dine receptors (RyR2) in situ in cardiac myocytes. Prior cryo-EM studies suggest the apoCaM and FKBP RyR binding sites are within ~9 nm of each other. To test this in the cellular environment, we measured FRET between fluorescently labeled FKBP and CaM in permeabilized rat cardiomyocytes. Single-cysteine FKBP mutants were labeled with fluorescein-5-maleimide or Alexa Fluor 488 maleimide as FRET donors (D), obtaining functionally-silent D-FKBP variants. Single-cysteine CaM variants were labeled with Alexa Fluor 568 as FRET acceptor (A), obtaining functionally-silent A-CaMs. Both D-FKBP and A-CaM were highly localized to the Z-line. Three methods were used to assess FKBP-CaM FRET in myocytes where intracellular [Ca]i was clamped at 10 nM. First, the addition of D-FKBP produced a robust FRET signal detected as an increase in the A-CaM fluorescence. FRET was abolished by rapamycin (due to dissociation of D-FKBP donor from its sites). Second, addition of A-CaM quenched direct D-FKBP fluorescence. The fractional decrease in donor signal upon acceptor addition reflects the amount of FRET. Third, after attaining steady state in the D-FKBP and A-CaM channels, we photobleached the acceptor and calculated FRET from the enhancement of D-FKBP fluorescence. The FRET efficiency between D-FKBP and A-CaM labeled in the N-lobe was 35.9 ± 1.8% (n= 12), corresponding to a distance of 6.9 ± 0.1 nm. This is identical to the distance measured in parallel determinations we carried out in isolated SR. These results are for CaM labeled at the N-lobe, but FRET between C-lobe labeled CaM and FKBP is substantially less. These results provide direct in situ measurements of FKBP-CaM localization on the functional RyR2.

2128-Pos The Effects of Ca^{2+}/ Calmodulin-Dependent Protein Kinase Phosphorylation of Single RyR2 Channels

Simon M. Carter, Rebecca Sitsapesan

University of Bristol, Bristol, United Kingdom.

Board B243

CaMKII phosphorylation of RyR2 has been reported to increase RyR2 channel activity (Witcher *et al.* J Biol Chem. 1991; Wehrens *et al.* Circ Res. 2004) but the phosphorylation site/s involved are disputed. We have therefore correlated the effects of CaMKII-dependent phosphorylation of RyR2 channels reconstituted into planar lipid bilayers with the phosphorylation state of S2809. Under voltage-clamp conditions, RyR2 channels incubated for 10 minutes in the presence of 12.5 units/ml of CaMKII (in the presence of 1μM CaM, 1mM ATP, 5mM Mg^{2+}) (P_o 0.269 ± 0.153 (SEM; *n*=6)) exhibited a small but non-significant increase in P_o compared with control channels (P_o 0.092 ± 0.032 (SEM; *n*=6)). This contrasts with the effects of PKA-dependent (10 units/ml) phosphorylation of RyR2 channels under similar experimental conditions, where P_o rose significantly from 0.126 ± 0.035 to 0.574 ± 0.106 (SEM; *n*=10; $p<0.01$). Using antibodies specific for the phosphorylated and dephosphorylated states of S2809 (Badrilla Ltd, Leeds), Western blot analysis demonstrated that both CaMKII and PKA treatment increases RyR2 phosphorylation at S2809. Thus, while both PKA and CaMKII phosphorylate RyR2 at S2809, only PKA treatment is effective at activating the channel. These results suggest that PKA phosphorylation at a site other than S2809 may be primarily responsible for the increase in P_o.

Supported by the British Heart Foundation.

IP_3 Receptors

2129-Pos Isoform-specific Modulation of Inositol (1,4,5)-trisphosphate Receptors by Cytosolic ATP

Matthew J. Betzenhauser, Larry E. Wagner, David I. Yule

University of Rochester, Rochester, NY, USA.

