

(InsP<sub>3</sub>). Recently, perturbations in the InsP<sub>3</sub>R1 receptor have been linked to a human neurodegenerative disorders. The slow, progressive neurological disease, Spinocerebellar Ataxia type15 (SCA15), is inherited through an autosomal dominant gene and causes degeneration of the cerebellum. A missense mutation P1059L in the regulatory and coupling domain of the receptor (P1073L in mice) has been suggested by linkage analysis to result in SCA15 in humans. A further mutation, Iptr1Δ18/Δ18, causes the deletion of 6 amino acids in InsP<sub>3</sub>R-1 and results in an ataxic phenotype in mice. We have created stable cell lines expressing corresponding mutations in the rodent InsP<sub>3</sub>R1 gene in DT40-3KO cells, an unambiguously InsP<sub>3</sub>R null background. Immunoblotting confirmed expression of the mutant channels at comparable levels to wild-type. In both "on-nuclear" single channel patch clamp experiments and Ca<sup>2+</sup> imaging both mutated InsP<sub>3</sub>R1 receptors are functional Ca<sup>2+</sup> channels. A comparison of the activity of the mutated receptors with wild type InsP<sub>3</sub>R1 will be presented.

#### 2661-Pos

##### Regulation of Inositol 1,4,5 Trisphosphate Receptors by InsP3 Receptor-Associated cGMP Kinase Substrate (IRAG)

Wataru Masuda, Matthew J. Betzenhauser, David I. Yule.

University of Rochester, Rochester, NY, USA.

Various factors interact with IP<sub>3</sub>R, regulating Ca<sup>2+</sup> release and thus serve to define the spatial and temporal characteristics of the cytosolic Ca<sup>2+</sup> signal. IP<sub>3</sub>R-associated cGMP kinase substrate (IRAG) has been reported to bind IP<sub>3</sub>R type-1 and inhibit Ca<sup>2+</sup> mobilization in smooth muscle cells. No information is however available as to whether IRAG interacts or has functional effects on other IP<sub>3</sub>R family members. In this study, we examined whether IRAG binds to and regulates Ca<sup>2+</sup> release via IP<sub>3</sub>R type-2 or type-3. cDNA encoding IP<sub>3</sub>R type-1, IRAG-GFP, and protein kinase G1β (PKG1β) were transiently transfected into COS cells. Following immunoprecipitation from cell lysates with an anti-GFP antibody, IP<sub>3</sub>R type-1 was detected by immunoblotting. In contrast, an IRAG-GFP construct (IRAGΔE12-GFP) in which 40 amino acids required for binding with IP<sub>3</sub>R was deleted, failed to interact with IP<sub>3</sub>R type-1, but was still capable of binding to PKG1β, an additional cognate binding partner of IRAG. Similarly, IP<sub>3</sub>R type-2 or IP<sub>3</sub>R type-3 could be shown to interact with IRAG-GFP but not IRAGΔE12-GFP in COS cells. Next, we investigated if IRAG regulates IP<sub>3</sub>-induced Ca-release using DT40-3KO cell lines stably expressing mammalian IP<sub>3</sub>R type-2 or type-3 in isolation. In DT40-3KO cells stably expressing IP<sub>3</sub>R type-2, and transiently expressing Muscarinic M3 receptor, IRAG-GFP and PKG1β, cell permeable PKG activators reduced the muscarinic agonist carbachol (CCh)-induced Ca<sup>2+</sup>-release. Ca<sup>2+</sup> oscillations induced by low concentrations of CCh or by stimulating the endogenous B cell receptor were similarly attenuated. No inhibitory effect was evident in cells expressing IRAGΔE12-GFP or in the absence of IRAG-GFP. Similar results were obtained with DT40-3KO cells stably expressing IP<sub>3</sub>R type-3. These results indicate that Ca<sup>2+</sup> release through all Inositol 1,4,5 trisphosphate receptors are inhibited by an interaction with IRAG and PKG1β.

#### 2662-Pos

##### CaMKII-Mediated Phosphorylation of InsP<sub>3</sub>R2 at Serine-150 Results in Decreased Channel Activity

Joshua T. Maxwell, A.S. Aromolaran, Gregory A. Mignery.

Loyola University Medical Center, Maywood, IL, USA.

InsP<sub>3</sub> mediated calcium release through the type-2 inositol 1,4,5-trisphosphate receptor (InsP<sub>3</sub>R2) in cardiac myocytes results in the activation of associated CaMKIIδ (Bare et al, 2005, *JBC*; Wu et al, 2006, *JCI*), enabling the kinase to act on downstream targets, such as histone deacetylases 4 & 5 (HDAC4 & HDAC5) (Zhang et al, 2007, *JBC*). The CaMKII activity also feedback modulates InsP<sub>3</sub>R2 function by direct phosphorylation and results in a dramatic decrease in the receptor-channel open probability ( $P_o$ ). The results of this study show that in planar lipid bilayers the channel activity of InsP<sub>3</sub>Rs can be inhibited by CaMKII-mediated phosphorylation, and that effect can be reversed by addition of protein phosphatases. Furthermore, we have used fragments of the InsP<sub>3</sub>R2 and site-directed mutagenesis to determine that Serine at residue 150 is the CaMKII phosphorylation site responsible for modulation of channel activity. Non-phosphorylatable (S150A) and phospho-mimetic (S150E) mutations were constructed in the full-length InsP<sub>3</sub>R2, expressed in COS cells and studied in planar lipid bilayers. Upon treatment with CaMKII, the non-phosphorylatable channel showed no decrease in activity. Conversely, the phosphomimetic channel displayed a very low  $P_o$  under normal recording conditions in the absence of CaMKII (2μM InsP<sub>3</sub> and 250nM [Ca<sup>2+</sup>]<sub>FREE</sub>), thus mimicking a channel that has been phosphorylated by CaMKII. The results of this study show that Serine-150 of the InsP<sub>3</sub>R2 is phosphorylated by CaMKII and results in a decrease in the channel's open probability. The mechanism for the regulation of the InsP<sub>3</sub>R2 appears to be a consequence of altered affinity for InsP<sub>3</sub> at

the ligand binding site and/or perturbation of the receptor amino to carboxyl-terminal interaction and is currently being examined.

