channels, whose primary contribution is to the upstroke and plateau of the action potential; Nav1.5 [I_{Na}] (heterologously expressed) and the native cardiac L-type Ca channel, Cav1.2 [I_{CaL}] (cardiac myocytes). The biophysical properties of these two cardiac ion channels have been extensively characterized and each ion channel assay has been pharmacologically validated with reference compounds. In conclusion, this approach is part of our continuing effort to move to a cellular based *in vitro* safety approach to provide mechanistic SAR for solving cardiac safety issues. To date, our ion channel toolbox includes: hERG, Kir2.1, KvLQT1/minK, Nav1.5 and the native cardiac L-type Ca channel (Cav1.2); to be used on an as needed basis for cardiac safety evaluation.

1343-Pos Board B187

Do hERG Enhancers and Blockers Compete?

Xiaoqin Liu, James T. Limberis, Zhi Su, Kathryn A. Houseman, Gilbert J. Diaz, Gary A. Gintant, Bryan F. Cox, Ruth L. Martin.
Abbott, Abbott park, IL, USA.

HERG (human ether a go-go related gene) encodes a cardiac potassium channel that has been linked to delayed repolarization. Due to the large vestibule of the hERG channel pore, many structurally dissimilar compounds are able to block the hERG channel. This, along with specific requirement of hERG data by regulatory authorities, has added to the difficulty of drug discovery. Recently, we have discovered a series of compounds (hACTs) that do not block hERG, but actually enhance hERG current. hACT-1({4-[4-(5-trifluoromethyl-1H-pyrazol-3-yl)-phenyl]-cyclohexyl}-acetic acid) enhanced hERG current by 50 % at 60 μM. In addition, hACT-1 caused concentration-dependent shortening of the action potential duration in canine Purkinje fibers and guinea pig atrial tissue. Preliminary studies suggest that binding of hACT-1 (60 μM) does not overlap sites of typical hERG blockers. hACT-1 did not displace radio labeled dofetilide. Also, in whole-cell voltage clamp studies, combination of hACT-1 with known hERG blockers (i.e., sotalol and terfenadine) suggest that the compounds are not competing for the same binding site. When applied simultaneously with a hERG blocker, the onset of hERG enhancement with hACT-1 occurs prior to block with either sotalol or terfenadine. Block with sotalol (150 μM) occurs at the same magnitude when used alone (42 %), or in combination with hACT-1 (44 %). Similarly, the enhancement of hERG current by hACT-1 is independent of sotalol block, just as the block of hERG current by sotalol is independent of hACT-1 current enhancement. These effects demonstrate that the binding site for enhanced hERG current is different than the binding site for block.

1344-Pos Board B188

A Novel *SCN5A* Mutation Associated With Brugada Type ECG And Intraventricular Conduction Defects

Kaveh Samani[1], Tomohiko Ai[1], Jeffrey A. Towbin[2], Ramon Brugada[3], Yutao Xi[1], Jie Cheng[1], Matteo Vatta[2].
[1]Texas Heart Institute/St Luke's Episcopal Hospital, Houston, TX, USA, [2]Pediatric Cardiology/Texas Children's Hospital/Baylor College of Medicine, Houston, TX, USA, [3]Montreal Heart Institute, Montreal, QC, Canada.

Background: Mutations of SCN5A, gene encoding α-subunit of cardiac sodium channel, can cause mixed phenotypes of Brugada syndrome (BrS) and cardiac conduction diseases (CCDs).
Methods: We have identified a novel nucleotide change of *SCN5A* (4178T>G) which results in a nonsense mutation, L1393X, in a 36 year-old Caucasian male who presented with intraventricular conduction delays and BrS type ECG change. To study biophysical characteristics of L1393X-SCN5A, electrophysiological and immunostaining studies were performed using mammalian expression systems.
Results: While WT-SCN5A showed significant currents (93.3 ± 10.6 pA/pF; 1 μg plasmid), L1393X (5 μg) did not generate any significant currents in NIH-3T3 cells. The cells co-transfected with WT (0.5 μg) and L1393X (0.5 μg) showed approximately 50% current amplitudes compared to the WT (1 μg). Voltage-dependency of the steady-state activation and inactivation was not affected by the co-transfection of L1393X. Immunohistochemical stainings demonstrated that L1393X proteins were expressed in the plasma membranes.
Conclusion: Our study demonstrated that L1393X-SCN5A does not form functional channel proteins, which might account for the patient's mixed phenotypes of BrS and CCDs.

1345-Pos Board B189

Effects Of Silencing Synapse Associated Protein-97 On Cardiac Potassium Currents

Ravi Vaidyanathan[1], Steven M. Taffet[1], Karen L. Vikstrom[2], Justus M.B. Anumonwo[2].
[1]SUNY Upstate Medical University, Syracuse, NY, USA, [2]University of Michigan, Ann Arbor, MI, USA.

