1253-Pos Physical Coupling Supports Local Ca^{2+} Transfer Between SR Subdomains And The Mitochondria In Heart Muscle

Gyorgy Csordas, Cecilia Garcia Perez, Gyorgy Hajnoczky

Thomas Jefferson University, Philadelphia, PA, USA.

Board B229

Mitochondrial Ca^{2+} signals associated with ryanodine receptor (RyR) dependent SR Ca^{2+} release are commonly supported locally by high [Ca2+] microdomains at close contacts between the organelles. Here we studied if the SR-mitochondrial interface underlying the local Ca^{2+} communication was secured via inter-organellar physical links.

Rat heart homogenates were tested for mitochondria-associated SR fragments capable for RyR-dependent Ca^{2+} mobilization and mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_m$) signal generation. Ample presence of SR markers (calsequestrin, SERCA2a, phospholamban) was detected by western blotting of crude mitochondria, most of which disappeared after percoll purification. 'Immunomitochemistry' revealed numerous SR particles in the crude fraction co-localized with mitochondria, while failed to show SR markers in the percoll-purified fraction. Sub-fractionation of crude mitochondria on a linear sucrose density gradient following osmotic lysis and sonication resulted in fractions of outer mitochondrial membrane (OMM) devoid of inner mitochondrial membrane (IMM) and also lacking SR markers. Fractions containing both OMM and SR markers also contained IMM. Fluorescence Ca^{2+}-imaging in rhod2-loaded adherent particles revealed $[Ca^{2+}]_m$ responses to caffeine stimulation, which were prevented by Ca^{2+}-predepletion of the SR, or by inhibitors of mitochondrial Ca^{2+} uptake. Also, no concomitant rise of $[Ca^{2+}]$ occurred in the gross volume of the incubation medium, indicating local Ca^{2+} transfer between the particles. Surprisingly, even the percoll-purified 'heavy' mitochondrial fraction displayed Tg-sensitive Caffeine-induced $[Ca^{2+}]_m$ rise despite the hardly detectable SR markers.

Our data suggest that physical links support the SR-mitochondrial associations where the local Ca^{2+} transfer occurs. Mitochondria bind a relatively small fraction of the total SR and utilize domains where the OMM also binds the IMM (contact points) for the SR anchorage.

1254-Pos Isoflurane enhances buffer Ca -induced mitochondrial Ca ion flux: Role of mitochondrial Ca ion uniporter?

Johan Haumann, Maria Herrera, Juan L. Vega, Mohammed Aldakkak, Amadou KS Camara, Martin Bienengraeber, Ranjan K. Dash, David F. Stowe

Medical College of Wisconsin, Milwaukee, WI, USA.

Board B230

Background: There is ample data suggesting that cardiac preconditioning against ischemia/reperfusion (I/R) injury is initiated within mitochondria, possibly on proteins involved in electron transport and or cation channels and exchangers. We tested if the anesthetic isoflurane directly modulates mitochondrial matrix $[Ca^{2+}]$ via the mitochondrial Ca^{2+} uniporter (CaU), which along with ROS, may participate in the triggering phase that leads to the cytosolic signaling cascade necessary to protect the mitochondrion from injury.

Methods: We examined effects of isoflurane on mitochondrial respiration, mCa^{2+} dynamics (by indo 1 AM), redox state (NADH), and membrane potential ($\Delta\Theta m$, by rhodamine 123/HRP) using spectrofluorometry in guinea pig heart isolated mitochondria energized with complex I substrate, Na-pyruvate, or complex II substrate Na-succinate in Ca^{2+} free buffer. The direct effect of isoflurane on mCa^{2+} handling was assessed by challenging mitochondria ± isoflurane (0.5–2 mM) with 0.5 to 3.0 mM $CaCl_2$ in 2.5 mM EGTA buffer (ionized $[Ca^{2+}]$ about 25–500 nM). Ruthenium red (RuR) was given to block CaU.

Results: Adding $CaCl_2$ caused step-wise increases in matrix $[Ca^{2+}]$ of 25–500 nM; this effect was blocked completely by RuR indicating Ca^{2+} uptake was through the CaU rather than a cation exchanger. Isoflurane promoted added matrix Ca^{2+} uptake (by 50–100 nM) in a dose-independent manner, while it decreased states 3/4 respiration (control index), and $\Delta\Theta m$ with no change in redox state.

