interactions significantly affect the VDAC properties [1]. The present study further explores these lipid-protein interactions with special emphasis on the effect of phospholipid headgroups. The VDAC was reconstituted in planar lipid bilayer formed from either DOPC or DOPE or a DOPE/DOPC mixture. The main findings of this study are: (a) DOPE is essential for the channel gating at salt concentration prevailing *in vivo* and the voltage-dependence is inhibited when DOPE is replaced by DOPC (b) in the presence of a DOPC/DOPE mixture, the DOPE molal fraction require to restore the VDAC gating is lower than that required to change the membrane viscosity, c) the unitary conductance is not affected by the DOPC or DOPE, (d) both selectivity and voltage-dependence are modulated by the degree of methylation of the DOPE but not the single channel conductance and the gating parameters. The high resolution structure of mammalian VDAC was used as a template to identify putative residues able to interact with PE headgroups. All together these results indicate that there is a direct interaction between PE and the VDAC and that PC locks the VDAC in its open state inhibiting transitions of the VDAC to the subconductance states.

[1] Mlayeh et al. Biophys.J. (2010),doi:10.1016/j.bpj.2010.07.067

551-Pos Board B351

Activation and Complex Regulation of the Kef Potassium Efflux System During Protection of Bacteria Against Toxic Electrophiles

Tim Rasmussen, Christos Pliotas, Lisbeth Lyngberg, Jess Healy, Wendy Bartlett, Samantha Miller, Tarmo P. Roosild, Samantha Castronovo, Stuart J. Conway, Ian R. Booth.

Electrophiles react rapidly with DNA and proteins and are therefore toxic. Bacteria protect themselves from the immediate effects of electrophiles by lowering the cytosolic pH before the compounds are detoxified. This is achieved by the Kef systems, which mediate potassium efflux, in turn coupled to an influx of protons. We investigated the activation and complex regulation of this system. When electrophiles enter the cell, the most likely reaction partner is the nucleophile glutathione which is present at high concentration in the cytosol. The resulting glutathione adducts activate Kef while glutathione itself inhibits. The kinetics of activation was investigated *in vivo* using a potassium selective electrode. Activator and inhibitor bind to the same binding site on the regulatory KTN domain of Kef and cause differential structural changes, as shown by crystal structures of this domain [1]. We characterised the binding and these structural changes further using fluorescence and EPR spectroscopy in purified Kef proteins. A second binding site on the KTN domain binds nucleotides. Furthermore, Kef is regulated by a soluble protein which serves as activator [2]. We present data that this activator protein also has quinone oxidoreductase activity. The relationship between this enzymatic activity and its function as an activator of Kef was dissected using point mutations to disrupt enzyme activity but preserve structural integrity.

[1] Rossild et al., *Structure 17*, 893 (2009).
[2] Miller et al., *J. Bac.182*, 6536 (2000).

552-Pos Board B352

Involvement of Caveolin-1 in Probucol-Reduced hERG Membrane Expression

Jun Guo, **Shetuan Zhang**.

The human ether-a-go-go-related gene (hERG) encodes the pore-forming subunit of the rapidly activating delayed rectifier K^+ current (I_{Kr}) important for cardiac repolarization. Dysfunction of hERG channels causes Long QT Syndrome (LQTS). While diverse compounds reduce the hERG current (I_{hERG}) by blocking hERG channels, probucol, a cholesterol-lowering drug that causes LQTS, reduces I_{hERG} by decreasing hERG channel expression in the plasma membrane. In the present study, we investigated the mechanisms of probucol effects on hERG expression. Probucol did not block hERG forward trafficking; it instead accelerated degradation of mature hERG channels. Immunocytochemistry and co-immunoprecipitation (Co-IP) data demonstrated association between hERG channels and caveolin-1 (Cav-1). In HEK cells without hERG expression, probucol promoted endogenous Cav-1 degradation. In hERG-expressing HEK cells, overexpression of Cav-1 enhanced, whereas knockdown of Cav-1 impeded probucol-induced reduction of hERG channels. Thus, probucol reduces hERG expression through accelerating Cav-1 turnover. The effects of probucol on Cav-1 and hERG result from probucol's cholesterol-disrupting action, since low density lipoprotein (LDL), a potent cholesterol carrier, effectively prevented probucol-induced reduction of I_{hERG} in hERG-HEK cells and of I_{Kr} in cultured neonatal rat ventricular myocytes. In conclusion, our data provide evidence that targeting hERG-interacting protein, caveolin, represents a novel mechanism for drug-induced hERG reduction and LQTS. (Supported by the Canadian Institutes of Health Research)

553-Pos Board B353

Involvement of Caveolin-3 in the Endocytic Degradation of the Cell Surface hERG Channels

Xian Li, Jun Guo, Shetuan Zhang.