Board B244

Inositol (1,4,5)-trisphosphate receptors (InsP3R) are the predominant route of calcium release in non-excitable cells and they play a major role in regulating calcium signaling in numerous physiological systems. There are three known isoforms (InsP3R-1, InsP3R-2 and InsP3R-3) and multiple splice variants of InsP3R expressed in mammalian cells. This sequence diversity along with varied tissue distributions hints at important isoform-specific regulatory mechanisms. One such regulatory mechanism is the modulation of calcium release from InsP3R by cytosolic free ATP. All three isoforms contain putative ATP binding domains, with InsP3R-1 expressing two such domains (ATPA, and ATPB) in the S2+ isoform and three sites (ATPA, ATPB and ATPC) in the S2- splice variant while InsP3R-2 and InsP3R-3 each express a single ATPB site. Functionally, ATP has been shown to positively regulate InsP3R-1 and InsP3R3 while InsP3R-2 is thought to be insensitive to ATP modulation. The purpose of this study was to examine the contributions of ATP binding sites to the calcium release properties of the individual isoforms. TNP-ATP binding assays using GST-fusion proteins containing the ATP binding domains were used to confirm ATP binding. Calcium release assays from permeabilized cells were used as a means of measuring the effects of ATP on endogenous InsP3R in native exocrine acinar cells and on individual wild type or mutant isoforms expressed in DT40-3ko cells. The results presented here demonstrate that, contrary to prior studies, InsP3R-2 can indeed be modulated by ATP. In addition, even though InsP3R-2 and InsP3R-3 contain identical ATP biding sites, they exhibit dramatically different sensitivities to ATP. The impact of this differential modulation can therefore depend on the metabolic state of the cell and on the relative abundance and localization of the three InsP3R isoforms.

2130-Pos Constructive Use Of Noise In Intracellular Calcium Oscillations

Alexander Skupin[1], Helmut Kettenmann[2], Maria Wartenberg[3], Stephen C. Tovey[4], Colin W. Taylor[4], Martin Falcke[1]

[1] *Hahn Meitner Institute, Berlin, Germany,*
[2] *Max Delbrück Center, Berlin, Germany,*
[3] *Forschungszentrum Lobeda, Jena, Germany,*
[4] *University of Cambridge, Cambridge, United Kingdom.*

Board B245

All processes in living cells are built from random molecular events, but the law of large numbers usually guarantees predictability. Intracellular Ca^{2+} oscillations mediated by inositol trisphosphate receptor channels (IP_3Rs) are widespread and important. Their apparent regularity and the large numbers of molecules involved have led to a perception that Ca^{2+} oscillations are deterministic and predictable. But we show that for four different cell types, Ca^{2+} oscillations are stochastic, consistent with theoretical predictions claiming that they are a sequence of random spikes. The existence of a minimal interspike interval allows for regular oscillations despite such fluctuations. Random molecular fluctuations usually destroy coherence and blur spatiotemporal structures, but Ca^{2+}-induced Ca^{2+} release deploys random fluctuations constructively to generate Ca^{2+} signals that spread throughout the cell. Ca^{2}+ signals use array enhanced coherence resonance to orchestrate noise into regular oscillations.

2131-Pos IP3 Induced Ca2+ Release In Endothelial Cells Of Single Coronary Arteries Of An Intact Beating Heart

Ariel L. Escobar[1], Josefina Ramos-Franco[2]

[1] *Texas Tech University Health Sciences Center, Lubbock, TX, USA,*
[2] *Rush University Medical Center, Chicago, IL, USA.*

