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COMMENTARY

Polyamine block of MthK potassium channels

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Polyamine molecules, such as spermine, spermidine, and putrescine, are polycations that are ubiquitous in cells and have been found to contribute to many physiological processes such as DNA stabilization (Igarashi and Kashiwagi, 2010), regulation of gene expression (Childs et al., 2003; Igarashi and Kashiwagi, 2010), cell proliferation (Gerner and Meyskens, 2004), and, last but not least, membrane excitability via regulating ion channel function (Bowie, 2018; Nichols and Lee, 2018). First evidence of polyamines interacting with ion channels came from experiments on eukaryotic inward rectifier potassium channels that display larger inward currents compared to outward currents, hence their name. In 1994, Lopatin and colleagues showed that the inward current was reduced because of a blocade by polyamine cations coming from the cytosol (Lopatin et al., 1994). At increasingly depolarized voltages across the membrane, the charged intracellular polyamine cations entered the channel pore just like the permeant potassium ions, but instead of escaping through the selectivity filter they got stuck due to their large size and obstructed further movement of permeant ions through the pore, thus reducing outward current through the channel. Since then, polyamines have been reported to inhibit many other ion channels, such as cyclic nucleotide-gated channels (Guo and Lu, 2000; Lu and Ding, 1999), voltage-gated calcium (Gomez and Hellstrand, 1995) and sodium (Fu et al., 2012) channels, BK channels (Zhang et al., 2006), glutamate receptors such as AMPA and kainate (Bowie and Mayer, 1995), and NMDA receptors (Huettner, 2015; Williams, 1997), and even prokaryotic channels such as the model channel MthK, used in the study by Suma et al. in this issue of the *Journal of General Physiology*.

The mechanism by which polyamines inhibit currents and the binding site(s) for polyamines in the channels have been heavily investigated since the original discovery, mainly with electrophysiology. Recently, the binding sites for spermine and spermidine inside the AMPA receptor channels have been unraveled with cryo-EM, where densities for spermine and spermidine have been found lodged inside the selectivity filter regions of these channels (Twomey et al., 2018). For other ion channels, such as the inward rectifier potassium channels, many different mechanisms for how and where polyamines bind to the channel in order to cause a block have been proposed using mainly electrophysiology, an approximate technique for structural inferences such as binding sites. The main reason for the different proposed binding site locations lies in the different mechanistic interpretations of the voltage dependence of polyamine block. At one extreme, there is the classical model where the voltage dependence of a charged blocker comes from it moving in the electric field to reach its binding site (Lopatin et al., 1995; Woodhull, 1973). At the other extreme, there are models where the binding site is not in the electric field at all, and the blocker binding step has no voltage dependence associated with it, per se (Guo et al., 2003; Shin and Lu, 2005). In the latter case, the apparent voltage dependence of the block comes from a secondary process, such as a voltage-dependent conformational change of the protein needed to uncover the binding site. These extreme models predict completely different sites for polyamine binding to the channel. At this time, however, there is no direct structural evidence of polyamine block in any channels other than the AMPA channels, and the receptor sites for polyamines inside potassium channels of any kind remain a mystery.

The article by Suma et al. (2020) uses MthK, a eukaryotic BK (large conductance calcium-activated potassium) channel homologue from *Methanobacterium thermoautotrophicum*, as a model to investigate in detail polyamine block in a potassium channel. MthK channels have only two transmembrane domains, similar to inward rectifier potassium channels and KcsA (Doyle et al., 1998) channels, and a selectivity filter containing the GYG signature sequence for potassium selectivity. The structure of MthK was first determined in 2002 using x-ray crystallography of the full-length channel with a mutation to eliminate the cytoplasmic RCK domain, in the presence of calcium (Jiang et al., 2002a, 2002b). A high-resolution structure of the MthK pore domain alone was determined in 2010 (Ye et al., 2010), and multiple structures of the full-length channel in the presence and absence of calcium were only recently determined with single-particle cryo-EM (Fan et al., 2020). Three aspects make MthK a great model for investigating polyamine block with electrophysiology and MD simulations. First, the electrophysiological preparation is robust and pure; purified MthK channels can be used directly in synthetic planar-lipid bilayers,

