Human Ether-a-go-go-Related Gene (hERG) encodes a potassium-selective voltage-gated ion channel essential for normal electrical activity in the heart. Genetic hERG mutations and blockage of the channel pore by drugs can cause long QT syndrome (LQTS). LQTS predisposes individuals to arrhythmia and puts them at risk for stroke or sudden cardiac arrest. A major problem in development of antiarrhythmic drug therapies is the proclivity for these drugs to promote fatal arrhythmias through hERG blockade. However, not all hERG blocking drugs are pro-arrhythmic, and their differential affinities to discrete channel states and/or their state stability modulations have been suggested to contribute to arrhythmogenicity. In this study, we used Rosetta density refinement and homology modeling to build complete structural models of wild-type and mutant variants of hERG channel voltage sensing and pore domains in open and closed states based on cryo-EM structures of hERG (pdb id: 5VA2) and EAG1 (pdb id: 5K7L), respectively. Here we present results from molecular docking studies using RosettaLigand to study drug interactions with hERG in open and closed states. Our results will provide structural insights into the molecular and state-dependent drug interactions with hERG that play a key role in differentiating safe and harmful hERG blockers.

1237-Pos

Beneficial Effect of Mitochondrial Calcium Uniporter Overexpression in a Guinea Pig Heart Failure and Sudden Cardiac Death Model

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Introduction: In the heart, excessive mitochondrial Ca^{2+} (mCa^{2+}) can cause permeability transition pore opening and cell death. On the other hand, insufficient mCa^{2+} can also compromise energy supply/demand matching and mitochondrial reactive oxygen species scavenging capacity, especially at high workloads. The effect of increasing mCa^{2+} uniporter (MCU) expression in the context of heart failure (HF) has not been tested.

Methods and Results: HF guinea pigs (ACi) were intramuscularly injected with adenoviral vectors expressing MCU with 3xFlag tag, leading to an increase of MCU-Flag levels by ~30% above endogenous MCU expression. MCU overexpression restored mCa^{2+} handling in failing myocytes (1-F/F0 of MityCam at 1hz stimulation in the presence of isoproterenol (ISO): 0.27 ± 0.02 in Sham, 0.21 ± 0.01 in ACi, and 0.29 ± 0.02 in ACi+MCU). Sarcomere shortening showed that the contractility of failing myocytes was significantly improved by MCU overexpression during high work load. MCU overexpression also potentiated cardiac output during β-adrenergic stimulation in perfused ACi hearts (the product of LV developed pressure and HR was increased by $36 \pm 22\%$ in ACi vs $152 \pm 47\%$ in ACi with MCU by ISO compared to baseline). To examined the effects of MCU overexpression *in vivo*, ACi heart was injected with vectors at 2w after aortic constriction, when cardiac decompensation usually accelerates. Cardiac function worsened in the untreated ACi in the following 2w (FS: $39 \pm 1\%$ at 2w and $32 \pm 1\%$ at 4w), whereas MCU overexpression significantly improved cardiac function ($36 \pm 1\%$ at 2w and 42 ± 2 at 4w). ECG telemetry recordings also showed a substantial decrease in the number of premature ventricular beats. Conclusions: The results indicate that MCU overexpression in ACi hearts enhances contractility and responses to β-adrenergic stimulation in isolated myocytes and whole hearts while mitigating HF and inhibiting arrhythmias *in vivo*.

