reduce or abolish the propensity for store-overload induced Ca release (SOICR) in HEK293 cells. In addition, single channel analysis showed that these mutations significantly reduce the response of the channel to activation by cystolic and luminal Ca. These observations demonstrate, for the first time, that suppression or loss of RyR2 function may be a common mechanism underlying idopathic ventricular fibrillation, which is opposite to the gain-of-function RyR2 mutations associated with CPVT. Thus, understanding the exact molecular defects of disease-causing RyR2 mutations will help to develop novel approaches to the diagnosis and personalized treatment of these lethal cardiac arrhythmias (Supported by CFI, CHIR, AIHS, and LCI).
interactions of electrostatic nature that induce coupled events (Supported by NIH R01 GM078665 and AHA-MWA 12180038).

577-Pos Board B332
Phosphorylation of Maurocalcin Strongly Modifies its Effect on Type 1 Ryanoide Receptor
Wei Feng 1, Michel Ronjat 1, Yao Dong 2, Eloi Bahemba 2, Michel De Waard 1, Isaac N. Packard 1 1University of California, Davis, Davis, CA, USA. 2LabEx Ion Channel Science and Therapeutics, Grenoble Institut des Neurosciences Inserm U836, UJF, Grenoble, France.

Maurocalcin (MCa) is a 33-amino acid peptide isolated from the venom of Scorpion maurus palmatus, a Tunisian scorpion. It possesses efficient cell penetration properties and has been shown to strongly modify ryanodine receptor (RyR1) channel behavior by increasing open probability and inducing a long-lasting subconductance state. The amino acid residue threonine at position 26 belongs to a putative phosphorylation site within MCA sequence. We investigated the effect of a) T26 phosphorylation (MCa T26Phospho) by protein kinase A and b) replacement of T26 by glutamic acid residue to mimic phosphorylation on the effect of MCa on RyR1 properties. Using [3H]ryanodine binding and single channel voltage-clamp measurements, we show that both MCa T26Phospho and MCa T26E analogs almost completely lose the ability to induce RyR1 activation. Neither MCa T26Phospho nor MCa T26E (up to 2μM) increase [3H]ryan binding. Small increase (~1.65-fold activation; p<0.01) was observed with high concentrations (10μM) of either MCa T26Phospho or MCa T26E. Single channel measurements revealed that neither MCa T26E nor MCa T26Phospho were able to induce the characteristic long-lasting subconductance state of RyR1 observed in presence of wild-type MCa. MCa T26Phospho altered RyR1 channel gating in a time-dependent biphasic manner with initial activation of Po followed by almost complete inactivation. These effects of MCa T26Phospho could be reversed by 200nM wt MCa. At concentrations ≥2μM, MCa T26E enhances Po~3 fold by shortening τx without altering τx. These results show that single phosphorylation of MCa modifies RyR1 activity. P011 AR52354.

578-Pos Board B333
Effects of Amino-Terminal Disease-Associated Mutations on the CICR Activity of RyR1 Channel
Takashi Murayama 1, Naogami Kurebayashi 2, Toshiko Yamazawa 2, Hitode Oyamada 1, Shigeru Takemori 1, Katsuji Oguchi 1, Takashi Sakurai 1 1University of Tokyo School of Medicine, Tokyo, Japan, 2Jikei University School of Medicine, Tokyo, Japan.

Type 1 ryanodine receptor (RyR1) is a Ca2+ release channel in the sarcoplasmic reticulum (SR) that plays a pivotal role in excitation-contraction coupling in skeletal muscle. RyR1 is the major target for muscle diseases, e.g., malignant hyperthermia (MH) and central core disease (CCD). To date, over 200 mutations in the RyR1 gene have been reported in MH patients. Most of those mutations have been found in three “hot spots” regions of RyR1. However, there were only a few experimental results confirming those mutations being responsible for the increment of the CICR sensitivities. We improved the method for making MH mutants in the cDNA of RyR1. We characterized the functional mutations on RyR1 in non-muscle cells, specifically HEK293 cells with tetracycline-regulated RyR1 expression. Rabbit RyR1 channels carrying corresponding mutations were expressed in HEK293 cells for functional assay. HEK293 cells were loaded at room temperature with fur-2 AM in physiological salt solution. Fluorescence images were acquired using an inverted microscope equipped with a objective, a cooled CCD camera and a polychromatic illumination system. We characterized the functional mutations on RyR1 in HEK293 cells. It was found that disease-associated mutations of the RyR1 resulted in enhanced Ca2+ release activity, therefore these mutations would be responsible for the MH incidence. These results suggest that exploration of the functional mutations of RyR1 is probably effective in preventive diagnosis of patients associated with MH disease.

580-Pos Board B335
Malignant Hyperthermia Associated Mutations in S2-S3 Loop of Type 1 Ryanoide Receptor Calcium Channel Alter Calcium Dependent Inactivation
Angela C. Gomez, Timothy Holford, Naohiro Yamaguchi 1
Medical University of South Carolina, Charleston, SC, USA.

Skeletal and cardiac ryanodine receptors (RyRs) are ~65% similar in their primary sequences, though differences exist in their regulatory properties and roles in disease. RyR mutations have been reported in MH and central core disease (CCD) to date, over 200 mutations in the RyR1 gene have been reported in the literature. Of these, mutations at amino-terminal region in HEK cells and tested their CICR by immunofluorescence and [3H]ryanodine binding. Our results suggest that exploration of the functional mutations of RyR1 is probably effective in preventive diagnosis of patients associated with MH disease.

581-Pos Board B336
Ryanodine Receptor Interaction with FKBP12 is Modulated by the RyR N-Terminus Repeat Region
Polly Marino, F. Anthony Lai, Lynda Blayney 1, School of Medicine, Cardiff Univ, Cardiff, United Kingdom.

The ryanodine receptor (RyR) is an ion channel involved in the release of Ca2+ from intracellular stores (sarcoplasmic reticulum) in muscle cells. RyR plays a pivotal role in excitation-contraction coupling and is modulated by several accessory proteins in vivo, including the immunophilin FK506 binding protein 12 (FKBP12) which, our previous studies demonstrate, binds to RyR with extremely high affinity. The FKBP12 binding site within the RyR sequence has not been definitively identified and a number of candidate regions have been proposed. A central domain phosphorylation region of RyR (CDR) has been crystallized (Sharma, P. et al, FEBS (2013)288, 903-914; Yuchi, Z. et al., Structure (2012) 20, 1210-1211). This domain has considerable sequence and structural homology to its repeat region at the N-terminus (NTR) and a pseudo atomic model of the two repeat regions, docked onto a topology model of RyR, positions NTR adjacent to the previously proposed FKBP12 binding domain and suggests a potential interaction (Zhu, L. et al, JBC (2013) 288, 903-914). The RyR interaction with FKBP12 was tested using [3H]ryanodine binding to measure the Po of the channel of solubilised pig RyR preparations and microsomal preparations. A dose response to FKBP12 reduced activity...