Ca^{2+} release-activated Ca^{2+} channels are regulated by Calpain

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Calcium release-activated calcium (CRAC) channels provide a major pathway for store operated Ca^{2+} entry (SOCE) in non-excitable cells (Parekh & Putney, 2005). Patients with non-functional CRAC channels suffer from severe immunodeficiency, and succumb due to infection in the first few years of life. CRAC channel dysfunction has been associated with a range of disorders including myopathy, autoimmunity, pulmonary disease, and anhidrotic ectodermal dysplasia (Feske, 2010). Given the importance of CRAC channels in cell homeostasis and Ca^{2+} signalling there is keen interest in uncovering the multitude of ways that CRAC channels are regulated in order to form a platform for the development of possible clinical interventions to treat CRAC channelopathies.

Studies in our lab have indicated that the expression levels of the molecular components of CRAC channel, STIM1 and Orai1 proteins, are regulated by cytoplasmic Ca^{2+}. A recent paper by Prins and Michalak (2015) demonstrated that STIM1, the Ca^{2+} sensor and pore activation molecule of the CRAC channel, is cleaved by calpain, a Ca^{2+}-dependent protease. This suggests that calpain may regulate the levels of functional STIM1 in the cell, thus regulating CRAC channel activity (Prins & Michalak, 2015).

In this work we used H4IIE cells, endogenously expressing CRAC channels, and HEK293T cells transfected with STIM1 and Orai1 plasmids to investigate whether inhibition of calpain by Calpain Inhibitor 2 or PD150606 affects SOCE and CRAC channel current (I_{CRAC}). Measurements of free cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) using Fura-2 AM intracellular Ca^{2+} indicator, SERCA inhibitor 2,5-Di-tert-butylhydroquinone (DBHQ) and Ca^{2+} add-back protocol showed that DBHQ-induced SOCE was potentiated by approximately 25% in H4IIE cells incubated with calpain inhibitors for 24 h compared to the control cells. Increasing baseline $[Ca^{2+}]_{cyt}$ in H4IIE cells, and therefore the activity of calpain, by adding thapsigargin to the incubation medium resulted in a stronger effect of calpain inhibitors on SOCE.

The results obtained using whole-cell patch clamping and HEK293T cells heterologously expressing Orai1 and STIM1 proteins were consistent with the measurements of free $[Ca^{2+}]_{cyt}$ by Fura-2 AM. The amplitude of I_{CRAC} recorded in the cells incubated with PD150606 calpain inhibitor for 24 h was significantly larger than I_{CRAC} amplitude in the control cells, suggesting that inhibition of calpain results in higher expression levels of both STIM1 and Orai1. Analysis of the apparent open probability (P_o) of CRAC channels revealed that incubation of the transfected cells with PD150606 promotes I_{CRAC} potentiation at negative potentials. Previously we have shown that increase in I_{CRAC} potentiation at negative potential is associated with higher expression of Orai1, relative to STIM1 (Scrimgeour *et al.*, 2009). This suggests that inhibition of calpain alters the expression ratio of STIM1:Orai1, increasing levels of functional Orai1 protein in the cell more than it increases the amounts of STIM1. In conclusion, these data indicate that the dependence of the cellular levels of STIM1 and Orai1 proteins on intracellular Ca^{2+} is mediated, at least in part, by calpain.

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