1238-Pos

Sirtuins Positively Regulate K_{ATP} Channels, Which Contributes to their Cardioprotective Role

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Sirtuins are NAD^+-dependent enzymes that deacetylate proteins specific lysine(K) residues and are involved in aging, energy metabolism, inflammation, and stress resistance. We hypothesized that sirtuins positively regulate K_{ATP} channels, thereby contributing to stress resistance. We increased cytosolic NAD^+ by preincubation with the NAD^+ precursor nicotinamide-mononucleotide (NMN) (2 mM). After 24 hours, K_{ATP} channel currents were recorded in excised patches from HEK293 cells expressing Kir6.2/SUR2A, rat cardiomyocytes, or INS-1 cells. In all cell types, K_{ATP} current density was increased by NMN and the inhibitory response of ATP was decreased. We confirmed that NMN increases cytosolic NAD^+ levels and tested the roles of the cytosolic sirtuins (SIRT1 and SIRT2). Inhibiting their activities with EX5720 and AGK2 abolished the ability of NMN to increase K_{ATP} channel density. We performed biotinylation assays and found that the increased channel density was due (at least in part) to elevated surface expression. To determine if K_{ATP} channel subunits are K-acetylated, we immunoprecipitated K-acetylated proteins with anti-K-Ac antibodies and immunoblotted with K_{ATP} channel antibodies. Kir6.2 and SUR2A were both constitutively K-acetylated, and the K-acetylated levels were decreased by NMN in a sirtuin-dependent manner. Kir6.2ΔC36, expressed in the absence of SUR2A, was also K-acetylated, but not functionally modulated by NMN, suggesting a key role for SUR2A. We are in the process of identifying specific K-residues responsible for function. To determine the pathophysiological relevance, we preformed cellular ischemia assays using isolated rat ventricular myocytes. Cardiomyocyte contracture in an oil-overlay ischemia assay was mitigated by pharmacological preconditioning with phenylephrine or by NMN. The protective effect of NMN was reversed by inhibiting sirtuins or by K_{ATP} channel block with glibenclamide. In summary, sirtuin-dependent K-deacetylation positively regulates K_{ATP} channels and has a cardioprotective role that depends on K_{ATP} channels (Supported by TUBITAK-SBAG117S386).

1239-Pos

Probing Kv1.3 Interactome with Proximity-Dependent Biotinylation

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Protein-protein interactions (PPIs) are central to the proper functioning of the most basic molecular mechanisms underlying cellular life and are often perturbed in disease states. Traditional techniques contributed to the improvement of knowledge in this field but they demonstrated significant limitations. Recently, a new method called BioID (proximity dependent biotin identification) based on proximity-dependent labeling of proteins has been developed (Ruox *et al.*, 2012 doi.org/10.1083/jcb.201112098). The technique exploits a promiscuous biotinylating enzyme, BirA, that can be fused to the protein of interest. In this work, we applied the BioID protocol to study the PPIs of the voltage gated potassium channel Kv1.3, that displays subcellular localization to various organelles in addition to being present in the plasmamembrane. Proteins identified by BioID are candidate interactors for the channel and represent the starting point to improve our knowledge about the role this protein plays in human diseases and to possibly to identify new pathways in which Kv1.3 is involved at the cellular level.

1240-Pos

Inhibition of Connexion Hemichannels by New Aminoglycosides without Antibiotic Activity

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Connexins hemichannels (HCs) from adjacent cells form gap junctional channels that mediate cell-to-cell communication. Abnormal opening of ''free'' undocked HCs can produce cell damage and participate in the mechanism of disorders such as cardiac infarct, stroke, deafness, skin diseases, and cataracts. Therefore, inhibitors of connexin HCs have great pharmacological potential. Antibiotic aminoglycosides (AGs) have been recently identified as connexin HC inhibitors, but their antibiotic effect is an issue for the treatment of disorders where infections do not play a role. Herein, we synthesized and tested several amphiphilic AGs without antibiotic effect for their inhibition against connexin HCs using a newly developed cell-based bacterial growth complementation assay. Several leads with superior potency than the parent compound, kanamycin A, were identified. Unlike traditional AGs, these amphiphilic AGs are not bactericidal and are not toxic to mammalian cells, making them better than traditional AGs as HC inhibitors for clinical use and other applications.This work was supported in part by This work was supported in part by NSF Award CHE-1429195 for a 500 MHz Bruker NMR, American Heart Association Texas Affiliate Inc. grant 14GRNT18750014, and a TTUHSC Preliminary Data Grant.