

## Mechanism of Inward Rectification in Kir Channels

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Two recent papers published in the *Journal of General Physiology* (Guo and Lu, 2003; Guo et al., 2003) address the mechanism of inward rectification by polyamines in Kir2.1 channels. In these two papers, Guo et al. (2003) extensively analyze channel block by monoamines, diamines (DAs), and polyamines of varying length and charge, using thermodynamic mutant cycles to calculate interaction energies of these compounds with acidic residues in the channel. Based on their findings, a physical model for the blocking action of polyamines is proposed that incorporates the most recent structural information determined for Kir channels.

We write to suggest a different and, in our view, more plausible physical interpretation, which we believe better reconciles their results with previous electrophysiological as well as structural findings (Pearson and Nichols, 1998; Kubo and Murata, 2001; Xie et al., 2002, 2003; Chang et al., 2003; Kuo et al., 2003).

DAs are alkyl chains with positively charged amine groups at each end, whereas polyamines such as spermidine and spermine have amine groups at both ends and interspersed within. In their model, Guo et al. (2003) propose that when intracellular DAs or polyamines enter the pore, the leading amine group first interacts with a ring of negative charges formed by E224 and E299 in the cytoplasmic pore, occluding the ion-conducting pathway with a shallow voltage dependence. The leading amine then moves deeper into the transmembrane pore toward the negative charge at D172, with the trailing amine group remaining stabilized by an electrostatic interaction with E224 and E299. They postulate that as the alkyl chain length of the DA increases, the leading amine approaches more closely to D172, displacing more K ions from the pore and thereby increasing the voltage dependence of block. For DAs, this effect plateaus at a chain length of nine alkyl groups (DA9), which is also the DA length at which the interaction energy (relative to DA2) peaks with respect to the D172N mutant. Although this schema explains their findings from the biophysical standpoint, a problem arises when the recent structural information is considered. From the crystal structure of closed KirBac1.1 (Kuo et al., 2003), the distance between D172 and E224/E299 is  $\sim 35$  Å, whereas DA9 has

a total extended length of only  $\sim 12$  Å. Assuming similar dimensions for Kir2.1, then if the leading amine of DA9 binds close at D172 (as required for it to sweep K ions from the transmembrane pore), then its trailing amine would be some 23 Å away from E224 and E299. To rationalize this distance problem, Guo et al. (2003) hypothesize a long-range electrostatic interaction between the trailing amine group of D9 and the negative ring of charges at E224 and E299, so that the actual position of the trailing amine is only 12 Å or so from D172. Thus, longer or shorter DA cannot be as effectively stabilized with their leading amine close to D172 to sweep K ions from the pore.

We suggest that an alternative scenario for polyamine binding is equivalently consistent with the biophysical evidence, but, in our view, more compatible with available structural information (Fig. 1) and previous observations by other investigators (Pearson and Nichols, 1998; Kubo and Murata, 2001; Xie et al., 2002, 2003; Chang et al., 2003). This model was previously sug-

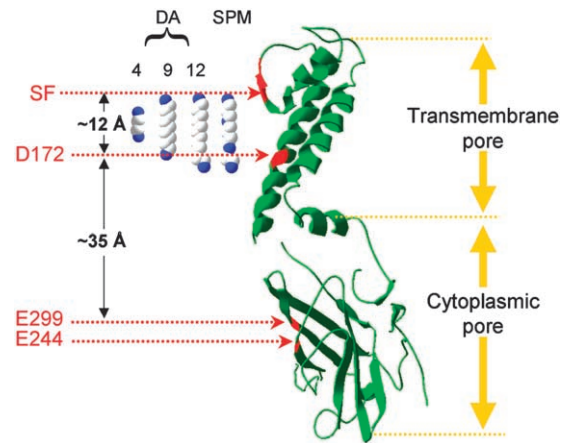


FIGURE 1. Model of inward rectification. Structural model of the closed KirBac1.1 channel, outlining the transmembrane and cytoplasmic pore regions. For comparison with channel pore dimensions, DA4, DA9, DA12, and spermine (SPM) are shown from left to right as space filling molecules. Relative distances (in angstroms) between the selectivity filter (SF), D172, and E224/E229 in Kir2.1 are assumed to be the same as between the corresponding positions in KirBac1.1.

