peptides did not interact with SERCA at all, some bound weakly, and some bound with a high affinity. The highest affinity candidates bound SERCA as avidly as some native regulatory micropeptides. The candidate peptides tested here include transmembrane fragments of phospholamban (PLB), phospholemman (PLM), calcium voltage-gated channel subunit alpha1 E (CACNA1E) and sarcolemma associated protein (SLMAP). We also tested whether the putative poison peptides altered the structure of SERCA, using time-correlated single photon counting to quantify intramolecular FRET in a "2-color SERCA" construct labeled with mCyRFP-1 and mMaroon1. Overall, the results suggest that transmembrane peptide fragments generated in heart failure can interact with SERCA and may disrupt calcium handling in the failing heart.

2565-Pos

Alzheimer's disease-related presenilin 1 M146L mutation disrupts SERCA2a regulation and increases propensity of Ca waves in ventricular cardiomyocytes

Elisa Bovo[1], Roman Nikolaienko[1], Daniel Kahn[1], Jody L. Martin[2,3], John Q. Yap[1], Seth L. Robia[1], Aleksey V. Zima[1].
[1]Department of Cell and Molecular Physiology, Loyola University Chicago, Maywood, IL, USA, [2]Department of Pharmacology, University of California Davis, Davis, CA, USA, [3]Cardiovascular Research Institute, University of California Davis, Davis, CA, USA.

Presenilin 1 (PS1) is a transmembrane protein expressed in the endoplasmic reticulum (ER) of different cell types. It has been shown that Ca mishandling due to PS1 mutations contributes to the Alzheimer's disease (AD)-related neurodegeneration. PS1 is also expressed in the heart. Several clinical studies revealed that PS1 mutations are associated with cardiomyopathies. We previously showed that PS1 interacts with the cardiac ER Ca ATPase (SERCA2a) and regulates its function. Here, we studied the role of AD-related $PS1^{M146L}$ mutation in regulation of intracellular Ca homeostasis. Fluorescent resonance energy transfer (FRET) experiments in HEK293 cells transfected with fluorescently labeled SERCA2a and $PS1^{M146L}$ revealed that the mutation does not disrupt the interaction between these two proteins. Measurements of SERCA2a-mediated Ca transport showed that at low ER Ca loads ($[Ca]_{ER} \leq 0.15$ mM), both $PS1^{WT}$ and $PS1^{M146L}$ enhance ER Ca uptake by a similar level (~40%) compared to control (no PS1 expression). At high ER Ca loads ($[Ca]_{ER} \geq 0.35$ mM), however, $PS1^{WT}$ reduced ER Ca uptake and ER Ca load, whereas $PS1^{M146L}$ was less effective in regulating SERCA2a function. We used *in vivo* gene delivery in mouse cardiac wall to study the effect of $PS1^{WT}$ and $PS1^{M146L}$ overexpression on Ca regulation in ventricular myocytes. We found that both $PS1^{WT}$ and $PS1^{M146L}$ localized predominantly at the sarcoplasmic reticulum. Analysis of cytosolic Ca dynamics revealed that $PS1^{WT}$ overexpression in cardiomyocytes increases diastolic Ca and decreases Ca transient amplitudes. In contrast, $PS1^{M146L}$ expression increased propensity of spontaneous Ca waves. These results illustrate that the lack of SERCA2a regulation by $PS1^{M146L}$ at high ER Ca loads can lead to ER Ca overload and Ca waves. These defects in Ca regulation might contribute to the onset of cardiomyopathies in patients with PS1 mutations.

2566-Pos

Calcium-dependent shifts between phospholamban (PLB) monomers and homo-pentamers reveal the interactions between PLB and SERCA in native cardiac membranes

Jin Guo, **Zhenhui Chen**.
Krannert Cardiovascular Research Center, Indiana University School of Medicine, Indianapolis, IN, USA.

Phospholamban monomer (PLB1) binds to the cardiac calcium pump (SERCA2a) and inhibits the enzyme. PLB homopentamers (PLB5) are inactive species, but stable in SDS that correlate well to that in the native membranes. In contrast, SDS separates PLB1 from SERCA2a. Using this feature, we developed assays to probe the constant exchanging PLB1 with SERCA2a, and with PLB5, in native cardiac sarcoplasmic reticulum (SR) membrane vesicles. Dog cardiac SR was pre-incubated at 37°C in 1mM EGTA, allowing PLB1 binding to SERCA2a. Then, 8 μg samples were incubated for 1 min with control buffer, 1mM Ca (free [Ca] = 16.4 μM), 1 mM Ca first and then 5 mM EGTA (free [Ca] = 0.06 μM), or 10μM SERCA2a inhibitor thapsigargin. These reactions were stopped by 1% SDS before SDS-PAGE and immunoblotting to measure PLB5/PLB1 ratios. Addition of Ca significantly increased PLB5/PLB1 to 2.91 ± 0.38 from 1.22 ± 0.08 in the control, Ca-free condition, suggesting that Ca activation of the pump dissociates the whole PLB monomer from inhibitory site on SERCA2a in cardiac membranes. Furthermore, subsequent addition of EGTA reversed Ca-induced increased in PLB5/PLB1 back to 1.38 ± 0.15. Meanwhile, thapsigargin virtually eliminated PLB1 binding to SERCA2a, increasing PLB5/PLB1 to 3.13 ± 0.40. In control experiments, none of these factors affected PLB5/PLB1 of WT-PLB expressed alone in insect cells. Therefore, the detected changes in PLB5/PLB1 must occur in the native SR membranes, reflecting fully reversible processes of Ca-dependent PLB1 association and dissociation from the inhibitory site on SERCA2a, followed by disassembling and assemblage into PLB5, respectively. Such assays will allow further studies to determine the effect of allosteric factors and proteins phosphorylation on the interactions between PLB and SERCA2a directly in native cardiac SR membranes.

