transmembrane flux/exchange of the metabolites. These transporters may possess novel and as yet unidentified characteristics that are in proportion to their critical role in cell metabolism. Evidence has been presented that they function as homodimers *(1)* even though each subunit appears to posses its own metabolite transport path *(2)*. We have expressed some of these transporter subunits as bacterial inclusion bodies. Homodimers but not complexes with more than two subunits can be formed and such dimers are able to catalyze metabolite transport or exchange when incorporated into liposomes. We find now that dimers can also be formed between subunits of different transporters (carriers), i.e. between phosphate and oxaloacetate or between phosphate and dicarboxylate. These experiments suggest that the subunit interface of different transporters must be very much alike. Heterodimers have never been purified from or identified in mitochondria. The homodimers are formed during insertion into the mitochondrial membrane *(3)*. These membrane insertion steps are very important since a heterodimer of different transporter subunits can lead to an inappropriate exchange of metabolites that can be deleterious to oxidative phosphorylation and to the link between metabolic reactions of the matrix and the cytosol.

(1) J. Biol. Chem. (1998) 273 14269.
(2) Nature (2003) 426 39.
(3) Science (2003) 299 1747.

1393-Pos Board B237

Role of Zinc Transporter ZnT5 In PKC Signaling And Cardiac Cell Survival

Irina Korichneva, Helen Khanova, Nashaba Khan, Camie Doyle.
UMDNJ-RWJMS, New Brunswick, NJ, USA.

Zinc transporter ZnT5 was determined to protect from intracellular zinc overload. We asked whether ZnT5 participates in intracellular signaling and regulation of cell function. Using adult rat cardiomyocytes and cultured atrial cells, HL-1, we investigated interaction of ZnT proteins with protein kinase C (PKC). ZnT5 was found to co-precipitate with PKC isoforms δ and ϵ. Complex formation was zinc-dependent: zinc-depletion with the specific chelator TPEN promoted interaction. PKC activators increased ZnT5 association with PKC ϵ and dissociated PKC δ from the complex. To determine functional significance we assessed translocation of PKC isoforms in HL-1 cells. The cells were transfected with ZnT5cDNA or the mutated dominant negative (DN) form. When ZnT5 expression was modified, localization of both PKC isoforms was altered, particularly pronounced with DN ZnT expression that resulted in disappearance of PKCδ from the Golgi complex. ZnT5 was found to localize in the Golgi in proliferating cultured HL-1 cells but not in adult cardiomyocytes where sarcomeric pattern was observed. Overexpression of ZnT5 enhanced proliferation of HL-1 cells. High demand for ZnT5 in the cells with high rate of proliferation was confirmed in the developing embryos and embryonic bodies. ZnT5 was prevalent in the areas of highly proliferating cells belonging to inner cell mass and not the differentiated ones surrounding basement membrane stained for laminin. Biochemical experiments confirmed that highly proliferating cells in embryonic bodies at stage D2 have higher expression of ZnT5 but not α-actin as compared to D7 stage of development. The expression of α-fetal protein (α-FP) at day 7 indicates differentiation. The data suggest that in addition of controlling zinc homeostasis, ZnT5 zinc transporter plays an important role in signaling. Both functions are likely required for cell survival, proliferation and therefore cardioprotection and embryonic development.

1394-Pos Board B238

Mrp4 Is A Transmembrane Export Pump Acting As An Endogenous Regulator Of Cyclic- Nucleotides Dependent Pathways

Yassine Sassi[1], Larissa Lipskaia[1], Gregoire Vandecasteele[2], Viacheslav O. Nikolaev[3], Stephane Hatem[1], Fleur Cohen Aubart[1], Frans G. Russel[4], Nathalie Mougenot[1], Cedric Vrignaud[1], Philippe Lechat[1], Anne-Marie Lompre[1], Jean-Sebastien Hulot[1].
[1]Université Pierre et Marie Curie-Paris6, INSERM UMR S 621, Paris, France, [2]Université Paris XI, INSERM U769, Chatenay Malabry, France, [3]University of Würzburg, Würzburg, Germany, [4]University Nijmegen Medical Centre, Nijmegen, Netherlands.

Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are second messengers which regulate many biological processes. They can be eliminated by active efflux transporters, namely the multidrug resistance proteins MRP4 and MRP5. To delineate the role of MRP4/5, we studied arterial smooth muscle cells in which the role of cyclic nucleotide levels on proliferation has been well-established.

Methods: Human SMCs were isolated from the coronary artery media from patients. Small interfering RNAs (siRNA) specific for MRP4 mRNA were designed and validated. Adenovirus encoding MRP4 short hairpin RNA (Ad-shMRP4) were used for *in vivo* studies.

