

S4-S5 Linker is Involved in Voltage-Dependent Gating of Human Transient Receptor Potential Ankyrin 1 Channel

Katja Witschas, Lucie Sura, Vlastimil Zima, Anna Hynkova, Ivan Barvik, Viktorie Vlachova

Biophysical Journal, Vol. 104, Issue 2, p453a–454a

The transient receptor potential ankyrin 1 channel (TRPA1) is a versatile sensory channel that is gated by depolarizing voltages, deep cooling, membrane deformation, and structurally diverse compounds which include proalgesic agents such as allyl isothiocyanate. How these disparate stimuli converge on the channel protein to open the ion-conducting pore has not yet been fully resolved. The overall architecture of TRP channels shows clear similarities to that seen in the well characterized voltage-gated potassium channels. Here, activation of the voltage sensors in the fourth transmembrane segment (S4) is transduced into pore opening via coupling of the S4-S5 linker to the C-terminal S6 segment. In TRPA1, the gain-of-function mutation N855S located in the S4-S5 region has been associated with familial episodic pain syndrome. In an attempt to elucidate the role of the S4-S5 linker and its putative interaction(s) with S6 or the first C-terminal helix in the voltage-dependent gating of TRPA1, we used site-directed mutagenesis, whole-cell electrophysiology, single-channel recording, and molecular dynamics simulations. The charge-reversal mutations K868E and K969E resulted in a decrease in the rectification index compared to wild-type TRPA1 channels, and a virtually voltage-independent conductance-voltage (G-V) relationship. This effect was also observed in the adjacent charge-neutralizing mutant H970A, but was less pronounced in charge-reversal H970D. These results indicate that positively charged residues in the S4-S5 linker and the helix adjacent to the C-terminal S6 segment play a vital role in the voltage-dependent gating of TRPA1.

This work was supported by Czech Science Foundation Grant 305/09/0081, Research Project Fund of the Academy of Sciences of the Czech Republic Grant RVO:67985823, and Ministry of Education, Youth and Sports of the Czech Republic Grants OP VK CZ.1.07/2.3.00/30.0025, MSM0021620835, SVV-2010-261 304, and GAUK 426311.

2321-PosB34