Regulation of Ca^{2+} release activated Ca^{2+} channels by intracellular pH

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Depletion of intracellular Ca^{2+} stores in mammalian cells results in activation of Ca^{2+} entry pathway mediated primarily by Ca^{2+} release-activated Ca^{2+} (CRAC) channels. Functional CRAC channel is composed of a hexamer of a protein called Orai1, which forms the channel pore, and a protein called STIM1, a Ca^{2+} binding protein that plays the role of Ca^{2+} sensor in the endoplasmic reticulum (Parekh & Putney, 2005). Previously it has been shown that activity of CRAC channels formed by Orai1 and STIM1 proteins strongly depend on both extracellular and intracellular pH (Beck *et al.*, 2014). Structure-function studies have identified glutamate 106 in the Orai1 pore and aspartates 110 and 112 in the first extracellular loop as the main sites mediating I_{CRAC} dependence on extracellular pH (Scrimgeour *et al.*, 2012; Beck *et al.*, 2014). Recently histidine 155 in the intracellular loop of Orai1 has been suggested as CRAC channel intracellular pH sensor. However, H155F mutation of Orai1 was only shown to abolish I_{CRAC} potentiation by alkaline pH_i, whereas inhibition of I_{CRAC} mediated by this mutant by acidic pH_i remained largely unchanged (Tsujikawa *et al.*, 2015), suggesting that some other mechanisms may be involved.

In this study we investigated dependence of I_{CRAC} on pH_i using HEK293T cells heterologously expressing different ratios of Orai1 and STIM1 proteins and whole-cell patch clamping. The results showed that intracellular acidification to pH 6.3 inhibited I_{CRAC} by ~70%, compared to pH_i 7.3. At the same time low pH_i affected CRAC channel gating, promoting I_{CRAC} activation at negative potentials. Similar increase in I_{CRAC} activation could be achieved by increasing relative expression levels of Orai1 at neutral pH_i, which suggested that intracellular acidification affects Orai1/STIM1 interactions. Intracellular alkalinisation strongly potentiated I_{CRAC} amplitude, but only when EGTA was used as intracellular Ca^{2+} buffer. Replacing EGTA with BAPTA virtually abolished I_{CRAC} potentiation by high pH_i, suggesting that pH dependence of Ca^{2+} binding properties of intracellular Ca^{2+} buffers may contribute to CRAC channel dependence on pH_i. To elucidate the mechanism of CRAC channel gating dependence on pH_i we neutralised several negatively charged amino acids in STIM1 475DDVDDMDEE483 domain previously shown to modulate fast Ca^{2+} dependent inactivation (FCDI) of I_{CRAC}. The amplitude of I_{CRAC} mediated by Orai1 and either of two STIM1 double mutants, DD475/6AA or EE482/3AA, showed similar to WT CRAC channel dependence on pH_i. However, Ca^{2+} dependent gating of these mutant channels showed a significantly weaker dependence on pH_i and the relative expression levels of STIM1 and Orai1, compared to WT. These results suggest that the effects of pH_i on I_{CRAC} amplitude and gating are mediated through different mechanisms. The pH_i dependence of I_{CRAC} amplitude is likely to be mediated by protonatable residues in the Orai1 pore, whereas pH_i dependence of CRAC gating is likely to be mediated by the protonatable residues responsible for Orai1/STIM1 interactions. Considering almost ubiquitous expression of CRAC channels in mammalian cells, their regulation by pH may play an important role in pathophysiology of acidosis- and alkalosis-related conditions.

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