Board B246

The Ca^{2+} dynamics of endothelial cells has been usually addressed in cell lines or primary cultures. Under these circumstances however, the interaction between the endothelial tissue and the smooth muscle of a coronary artery is completely lost. Here, the Ca^{2+} dynamics of endothelial cells were studied for the first time at the single coronary artery level on an intact beating heart. Ca^{2+} dynamics were measured in a mouse heart mounted on horizontal Langendorff, on an upright confocal mycroscope (Zeiss 510 slm, Germany). The hearts were perfused with a Ca^{2+} indicator (either Fluo-4 or Rhod-2). Additionally, the heart was perfused with acetomethyl esther form of a caged IP_3 or nitrophenyl EGTA. These caged compounds were rapidly uncaged by applying 1 ms UV pulses generated by a frequency tripled Nd-Yag DSPP. The UV pulses were locally delivered through a multimode optical fiber micropositioned on the epicardial site of the left ventricular wall. Substantial changes in basal [Ca^{2+}] were obtained when the caged IP_3 was photo-hydrolyzed. These changes were not dependent on the extracellular Ca^{2+} concentration and were blocked by inhibiting the SERCA pump with thapsigargin. The mechanical response of a single artery was also evaluated. Our results demonstrate that this technique can be a unique tool to evaluate the endothelial function of single coronary arteries under normal or physiopatological conditions

Supported by NIH R01-HL-084487 & Cardiovascular SEED grant 7741-78-0420 to AE and NIH R01-HL-071741 to JRF

Epithelial Channels & Physiology

2132-Pos The Selectivity Mechanism of Aquaporins and Aquaglyceroporins

Jochen S. Hub, Bert L. de Groot

Max-Planck-Institute for biophysical Chemistry, Goettingen, Germany.

Board B247

Aquaporins (AQPs) constitute a family of pore proteins that facilitate the efficient flux of water across biological membranes. Related aquaglyceroporins additionally transport small organic solutes such as glycerol or urea. Within the last years substantial progress has been made in understanding the permeation mechanism through AQPs, however questions regarding their selectivity for different solutes remain challenging. Moreover, the role of aqua(glycero)porins in gas transport across membranes is still a matter of debate. Using molecular dynamics simulations, we studied the selectivity of Aquaporin-1 and the bacterial glycerol facilitator GlpF for a wide range of physiologically relevant solutes. We present potentials of mean force (PMFs) for solute permeation through the AQP channels and compare them to PMFs for the alternative route across the lipid membrane. In addition, the effects of point mutations on the channel characteristics have been studied. The results help to rationalize permeation experiments and allow to identify the molecular mechanisms underlying the selectivity of aquaporins and aquaglyceroporins.

2133-Pos Store-operated Ca^{2+} Channels (SOC) In Pancreatic Duct Epithelial Cells (PDEC)

Mean-Hwan Kim[1], Toan D. Nguyen[2], Bertil Hille[2], Duk-Su Koh[2]

[1] *POSTECH, Pohang, Republic of Korea,*
[2] *University of Washington, Seattle, WA, USA.*

Board B248

Ca^{2+} influx through store-operated Ca^{2+} channels (SOC) is activated by depletion of intracellular Ca^{2+} stores following agonist-mediated Ca^{2+} release. We previously established that Ca^{2+} entry *via* SOC contributes to exocytosis in pancreatic duct epithelial cells (PDEC) (Kim et al., 2007). We now characterize electrophysiological properties, modulation, and expression of the SOC on PDEC using Ca^{2+} imaging and patch-clamp techniques. The agonists ATP, UTP, acetylcholine, and epinephrine stimulated a Ca^{2+} influx dependent on extracellular Ca^{2+}. Inclusion of 100 μM IP_3 or 5 μM thapsigargin in the internal pipette solution elicited whole-cell currents, mediated by the SOC, of ~ 1 pA/pF (−100 mV) with 20 mM Ca^{2+} in the external medium. In a divalent cation-free medium, the SOC-mediated current (measured as a Na^{+} current stimulated by thapsigargin and inhibited by 10 μM $LaCl_3$) increased 6–7 fold. Ca^{2+} influx through SOC was completely blocked by 10 μM La^{3+} or 100 μM 2-aminoethoxydiphenyl borate (2-APB) but only partially by 50 μM SK&F 96365. Influx was also reduced by 100 μM W-7, an inhibitor of calmodulin, suggesting that Ca^{2+}-activated calmodulin modulated SOC. In polarized PDEC, thapsigargin-induced Ca^{2+}