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of 24-months old WT mice as compared to 4-months adult mice. In contrast, cardiac specific Cav3OE mice had normal cardiac function with no detectable fibrosis and cardiac hypertrophy at 24-months, which was similar to 4-months adult WT or Cav3OE mice. Whole-cell patch clamp analysis showed significantly increased APD50 (44.89 ± 8ms) and APD90 (66.5 ± 11ms) (n=6) in the ventricular myocytes of 24-month aged mice compared to 4-months adult mice (APD50: 8.9 ± 2ms; APD90: 17.88 ± 6ms; n=6). A re-expression of $I_{Ca,T}$ (−2.1 ± 1pA/pF) observed in ventricular myocytes from 24-months aged WT mice suggested possible cause for altered Ca^{2+} signaling and induction of ventricular hypertrophy. In contrast, ventricular myocytes from 24-months aged Cav3OE showed a normalized action potential (APD50:11 ± 3ms; APD90:47.5 ± 6ms; n=8) and negligible $I_{Ca,T}$ (−0.3 ± 0.5 pA/pF). We conclude that a reduced Cav3 expression in aged ventricle contributes to arrhythmia, altered Ca^{2+} signaling via re-expressed $I_{Ca,T}$ and cardiac dysfunction. Cardiac specific overexpression of Cav3 prevents aging induced arrhythmia, cardiac dysfunction and hypertrophy.

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Nucleoplasmic [Ca] Transients Alterations and Perinuclear Ca Stores Remodeling after Pressure Overload-Induced Hypertrophy in Adult Cardiac Myocytes

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Nucleoplasmic calcium concentration ([Ca]) in cardiac myocytes (CMs) regulates exitation-transcription coupling and is involved in remodelling processes. Perinuclear Ca stores contribute to the regulation of nucleoplasmic [Ca] transients (CaTs). We thus characterized alterations in perinuclear Ca stores and nucleoplasmic CaTs after pressure overload-induced hypertrophy in adult CMs.
Pressure overload was induced by transverse aortic constriction (TAC) in adult wild-type mice. Sham-operated mice served as controls. Ventricular CMs were isolated 1 and 7 weeks after TAC/Sham. Perinuclear Ca stores were visualized using confocal imaging and staining with Mag-Fluo-4/AM (10μM). CaTs were recorded in electrically-stimulated CMs loaded with Fluo-4/AM (8μM).
In Sham CMs, staining of perinuclear Ca stores revealed a nuclear envelope and tubular structures transversing the nucleus. Rapid application of caffeine (20 mM, n=7) reversibly abolished Mag-Fluo-4 fluorescence. Fluorescence recovery after depletion was identical in nuclear envelope and tubular structures. A significant increase in number of tubules per nucleus was observed during physiological growth (1week: 4.2 ± 0.2; 7weeks: 4.7 ± 0.2 (n=90)). Nuclear dimensions as well as cyto- and nucleoplasmic CaTs remained unaltered. In TAC CMs, the number of tubules progressively decreased (1week; 4.3 ± 0.2; 7weeks: 3.4 ± 0.2 (n=90)), whereas length and width of nuclei increased (1week: 13.3 ± 0.3 and 4.9 ± 0.2μm; 7weeks: 17.8 ± 0.7 and 5.5 ± 0.2μm). One week after TAC changes in the kinetics and amplitude of CaTs were found in the nucleus (TTP: 127 ± 2 vs 194 ± 5ms; RT_{50}: 360 ± 7 vs 380 ± 5ms; amplitude: 491 ± 19 vs 376 ± 28nM; Sham vs TAC (n=15); all $P<0.05$). Seven weeks after TAC similar changes also occurred in the cytoplasm.
Perinuclear Ca stores and nucleoplasmic CaTs undergo significant changes during hypertrophy progression, which appear to precede changes in cytoplasmic Ca regulation. These results raise the possibility that altered nucleoplasmic [Ca] may contribute to the development and/or progression of hypertrophy.