Discussion: Isoflurane-induced increases in matrix Ca^{2+} uptake (cation leak) down its large electrical and chemical gradient are associated with a small decrease in $\Delta\Theta m$ and slowed respiration. Isoflurane may increase matrix Ca^{2+} influx indirectly via changes in electrochemical potential or by a direct effect on CaU proteins to enhance conductance. Increased cell Ca^{2+} is known to initiate a cascade of protective kinases leading to cardioprotection.

Endoplasmic Reticulum & Protein Trafficking

1255-Pos Characterization Of Endosomal Insulin Receptor Complexes By Mass Spectrometry

Gabriel Collard-Simard[1], Sylvie Bourassa[2], Eric Winstall[2], Suzanne Fortier[1], Robert Faure[1]

[1] *CHUL Research Centre, Quebec, QC, Canada*
[2] *CHUL Research Centre, Proteomics Plateform, Quebec, QC, Canada.*

Board B231

Insulin binding to its cognate receptor at the cell surface triggers autophosphorylation of the β-subunit on regulatory tyrosine residues and rapid internalization of the complexes into the endosomal apparatus. Using highly purified hepatic endosomes, we characterized here, by mass spectrometry, IR immunocomplexes prepared at the peak time of internalization (2 min post-insulin injection). Mono-phosphorylated tyrosines residues located within the catalytic loop (Y1146,Y1150,Y1151), and the C-terminal domain (Y1316, Y1322) of the β-subunit were identified. Among the co-immunoprecipitated proteins, we identified unambiguously ATIC (5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase), an enzyme involved in the two last steps of de novo purine biosynthesis as well as the molecular adaptors grb7/grb14, coatomer subunits and VPS-1 like proteins. We con-

firmed here specific association of ATIC with the IR in vitro and in vivo. Therefore, characterization of endosomal IR complexes by mass spectrometry reveals new features and interactors involved in both IR kinase activation/signalling and endocytic trafficking in vivo.

1256-Pos N-terminal ER Retention Motif Regulates Trafficking of Kv4-Encoded Ventricular Ito, f Channels

Nicholas C. Foeger[1], Haodong Xu[2], Bin Ye[3], Jeanne M. Nerbonne[1]

[1] *Washington University in St. Louis, St. Louis, MO, USA*
[2] *University of Rochester, Rochester, NY, USA*
[3] *University of Wisconsin, Madison, WI, USA.*

Board B232

Voltage-gated K^+ (Kv) channels are the primary determinants of action potential repolarization in the myocardium. Although the roles of specific pore-forming and accessory subunits in the generation of cardiac Kv channels are well characterized, the molecular mechanisms controlling the trafficking of these channels remain poorly understood. For example, the differentially expressed, fast transient outward Kv current, $I_{to,f}$, reflects the assembly of Kv4 subunits with accessory KChIP2 and Kvbeta1 subunits. Voltage-clamp recordings from ventricular myocytes isolated from mice with a targeted deletion of Kv4.2 (Kv4.2-/-) or KChIP2 (KChIP2-/-) revealed that $I_{to,f}$ is eliminated. Western blot analyses further demonstrated that KChIP2 expression is reduced markedly (by ~90%) in Kv4.2-/- ventricles and that Kv4.2 expression is similarly reduced in KChIP2-/- ventricles, suggesting that the association of Kv4.2 and KChIP2 subunits is required for the stabilization and trafficking of $I_{to,f}$ channels. Sequence analysis revealed a putative endoplasmic reticulum (ER) retention sequence (RKR) in the N-terminus (residues 35–37) of Kv4.2, suggesting a potential mechanism for the regulation of the surface expression of $I_{to,f}$ channels. To test this hypothesis directly, a Kv4.2 point mutant (Kv4.2AAA), in which residues 35–37 were replaced with alanines, was generated and expressed (together with EGFP) in adult Kv4.2-/- ventricular myocytes. Whole-cell voltage-clamp recordings from EGFP-positive Kv4.2-/- myocytes revealed that mean ± SEM peak Kv current densities are significantly higher in cells expressing Kv4.2AAA (78.7±6.5 pA/pF; n=25) compared with cells expressing EGFP alone (35.7±2.3 pA/pF; n=43) or EGFP plus WT Kv4.2 (36.9±2.2 pA/pF; n=25). Additionally, there is a prominent, rapidly-inactivating Kv current in Kv4.2AAA-expressing cells with time and voltage-dependent properties that are similar to (endogenous) mouse ventricular $I_{to,f}$, suggesting a pivotal role for the Kv4.2 N-terminal RKR motif in the regulation of the trafficking of Kv4 α-subunit-encoded $I_{to,f}$ channels.