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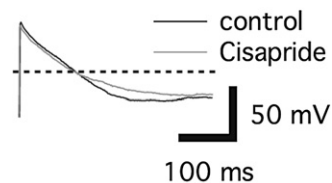
#### 2663-Pos

##### Excitement Over Automated Patch Clamp: Action Potentials from Cardiac Myocytes

Sonja Stoelzle<sup>1</sup>, Andrea Bruggemann<sup>1</sup>, David Guinot<sup>1</sup>, Alison Haythornthwaite<sup>1</sup>, Michael George<sup>1</sup>, Cecilia Farre<sup>1</sup>, Claudia Haarmann<sup>1</sup>, Ralf Kettenhofen<sup>2</sup>, Niels Fertig<sup>1</sup>.

<sup>1</sup>Nanon Technologies, Munich, Germany, <sup>2</sup>Axisogenesis AG, Cologne, Germany.

The use of cardiac myocytes is becoming increasingly important for drug safety testing. Unique features of certain planar patch clamp workstations, coupled with ease-of-use and higher data throughput, make these devices ideal tools for ion channel screening and safety testing. Using stem cell derived cardiac myocytes, recordings could be made not only in the voltage-clamp mode but also in the current-clamp mode on a planar patch clamp workstation. This demonstrates for the first time parallel current-clamp recordings on a planar patch clamp workstation. Ion channels important in drug discovery, such as hERG and voltage-gated Na<sup>+</sup>, Ca<sup>2+</sup> and K<sup>+</sup> channels in the voltage-clamp mode from stem cell derived cardiac myocytes will be shown. In addition, action potential recordings in the current-clamp mode at 35°C, and modulation of the action potentials by hERG active compounds, will also be shown.



#### 2664-Pos

##### In Silico Studies of Cardiac Inotropy using a New Model of Force Generation

Jose L. Puglisi<sup>1</sup>, Jorge A. Negroni<sup>2</sup>, Donald M. Bers<sup>1</sup>.

<sup>1</sup>University of California, Davis, Davis, CA, USA, <sup>2</sup>Universidad Favaloro, Buenos Aires, Argentina.

An improved model of force generation was incorporated into a complete mathematical description of action potential (AP), ionic currents and Ca<sup>2+</sup> transient of the rabbit ventricular myocyte (LabHEART 5.0). This new model reproduces the main events involved in Excitation-Contraction Coupling, namely the AP (excitation), the shortening (contraction) and the Ca<sup>2+</sup> transient as the link between them. LabHEART 5.0 was able to reproduce isotonic and isometric contractions and the classical curves of Force vs. Ca<sup>2+</sup> and Force vs cell length. Cardiac inotropy was investigated by simulating the application of isoproterenol (ISO). This effect was achieved by altering L-type Ca<sup>2+</sup> current, the slowly activating delayed rectifier K<sup>+</sup> current, sarcoplasmic reticulum (SR) Ca<sup>2+</sup> pump, SR Ca<sup>2+</sup> leak, myofilament Ca-sensitivity and cross-bridge cycling. The latter modification was essential for replicating the ISO-induced increase in force generation/shortening development experimentally observed. AP duration (APD, for 90% of repolarization) adaptation to pacing frequencies was also examined. ISO shortened APD at all frequencies with respect to control and flattened the adaptation curve, thus allowing an APD compatible with short cycle length (up to 5 Hz). ISO also increased the Ca<sup>2+</sup> transient dynamic range by keeping a low diastolic level while increasing the peak Ca<sup>2+</sup> at all the simulated frequencies (0.5 to 9 Hz). This model provides a useful framework to study cardiac inotropy and constitutes a starting point to investigate the electro-mechanical feedback in cardiac performance. The new version LabHEART 5.0 is freely available online at [www.labheart.org](http://www.labheart.org).

## Voltage-gated Ca Channels I

#### 2665-Pos

##### Monte Carlo Simulation of Free Energy Components: Energetics of Selective Binding in a Reduced Model of L-Type Ca Channels

Janhavi Giri<sup>1,2</sup>, Bob Eisenberg<sup>2</sup>, Dirk Gillespie<sup>2</sup>, Douglas Henderson<sup>3</sup>, Dezső Boda<sup>4</sup>.

<sup>1</sup>University of Illinois at Chicago, Chicago, IL, USA, <sup>2</sup>Rush University Medical Center, Chicago, IL, USA, <sup>3</sup>Brigham Young University, Provo, UT, USA, <sup>4</sup>University of Pannonia, Veszprém, Hungary.

A reduced model of voltage-gated L-type Ca channels is used to study the energetics of selective binding of Ca<sup>2+</sup> versus monovalent and divalent cations. Widom's particle insertion method is combined with Grand Canonical Monte Carlo simulations to compute the electrostatic and excluded volume components of the free energy difference between channel and bath. We have shown (in ~ 30 papers) that selectivity of the L-type Ca channel and voltage activated