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away from other cellular components, such as in a cell patch-clamp experiment, and the currents obtained are large, reproducible, and easy to recognize, as previously documented (Jiang et al., 2002a; Li et al., 2007; Pau et al., 2010; Pau et al., 2011; Posson et al., 2013; Thomson et al., 2014; Thomson and Rothberg, 2010; Ye et al., 2006; Zadek and Nimigean, 2006). Second, the channels are blocked by physiologically relevant concentrations of both Mg^{2+} and polyamines (Thomson et al., 2014). Third, the availability of a very high resolution (>1.5 Å) structure of the pore-only MthK allows for meaningful interpretations of the MD simulations.

First, Suma et al. (2020) measured intracellular spermidine and spermine block of macroscopic potassium currents through maximally activated MthK channels over a large range of voltages. They found that spermine blocks with ~10-fold higher apparent affinity than spermidine. They also found that the block is voltage dependent, not surprising considering that the polyamines are positive cations, and that the voltage dependence of the block (zδ) is roughly similar between the two polyamines. The zδ values for spermine and spermidine (with four and three positive charges, respectively) are in the same range as those found previously for quaternary amine compounds (0.7–0.9, with only one positive charge; Posson et al., 2013), suggesting that the number of charges of the polyamine molecule is not the sole determinant of the voltage dependence of the block. Thus, the simplest model of block, where the voltage dependence arises exclusively from movement of the charged blocker in the electric field to reach its binding site (Fig. 1 A) is unlikely to be sufficient to describe spermine and spermidine block of MthK channels. In addition, this model predicts that at sufficiently high voltages, all current through MthK channels will be blocked regardless of the blocker concentration, while the data in Suma et al. (2020) deviates from these predictions for both polyamines. Indeed, a global fit of the data with the model in Fig. 1 A failed to capture the plateau the current reaches at every blocker concentration at very depolarized voltages.

To capture this aspect of the data, the authors proposed a new model where they made the polyamine binding itself voltage independent, but introduced an earlier step where the channel needs to undergo a voltage-dependent change in order to become "permissive" to polyamine binding (Fig. 1 B). This earlier step is proposed to be an outward movement of permeant ions in the electric field, a step that is clearly voltage dependent, which is necessary to free up the site where the polyamine needs to bind. This new model is superior to the classical one as it better fits both the spermine and the spermidine block of MthK channels in Suma et al. (2020). Importantly, such a model allows for better fit of the current at extreme depolarizations since, in these conditions, the current becomes voltage independent, and only dependent on the polyamine concentration and the intrinsic affinity of the blocker to the permissive form of the channel (Fig. 1 B). Global fits with this model indicated that the voltage dependence is similar for both spermine and spermidine, reinforcing the assumption that this step is polyamine-type agnostic. The fits also yield a higher affinity for the spermine compared with spermidine, as also visible in the model-independent fits, indicating that the interactions between the channel and the polyamine is polyamine specific.