1257-Pos The NH_2 Terminus Promotes ER Export and Inhibits Degradation of HCN2 Channels

Sarah Chow, Leisha Leclair, Gina Whitaker, Damiano Angoli, Vincenzo Macri, Eric Accili

University of British Columbia, Vancouver, BC, Canada.

Board B233

Hyperpolarization activated cyclic nucleotide-gated "HCN" or 'pacemaker' channels are important for the regulation of automaticity in the heart. Like voltage-gated K+ (Kv) channels, HCN channel subunits are thought to have six transmembrane helices with cytoplasmic amino and carboxyl termini, and to co-assemble as tetramers which form functional channels. Similar to Kv channels, HCN channels are likely synthesized folded and assembled in the endoplasmic reticulum (ER) prior to movement to the Golgi and eventually to the plasma membrane. The efficiency of this process is variable among the four different mammalian HCN isoforms (HCN1-4) and may be influenced by mutations in the primary sequence - including mutations associated with cardiac arrhythmias. HCN2 subunits undergo complex glycosylation, which is a prerequisite for cell surface expression. We find that HCN2 subunits that lack either partial or complete N-terminus do not express on the plasma membrane or undergo complex glycosylation and thus they are likely retained in the ER. Increasing truncations of the N terminus produced increasing amounts of a C-terminal fragment. This fragment was also associated with wild-type subunits, but the amounts were significantly lower than those produced by the N-terminal truncations. This suggests that entry into degradation pathways is inhibited by the N terminus. Together the data are consistent with a role for the N-terminus in enhancing HCN2 channel cell surface expression by a mechanism that inhibits intracellular degradation and promotes exit from the ER.

Acknowledgement

Canadian Institute of Health Research and the Heart and Stroke Foundation of Canada.

1258-Pos A Model For The Formation Of Exit Sites In The Endoplasmic Reticulum

Matthias Weiss

German Cancer Research Center, Heidelberg, Germany.

Board B234

Exit sites (ES) are specialized domains in the endoplasmic reticulum (ER) at which cargo proteins of the secretory pathway are packaged into COPII-coated vesicles. We recently have quantified the sequential binding kinetics of the essential COPII proteins (Sar1p, Sec23/24p, Sec13/31p) to ERES in vivo [1]. However, the basic processes that govern the self-assembly and spatial organization of ERES have so far remained elusive. Here, a generic computational model is presented that describes the non-equilibri-

um ERES formation on a mesoscopic scale. The model predicts that ERES are arranged in a quasi-crystalline pattern while their size strongly depends on the cargo-modulated COPII turnover kinetics, i. e. a lack of cargo leads to smaller and more mobile ERES. These predictions are in favorable agreement with experimental data obtained by fluorescence microscopy. The model further suggests that a cooperative binding of COPII components, e.g. mediated by regulatory proteins, is a key factor for the experimentally observed organism-specific ERES pattern. Moreover, the anterograde secretory flux is predicted to grow when the average size of ERES is increased while an increase in the number of (small) ERES only slightly alters the flux.

References

[1]. Forster, Weiss, Zimmerman, Reynaud, Verissimo, Stephens & Pepperkok, Curr. Biol. *16*, 173 (2006).

