Calcium Signaling Proteins

456-Pos Board B256
Expression and Subcellular Localization of TRPC3, Orai1, and STIM1 after Pressure Overload-Induced Hypertrophy in Adult Cardiac Myocytes
Senka Ljubojevic, Simon Sedej, Michael Holzer, Gunther Marsche, Vanja Marijanski, Jens Kockskämper, Burkert Pieske.
Recent studies have suggested that store-operated Ca2+ entry (SOCE) regulates physiological and pathological cardiac growth. Molecular players identified to contribute to SOCE are TRPC family members and the Orai1/STIM1 complex found in neonatal cardiac myocytes (CMs). However, whether the expression and subcellular distribution of TRPC3, Orai1 and STIM1 are changed in adult CMs during the development and progression of hypertrophy, remains unknown. Pressure overload-induced hypertrophy was induced by minimally invasive transverse aortic constriction (TAC) in adult wild-type mice. Sham-operated mice served as controls. Left ventricular tissue homogenates obtained 1, 3, and 6 weeks after TAC were subjected to immunoblotting. Immunocytochemistry was performed in isolated CMs 6 weeks after TAC. TRPC3, Orai1, and STIM1 were constitutively expressed in Sham and TAC hearts. Co-immunoprecipitation revealed association of STIM1 with Orai1 and TRPC3. Following TAC, mice developed progressive hypertrophy as evidenced by echocardiographic parameters and gravimetric analysis. In TAC hearts, TRPC3 expression progressively increased (1 week: +13 ± 3%, 3 weeks: +32 ± 1%, 6 weeks: +32 ± 1%), whereas Orai1 expression decreased (1 week: −32 ± 1%, 3 weeks: −38 ± 2%, 6 weeks: −38 ± 2%) during the progression of hypertrophy. STIM1 expression remained unchanged (n=7). In Sham CMs, TRPC3 was distributed in a striated pattern throughout the cells. Orai1 was expressed on the plasma membrane and in a striated pattern throughout the cells, with the higher density in the cell periphery. STIM1 appeared in a punctate pattern throughout the cells. In TAC CMs, by contrast, TRPC3 was predominantly found in the cell periphery. Orai1 and STIM1 subcellular distribution appeared unaffected during pressure overload.
In summary, we observed altered expression of TRPC3 and Orai1 and altered subcellular distribution of TRPC3 in pressure overload-induced hypertrophy in mice. The results raise the possibility that altered SOCE may contribute to the development and/or progression of hypertrophy.

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Calcineurin a Subunit Silencing Reduces Sera2 Expression in Cardiac Myocytes
Anand M. Prasad, Giuseppe Inesi.
Resting intracellular Ca2+ can be raised, in neonatal rat cardiac myocytes, by exposure to very low concentration of thapsigargin (TG). Such a Ca2+ rise yields calcineurin (CN) activation, demonstrated by increased expression of transfected luciferase cDNA under control of nuclear factor of activated T-cells (NFAT) promoter. We found that exposure of cardiac myocytes to TG is folowed by increase of SERCA2 expression, which is further increased when CN inactivation by CAMKII (calmodulin dependent kinase) is prevented with Kemptide (tissue calcineurin inhibitor). On the other hand, SERCA2 expression and luciferase activity can be prevented by CN inhibition with cyclosporine. We have now induced calcineurin A (CNA) alpha or beta subunit gene silencing with siRNA, and observed strong interference with expression of SERCA2, both in control myocytes and following exposure to TG. Such interference is also obtained following NFAT displacement from calcineurin with INCA-6. We have also observed analogous effects on expression of phospholamban (PLB) and Na+/Ca2+ exchanger (NCX). Pertinent to these findings, we have identified, by in-silico analysis, NFAT binding sites in SERCA2, PLB and NCX promoters. Our experiments indicate that activation of the calcineurin/NFAT pathway by rise of resting cytosolic Ca2+ elevates transcription/expression of SERCA2, PLB and NCX, providing a homeostatic mechanism for long term control of cytosolic Ca2+. (Supported by 5 R01 HL069830-08).

