STIM1 C-terminus, which in line substantially increased binding to Orai1. In aggregate, our data support the theory of flexible regions within STIM C-terminus that undergo conformational rearrangement upon coupling to Orai1. (Supported by FWF-P21118)

3529-Pos

Cch1 Restores Intracellular Calcium in Fungal Cells during ER Stress

Min-Pyo Hong, Kiem Vu, Jennifer Bautos, Angie Gelli.
University of California, Davis, CA, USA.

Pathogens endure and proliferate during infection by exquisitely coping with the many stresses imposed by the host as a means to prevent pathogen survival. Recent evidence has shown that fungal pathogens and yeast respond to insults to the ER (endoplasmic reticulum) by initiating Ca^{2+} influx across their plasma membrane. Although the high-affinity Ca^{2+} channel, Cch1 and its subunit Mid1, have been suggested as the protein complex responsible for mediating Ca^{2+} influx, a direct demonstration of the gating mechanism of the Cch1 channel remains elusive. In this first mechanistic study of Cch1 channel activity we show that the Cch1 channel from the model human fungal pathogen, *Cryptococcus neoformans*, is directly activated by the depletion of intracellular Ca^{2+} stores. Electrophysiological analysis revealed that agents that enable ER Ca^{2+} store depletion promote the development of whole-cell inward Ca^{2+} currents through Cch1 that are effectively blocked by La^{3+} and dependent on the presence of Mid1. Cch1 is permeable to both Ca^{2+} and Ba^{2+} however, unexpectedly, in contrast to Ca^{2+} currents, Ba^{2+} currents are steeply voltage-dependent. Cch1 maintains a strong Ca^{2+} selectivity even in the presence of high concentrations of monovalent ions. Single channel analysis indicated that Cch1 channel conductance is small, similar to that reported for the Ca^{2+} current I_{CRAC}. This study demonstrates that Cch1 functions as a store-operated Ca^{2+}-selective channel that is gated by intracellular Ca^{2+} depletion. In ER stress conditions, Cch1 is poised to restore Ca^{2+} homeostasis and consequently fungal pathogens like *C. neoformans* require Cch1 activity for survival and colonization of the host.

3530-Pos

SERCA and IP_3R Expression and Function in Vascular Smooth Muscle is Altered Throughout Atherosclerotic Progression

Marie-Ann Ewart[1], John G. McCarron[2], Simon Kennedy[1], Susan Currie[2].
[1]University of Glasgow, Glasgow, United Kingdom, [2]University of Strathclyde, Glasgow, United Kingdom.

Peroxynitrite, the reaction product of superoxide and nitric oxide, forms in diseased vessels and has been shown to induce relaxation in vascular smooth muscle (SM). Here we demonstrate that relaxation of isolated aorta to peroxynitrite (30μM) is altered between healthy C57/BL-6 [25.2+/-6% (*n=10*)] and atherosclerotic $ApoE^{-/-}$ mice [59.3% and 22.4% after 2 (*n=13,p<0.05*) and 4 months (*n=13*) high fat diet (hfd)]. Inhibition of peroxynitrite relaxation by 3μM SERCA inhibitor thapsigargin (TG) or 60μM IP_3 receptor blocker 2-aminoethoxydiphenyl borate (2-APB) is also changed in atherosclerotic vessels [% reduction TG:30.1, 51.7 and 31.2 in C57 (*n=7*), 2mo (*n=13,p=0.05*) and 4mo (*n=11*)hfd $ApoE^{-/-}$; % reduction 2-APB:7.2, 60.3 and 26.7 in C57 (*n=9*), 2mo (*n=13,p<0.05*) and 4mo (*n=13*) hfd $ApoE^{-/-}$], potentially indicating altered SERCA and IP_3R Ca^{2+} handling mechanisms. Aorta expression levels of SERCA2b and IP_3R1 were found to be significantly down-regulated in atherosclerotic mice [56% SERCA (*n=8,p<0.05*) and 39% IP_3R (*n=9,p<0.05*)] confirming changes at the protein level. Further characterisation of functional changes was performed by estimation of SERCA activity. This was done by measuring calcium levels and rise rates following the addition of 10mM caffeine +/- 1μM TG. Increases in cytosolic calcium were found to be larger in $ApoE^{-/-}$ [ΔF/F0 caffeine:1.88+/-0.24, 1.96+/-0.22 and 2.62+/-0.24 in C57 (*n=6*), 2mo (*n=4*) and 4mo (*n=3,p<0.05)* hfd $ApoE^{-/-}$; ΔF/F0 TG:1.05+/-0.2, 1.01+/- 0.08, 1.41+/-0.2 in C57 (*n=6*), 2mo (*n=4*) and 4mo (*n=3*) hfd $ApoE^{-/-}$]. Calcium rise rates were also calculated [ΔF/ms:4.28· 2.95 and 5.82 x10^{-5} in C57 (*n=4*), 2mo (n=4) and4mo hfd $ApoE^{-/-}$ (*n=3*)] and indicate altered SERCA activity in atherosclerotic vessels. These data suggest that the changes observed in SM Ca^{2+} handling in atherosclerosis may be largely due to modulation of SERCA and IP_3R expression and function.

