**2650-Pos**

**Enhanced Beta-Adrenergic Response, Spontaneous Calcium Release, and Arrhythmogenic Propensity in Mice with a Phosphomimetic Mutation of a PKA Site (S2030D) in the Cardiac Ryanodine Receptor**

**Qiang Zhou**, Yijun Tang, Ruiwu Wang, S.R. Wayne Chen.
University of Calgary, Calgary, AB, Canada.

The phosphorylation of the cardiac calcium release channel/ryanodine receptor (RyR2) by protein kinase A (PKA) has been extensively investigated, but its functional consequences remain poorly understood and controversial. We have previously shown that S2030 is a major PKA site in RyR2 responding to beta-adrenergic stimulation, and that phosphomimetic mutation of this PKA site (S2030D) enhances luminal calcium activation of single RyR2 channels and increases the propensity for spontaneous calcium release in HEK293 cells during store calcium overload. To further investigate the physiological and pathophysiological significance of PKA phosphorylation of S2030, we generated a knock-in mouse model harboring the phosphomimetic S2030D mutation in RyR2. We found that S2030D mutant mice displayed higher isoproterenol-induced heart rate increase than wt mice. ECG recordings revealed that S2030D mutant mice were more susceptible to bidirectional ventricular tachycardia induced by the injection of epinephrine plus caffeine as compared to wt mice. To determine the impact of the S2030D mutation on sarcoplasmic reticulum (SR) calcium handling, ventricular myocytes isolated from wt and mutant mice were loaded with fluo-4 and field-stimulated. Calcium imaging showed that S2030D mutant cells exhibited enhanced spontaneous calcium release activity in the presence of isoproterenol. These observations indicate that the S2030D mutation in RyR2 enhances the beta-adrenergic response, and suggest that excessive PKA phosphorylation of S2030 in RyR2 may increase the propensity for stress-induced spontaneous SR calcium release and ventricular arrhythmias (supported by CIHR).

**2651-Pos**

**Mice Expressing Heterozygous and Homozygous RyR1-T4826I Mutation Reveal Gender-Dependent Phenotypic Penetrance to MH Triggering Agents and Altered Temperature Regulation Following Glucose Challenge**

**Benjamin T. Yuen**[1], Genaro Barrientos[1], Diptiman D. Bose[1], Tianzhong Yang[2], Samuel R. Goth[1], Paul D. Allen[2], Isaac N. Pessah[1].
[1]University of California, Davis, VM: Molecular Biosciences, Davis, CA, USA, [2]Bringham and Women's Hospital, Anesthesiology, Boston, MA, USA.

MH is an adverse reaction to general anesthetics characterized by increased temperature, muscle rigidity, general metabolic acidosis, and is fatal if left untreated. Knock-in mice expressing either heterozygous (HET) or homozygous (HOM) human MH mutation RyR1-T4826I are fully viable under typical rearing conditions. HOM mice trigger within 20 min of heat stress (40°C) or within 40 min of exposure to halothane (2% ≅37°C), whereas HET mice do not. All HET males exposed to a single halothane anesthetic (2% ≅40°C) trigger with MH, whereas up to 50% of the females survive multiple anesthetics. Fasted mice were challenged with glucose i.p., and glucose uptake rates measured. Our results indicate that at 23°C, HOM and WT males exhibit similar glucose uptake, whereas HOM females displayed lower glucose uptake than their WT counterparts (regardless of gender, n = 4). However, at 30°C, HOM males and females exhibit enhanced glucose uptake compared to corresponding WT (n = 4). WT male and female animals maintain stable temperatures during testing, whereas HOM mice show an inverse relationship (decreasing body temperature with increasing blood glucose following challenge). MH mutation RyR1-T4826I confers genotype- and gender-dependent susceptibility to triggering. These data reveal that RyR1-T4826I mice have pronounced metabolic impairments that may contribute to the fulminant MH response. Supported by NIH AR43140 and AR52354.

**2652-Pos**

**Role of Oxidative Stress, Autophagy and Apoptosis in the I4895T RyR1 Knockin Mice**

**Aditya D. Joshi**, Ruirui Ji, Jianjun Xu, Cheng Long, Susan L. Hamilton.
Baylor College of Medicine, Houston, TX, USA.

I4898T mutation in the skeletal muscle ryanodine receptor (RyR1) in humans has been suggested to underlie Central Core Disease (CCD). CCD is the congenital myopathy characterized by muscle weakness of lower extremities leading to delayed attainment of motor skill milestones. We created mice with a corresponding RyR1 I4895T mutation. Although no central cores were observed in the muscle of these mice, this uncoupling mutation dramatically reduces voluntary running. The soleus muscle from the I4895T mice displays decreased ability to generate force, increased oxidative stress, decreased antioxidant capacity and decreased cytochrome c oxidase activity (suggesting mitochondrial damage). We also find evidence of both autophagy and apoptosis in the mutant muscle since Atg5, Atg6 (Beclin1), Atg9, Atg12, Bcl2, caspase family members, C/EBP homologous protein (CHOP) are all upregulated in the soleus. Upregulation of several ER stress related proteins (BiP, IRE1α, and PDI) was also observed. We propose that the I4895 mutation is associated with the oxidative stress, an unfolded protein response (UPR), autophagy and apoptosis, all of which may contribute to mitochondrial destruction associated with this disease.