The human ether-a-go-go-related gene (hERG) encodes the pore-forming subunits of the rapidly activating delayed rectifier potassium channel (I_{Kr}) that is important for cardiac repolarization. We have discovered that hERG channels rapidly internalize in low extracellular K^+ conditions ($[K^+]_o$), known clinically as hypokalemia. In cell culture, this internalization process is driven by endocytic protein, caveolin-1 (Cav-1), which is an integral player in the caveolae-dependent endocytosis pathway. However, Cav-1 is tissue specific; its homolog, Caveolin-3 (Cav-3), is the predominant form in muscles and thus may play a direct role in regulating hERG expression in cardiac myocytes. To investigate the roles of Cav-3 on hERG endocytosis, we overexpressed Cav-3 in human embryonic kidney (HEK) 293 cells stably expressing hERG channels. Cav-3 overexpression decreased both the hERG current and the channel expression levels by more than 50% in normal culture conditions. In addition, Cav-3 overexpression exacerbated hERG endocytosis under hypokalemic (0 mM K^+_o) culture conditions. Since hypokalemia has also been shown to reduce hERG conductance in cardiomyocytes, we demonstrated that knockdown of Cav-3 using siRNA can significantly impede such reduction. Co-immunoprecipitation analysis and confocal imaging also demonstrated an association between hERG and Cav-3 in rat and rabbit cardiomyocytes. Our data indicate that Cav-3 participates in hERG trafficking, and is an important regulator of hERG expression in cardiac myocytes. (Supported by the Heart and Stroke Foundation of Ontario and Canadian Institutes of Health Research)

554-Pos Board B354

Cardiac Potassium Channel hERG Is Regulated by Ubiquitin Ligase Nedd4l

Heidi Shallow, Jun Guo, Shetuan Zhang.

The cardiac rapidly activating delayed rectifier potassium channel (I_{Kr}) is encoded by the human ether-a-go-go related gene (hERG), which is important for repolarization of the cardiac action potential. Reduction in hERG expression levels due to genetic mutations or drugs causes Long QT Syndrome (LQTS). Recently, we demonstrated that ubiquitination of hERG channels is involved in low K^+ induced hERG endocytic degradation. Since homeostatic degradation is an important pathway in maintaining hERG membrane expression levels, we investigated the molecular mechanisms for hERG degradation by focusing on the role and consequence of the ubiquitin (UB) ligase, Nedd4L. Ub plays a role in the internalization of cell-surface hERG channels, and we hypothesized that ubiquitination of hERG channels is facilitated through Nedd4L. To study the effects of Nedd4L on hERG channels, we overexpressed Nedd4L in human embryonic kidney (HEK) 293 cells that stably express the hERG channels. We performed electrophysiological recordings, Western blot, co-immunoprecipitation analysis, and confocal microscopy to identify Nedd4L's role in hERG expression. Our data from whole-cell patch clamp recordings demonstrated that, among hEAG, Kv1.5 and hERG, Nedd4L specifically eliminates the hERG channel current. Western blot and confocal imaging analyses showed that Nedd4L overexpression led to significant reduction in mature hERG channels in the plasma membrane. Data obtained using co-immunoprecipitation indicated that Nedd4L significantly increases ubiquitinated hERG channels. Our data indicate that Nedd4L may play a role in hERG homeostatic degradation. (Supported by the Canadian Institutes of Health Research and Heart and Stroke Foundation of Ontario).

555-Pos Board B355

Structural Basis of Action for a Novel hERG1 Channel Activator

Vivek Garg, Frank Sachse, Michael Sanguinetti.

The rapid delayed rectifier K+ current (IKr) is conducted by human ether-a-go-go-related gene 1 (hERG1) channels and plays a predominant role in the repolarization of cardiac action potentials. Long QT syndrome caused by loss-of-function mutations in hERG1, or unintended blockade of hERG1 channels by commonly used drugs, can lead to severe arrhythmia and sudden death. Here, we investigated the structural basis of action of ICA-105574 (ICA), a novel hERG1 agonist previously reported to activate current by causing a large shift (>180 mV) in the V0.5 for P-type inactivation. Here we show that ICA is a partial agonist and has decreased activity in inactivation-impaired mutants of hERG1. Alanine-scanning mutagenesis of the S5, pore-helix and S6 segments of hERG1 and voltage clamp analysis of mutant channels expressed in Xenopus oocytes was used to define residues critical for current activation by ICA. The effects of 30 μM ICA were tested on 43 different mutant channels. Nine mutations attenuated ICA-mediated hERG1 activation by >10-fold. Two mutations, F557L (S5) and L622 (pore helix) completely prevented hERG1 activation by ICA. Residues identified as critical for ICA activity