3531-Pos

Extracellular Ca2+ and Glutamate Modulating the Function of Metabotropic Glutamate Receptor 1 Alpha (mGluR1α)

Yusheng Jiang.
Georgia State University, Atlanta, GA, USA.

Metabotropic Glutamate Receptor subtype 1 alpha (mGluR1α) mediates the accumulation of IP3 and DAG, release of intracellular Ca^{2+} from ER, and activation of PKC and PKA, by interacting with extracellular glutamate and Ca^{2+}. While more than 12 X-ray Structures of different forms of the extracellular domain (ECD) has been determined, the detailed binding locations of Ca^{2+} still remain unidentified. In this study, we first report our prediction of several Ca^{2+} binding sites in the ECD of mGluR1a using our newly developed computational algorithms. Putative residues involved in the calcium binding were verified using a grafting approach. Their capability to bind Ca^{2+} and its trivalent analog, Tb^{3+} were determined using fluorescence energy transfer and NMR. The substitution of charged or polar ligand binding residues with Ile at Site 1 resulted in up to 99-fold decrease in the Tb^{3+} binding affinity. In transiently-transfected HEK cells, mutations on these proposed ligand binding residues in the predicted calcium binding site either resulted in increase or decrease of intracellular Ca^{2+} response toward changes in $[Ca^{2+}]_o$. When treated mGluR1α with glutamate and Ca^{2+} together, the intracellular Ca^{2+} response is significantly greater than individual effects by glutamate or Ca^{2+}, suggesting a cooperative effects by calcium binding and glutamate.

3532-Pos

Expression and Regulation of Shark NCX Gene in Transgenic Mouse Heart

Sarah Haviland[1], Lars Cleemann[2], Tim McQuinn[3], Michael Kern[3], Martin Morad[2].
[1]Georgetown University, Washington, DC, USA, [2]University of South Carolina, Charleston, SC, USA, [3]Medical University of South Carolina, Charleston, SC, USA.

Inward currents generated by the mammalian cardiac Na^+-Ca^{2+}-exchanger (NCX1.1) have arrhythmogenic potential, especially in the failing heart where expression of NCX is up-regulated. We hypothesize that arrhythmogenesis might be alleviated if NCX were subject to the same cAMP mediated regulation (suppression of Ca^{2+}-influx, but enhancement of Ca^{2+}-efflux on NCX) as found in the native shark ventricle. To test this hypothesis, we created heterozygous transgenic mice that express the shark NCX protein with a myc-tag, under the control of the alpha-myosin heavy chain promoter (α-MHC). The construct was evaluated by expression and functionality prior to production of transgenic lines. The expression of the transgene was confirmed by immunocytochemistry staining using DAPI and myc-FITC antibody on transfected HL-1 cells. Using dual laser confocal microscopy, the pattern of staining was consistent with NCX expression at the protein level. To determine the functionality of the transgene, HL-1 cells were co-transfected with the shark NCX transgene and GFP. GFP positive cells were incubated with Fluo-4 AM and imaged confocally. These cells showed NCX activity in response to withdrawal and readmission of $[Na^+]_o$ consistent with electrophysiological data of native shark myocytes.
Echocardiography and ECG studies on transgenic mice showed no remarkable cardiac phenotype, but analysis of initial voltage-clamp and western blot studies verify robust exchanger currents and expression levels. Our findings show that shark NCX is functional in the transgenic mouse producing no discernable cardiac pathology, but it remains to be determined as yet whether shark NCX can confer anti-arrhythmic properties to the mammalian heart.

Exocytosis & Endocytosis II

3533-Pos

Chemomechanical Regulation of Snare Proteins Studied with Molecular Dynamics Simulations

Lars Bock[1], Brian Hutchings[2], Helmut Grubmüller[1], Dixon J. Woodbury[2].
[1]MPI for biophysical chemistry, Goettingen, Germany, [2]Brigham Young University, Provo, UT, USA.

SNAP-25B is a neuronal protein required for neurotransmitter (NT) release and is the target of Botulinum Toxins A and E. It has two SNARE motifs that form a four helix bundle when combined with syntaxin1A and synaptobrevin. Formation of the three protein complex requires both SNARE motifs of SNAP-25B to align, stretching out a central linker. The N-terminal of the linker has four cysteines within eight amino acids. Palmitoylation of these cysteines are thought to target SNAP-25B to the membrane, however these cysteines are also an obvious target for oxidation, which has been shown to decrease SNARE complex formation and NT secretion. We hypothesize that since the linker is not much longer than the SNARE complex, formation of a disulfide bond between two cysteines could shorten it sufficiently to reduce secretion by limiting complex formation.
Molecular Dynamics simulations of the SNARE complex, including a modeled linker in the oxidized and reduced state, respectively, reveal drastic conformational differences and a reduction of helical content in SNAP-25B upon oxidation. Further, the conformations of three hydrophobic layers, crucial for the helix association are significantly different. We therefore suggest that oxidation of the cysteines leads to a dysfunctional SNARE complex, thus down-regulating NT release during oxidative stress.