**2653-Pos**

**Does Central Core Disease have a Neuronal Component?**

**Valerie De Crescenzo**[1], Kevin E. Fogarty[1], Karl D. Bellvé[1], Richard A. Tuft[1], Elena Zvaritch[2], David H. Maclennan[2], John V. Walsh[1].
[1]UMASS med school, Worcester, MA, USA, [2]University of Toronto, Toronto, ON, Canada.

Type 1 ryanodine receptors (RyR1) are the second most common isoform found in neurons after RyR2. We have provided evidence that in nerve terminals from neurohypophysis, L-type Ca2+ channels are coupled to RyR1 in the same way found in EC coupling in skeletal muscle. In these nerve terminals (J.Neuroscience 2006, 26 −7565) L-type channels are the sensors of membrane potential for Voltage Induced Ca2+ Release (VICaR), independently of their role as Ca2+ current carriers, and RyRs of uknown type are the effectors through which Ca2+ is released into the cytosol. Here we wished to study whether RyRs of type 1 are mediators of VICaR in these nerve terminals. We employed a RyR1 mutant mouse (I4895T) with a loss of function phenotype that causes Central Core Disease in humans. We studied both, global [Ca2+] with Fura-2 and focal [Ca2+] in the form of Ca2+ syntillas with fluo3. When the nerve terminals were depolarized to 0mV, the mutant showed a 4-fold decrease in the global Ca2+ transient compared to WT. This very large decrease in the Ca2+ transient occurred in the absence of any change in the Ca2+ current. In the absence of external Ca2+, we also demonstrated the presence of VICaR in WT. Moreover, in the WT depolarization to 0mV from −80mV induced a 3 fold increase in syntilla frequency due to VICaR (i.e. in the absence of external Ca2+). VICaR was totally absent in the mutant. These data show for the first time the involvement of type 1 RyRs during depolarization of nerve terminals. This suggests that Central Core Disease has a neuronal component as well as a skeletal muscle component.( Supported by grants from NIH GM087580-01 to JW and AHA 0835580D to VD.)

**2654-Pos**

**Functional Role of Mitochondrial Ryanodine Receptor Type 1 in the Heart**

Sung Hyun Kang[1], Sergiy M. Nadtochiy[1], George Porter[1], Gisela Beutner[1], Karen Bentley[1], Paul S. Brookes[1], Robert T. Dirksen[1], Susan L. Hamilton[2], Winston E. Gaum[1], **Shey-Shing Sheu**[1].
[1]University of Rochester Medical Center, Rochester, NY, USA, [2]Baylor College of Medicine, Houston, TX, USA.

We recently demonstrated the existence of type 1 ryanodine receptors (RyR1) in the mitochondria of cardiac muscle cells (Beutner et al, 2001, 2005, Altschafl et al, 2007). However, the functional significance of mitochondrial type 1 ryanodine receptors (mitoRyR1), especially in the whole heart preparations, remains unclear. We determined the cardiac phenotype of knock-in mice harboring an RyR1 mutation ($RyR1^{Y522S}$) that promotes RyR1 $Ca^{2+}$ leak and results in malignant hyperthermia and central cores in humans. In Langendorff perfused hearts, isoproterenol (10-50 nM) infusion induced a significantly ($p<0.01$) larger increase in heart rate (HR) and left ventricular end-diastolic pressure (LVDEP) in $RyR1^{Y522S/\ WT}$ mice (HR, 593.1 ± 24.0 / min; LVDEP, 56.2 ± 1.5 mmHg) compared with littermate control wild type mice (HR, 518.6 ± 11.4/ min; LVDEP, 24.8 ± 7.4 mmHg).In anesthetized mice, electrocardiogram recordings showed isoproterenol-induced arrhythmia and tachycardia in the $RyR1^{Y522S/WT}$ but not wild type mice (n>8). We studied the morphology of cardiac mitochondria using electron microscopy. Cardiac mitochondria in $RyR1^{Y522S/WT}$ mice (3-18 months old) exhibit a lower density of cristae, a higher incidence of mitochondrial clustering and misalignment with sarcomeres, and a dissociation of mitochondria from myofilaments, as routinely observed in controls (n>5). Finally, we examined the heart structure of homozygous $RyR1^{Y522S/Y522S}$ embryos, which die prior to birth. Compared to $RyR1^{Y522S/WT}$ and wild-type littermates, $RyR1^{Y522S/Y522S}$ embryos developed normally until approximately embryonic day (E) 14.5, after which their hearts exhibit significant myocyte disarray, lack ventricular lumen and display evidence of heart failure. These results are consistent with the hypothesis that mitoRyR1 plays an important role in the proper development and regulation of cardiac structure and function.