ophores populations that occur during changes in metabolism. To evaluate the impact of these changes, we have recently employed two-photon excited NADH Fluorescence Lifetime Imaging. Treatment with both metabolic uncouplers and inhibitors caused systematic shifts in both the lifetime and the free to bound ratio of NADH. Assessment of metabolic state by the intensity-based and lifetime-based techniques will be compared for both monolayer cultures and the hair cells of the excised organ of Corti.

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Store-operated Ca^{++} Channels

2933-Pos Store-Operated Ca^{2+}-Induced Ca^{2+} Release Amplifies Cytosolic Ca^{2+} Signaling and Prevents Store Refilling in Jurkat T Cells

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The precise control of many T cell functions relies on cytosolic Ca^{2+} dynamics which is shaped by the release of Ca^{2+} from intracellular store and extracellular Ca^{2+} influx via plasmalemmal store-operated Ca^{2+} (SOC) channels. It is presumed that Ca^{2+} influx via SOC channels is required for store refilling. However, using Fura2 Ca^{2+} probe to simultaneously assess the store content and Ca^{2+} dynamics in the cytosol, we demonstrated that in T lymphocytes the store refilling was inhibited in the presence of extracellular Ca^{2+} and SOC channels activated by store depletion with cyclopiazonic acid (CPA), a reversible blocker of the sarco-endoplasmic reticulum Ca^{2+}-ATPase. Pretreating cells with xestospongin C (10 μM) or ryanodine (400 μM), the antagonists of inositol 1,4,5-trisphosphate (IP3R) or ryanodine (RyR) receptors, respectively, facilitated store refilling while reducing cytosolic Ca^{2+} transients associated with SOC channel activation. These data indicate that Ca^{2+}-induced Ca^{2+} release (CICR) is an essential source of Ca^{2+} for elevation of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) upon stimulation. Consistently, inhibition of IP3R or RyR significantly inhibited T cell proliferation and IL2 production. Finally, we observed that store refilling persisted when store-operated Ca^{2+} entry (SOCE) was completely inhibited by 1 μM extracellular La^{3+} (IC_{50} ~ 30 nM), confirming that store can be refilled in the absence of SOCE and global $[Ca^{2+}]_i$ elevation. Store refilling was inhibited by higher $[La^{3+}]$ (IC_{50} ~ 5 μM) suggesting the existence of a store-independent store replenishing pathway that may sustain CICR. We conclude that CICR is essential mechanism regulating $[Ca^{2+}]_I$ dynamics in T cells and that IP3R and/or RyR may represent novel pharmacological targets for manipulation of Ca^{2+}-dependent functions of T lymphocytes.

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WITHDRAWN

2935-Pos Different STIM to Orai1 ignaling pathways between *C. elegans* and human

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Endoplasmic reticulum (ER) Ca^{2+} depletion evokes Ca^{2+} entry through Ca^{2+} release activated Ca^{2+} (CRAC) channel in the plasma membrane (PM). Recent studies have identified STIM1 and Orai1 as essential components of CRAC channel. The molecular mechanism by which STIM1 is translocated and targeted on the plasma membrane (PM) and convey signals from the ER lumen to PM is not yet understood. Here we report that in HEK293 cells the nematode *Caenorhabditis elegans* STIM1 (C.STIM1) are pre-oligomerized in puncta at the cell periphery before store depletion, and this oligomization of STIM1 is not sufficient for the aggregation of Orai1 on the opposing PM and CRAC activation. Therefore, C.STIM1 does not have the store-depletion induced translocation steps of human STIM1 (H.STIM1). In contrast, the C.STIM1 puncta and C. elegans Orai1 functioned as a pair that can locally respond to ER store depletion and lead to activation of CRAC channel. By switching the N- and C- termini of C.STIM1 to H.STIM1, we prove that the STIM1 C-terminus decides its resting localization in different organisms. A mutant H.STIM1 lacking the C terminal proline-rich domain and the polybasic sequence motif (PPK) that are absent in C. STIM1 shows the same distribution as C.STIM1 and colocalized with C.STIM1 near the PM before store depletion, proving that targeting STIM1 on the PM is dispensable of PPK domain. Taken together, our results suggest that: 1) *C. elegans* uses a different signaling pathway of STIM1 compared with human, but uses conserved targeting machinery in the C-terminus to trap STIM1 to the cell periphery. 2) The mammalian C-terminal PPK domain evolves as an autoinhibitory mechanism by which STIM1 conformation changes after Ca^{2+} store depletion and exposes the PM-targeting motif for its targeting on the PM.

