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# Functional Analysis of the $K^+$ Channel Complex that Regulates $K^+$ Recycling in Epithelia

Go Kasuya

Division of Integrative Physiology, Department of Physiology, Jichi Medical University

Summary

$Cl^-$ secretion from epithelial cells in the intestine and lung is important for fluid homeostasis. For the proper $Cl^-$ secretion by CFTR channels expressed on the apical side of epithelial cells, excess intracellular $K^+$, which is imported by $Na^+/K^+/Cl^-$ cotransporters and $Na^+/K^+$ pumps on the basolateral side of epithelial cells, needs to be properly exported. This process is called "$K^+$ recycling". Malfunction of $K^+$ recycling leads to impaired $Cl^-$ secretion, resulting in secretory diarrhea, pulmonary edema, and cystic fibrosis. The KCNQ1-KCNE3 channel complex expressed on the basolateral side of epithelial cells is a $K^+$ channel responsible for $K^+$ recycling. The KCNQ1-KCNE3 channel complex is composed of two membrane proteins, KCNQ1 and KCNE3. KCNQ1 is a voltage-gated $K^+$ channel and KCNE3 is a single transmembrane protein. KCNE3 binds to the voltage sensor domain (VSD) of KCNQ1 and modulates VSD position and movement to change KCNQ1 to a constitutive open $K^+$ channel at physiological voltages. The cryo-EM structure of the KCNQ1-KCNE3-calmodulin (CaM) complex reported in 2020 revealed the amino acid residues involved in the interaction between KCNQ1 and KCNE3. However, it is unclear how the interaction between KCNQ1 and KCNE3 modulates the VSD position to form a constitutive open channel at physiological voltages.

In this study, based on the KCNQ1-KCNE3 structure, we created a series of KCNQ1 and KCNE3 mutants to investigate the functional role of the interaction between KCNQ1 and KCNE3 and conducted electrophysiological experiments using two-electrode voltage clamp (TEVC) method. The results revealed that the interaction between the first transmembrane segment (S1 segment) of KCNQ1 and the transmembrane segment of KCNE3 is optimized at the size level of the side chains of amino acid residues.

Furthermore, from the voltage-clamp fluorometry (VCF) analysis, we found that the interaction between the S1 segment of KCNQ1 and the transmembrane segment of KCNE3 keeps the position of the fourth transmembrane segment of KCNQ1 (S4 segment), which moves up and down depending on the membrane voltage change and is involved in opening and closing the ion permeability pore, in an intermediate state and thus allows the formation of a constitutive open $K^+$ channel at physiological voltages.