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Changes in Ca2+/C02-Mediated Dependent Protein Kinase (CaMKII) During Development of Hypertension-Induced Hypertrophy and Heart Failure
David Yu, Julie Bossuyt, Jeffery Erickson, Byron Norton, Marius P. Sumaneda, Leighton T. Izu, Donald M. Bers, Ye Chen-Izu.
CaMKII is important in cardiac function and disease, and the two main cardiac isoforms (βc and βb) are targeted to cytosol and nucleus respectively. They regulate many ion channels and Ca2+ handling molecules in excitation-contraction coupling and also participate in transcriptional control and hypertrophic/heart failure signaling. Here we investigate changes in βb and βc expression and activity during the development of Hypertensive Heart Diseases (HHD) through four progressive stages: (1) pre-hypertension, (2) onset of hypertension prior to hypertrophy, (3) overt hypertrophy, and (4) heart failure. Method: Western blots were used to measure the βb and βc expression and phosphorylation during Th286/Thr305 (indicating enzyme activity) in the left ventricle of spontaneously hypertensive rat (SHR) at distinct stages of HHD vs. age-matched normotensive Wistar-Kyoto rat (WKY). MAJOR RESULTS: (1) βb and βc expression and phosphorylation in WKY gradually increased with age. (2) SHR show different βb and βc expression and phosphorylation from WKY, revealing disease-related changes apart from age-related changes. (3) Both βb and βc activity increased at the onset of hypertension, and persisted during hypertrophy and heart failure. (4) Interestingly, βb shows larger changes at earlier stages of hypertension and hypertrophy than βc, while βc shows larger changes at heart failure, implicating differential roles of each in regulating hypertrophy and transition to heart failure. (5) ACE inhibitor treatment of SHR at each disease stage effectively reduced hypertension and also reversed changes in βb and βc. CONCLUSION: CaMKII βb and βc are immediate responders to the onset of hypertension and mediate the development of hypertrophy and heart failure during chronic hypertension.

459-Pos Board B259
Calcium Signaling in Mouse Ventricular Myocytes with Impaired Calmodulin Regulation of RyR2
Juan José Arnáiz-Cot, Susannah L. Stone, Gerhard Meissner, Naohiro Yamaguchi, Martin Morad.
The cardiac muscle ryanodine receptor ion channel (RyR2) is a 560 kDa homotetramer that is regulated by calmodulin (CaM), which decreases the open probability of RyR2 at diastolic and systolic Ca2+ concentrations. Point mutations in the CaM binding domain of RyR2 (W3578A/L3591D/F3603A, RyR2ADA) results in lower cardiac load and reduced RyR2 expression and death by postnatal day 16, which suggests that CaM inhibition of RyR2 is required for normal cardiac function. Little is known, however, about Ca2+ signaling of the mutant mouse hearts. Here, we report on Ca2+ signaling of enzymatically isolated cardiac myocytes of mutant mice-11 1 day old wild type and homozygous RyR2ADA/ADA mouse hearts. Cardiomyocytes, dialyzed with the fluorescence probe Fluo-4, were voltage-clamped and subjected to 2 segmented rapid con-focal imaging. At holding potentials of ~80 mV, the Ca2+ spark frequency was 7-fold lower in mutant (0.61 ± 0.40 sparks/sec, n=7) compared to wild type (4.01 ± 1.07/sec, n=7) myocytes. Further, preliminary data indicate that the amplitude of the Ca2+ transients, triggered by membrane depolarization, was lower in voltage-clamped mutant cells than in wild type cells, even though caffeine-triggered Ca2+ release was larger in mutant cardiomyocytes. The data suggest that RyR2ADA mutation reduces resting Ca2+ spark frequency and depolarization-induced SR Ca2+ release in presence of increased SR Ca2+ store size. The reduction of RyR2 expression in RyR2ADA/ADA hearts may, in part, contribute to this phenotype. Supported by NIH (HL16152 and HL07305), NSF (EPS-0903739) and AHA (10SDG350001).

460-Pos Board B260
Molecular Mechanism of Alpha-CaMKII Self-Association
Nicole M. Ashpole, Derrick E. Johnson, Andy Hudmon.
Calcium/calmodulin-dependent protein kinase II, CaMKII, is a calcium-dependent serine/threonine kinase associated with ischemia and other neurodegenerative diseases. Biochemical studies have shown that CaMKII undergoes an activity-dependent aggregation, termed self-association, when activated in an ischemic-like environment - reduced pH, reduced ATP availability. To date, the molecular mechanisms differentiating CaMKII self-association from targetting are not known, as mutants such as Thr286Ala which disrupt self-association also disrupt targeting. Interestingly, self-association is only seen with αCaMKII and not βCaMKII. While these isoforms are highly conserved, sequence alignment of the catalytic and autoregulatory domains highlights several amino acids that vary between αCaMKII and βCaMKII, including residues which may serve as pH sensors (βH38, βH84, βH73) or potential crosslink sites (βC35, βC148, βC272). Thus, we constructed zCaMKII/zCaMKII chimeras to identify mutations in zCaMKII that would prevent self-association. Unlike wild-type αCaMKII, GFP–zCaMKII chimeras failed to self-associate in HEK293 cells subjected to ionomycin/calcinium at pH 6.5 - a cell-based model where self-association is monitored in real-time using fluorescent microscopy. Recombinant zCaMKII, βCaMKII, and zβCaMKII chimeras were expressed, purified, and their enzymatic properties and ability to self-associate were measured in controlled conditions in vitro. No differences were observed for any of the wild-type or mutant enzymes to bind or phosphorylate substrates, suggesting that the zβCaMKII chimeras are enzymatically identical to wild-type protein. Using a style-light microscopy to monitor CaMKII activation in vitro, the zβCaMKII chimera, like βCaMKII, did not undergo self-association. These data suggest that key residues in the catalytic and autoregulatory domain

