Video Article

Whole-cell recording of Calcium Release-activated Calcium (CRAC) currents in human T lymphocytes

Pratima Thakur[1], Alla Fomina[1]
[1]

Correspondence to: Alla Fomina at affomina@ucdavis.edu

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Abstract

In T lymphocytes, depletion of Ca2+ from the intracellular Ca2+ store leads to activation of plasmalemmal Ca2+ channels, called Calcium Release-activated Calcium (CRAC) channels. CRAC channels play an important role in the regulation of T cell proliferation and gene expression. Abnormalities in CRAC channel function in T cells were linked to severe combined immunodeficiency and autoimmune diseases 1, 2. Studying CRAC channel function in human T cells may uncover new molecular mechanisms regulating normal immune responses and unravel the causes of human diseases. Electrophysiological recordings of membrane currents provide the most accurate assessment of properties and regulation of functional channels. Although electrophysiological assessment of CRAC channel currents in Jurkat T cells, a human leukemia T cell line, was first performed more than 20 years ago,3 recording of CRAC currents in normal human T cells remains a challenging task. The difficulties in recording CRAC channel currents in normal T cells are compounded by the fact that blood-derived T lymphocytes are much smaller in size than Jurkat T cells and, therefore, the endogenous whole-cell CRAC currents are very low in amplitude. Here we provide a step-by-step procedure that we routinely use to record the Ca2+ or Na+ currents via CRAC channels in resting human T cells isolated from the peripheral blood of healthy volunteers. The method described here was adopted from the procedures used for recording the CRAC currents in Jurkat T cells and activated human T cells.4-8

Disclosures

No conflicts of interest declared.

