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RECEPTOR-MEDIATED REGULATION OF GIRK CHANNELS BY LIPID SIGNALING

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Phosphatidylinositol 4,5-bisphosphate (PIP_2) is well known as a central molecule in the phosphoinositide cycle, by serving as the precursor of important signaling molecules such as inositol trisphosphate (IP_3), diacylglycerol (DAG) or Phosphatidylinositol 3,4,5-trisphosphate (PIP_3). Recently, it was shown that PIP_2 is not just a precursor, but also exerts a direct role in the regulation of various ion channels, including G protein-gated inward rectifying K^+ (GIRK) channels. We investigated whether the change in PIP_2 concentration induced by Gq protein-coupled receptors (GqPCRs) activation can regulate GIRK channel activities. In atrial myocytes, phenyleprine, endothelin-1 and prostaglandin-F2α caused inhibition of GIRK currents, and this inhibition was further potentiated by wortmannin (an inhibitor of phosphatidylinositol kinase) and attenuated by addition of PIP_2 in the pipette solutions. These results suggest that these agonists inhibit GIRK currents via PIP_2 depletion. In contrast, stimulation of bradykinin receptors or M_1/M_3 muscarinic receptors had no effect on GIRK channels. In hippocampal neurons, GIRK currents were inhibited by carbachol, a M_1/M_3 muscarinic receptor agonist, or DHPG, a group I metabotropic glutamate receptors, which are known to be GqPCRs. However, this inhibition was not affected by wortmannin or addition of PIP_2 in the pipette solutions. We found that carbachol-induced inhibition of GIRK currents was attenuated by PKC inhibitors in a Ca^{2+}-dependent manner. On the other hand, DHPG-induced inhibition of GIRK currents was attenuated by a phospholipase A_2 inhibitor. Arachidonic acid also directly inhibited GIRK currents, suggesting that the effect of DHPG was mediated by phospholipase A_2 dependent production of arachidonic acid. Taken together, it was concluded that various GqPCRs inhibit GIRK currents in a receptor- and cell-specific manner.

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COMPARTMENTALIZED MOVEMENT OF LIPIDS IN THE CELL MEMBRANE AS REVEALED BY SINGLE MOLECULE TECHNIQUES

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The movements of phospholipid (DOPE) molecules in the live cell membrane were detected using single fluorescent molecule tracking and high-speed single particle tracking techniques. It was found that the cell membrane is partitioned into many small compartments, and individual molecules undergo short-term confined diffusion within a compartment, and long-term hop diffusion between the compartments. For the compartmentalized movement of phospholipids, we propose an "anchored membrane-protein picket" model in which various transmembrane proteins anchored to the membrane skeleton meshwork effectively act as rows of pickets against the free diffusion of lipids, due to steric hindrance as well as hydrodynamic-friction like effects. This picket model for lipid diffusion is based on the "membrane-skeleton fence" model for the diffusion of transmembrane proteins. Transmembrane proteins are protruding into the cytoplasm, and their cytoplasmic domains collide with the membrane skeleton, which induces temporary confinement or corralling of transmembrane proteins. In all of the 5 cell types (NRK, T24, HeLa, HEPA-OVA, and PtK2) examined thus far, although the compartment size varied from cell to cell (40~260 nm), phospholipid (DOPE) and transmembrane protein (transferrin receptor) sensed the same compartment size. This result further supports the coupling of "picket" and "fence" effects.

Key Words: single molecule, phospholipid, cell membrane, membrane skeleton