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Lipid-protein Interactions in Ion Channel Regulation: The Role of Anionic Lipids in Kcsa

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In this study, we explore how anionic lipids modulate the activity of the potassium channel KcsA. Using a combination of electrophysiological experiments, structural analysis, and site-directed mutagenesis, we demonstrate that anionic lipids affect both the inactivation through the outer gate, and the opening through the inner gate of the channel. Specific mutants, alongside advanced fluorescence anisotropy techniques to monitor conformational changes in the selectivity filter (SF) have revealed that anionic lipids, such as phosphatidic acid (PA), reduces inactivation by stabilizing the conducting conformation of the SF, which results in increasing its affinity for K+. Moreover, our results suggest that anionic lipids facilitate the opening of the inner gate, shifting the pKa of activation to higher values of pH (from 4.5 to 6). Notably, our findings indicate that the modulation of both gates by PA occurs without requiring the formation of a continuous lipid bilayer, indicating a direct effect on the channel. Specifically, our experiments suggest that anionic lipids act on the inner gate to facilitate its opening, while decreasing inactivation at the outer gate, contributing to prolonged conductance. This study provides new insights into the modulation of ion channel activity by lipids, highlighting the importance of direct lipid-protein interactions in membrane dynamics.