Results: MRP4 was over-expressed in serum-induced proliferating SMC as well as in atherosclerotic plaques in human coronary arteries and in neo-intima of injured rat carotid arteries. Inhibition of MRP4 by siRNA blocked VSMC proliferation *in vitro*. In balloon-injured rat carotid arteries, intima/media ratios were significantly lower in Ad-shMRP4-infected arteries than in Ad-shLuciferase-infected arteries (0.65 ± 0.1 *vs* 1.05 ± 0.2; $p<0.03$). *In vitro*, MRP4 inhibition significantly increased intracellular cAMP and cGMP levels. A PKA inhibitor (PKI) but not the PKG inhibitor (KT5823) completely reversed the anti-proliferative effect of MRP4 inhibition. The level of pCREB increased by $329 \pm 18.8\%$ ($p=0.003$) on MRP4 inhibition. **Conclusion**: We provide first evidences that MRP4 acts as an independent endogenous regulator of cyclic nucleotides intra-cellular levels in vascular smooth muscle cells

Calcium Fluxes, Sparks, and Waves I

1395-Pos Board B239

Synchronized Spontaneous Calcium Release Events Throughout The Intact Heart

Bradley N. Plummer, Michael J. Cutler, Kenneth R. Laurita.
Case Western Reserve University, Cleveland, OH, USA.

Intracellular calcium (Ca) dysregulation associated with cardiac disease (e.g. heart failure) has been linked to mechanisms of ventricular arrhythmias. Such arrhythmias can arise at the sub-cellular level from delayed afterdepolarizations that are due to spontaneous calcium release from the sarcoplasmic reticulum. The mechanisms that govern an aggregate of sub-cellular spontaneous calcium release events at the tissue level (i.e. an SCR) are not well understood. We hypothesize that in tissue, an SCR can be significantly influenced by ryanodine receptor (RyR) function. **Methods:** High resolution optical mapping of Ca (Indo-1AM) from the anterior surface of the Langendorff perfused guinea pig heart (n=4) was performed in hearts under high Ca conditions ($[Ca^{2+}]_e$=5.5mM), with and without caffeine (CAFF, 1mM) to enhance RyR open probability. Endocardial cryoablation was performed to eliminate Purkinje fibers and cytochalasin-D (7μM) was administered to remove motion artifact. Fifteen seconds of rapid pacing (400-160 ms cycle length) followed by a pause was used to induce SCR activity. **Results:** In all preparations**,** synchronized SCR activity was observed across the entire anterior surface of the heart with and without CAFF. SCR activity increased in magnitude and occurred earlier with decreasing pacing cycle length. With CAFF, the amplitude of SCR activity increased (+10.9%, $p < 0.05$) and occurred earlier (+15.6%, $p < 0.05$). CAFF also decreased the spatial heterogeneity of SCR onset across the mapping field (-37.4%, $p < 0.05$), suggesting that increased RyR open probability enhances the synchronization of SCR activity. **Conclusions:** These results demonstrate that sub-cellular spontaneous calcium release events (an SCR in tissue) occur over a broad region of the intact heart and are enhanced when RyR open probability is increased. SCR activity may be an important mechanism of arrhythmogenesis in heart disease associated with calcium dysregulation.

1396-Pos Board B240

Investigating the Ca^{2+}-Cycling Basis of Rhythmicity and Synchronicity in Coupled Cardiomyocyte Monolayers

Nicole C. Silvester, Steven R. Barberini-Jammaers, Phillip M. Ashton, Archana Jayanthi, Wai Yin Yeung, Hela Jundi, Anish Kotecha, F. Anthony Lai, Christopher H. George.
Wales Heart Research Institute, Cardiff University, Cardiff, United Kingdom.

Arrhythmia arises from the breakdown of synchronous ion handling. Abnormal intracellular Ca^{2+} cycling has emerged as a primary driver for subsequent arrhythmogenic perturbations in sarcolemmal Na^+ and K^+ fluxes. Consequently, therapeutic manipulation of intracellular Ca^{2+} handling represents a key target for new anti-arrhythmic strategies. In order to better understand the potential modes of modulating Ca^{2+} cycling that may underpin feasible anti-arrhythmic approaches, we focussed on dissecting the relationships between the spatiotemporal aspects of Ca^{2+} release and intercellular (dys)synchrony. Ca^{2+}-dependent fluo-4 signals recorded in spontaneously oscillating, electrically-coupled cardiomyocytes were decoded using the **S**ynchronicity-**A**mplitude-**L**ength and **V**ariability of **O**scillation (SALVO) program that describe 'contractile' and 'non-contractile' aspects of Ca^{2+} handling. In 40 separate experiments (n> 500 cells), intercellular synchronisation of Ca^{2+} release and sequestration was relatively constant ($42.7 \pm 2.5\%$) over wide ranges of Ca^{2+} transients profiles (transient areas of 7 - 117 units) and oscillatory frequencies (0.026 - 2.17Hz). Surprisingly, the relative areas of Ca^{2+} transients (reflecting the amplitude and kinetics of Ca^{2+} release and sequestration) were not linked to the oscillatory frequency. Although high levels of intercellular synchrony persisted despite a remarkable plasticity in both the frequencies and shapes of Ca^{2+} transients, we found that the transients became more uniformly ordered at