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account for either the ability of αCaMKII to self-associate or βCaMKII to be unable to self-associate. Identification of these mutations that disrupt self-association and still allow targeting affords the opportunity to definitively determine the role that self-association plays in the subcellular localization of CaMKII during physiological and pathological conditions.

461-Pos Board B261
Nucleobindin 1 is a Calcium Regulated Guanine Nucleotide Dissociation Inhibitor of Gi1
Neeraj Kapoor, Ruchi Gupta, Santosh T. Menon, Ewa folta-Stogniew, Daniel P. Raleigh, Thomas P. Sakmar.
Nucleobindin 1 (NUCB1) is a widely expressed multi-domain calcium-binding protein whose precise physiological and biochemical functions are not well understood. We engineered and heterologuously expressed a soluble form of NUCB1 (sNUCB1) and show that sNUCB1 exists as a Calcium binding dimer in solution and binding to Calcium causes conformational changes in sNUCB1. Earlier reports suggested that NUCB1 might interact with heterotrimetric G protein z subunits. We show that dimeric sNUCB1 binds to Gi3 and that calcium-binding inhibits the interaction. The binding of sNUCB1 to Gi3 inhibits its basal rate of GDP release and slows its rate and extent of GTPyS uptake. Additionally, our tissue culture experiments show that sNUCB1 prevents receptor-mediated Gi3-dependent inhibition of adenyl cyclase (AC). Thus, we conclude that sNUCB1 is a calcium dependent guanine-nucleotide dissociation inhibitor (GDI) for Gi3. To our knowledge sNUCB1 is the first example of a calcium-dependent GDI for heterotrimetric G proteins. We also show that the mechanism of GDI activity of sNUCB1 is unique and does not arise from the consensus Goloco motif found in RGS proteins. We propose that cytoplasmic NUCB1 might function to regulate heterotrimetric G protein trafficking and signalling.

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Conformational Dynamics in Dream Protein
Jaroslava Milkovska.
Downstream Regulatory Element Antagonist Modulator (DREAM), also known as calsenilin or Kþ channel interacting protein 3 (KChIP-3) belongs to neuronal calcium sensor proteins that are found predominantly in neuronal cells where they regulate diverse aspects of neuronal function ranging from neurotransmitter release to neuronal growth and apoptosis. DREAM is a highly multifunctional protein that binds to preselinin, acts as a transcriptional repressor for a number of genes including c-fos gene and prodynorphin gene in Caþ dependent manner, and interacts and modulates the activity of A-type of Kþ channels. To obtain insight into the Ca2þ signaling mechanism, the impact of binding of metals and DNA on conformational heterogeneity and dynamic motion of DREAM was probed using steady-state and time-resolved fluorescence and anisotropy techniques. In addition the role of the individual EF-hands in the Ca2þ signal transduction was determined by investigating DREAM mutants with the impaired EF-hand 3 and EF hand 4.

463-Pos Board B263
The Interaction of S100A1 with Type-2b Regulatory Subunit of Protein Kinase A
Brian Cannon, Erick Hernández-Ochoa, Kristen Varney, Martin Schneider, David Weber.
The S100 family of proteins consists of 24 calcium-activated signaling molecules that are involved in a variety of biological processes. They are expressed exclusively in vertebrates in a tissue-specific manner. One member of this family, S100A1, is involved in several biological processes. In the heart, S100A1 has been shown to be essential for cardiac function (Pleger et al, 2007). In skeletal muscle, S100A1 is known to help modulate excitation-contraction coupling through interaction with the ryonodine receptor (Prosser et al, 2008). Furthermore, in ganglion neurons exogenous S100A1 increases sympathetic output by enhancing Cav1 channel currents (Hernández-Ochoa et al, 2009). This effect is occluded by the inhibition of PKA, suggesting a PKA-dependence of this process. Our laboratory has now used ITC and NMR to demonstrate that S100A1 interacts with the type-2b regulatory subunit of PKA.

