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RESEARCH NEWS

Modeling GIRK channel conductance

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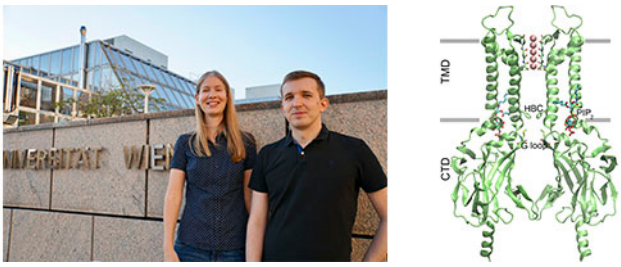
***JGP* study uses MD simulations to investigate the gating and conductance of the inwardly rectifying potassium channel GIRK2.**

GIRK proteins are a family of G protein–regulated inwardly rectifying potassium channels that, in response to dopamine and other neurotransmitters that stimulate G protein–coupled receptors, generate small, hyperpolarizing outward K^+ currents that inhibit neuronal activity. Like all other inwardly rectifying potassium channels, however, GIRK activity is also regulated by the phospholipid PIP_2, and the precise details of how these channels are gated and how they conduct K^+ ions remain uncertain. In this issue, Bernsteiner et al. use MD simulations to uncover new details about the mechanisms controlling K^+ flux through GIRK2, a GIRK family member also known as Kir3.2 (1).

Experiments on GIRK2 embedded in planar lipid bilayers suggested that channel opening requires both PIP_2 and $G_{\beta\gamma}$ (2), whereas studies on GIRK2 incorporated into liposomes indicated that PIP_2 is sufficient to activate the channel (3). Crystal structures have been obtained for wild-type GIRK2 in the presence and absence of both PIP_2 and $G_{\beta\gamma}$ (4, 5). "However, none of these structures appear to be in an open state," explains Anna Stary-Weinzinger, an assistant professor at the University of Vienna.

To learn more about the channel's gating, Stary-Weinzinger and colleagues, including first author Harald Bernsteiner, performed MD simulations based on the crystal structure of GIRK2 bound to PIP_2 but not $G_{\beta\gamma}$, in which the channel's helix bundle crossing (HBC) and G-loop gates both appear to be closed. "In our simulations, however, we were quite surprised to see that, when embedded in a membrane, the channel spontaneously opens and allows K^+ ions to pass through the gates," Stary-Weinzinger says.

This spontaneous opening was facilitated by the wetting of the HBC and G-loop gates by water molecules diffusing into the channel, opening them wide enough to permit the passage of K^+ ions. The gates remained closed when the researchers removed PIP_2 from the simulations, indicating that the phospholipid is sufficient to activate GIRK2. One possibility is that, in vivo, $G_{\beta\gamma}$ promotes channel opening by acting as an allosteric modulator, increasing GIRK2's affinity for PIP_2.



Anna Stary-Weinzinger (left), Harald Bernsteiner (right), and colleagues use MD simulations to provide new details about the gating and conductance of the inwardly rectifying K^+ channel GIRK2. The crystal structure of the channel bound to its activator, PIP_2, shows the position of the HBC and G-loop gates, as well as five K^+ ions (pink spheres) passing through the selectivity filter via what the simulations suggest is a direct knock-on mechanism.

The researchers then applied an electric field to their simulations to mimic membrane potential and, in a series of microsecond-long simulations, saw that, after diffusing through the G-loop and HBC gates, K^+ ions move through the channel's selectivity filter via a "direct knock-on" mechanism. Such a mechanism, in which K^+ ions entering the selectivity filter directly push the ions ahead of them through to the other side, has recently been demonstrated in voltage-gated K^+ channels. But the subject remains controversial, with many studies supporting a "soft knock-on" mechanism in which the permeating K^+ ions are separated by intervening water molecules. "Further studies are needed, but maybe all K^+ channels can conduct in both ways," Stary-Weinzinger suggests.

Finally, free energy profiling revealed that, rather than the HBC or G-loop gates, the selectivity filter represents the main barrier to K^+ ion movement through GIRK2. In voltage-gated K^+ channels, a phenomenon known as C-type inactivation can restrict ion movement by narrowing the selectivity filter. Surprisingly, however, Bernsteiner et al. found that the selectivity filter of GIRK2 was dilated in MD simulations showing little or no conductance.

"If the filter gets as little as 1 Å wider, it appears to stop conductance," Stary-Weinzinger says. "In contrast, we did not find any correlation between conductance and the diameters of the HBC or G-loop gates."

Mutations in the selectivity filter of GIRK channels have been linked to the rare developmental disorder Keppen-Lubinsky syndrome as well as cases of aldosterone-producing adenomas. "We now want to investigate the effects of these disease-causing mutations in our MD simulations," Stary-Weinzinger says.

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