Analysis of subcellular calcium signals in T-lymphocytes

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Stimulation of T-lymphocytes via the T cell receptor/CD3 complex (TRC/CD3) activates a multitude of intracellular signalling pathways. Among these, Ca^{2+} signalling is one of the essential events involving Ca^{2+} release by intracellular second messengers, but also Ca^{2+} entry via the plasma membrane [reviewed in 1]. In the last years we have demonstrated the involvement of D-myo-inositol 1,4,5-trisphosphate (IP_3), cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate as Ca^{2+} releasing intracellular messengers [2,3]. Objective of the current study was to analyze subcellular Ca^{2+} signals at high temporal and spatial resolution in living human T-lymphocytes.

Stimulation of the TCR/CD3 complex of human T-lymphoma Jurkat cells resulted in a series of subcellular pacemaker Ca^{2+} signals preceding the first global Ca^{2+} signal. The pacemaker signals occurred in a cytosolic “trigger” zone, which was localized close to the plasma membrane. The pacemaker signals were almost independent of extracellular Ca^{2+} as shown by measurements in the absence of extracellular Ca^{2+}, or in the presence of the Ca^{2+} channel blocker SK-F 96365. This indicates that Ca^{2+} release involving the above mentioned Ca^{2+} mobilizing compounds is the major process during this phase. Analysis of the pacemaker Ca^{2+} signals revealed characteristic amplitudes of 82±30 to 109±21nM, signal diameters between 2.5±0.9 and 3.5±1.5μm and frequencies between 0.235 and 0.677 s^-1. However, due to the great variety of values published, it was impossible to assign the subcellular signals in T cells to IP_3 receptor- or ryanodine receptor(RyR)-mediated events. Similar results were also obtained using freshly isolated peripheral human T cells indicating that the signals obtained in the lymphoma cell line were representative also for normal T cells.

For spatial comparison the subcellular distribution of proteins and organelles involved in Ca^{2+} release was investigated by confocal immunohistochemistry in both the Jurkat T cell line and peripheral T cells. The RyR was detected close to the plasma membrane, but also within the inner cytosolic regions, whereas staining of the IP_3 receptor was observed in spot-like structures close to the plasma membrane in Jurkat cells. In peripheral T cells, both the IP_3 receptor and the RyR were evenly distributed in the small cytosolic part of the cells.

Taken together, our data constitute the first analysis of subcellular Ca^{2+} signals in T cells and indicate that the pacemaker Ca^{2+} release events, which are necessary for the development of the global Ca^{2+} signal, are composed of Ca^{2+} release both from IP_3 receptors and RyR.

References