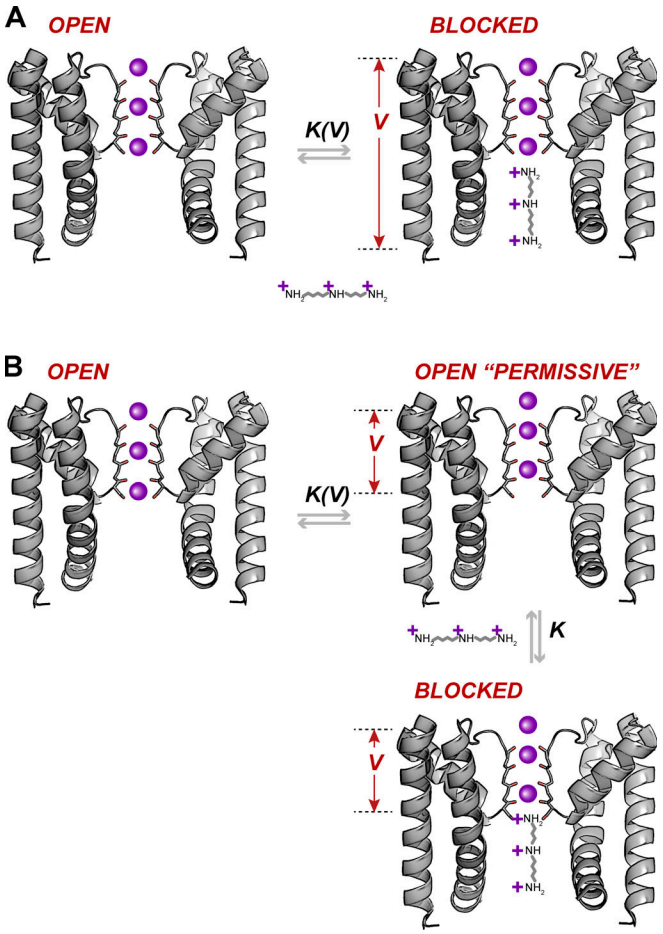

Figure 1. **Polyamine block models. (A)** Classical model where voltage dependence of the block comes from charged polyamines moving in the membrane electric field to reach the blocking site in the pore (right). **(B)** Model from Suma et al. (2020), where voltage-dependent movement of permeant ions upward in the selectivity filter (top right) is needed to free up the blocking site (S4) for the polyamine to bind (bottom). Voltage drop (V) across the whole channel is shown in A, while in B a more realistic depiction of the voltage across the membrane dropping mostly across the selectivity filter. K ions are shown in purple. Spermidine in chemical representation and MthK structure in gray (PDB accession no. 3LDC), where only two opposing subunits are shown.

Although the second model (Fig. 1 B) describes the data much better than the first, classical model, the binding site for the polyamines is still undetermined. The second model intimates that the polyamine need not travel in the electric field to reach its binding site, and one may conclude that this binding site is outside of the channel pore or somewhere very near to the intracellular pore mouth. However, MthK has a very large vestibule near the intracellular pore mouth, and it has been previously proposed that only a negligibly small fraction of the electric field across the membrane falls across it, while most of it falls across the selectivity filter (Fig. 1 B; Posson et al., 2013). Thus, the blocking site can be anywhere in the vestibule and still fall within the bounds of the second model.

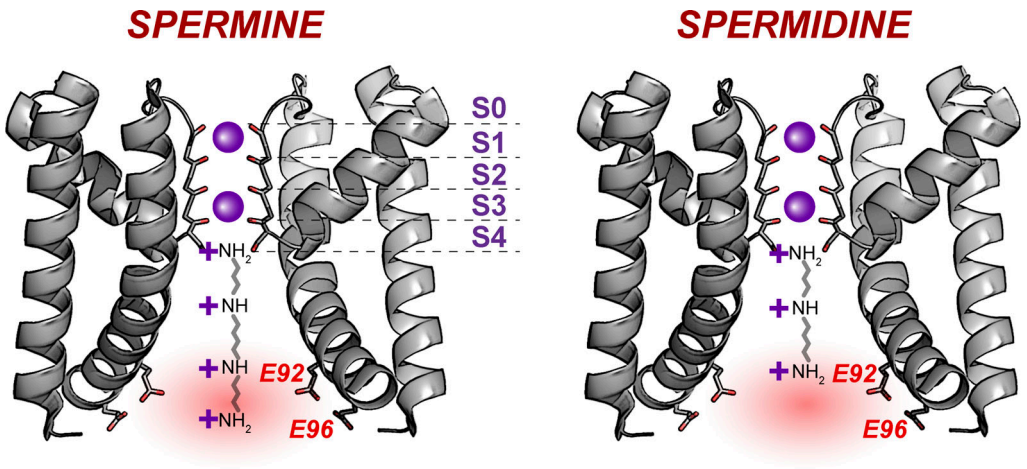

Figure 2. **Spermine and spermidine interactions with the pore.** Left: The third and fourth nitrogens of spermine interact with E92 and E96, shown in stick representation with oxygens in red. Right: The third nitrogen of spermidine interacts with only E92.