2936-Pos Functional Comparison Of ORAI Homologs In Regulating Mammalian CRAC Channel Activity

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RNAi screening has identified a requirement for Stim and Orai and their mammalian homologs to produce store-operated Ca^{2+} entry (SOCE) and CRAC channel activity. Here, we evaluate the three human homologs of Orai expressed together with STIM1 separately and in combinations in HEK cells. Promiscuous heteromultimerization within Orai members was found by co-immunoprecipitation. Coexpressed with STIM1, Orai1 induced a large inwardly rectifying Ca^{2+} current with a form of Ca^{2+}-induced slow inactivation. A point mutation of Orai1 (E106D) drastically altered the ion selectivity of the induced CRAC current and eliminated slow inactivation while retaining an inwardly rectifying I–V characteristic. Using the amplified current model of co-expressed Orai1 + STIM1, we show that CRAC current can be recorded in cell-attached and inside-out patches. A C-terminal portion of Stim lacking the putative Ca^{2+}-binding motif and the transmembrane segment interacted with Orai by co-immunoprecipitation and effectively activated Ca^{2+} influx in S2 cells in the absence of store depletion. Expression of the corresponding STIM1 C-terminus, coexpressed with Orai1, was sufficient to generate CRAC current without store depletion. Furthermore, 50 μM 2-APB is able to sensitize/activate exogenous Orai3 channel activity without store depletion. The structural determinants responsible for the different Orai channel activities are being revealed by analyzing a series of Orai chimeras and point mutants.

2937-Pos Differential Pharmacology of 2-aminoethoxydiphenyl borate (2-APB) on CRAC Channels

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STIM1 in the endoplasmic reticulum (ER) and CRACM1 (or Orai1) in the plasma membrane are essential molecular components for controlling store-operated Ca2+ entry (SOCE) via CRAC channels. Combined over-expression of both proteins reconstitutes amplified CRAC currents and all three mammalian CRAC channel homologs (CRACM1, CRACM2 and CRACM3) represent functional store-operated channels with distinctive properties in terms of kinetics of activation, selectivity for Ca2+, Ba2+, and different feedback regulation by intracellular Ca2+. The dissection of the various molecular components of SOCE is complicated and only few pharmacological tools are available to address this problem. Although 2-aminoethoxydiphenyl borate (2-APB) cannot be considered a SOCE-specific compound, it has previously been found to affect native CRAC channels by potentiating CRAC currents at low concentrations (< 5 μM) and inhibiting them at high concentrations (> 10 μM). We have previously reported that 50 μM 2-APB completely blocks CRACM1, reduces CRACM2 by ~50% and fails to suppress CRACM3, but enhances it ~7-8 fold. We now have qualitatively and quantitatively assessed the effects of 2-APB on both store-operated and store-independent activation of CRAC channels and reveal that the three homologs exhibit strikingly different pharmacological responses to different concentrations of 2-APB that are mediated by differential effects on the CRAC channel itself as well as through its interaction with STIM1.

2938-Pos ADAR-mediated RNA Editing of Orai1 is A Key to Different Selectivity of Store-operated Channels

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Store-operated Ca^{2+} entry (SOCE) is known to be mediated by store operated channels (SOCs) that may have different Ca^{2+} selectivity. While Orai1 is thought to encode Ca^{2+}-selective SOC (CRAC) in nonexcitable cells, the molecular nature and the reasons for a poor cation selectivity of SOCs in other cell types remain a mystery. Here we present first evidence that Orai1 may encode different types of SOC, and the variations in their selectivity may result from post-translational modification of Orai1, with ADAR-mediated RNA editing being a key to this process. Patch-clamp, Ca^{2+} imaging and molecular approaches were used to study Ca^{2+}-selective CRAC in RBL-2H3 cells and cation-selective SOC (cat-SOC) in SMC. We discovered that: 1) siRNA knock down of Orai1 impairs SOCE and whole-cell SOC currents in both, RBL and SMC cells; 2) ADAR1 expression in RBL cells is significantly lower than in SMC; 3) overexpression of functional ADAR1 in RBL results in transformation of inwardly rectifying I_{CRAC} into a linear current, and appearance of significant Mn^{2+} influx through SOCE pathway; 4) siRNA knock down of endogenous ADAR1 in SMC results in disappearance of significant Mn^{2+} influx, and appearance of inwardly rectifying I_{CRAC} component of the whole-cell current. Thus, expression and functional activity of ADAR1 may change the selectivity of SOC channels. We propose that while the original (un-edited) Orai1 molecule forms Ca^{2+}-SOC(CRAC), ADAR-mediated RNA editing of specific residues may create post-transcriptional modified variants of Orai1 that form cat-SOCs with different cation selectivity. These important findings resolve the long lasting controversy about the relationship between SOC channels with different cation selectivity, and demonstrate how one gene (Orai1) may encode different SOCs, with ADAR-dependent RNA editing adjusting their selectivity to the specific needs of different cell types.

Membrane Receptors & Signal Transduction - I

2939-Pos Endothelial Cells Increase Tumor Cell Invasion

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