Altered CRAC channel gating in the Orai1 E106D mutant

N.R. Scrimgeour and G.Y. Rychkov, Discipline of Physiology, University of Adelaide, Adelaide, SA 5005, Australia.

Ca^{2+}-release activated Ca^{2+} (CRAC) channels represent the main avenue for Ca^{2+} entry in non-excitable cells, and play an important role in Ca^{2+} dependent processes of both excitable and non-excitable cells, such as immune cell differentiation, regulating vascular smooth muscle tone, and exocytosis in neurons. The minimal molecular components of functional CRAC channels are Stromal interaction molecule 1 (STIM1), a Ca^{2+} binding protein that plays the role of Ca^{2+} sensor, and Orai1, the pore forming subunit. Investigation of the structure of Orai1 has identified key residues in the transmembrane domains that control the selectivity and gating of CRAC channels (Yamashita *et al*., 2008). In this work we have generated and expressed V102I, E190Q and E106D mutants along with STIM1 in H4IIE rat liver cells to investigate their selectivity and gating. In contrast to previous reports we have found that that V102I and E190Q mutant channels retain normal fast Ca^{2+}-dependent inactivation when co-expressed with saturating amounts of STIM1, similarly to WT (Scrimgeour *et al*., 2009). Compared to WT Orai1, E106D mutation, however, shifted voltage dependence of gating to more positive potentials by 80 mV ($p<0.01$, $n=10$) and significantly reduced the time constant of current inactivation at negative potentials ($p<0.01$, $n=6$). Furthermore, while WT Orai1 is highly selective for Ca^{2+} over Na^{+} and is completely blocked at pH 6.0, we have found that E106D conducts Na^{+} in the presence of Ca^{2+} and is not blocked by low pH. These results show that glutamate at position 106 in WT Orai1 determines selectivity of the channel and its dependence on external pH.

Scrimgeour *et al*. (2009) *J. Physiol*. **587**:2903-18.
Yamashita *et al*. (2008) *J. Gen. Physiol*. **13**:525-40.