To gain further physical insight into the blocking process and understand the determinants of the higher spermine binding affinity, as well as the nature of the permissive channel conformation, the authors used MD simulations. The simulations were performed in lipid bilayers with K^+ ions in the selectivity filter at positions S0, S2, and S4 (Fig. 2), and either spermine or spermidine in the vestibule of the MthK pore. The authors found that while a K^+ was residing in S4, the polyamines were delocalized in the large MthK vestibule, but the moment the K^+ in S4 moved upwards into the adjacent site S3, the leading amine of the polyamine snuck up to S4, hijacking the hydroxyls of the S4-forming threonine, and was able to move even deeper into S4 as the K^+ went further up to S2. This behavior was seen with both spermine and spermidine (Fig. 2). These simulations are consistent with the previous assumption where the permissive channel has a vacancy at the S4 K^+ binding site after K^+ ions moved upwards in the selectivity filter, thus creating the binding site for the polyamines. This step is voltage dependent, as it involves movement of ions in the selectivity filter, across which most of the electric falls; with depolarization, K^+ moves more frequently upwards in the filter allowing a polyamine to get into its binding site in S4.

Since the binding site in S4 only accommodates the leading amine, which is identical between the two polyamines, this could not account for the differences in affinity between spermine and spermidine. The authors then examined if there are other interactions between the polyamine molecule and the channel pore while the leading amine is in its binding site in S4. Spermidine and spermine have two and three additional positive charges, respectively. MthK channel pore displays two rings of negative charges at the intracellular mouth entrance composed by glutamates at positions 92 and 96 (Fig. 2). They found that the third amine of spermidine is coordinated by the side-chain oxygens of E92, while both the third and the fourth amines of spermine are coordinated by E92 and E96, providing a molecular reason for the increased affinity for spermine block (Fig. 2).

The article by Suma et al. (2020) determined the molecular mechanism of an important regulator of membrane excitability, polyamine block, in a model potassium channel. The polyamines bind so that the leading amine is in the S4 bottommost site of the selectivity filter and the other lower amines interact with the rings of glutamates at the intracellular mouth of the MthK pore (Fig. 2). While the binding of the polyamines is not voltage dependent, per se, the voltage dependence associated with the block arises from the need to vacate the S4 site in order to allow the polyamine to bind via pushing permeant ions upwards in the selectivity filter. A similar mechanism was previously proposed for eukaryotic inward rectifier potassium channel (Guo et al., 2003; Shin and Lu, 2005), with the difference being that in the case of inward rectifiers, the voltage dependence of polyamine block is much larger (approximately three to four compared with approximately one for MthK; Nichols and Lee, 2018). Since the inward rectifier potassium channels have an extra-long pore, which extends deep into the intracellular side (Nichols and Lee, 2018), more ions need to be presumably pushed outwards in order for polyamines to bind, a potential explanation for their larger voltage dependence in the context of this model. Thus, even if the vestibules of these channels are different, it is intriguing to contemplate that the broad principles of the polyamine block may be conserved from archaea to eukaryotes. Unlike the voltage dependence, the polyamine binding affinity depends on the polyamine type, partly because of the presence of negatively charged residues lining the pore, a feature of channels displaying polyamine block: inward-rectifying K^+ channels have aspartates (Nichols and Lee, 2018), and MthK and BK channels have rings of glutamates in the pore (Suma et al., 2020; Zhang et al., 2006).

In addition to the importance of this study, as it may apply toward understanding a fundamental regulatory mechanism in closely related eukaryotic channels, the intracellular polyamine block may also physiologically regulate ion flux through MthK channels in *M. thermoautotrophicum*, as concentrations of up to 250 and 500 μM of spermidine and spermine, respectively, are known to exist in these archaea (Hamana et al., 2007). This may turn out to be important in the future toward our understanding of channel function in these microorganisms.

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