channel (i.e. non-domain swapped arrangement of the VSD and the pore domains; and the domain-swapped arrangement of the CTD and the VSD) is the source of inter-subunit cooperativity, shown in functional experiments: while RCK1 pulls its own S6 subunit, it also causes the displacement of the neighboring subunit (figure 2B).

Apparently, Ca^{2+} binding to either binding site induce the open conformation of CTD (i.e. tilting of the N-terminal lobe of RCK1), in which the other Ca^{2+} binding site is completed by an additional coordinating side chain. This mechanism is one of the structural bases for the positive cooperativity between the two Ca^{2+} binding sites. The other possible reason for the cooperativity is that the two binding sites of the same subunit are physically connected (see details in the

previous paragraph), therefore, Ca^{2+} binding to either site should influence the structure and the affinity of the other site.

In addition, Ca^{2+}-induced tilting of the N-lobe of RCK1 is also coupled to the transmembrane outward shift of gating charges (S4). This direction of displacement is the same as expected from the voltage sensor in response to membrane depolarization. Thus, membrane depolarization and Ca^{2+} control the position and shape of RCK N-lobe in the same way. Membrane depolarization induces a tilted conformation of RCK1, which enhances the Ca^{2+} affinity of the sensor. Similarly, Ca^{2+} promotes the activated position of the voltage sensor. The structure clearly shows, how voltage sensors communicate with the Ca^{2+} sensors. These results are in accordance with the HA model, because explains the allosteric coupling between voltage and Ca^{2+} during gating: the mutual enhancement of channel open probability by voltage and Ca^{2+}, and the reason why voltage increases Ca^{2+}-sensor affinity.

Apparently, the architecture of VSD in BK channel does not look like to be specialized to function as an effective voltage sensor, but rather to finely modulate the Ca^{2+} sensitivity of the channel (Horrigan and Aldrich, 2002; Tao et al, 2017; Hite et al. 2017).

Acknowledgements

This work was supported by the Hungarian National Research Development and Innovation Office (K115307 to PPN). JA is supported by the Lajos Szodoray Scholarship of the University of Debrecen. The publication was supported also by the GINOP-2.3.2-15-2016-00040 and EFOP-3.6.2-16-2017-00006 projects (to

PPN and JA), which are co-financed by the European Union and the European Regional Development Fund.

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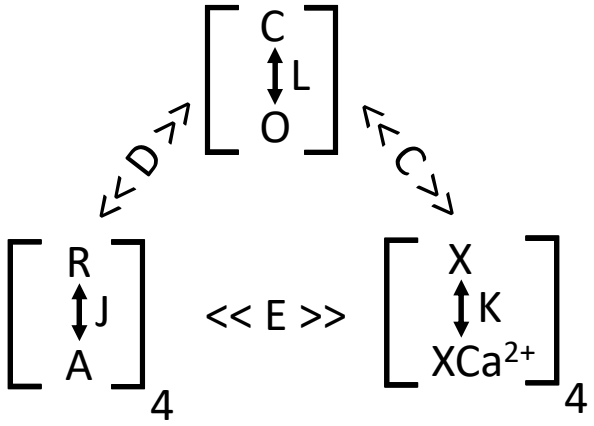
Figure legends

Figure 1. Horrigan-Aldrich model of BK channel gating

The gating mechanism includes an allosteric interaction between voltage sensor activation (R-A), a Ca^{2+} binding transition (X-XCa) and channel opening (C-O). L is the C-O equilibrium constant, J is the R-A equilibrium constant and K is the X-XCa equilibrium constant. D, C and E are allosteric factors between the transitions. Subscripts refer to 0-4 activated voltage sensors or Ca^{2+} binding sites.

Figure 2. Schematic structure of BK channel

A) Schematic representation of the assembly of RCK domains in the gating ring along with the atomic structure of Slo1 from the intracellular view (**A/i**). The atomic structure of the BK channel in the presence of Ca^{2+} and Mg^{2+} (5tj6.pdb) was visualized using the molecular graphics system PyMOL (Schrodinger, LLC). RCK1s are in red, RCK2s are in blue. Ca^{2+} is labeled in green. **B)** A cartoon, representing the gating mechanism of BK channel from the side-view. RCK1 and RCK2 are labelled in blue and red, respectively. The transmembrane helices are yellow. The Ca^{2+}- and Mg^{2+} binding sites are labelled with 1, 2 and M, respectively. **B/i)** Side-view of the atomic structure of the channel (TMD-transmembrane domain) **C)** Extracellular view of the atomic model, showing that the gating ring and the VSD is domain-swapped.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