activation (no $[Ca]_i$ increases even after repeated 0 Na steps), but activated after field stimulation (Ca influx via $I_{Ca}(L)$), and reverted over tens of sec on return to rest. Increasing $[Na]_i$ (strophanthidin, 100 μM) made NCX likely to self-activate (0 Na steps became effective) and remain activated or easily reactivated. Mouse cardiomyocytes, where Na pump/leak balance sets $[Na]_i$ higher *vs* rabbit, showed a propensity to self-activation and sustained activation. We simulated NCX activation dynamics in intact cells with a model having fourth-order fully cooperative (Hill) dependence on $[Ca]_i$, with activation $K_{0.5} = 375$ nM and forward rate constant 2.53E9 $mM^{-4}msec^{-1}$, incorporated into a rabbit ventricular cell framework (Shannon et al., Biophys J, 87:3351). The model predicts enhanced and prolonged NCX activation when Na flux balance is changed (↓pump and/or ↑leak) to increase $[Na]_i$ by a few mM. We conclude that NCX Ca-dependent regulation as well as transport are instrinsic to control of NCX (and by implication cardiac Ca handling) by $[Na]_i$. While our use of physiological conditions (no voltage clamp) requires us to infer NCX fluxes rather than observe them directly, it also allows us to follow Ca-dependent activation without artificial control of $[Na]_i$, which might perturb temporal dynamics.

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Quantification of Mlck Activation in Arteries of Living Mice That Express a Genetically Encoded Fret-Based Biosensor

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FRET (Forster resonance energy transfer)-based sensors are powerful tools for understanding physiological mechanisms at the molecular level. Here, we used transgenic mice that express, specifically in smooth muscle, a FRET-based, exogenous myosin light chain kinase biosensor ('exMLCK') to observe for the first time changes in [Ca^{2+}/Calmodulin] and MLCK activation within arteries *in vivo* ('intra-vital FRET imaging'). exMLCK biosensor mice were anesthetized (1.5% isoflurane) and placed on the stage of a 'macro' epi-fluorescence microscope (Olympus MVX 10) equipped with an image splitter and a digital CCD camera. Arterial blood pressure (AP) was recorded. Femoral or mesenteric arteries were exposed through cutaneous incisions. Quantification of exMLCK FRET *in vivo*requires precise assessment and removal of tissue intrinsic fluorescence and extraneous sources of light. Intrinisic fluorescence (of isolated arteries) was negligible in comparison to exMLCK fluorescence. *In vivo*, background fluorescence was minimized by dissection of surrounding tissues and the use of an appropriate field diaphragm. Artery diameter was determined using edge detection and spatially averaged exMLCK FRET ratio (CFP/YFP) was determined from regions of interest. In the basal state (mean AP of ~90 mm Hg), average exMLCK FRET ratio in femoral artery walls was 1.8 to 1.9, similar to that obtained by us in isolated arteries. Application of the α1-adrenoceptor agonist, phenylephrine (PE) directly to an artery increased exMLCK FRET ratio transiently to a peak of about 2.2 and caused a local vasoconstriction of ~33%, without changing AP. Intra-venous application of PE elevated AP and caused smaller changes in exMLCK FRET ratio and artery diameter (than direct application). In conclusion, quantitative intra-vital FRET imaging in arteries of transgenic animals is feasible and will permit observation of specific molecular events in tissues of living animals. (AHA, NIH-HL078870).

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Systematic Characterization of Initial Calcium Signaling in T Cells

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Elevation of intracellular free Calcium is part of the key signals during T-cell activation by antigens. Following activation a remarkable variety of this signals - ranging from infrequent spikes to sustained oscillations and plateaus - is shaped by the interactions of the different Calcium sources and sinks in the cell. We present an approach to study calcium signalling in T-cells at a large scale in parallel fashion that allow to extract proteins and their interactions involved in generating this Calcium signals. Briefly T cells, knock out T cell lines and T cells with proteins knocked down by siRNA were synchronized and exposed to surfaces, coated with stimulatory and non stimulatory antibodys. The assay yielded a data set of several thousand calcium traces from which parameters like number of spikes or length of plateaus were extracted and used to cluster the data hypothesis free. Based on this similarities and differences between signalling pathways are inferred, which may provide the basis to systematically explore proteins and their interactions governing calcium signalling pathways in T-cells.