Exocytosis & Endocytosis - I

1259-Pos Imaging of Antibody-Antigen Complexes Using Conical Electron Tomography

Guido A. Zampighi[1], Cataldo Schietroma[1], Sirus A. Kohan[1], Lorenzo M. Zampighi[1], Nick Fain[1], Salvatore Lanzavecchia[2], Ernest Wright[1]

[1] *UCLA School of Medicine, Los Angeles, CA, USA*
[2] *University of Milan, Milan, Italy.*

Board B235

Conical electron tomography allows the imaging of macromolecular assemblies in their cellular environments, with isotropic resolution (~3 nm) approximately two orders of magnitude higher than that achieved by optical methods. Furthermore, there is no need to impose symmetry or use averaging methods to increase signal-to-noise ratios. Here, we explore the possibility that proteins in these assemblies can be identified with monoclonal antibodies: in the tomographic reconstructions, antibodies are located on the basis of their size and shape, revealing the identity of their target protein. To test this possibility, we reconstructed two specimens:

(a) meshes of filamentous actin, decorated with monoclonal antibodies; and,
(b) synaptosomal preparations from mouse brain, decorated with antibodies against clathrin, the principal component of coated vesicles.

Conventional thin section microscopy methods were used to prepare both specimens. The maps of actin decorated with IgGs show a dense network of filaments, ~6 nm in diameter, with denser particles of 6–10 nm, jutting out at variable intervals along their length. These particles are absent in pure actin maps. While the shape of the particles varies as expected for flexible molecules oriented at random, many of them exhibit the classical tri-lobar (Y) structure characteristic of IgGs. The synaptosomal maps show the normal synaptic components, including coated vesicles characterized by their polyhedral cages comprised of clathrin triskelions. The antibody molecules appear as particles of 6–10 nm, decorating the clathrin coats. Unexpectedly, we also observe larger particles (15–20 nm), that we interpret as multiple IgGs (2–3) bound to epitopes located in close proximity within the clathrin triskelions. We thus conclude that conical electron tomography represents a novel experimental avenue for identifying proteins comprising key macromolecular assemblies in their cellular environments.

1260-Pos Probing the Endocytosis Pathway of Dendritic Cells Involved in Class I Antigen Presentation

Kenny K. Tran, Hong Shen

University of Washington, Seattle, WA, USA.

Board B236

Dendritic cells (DCs), also known as professional antigen presenting cells, are crucial mediators of the immune response and have emerged as prime targets for vaccination. Antigen, foreign or benign, are captured through endocytosis, routed, and processed in a tightly regulated network of compartments that ultimately determines the type of immune response elicited. The luminal pH of endocytic compartments is closely correlated to its trafficking. The kinetics of pH change, which may be regulated by redox reactions, has been implicated as a key mechanism by which DCs selectively process antigen for a particular immune response. In this work, we have developed a microprobe aimed at monitoring pH and redox reactions of the endocytic compartments of DCs in situ. By using this probe, we have examined the mechanisms by which size and pH-sensitivity of carriers affect class I antigen presentation.

1261-Pos Ca2+ Syntillas Decrease Spontaneous Exocytosis In Mouse Chromaffin Cells

Jason J. Lefkowitz, Kevin E. Fogarty, Lawrence M. Lifshitz, Karl D. Bellve, Ronghua ZhuGe, Richard A. Tuft, John V. Walsh, Valerie De Crescenzo

UMASS med school, Worcester, MA, USA.

Board B237

Ca^{2+} syntillas are spontaneous transient, focal increases in cytosolic [Ca^{2+}] in excitable secretory cells, resulting from a release from intracellular stores via ryanodine receptors (RyRs). Originally found in nerve terminals of hypothalamic magnocellular neurons (*scintilla*, L.spark, from a synaptic structure, a terminal), syntillas are also found in mouse chromaffin cells. There they do *not* cause exocytosis because they appear to arise in a microdomain different from the one where the final exocytotic steps occur (ZhuGe et al., 2006). What is the function of spontaneous Ca^{2+} syntillas? To find out we studied syntillas and amperometrically recorded spontaneous exocytosis in chromaffin cells in whole-cell patch configuration held at a membrane potential of –80 mV. We found that blocking the stores with ryanodine resulted in significant increases in the mean charge (0.088±0.006 vs 0.233±0.008 pC) and frequency