gested for Kir6.2 (Phillips and Nichols, 2003) and Kir3 (Dibb et al., 2003) channels as well as Kir2.1 channels (Chang et al., 2003), and is based on the leading amine group of the DA or polyamine first occluding the pore when it interacts electrostatically with D172 in the transmembrane pore, rather than at E224/E299. The leading amine then moves deeper toward the selectivity filter (SF), thereby displacing K ions from the pore, whereas the trailing amine is stabilized by electrostatic interaction with D172. The distance between I138 (D172 in Kir2.1) and V111 (V143 in Kir2.1), which is just at the intracellular side of the SF signature sequence (GYG) in KirBac1.1, is estimated at  $\sim 12$  Å, which matches almost exactly the length of DA9 ( $\sim 12$  Å). This explains why the interaction energy peaks for DA9, and falls off for longer or shorter DAs. For example, if the extracellular movement of the leading amine group of DA12 (length  $\sim 16$  Å) is physically obstructed by the SF, then its alkyl chain will be too long for the trailing amine to interact as effectively with D172. Like DA9, spermidine ( $\sim 11$  Å) has a nearly ideal length for its leading and trailing amine groups to bind between D172 and the SF. Spermine ( $\sim 16$  Å) is too long for the leading and trailing amine groups to fit between D172 and the SF, but the distance between its leading and third amine groups, which is the same as spermidine, is optimal. The middle amine groups in spermine appear to further increase its stability in the pore, because its interaction energy was higher than any of the DAs. If, as originally postulated by Pearson and Nichols (1998) and later supported by Guo et al. (2003) as well as by our findings (Xie et al., 2002, 2003), the voltage dependence of polyamine block arises chiefly from the polyamines displacing K ions from the pore, then the similar effective valences for DA9, spermidine, and spermine can also be readily explained in this model, based on the following assumptions: (a) that the transmembrane voltage field is centered near the SF and K binding sites (such that the initial blocked state has a shallow voltage dependence); (b) it is primarily the leading amine group of DA9, spermidine, or spermine that electrostatically repels K ions and sweeps them from their binding sites between D172 and external side of the SF. The trailing amine groups of DA9, spermidine, or spermine would have little effect on valence, since they are for the most part located outside of the transmembrane voltage field, and do not contribute greatly to K ion displacement.

In this model, the lower valence of monoamines (including M9) occurs for the following reason: if the monoamine enters the pore with its uncharged nonpolar end leading, so that its trailing amine group interacts electrostatically with D172, then the nonpolar end will be less effective at repelling K ions near the SF. Conversely, if its amine group leads and it penetrates

past D172 then the trailing nonpolar end cannot electrostatically stabilize the monoamine at D172, and the leading amine group will not penetrate as deeply toward the SF to repel K ions. In contrast, in their model, Guo et al. (2003) must postulate that monoamines can only enter the pore in the energetically less favorable orientation with the nonpolar end leading

The orientation of DAs in the pore between the D172 and the SF that we propose here is compatible, unlike the model proposed by Guo et al. (2003), with the considerable evidence that polyamines can bind to the negative ring of charges at E224 and E299 without occluding the pore (Kubo and Murata, 2001; Xie et al., 2002, 2003; Chang et al., 2003). We (Xie et al., 2003) have presented evidence that DAs and polyamines of length  $\geq 8$  alkyl groups bind efficiently to the negative charges at E224 and E299 in the cytoplasmic pore without occluding the ion permeation pathway. This conclusion was based on the observation that DAs and polyamines with  $\geq 8$  alkyl groups were effective at reducing single-channel conductance over a wide range of voltage, which we attributed to reduction of net negative surface charge in the cytoplasmic pore. The distance between E224 and E299 on adjacent subunits, estimated from the Kir3.1 cytoplasmic structure, is 9.2 Å, which corresponds closely to the length of DA8 (9.5 Å). Thus, we postulated that initial binding of DAs and polyamines longer than 9 Å, at E224 and E299 prepositions them in the wide (7–15 Å) cytoplasmic pore, facilitating their access to the pore-blocking site at D172. In our model, this accounts for the kinetically rapid component of pore block, while the slow component is due to diffusion of untethered polyamines into the pore. This role of the negative charge ring in the cytoplasmic pore is consistent with the findings of Guo et al. (2003) that the alkyl chain length had no effect on the low interaction energy between DAs and E224 or E299. (We agree with their interpretation that the higher interaction energy of spermine with E224 or E299 compared with diamines is likely due to the additional amines in spermine acting as pseudodivalent cations.)

In summary, we believe that the model of polyamine block proposed by Guo et al. (2003) is difficult to reconcile with all available structural and electrophysiological data. A caveat, of course, is that the structure of open Kir2.1 channels is not yet available to settle definitively which interpretation is correct. In addition, there are also controversies about the biophysical analyses (Ishihara and Ehara, 2004). Nevertheless, based on the available information, we agree fully with the comment by Stanfield and Sutcliffe (2003) in their accompanying editorial noting “how exquisitely well the channel and spermine match each other”, in the sense that our current best estimates of the distances between D172 and the SF, and between E224 and E299 on

adjacent subunits in Kir2.1 appear to be exquisitely matched to the dimensions of the key biomolecules that cooperate synergistically to facilitate inward rectification so efficiently.

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