Introduction: Synapse associated protein-97 (SAP97) is a scaffolding protein expressed in cardiac myocytes. Previous studies have suggested that SAP97 interacts with and modifies properties of ion channels. We have investigated the functional effect of silencing SAP97 on major repolarizing currents in adult rat ventricular myocytes (ARVMs). **Methods:** SAP97 was silenced using a shRNA expressing adenovirus. The standard patch clamp technique was used to investigate the effects of this silencing on potassium currents in ARVMs. Control experiments were carried out in ARVMs infected with a GFP expressing adenovirus. **Results:** Western blot analysis showed that SAP97 was silenced in ARVM after 3 days in culture. In SAP97 silenced ARVMs, I_{K1} density was reduced by ~50% when measured at -100 mV (Holding potential (HP) = -50 mV). Average current density was -1.85 ± 0.3 pA/pF, n=12 as compared to -3.76 ± 0.5 pA/pF, n = 6 in control cells. Depolarization-activated (at +60 mV, HP = -70 mV) currents in the ARVM were fitted with a two-exponential function for analysis. Amplitude and kinetic analysis of the fits showed that there was a 30% decrease in the current density of the first component in SAP97 silenced ARVMs (9 ± 1.9 pA/pF, n=5) as compared to control (13.5 ± 0.9 pA/pF, n=4). SAP97 silencing however did not significantly change the kinetics of the first component. Time constants averaged 68 ± 12 msec and 1.1 ± 0.1 sec in control versus 121 ± 24 msec and 1.47 ± 0.21 sec in SAP97 silenced cells. Compared to the control cells, there was no change in the current density of the second component in SAP97 silenced cells. Current amplitude averaged 3.1 ± 0.4 pA/pF (n=5) and 3.7 ± 0.2 pA/pF (n=4), respectively for SAP97 silenced ARVMs and control. These results suggest that the silencing of SAP97 has differential effects on potassium currents in adult cardiac myocytes.

1346-Pos Board B190

Evidence And Functional Impact Of A New K+ Channel In Mouse Ventricular Fibroblasts

Najate Benamer[1], Hamid Moha ou maati[1], Sophie Demolombe[2,3], Patrick Bois[1], Jocelyn Bescond[1], Jean-François Faivre[1].
[1]University of Poitiers, POITIERS, France, [2]INSERM, UMR915, l'institut du thorax, NANTES, France, [3]CNRS, ERL3147, Nantes, France.

In the heart, fibroblasts represent the major cell type. They contribute to the production of the extracellular matrix. Cardiac remodelling during pathological injury is associated with differenciation of fibroblasts into myofibroblasts. The aim of this study was to characterize at molecular and functional levels a new K conductance in these cells.
Among K channel transcripts which were screened by high-throughput real-time PCR, SUR2 and Kir6.1 mRNAs were found to be the most abundant. Western-blots showed that SUR2 and Kir6.1 protein expression levels increased with culture duration as fibroblasts differenciated into myofibroblasts. In the inside-out configuration of the patch-clamp technique, SUR2/Kir6.1 K channels were recorded and showed insensitivity to ATP, inhibition by glibenclamide and activation by pinacidil and UDP. These properties are similar to those reported by Yamada et al (1998) for the SUR2/Kir6.1 molecular signature. In the whole cell configuration, these channels gave rise to a macroscopic glibenclamide-sensitive current which was activated by pinacidil and which amplitude increased with culture duration. This current was also activated by the endogenous sphingolipid sphingosine-1-phosphate (S1P) at the nM concentration range. The activation of this current was found to stimulate cell proliferation and to decrease IL-6 secretion. All these functional effects occurred for culture duration greater than 5 days.
In conclusion this work shows for the first time the presence of a glibenclamide-sensitive current which appears during differenciation of fibroblasts into myofibroblasts. This SUR2/Kir6.1 current, which may be activated in pathological conditions where fibroblasts differentiate into myofibroblasts and where S1P level increases, may modulate cardiac ventricular function.

TRP Channels

1347-Pos Board B191

The TRP Domain Of TRPC3 Is Essential But Not Sufficient For Erythropoietin- Regulated Activation Of TRPC3

Iwona M. Hirschler-Laszkiewicz.
Penn State University, Hershey, PA, USA.

TRPC3 and TRPC6 are nonselective calcium channels and two members of the canonical transient receptor potential (TRPC) subfamily expressed on human erythroblasts. Although they are 73% identical in their amino acids sequence, they respond differently to erythropoietin (Epo) stimulation. Epo stimulates a significantly greater increase in calcium influx through TRPC3 (236 ± 7% increase above baseline) compared to TRPC6 (74 ± 5% above baseline). TRPC6 also inhibits Epo-stimulated calcium influx in cells cotransfected