The mechanism of Orai channels dependence on intracellular pH

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The activity of store-operated Ca^{2+} channels formed by Orai1 and STIM1 proteins has been shown to strongly depend on the changes of intracellular pH (pH_i) within the physiological ranges, however, the amino acids responsible for this dependence have not been yet identified (Beck *et al.*, 2014, Gavriliouk *et al.*, 2017, Tsujikawa *et al.*, 2015). Furthermore, it is not known whether these amino acids are localized in Orai1 or STIM1 polypeptides, and whether Ca^{2+} channels made of Orai1 homologues, Orai2 and Orai3, exhibit pH_i dependence similar to that of Orai1.

In this study we investigated dependence of Orai2- and Orai3-mediated Ca^{2+} currents on pH_i using whole-cell patch clamping of HEK293T cells heterologously expressing STIM1 and either Orai2 or Orai3 proteins. Intracellular pH has been varied by application of different concentrations of sodium propionate (C_2H_5COONa) or ammonium chloride (NH_4Cl) to the bath solution.

It was found that intracellular acidification achieved by applying 60 mM propionate to the bath solution inhibited Orai2-mediated Ca^{2+} current by ~80-90%, compared to the current recorded under control conditions (pH_i 7.3). Intracellular alkalinisation using 15 mM NH_4Cl in the bath strongly potentiated Orai2 current amplitude, but only when EGTA was used as intracellular Ca^{2+} buffer. This dependence of Orai2 amplitude on pH_i was very similar to that exhibited by Orai1-mediated current (Gavriliouk *et al.*, 2017). However, unlike in Orai1, the kinetics of Orai2 fast Ca^{2+} dependent inactivation was not affected by intracellular acidification. In contrast, Orai3-mediated store-operated Ca^{2+} current exhibited no dependence on pH_i, suggesting that amino acids that mediate pH_i dependence of Orai1 and Orai2 are localized in intracellular C- or N-termini, or the intracellular loop of these proteins, and are absent in Orai3. Replacement of Orai1 C- and N-termini with those of Orai3 did not affect either Orai1 amplitude or kinetics dependence on pH_i. Replacement of Orai1 intracellular loop with that of Orai3 did, however, abolish Orai1 dependence on pH_i. Surprisingly, mutating the only potentially protonatable glutamates E162 and E164 in the intracellular loop of Orai1, which are absent in Orai3, to glutamine, had no effect on pH_i dependence of Orai1 current, suggesting that protonatable site is localized in STIM1.

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