2567-Pos

Deep learning identification of cardiac transporters as targets for approved drugs

Carlos Cruz Cortes[1], Manuel Andres Velasco Saavedra[1], Eli Fernandez-De Gortari[1], Guadalupe Guerrero-Serna[2], Rodrigo Aguayo-Ortiz[3], L. Michel Espinoza-Fonseca[1].
[1]Internal Medicine, University of Michigan, Ann Arbor, MI, USA, [2]Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA, [3]Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, MI, USA.

We re-tasked a novel ligand-centered deep learning drug discovery method to identify molecular targets for approved drugs in the heart. This approach uses information about small-molecule effectors to map and probe the pharmacological space of functionally relevant targets in the heart. Here we studied the calcium pump (Sarcoplasmic reticulum Ca^{2+}-ATPase, SERCA) as a proof-of-principle target to test our method. We chose SERCA because it plays a major role in the excitation-contraction-relaxation cycle in normal and pathological muscle, and it represents a major pharmacological target in the heart. We applied this method to demonstrate that SERCA is a pharmacological target for statins, a group of FDA-approved HMGCoA inhibitors used as lipid-lowering medications. We used in situ enzymatic assays and atomistic simulations to demonstrate that that these approved drugs are SERCA inhibitors at micromolar concentrations, inhibiting the pump by binding to two different effector sites. These proof-of-concept studies support the applicability of our approach for off-target identification and drug repurposing, ultimately minimizing the translational gap in drug development targeting the heart.

2568-Pos

Functional analysis of plasma membrane Ca^{2+} ATPase 3 and its primary aldosteronism-associated mutations

Victoria C. Young[1], Marilina de Sautu[2], Irene C. Mangialavori[3], Pablo Artigas[1].
[1]Cell Physiology & Molecular Biophysics, Texas Tech University Health Sciences Center, Lubbock, TX, USA, [2]Boston Children's Hospital, Harvard Medical School, Boston, MA, USA, [3]Instituto de Química y Fisicoquímica Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.

Primary aldosteronism (PA) is the most common form of secondary hypertension and can lead to higher rates of cardiovascular complications and death than essential hypertension. Aldosterone-producing adenomas (APAs) are the most frequent cause of PA. Most APAs have mutations in plasma membrane ion-transport proteins with 0.6%-9% carrying a mutation in the Plasma Membrane Ca^{2+} ATPase 3 (PMCA3). We have developed a *Xenopus* oocyte expression system to measure the function of PMCA3 wildtype ($PMCA3^{WT}$) and an APA associated deletion mutant ($PMCA3^{L425\_V426del.}$) with minimal endogenous contamination. We measured ATPase activity at 37 °C in plasma membrane preparations from oocytes expressing PMCA3. Ca^{2+}-dependent ATPase was observed in preparations from oocytes expressing $PMCA3^{WT}$, with a $[Ca^{2+}]$ dependence well described with a rectangular hyperbola ($K_{0.5}$ = 25.6 ± 5.1 μM) (triplicates from three independent membrane preparations). In contrast, membrane preparations from $PMCA3^{L425\_V426del.}$ expressing oocytes lacked Ca^{2+}-dependent ATPase activity, indicating loss of enzymatic activity in the mutant. We used two-electrode voltage clamp electrophysiology to evaluate the presence of PMCA3-variant mediated currents. At resting intracellular $[Ca^{2+}]$, $PMCA3^{WT}$-injected oocytes had currents indistinguishable from those in uninjected oocytes, at all voltages. When bathed by extracellular 125 mM Na^+, $PMCA3^{L425\_V426del.}$-injected oocytes presented inward currents at negative membrane potentials and outward currents at positive ones, consistent with induction of an aberrant channel-like current. The currents were outwardly directed upon substitution of external Na^+ with N-methyl D-glucamine$^+$ and were insensitive to changes in extracellular $[Ca^{2+}]$. These results indicate that $PMCA3^{L425\_V426del.}$ causes hyperaldosteronism due to the concomitant loss of active Ca^{2+} transport and induction of a depolarizing Na^+-mediated current. Current experiments are evaluating the ATPase and physiological characteristics of other PA-associated PMCA3 mutations.
Funded by NSF-MCB 2003251.