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Structural Determinants of Ion Permeation in Crac Channels

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CRAC channels generate Ca2+ signals critical for the activation of immune cells and exhibit an intriguing pore profile distinguished by extremely high Ca2+ selectivity, low Cs+ permeability, and small unitary conductance. To identify the conduction pathway of the transported ions and gain insight into the structural bases of these characteristics, we introduced cysteine residues in the CRAC channel pore subunit, Orai1, and probed their accessibility to various thiol-reactive reagents. Our results indicate that the architecture of the ion conduction pathway is characterized by a flexible outer vestibule formed by the TM1-TM2 loop, which leads to a narrow pore flanked by residues of a helical TM1 segment. Residues in TM3, and specifically, E190, a residue considered important for ion selectivity, are not close to the pore. Moreover, the outer vestibule does not significantly contribute to ion selectivity, implying that Ca2+ selectivity is conferred mainly by E106 in the TM1 segment. The pore is sufficiently narrow along much of its length to permit stable coordination of Cd2+ by several TM1 residues, which likely explains the slow flux of ions within the restrained geometry of the pore. Together, these results reveal new insights into the long-sought structural basis for the unique permeation properties of CRAC channels.

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Orai1 Expression, Mitosis and Cell Cycle Progression in HEK293 Cells

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Calcium influx is needed for cell proliferation and recent identification of Orai1 as the main constituent for both store-operated (SOCE) and non capacitative (NCCE) calcium entries led us to investigate the role of Orai1 in mitosis and cell cycle progression in HEK293 cells. 10μM RO-3306, a cyclin dependent kinase 1 (cdk-1) inhibitor was applied for 24 hours to block 90% of HEK293 cells in G2/M phase. Mitotic index was measured every 15 minutes for an hour after release from cell cycle block. Mitosis was observed after 15 minutes and reached a maximum of 50% of the total cells after 45 minutes while no mitosis was observed in the presence of RO-3306. Cell cycle progression after release from RO-3306 block was assessed using FACS analysis and 20% of the cells entered G1 phase after 1 hour. We monitored cell cycle progression over a period of 24 hours in control and Orai1 knock down (siOrai) cells and we observed that progression through G1 phase depended on the presence of Orai1, as the number of cells in S phase 15 hours after release was twice lower in siOrai cells. We have observed that Orai1 expression was reduced by 70% in the presence of RO-3306 with full reversion within 4 hours after release from block. Calcium imaging and whole-cell voltage clamped recordings showed that SOCE was reduced by 60% in the presence of RO-3306, that no change was observed one hour after release from block, and that full recovery was achieved in less than 4 hours. Our results indicated that mitosis occurs even at low Orai1 expression and little calcium influx, while larger calcium influx is needed to speed up cell cycle progression.

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Biophysical Properties of Calcium Homeostasis Modulator 1 (CALHM1) Ion Channel

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CALHM1 was identified recently as a membrane protein expressed in the hippocampus and linked to late onset Alzheimer's disease (*Cell,133:1149(2008)*). Here, we demonstrate its permeation and gating properties in plasma membrane. The relative permeability sequence determined from reversal potentials is: P_{Na^+}: P_{K^+} : $P_{Ca^{2+}}$: P_{Cl^-} = 1: 1.2: 4.4: 0.52, indicating that CALHM1 is a Ca^{2+}-permeable channel. Inward currents elicited by lowering extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) were inhibited by Gd^{3+}, but not by blockers of connexin or NMDA receptors. CALHM1 channels are activated by voltage and by lowering $[Ca^{2+}]_o$ with an IC_{50} of 250 μM at a holding potential of −15 mV. 5 mM Ca^{2+} shifts the G-V relation by +150 mV and increases the slope (Z_e) by 3-fold without reducing G_{max} (Boltzman fits in 0 Ca^{2+}: $V_{0.5} = -70$ mV, $Z_e = 0.54$; in 5 mM Ca^{2+}: $V_{0.5} = +82$ mV, $Z_e = 1.48$), indicating that CALHM1 has an intrinsic voltage-dependent gate, although it lacks an S4-like domain, and that $Ca^{2+}{}_o$ regulates voltage-dependent gating by voltage-dependent conformation changes rather than by voltage-dependent blockage, with high selectivity for Ca^{2+} over Mg^{2+} (IC_{50} 0.25 vs 3 mM). Mild oxidation of CALHM1 by 0.1% H_2O_2 alters this Ca^{2+}-regulation, resulting in channel more leaky at physiological conditions. Biochemical and single molecule bleaching measurements suggest that CALHM1 is oligomeric with six monomers comprising the pore. Together these properties suggest that CALHM1 is a novel Ca^{2+}-permeable cation channel, which is regulated by voltage, $Ca^{2+}{}_o$ and oxidative stress. Insights into the properties of CALHM1 channels may help us to understand the mechanism of Ca^{2+} influx through CALHM1 in physiological and pathological conditions, and to facilitate therapeutic interventions in Alzheimer's disease.