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Histamine-Evoked Signaling in Human Primary Keratinocytes

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Itch, the unpleasant sensation that evokes a desire to scratch, is mediated by a subset of primary afferent C-fibers. Several lines of evidence suggest that keratinocytes also play an active role in itch signaling. First, keratinocytes release nerve growth factor (NGF), a molecule implicated in chronic itch. Second, keratinocytes secrete a number of pruritogens, including histamine, endothelin-1 (ET-1) and leukotriene B_4. Third, keratinocytes are directly activated by a number of pruritogens. To elucidate the role of keratinocytes in the transduction of itch, we are probing the mechanisms underlying itch-evoked calcium signaling in primary human keratinocytes. Using ratiometric Ca^{2+} imaging, we found that keratinocytes respond robustly to three pruritogens: histamine (10 μM), ET-1 (100 nM) and the peptide activator of PAR2, SLIGRL (10 μM). These pruritogens trigger both Ca^{2+} release from stores and Ca^{2+} influx across the plasma membrane. Histamine signaling requires the histamine receptor 1 (H1R), as the H1R-specific antagonist, diphenhydramine (10 μM), completely blocks all calcium signals. Pruritogen-evoked calcium signals also require functional store-operated channels (SOCs), as 2-Aminoethoxydiphenyl borate (2-APB; 50 μM), Gd^{3+} (10 μM) and La^{3+} (50 nM) inhibit pruritogen-evoked calcium influx. Consistent with our pharmacological findings, real time qPCR and microarray gene expression analysis demonstrate that Orai1 is highly expressed in both human and mouse primary keratinocytes. Thus, Orai1 may be a channel onto which multiple itch pathways converge to trigger pruritus.

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Quantitative Characterization of Local Chemical Delivery through Nanopipette

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Scanning Ion Cunductance Microscope (SICM) produces 3D images of live cells with nanometer resolution. In the past, SICM nanopipette probe was used for chemical agent delivery(1) and also to map potassium channels in cardiomyocytes(2). However these applications had little control over the concentration of applied agent that was only estimated from the geometry of the pipette tip. We have used two different methods to quantify and monitor chemical delivery. A highly sensitive electrochemical substrate sensor was fabricated, to quantify the spatiotemporal distribution of delivered electrochemical mediators by SICM nanopipette. The amount of substance released at different pressures and voltages were measured and calibrated by the electrochemical sensor. A double barrel nanopipette with integrated detection sensor was also fabricated to monitor the concentration of mediator near the tip. One barrel was filled with carbon as electrochemical sensor and the other barrel was filled with the mediator. Using this approach and by applying different pressures and voltages to the nanopipette, concentration of chemicals at the tip can be controlled. We were able to trigger localised receptor mediated responses in neurons, by varying local concentration of agonists under the SICM nanopipette tip. This will open the possibility for functional mapping of receptor mediated responses in cells.

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Cytosolic Calcium Dependency of STIM1 De-Oligomerization

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Store operated calcium entry (SOCE) is a universal mechanism that cells use to replenish their intracellular calcium stores. SOCE activation is regulated by STIM1, an endoplasmic reticulum (ER) transmembrane protein with calcium binding EF-hand domains protruding into the ER lumen. Upon depletion of ER calcium stores, the dissociation of calcium ions from STIM1 EF-hands triggers a conformational change that leads to the formation of STIM1 oligomers, which translocate to the plasma membrane to bind and activate calcium channels of the Orai family. Once ER stores are replenished, STIM1 oligomers detach from plasma membrane channels and subsequently de-oligomerize. Whether changes in ER calcium levels regulate STIM1 oligomerization and de-oligomerization in a similar fashion is not known. In this study, we used reversible SERCA inhibitors to deplete and refill ER calcium stores in HeLa cells and measured by FRET the kinetics of oligo- and de-oligomerization between CFP-STIM1 and YFP-STIM1. We found that the refilling of ER calcium stores was not sufficient to de-oligomerize STIM1 molecules. Using BAPTA-AM to increase the cytosolic calcium buffering capacity of cells, we could fully refill ER stores without inducing the de-oligomerization of STIM1. De-oligomerization of STIM1 in BAPTA-loaded cells occurred upon readmission of Ca2+ to the extracellular medium, and was prevented by SOCE channel inhibitors (La3+ and Gd3+). Applying divalent ion Ba2+ in substitution for Ca2+ at the readmission stage induced only a partial and temporal STIM1 de-oligomerization. These data indicate that an elevation of the cytosolic Ca2+ concentration is required for the induction of the de-oligomerization process while ER refilling is not sufficient. Our findings thus indicate that STIM1 oligomerization exclusively depends on ER Ca2+ content, while de-oligomerization depends both on ER and cytosolic Ca2+ concentrations.

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Conformational Rearrangements of STIM1 Cytosolic Portion Sensed by Altered Coiled-Coil Accessibility

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STIM1 and Orai1 are key players of the Ca2+-release activated Ca2+ (CRAC) current that plays an important role in T cell activation as well as mast cell degranulation. Activation of the CRAC channel forming subunit