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Differential Activation of Intracellular Calcium Oscillations by Multiple Calcium and L-Phenylalanine Binding Sites Located at the Extracellular Domain of Calcium-Sensing-Receptor
Chen Zhang, Yun Huang, Yusheng Jiang, Hing Wong, Xue Wang, Adriana Castiblanco, Ling Wei, Edward Brown, Jenny J. Yang.
Calcium sensing receptor (CaSR), along other members of the family C G protein-coupled receptors (GPCRs), play very important roles in responding to changes in the extracellular calcium concentrations and in regulating levels of amino acids and integrating these extracellular signals into alterations in intracellular signaling pathways. We have reported several potential calcium-binding sites located within the CaSR’s extracellular domain using our developed computational algorithms based on geometric factors and surface electrostatic potentials. In the present study, we first report the differential effects of several disease-related mutations located at the predicted calcium binding sites on the inhibition and activation of intracellular calcium responses using both a cell population assay and single cell imaging. We then identify a potential amino acid binding site using computational methods and site-directed mutagenesis. The effect of amino acid binding in altering intracellular calcium responses, especially calcium oscillations, and its synergistic interaction with the effects of extracellular calcium on these parameters are also investigated. A common activation mechanism for CaSR and other family C GPCRs, such as mGluR1 by extracellular calcium and amino acid is been proposed. These results could have important implications for our understanding of how the CaSR integrates information about these two completely different classes of agonists—one an inorganic divalent cation, the other a nutrient—and how the receptor senses these agonists in health and in disease states.

Epithelial Channels & Physiology

465-Pos Board B265
Living on the Edge: Mechanisms of Single Cell Responses at Air-Liquid Interfaces
Nina Hobi, Andrea Ravasio, Thomas Haller.
Many epithelia have contact with air-liquid interfaces. This applies particularly to the lung, where one of the epithelial cell types, the surfactant secreting AT II cells, even project into the air-filled alveolar lumen. This specific environment may be of considerable physiological relevance; however, only few data exist to provide a satisfying description. This is mainly due to the experimental difficulty to manipulate cell-air contacts in a specific way. In previous investigations, using new microscopic approaches, we found that the presence of an air-liquid interface leads to a paradoxical situation: it is a potential threat that causes cell injury, but also a potent stimulus: AT II cells respond promptly, and show sustained Ca2þ-signals that activate exocytosis. Excocytosed surfactant, in turn, clearly prolonged the time to irreversible cell damage, and may be an adaptive defense against the harmful nature of surface forces. The strength of this stimulus became also apparent by a rapid and significant change on the transcriptional level: cellular pathways that are involved include e.g. defense response and lipid metabolism, and a Pubmatrix search identified genes associated with several lung diseases and injuries. Furthermore, we found that the signalling mechanisms underlying sensation of an air-liquid interface can be sufficiently explained by mechanical forces. These forces trigger cellular events that are closely related with classical concepts in mechanotransduction. In conclusion, we suggest that an air-liquid interface has to be regarded as a specialized form of an extracellular matrix. This matrix, probably an important constraint in the evolution of air-exposed biological surfaces, exerts distinct physical stimuli which are very well perceived by the cells.

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Electrodiffusion and Osmotic Water Flow and its Variational Structure
Yoichiro Mori, Chin Liu, Robert S. Eisenberg.
We propose a system of partial differential equations (PDE) that describe electrodiffusion and osmotic water flow. From a physical standpoint, this is a far-reaching generalization of the standard treatment of osmosis and electrodiffusion in irreversible thermodynamics to spatially extended systems. As far as we know, this is the first mechanically and thermodynamically consistent model of osmotic water flow and electrodiffusion in systems with deformable cells and membranes with capacitance and conductance. We use an energetic variational approach to enforce consistency and derive a field theory describing the flow, diffusion, and migration of ions, water, and the solution itself. The variational approach is particularly useful because it treats interactions automatically and consistently with a minimal number of arbitrary parameters. Electrodiffusion and osmotic water flow are involved in a wide range of biological functions of organs, tissues, cells, and organelles, including the homeostasis of ions in the brain, fluid secretion by epithelial systems, electrolyte...