

interactions of electrostatic nature that induce coupled events (Supported by NIH R01 GM078665 and AHA-MWA 12180038).

#### 577-Pos Board B332

##### Phosphorylation of Maurocalcine Strongly Modifies its Effect on Type 1 Ryanodine Receptor

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Maurocalcine (MCA) is a 33-amino acid peptide isolated from the venom of *Scorpio 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 [<sup>3</sup>H]ryanodine ([<sup>3</sup>H]Ry) 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 [<sup>3</sup>H]rya 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 (up to 2μM) 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 τ<sub>c</sub> without altering τ<sub>o</sub>. These results show that single phosphorylation of MCA modifies RyR1 activity. IP01 AR52354.

#### 578-Pos Board B333

##### Effects of Amino-Terminal Disease-Associated Mutations on the CICR Activity of RyR1 Channel

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Type 1 ryanodine receptor (RyR1) is a Ca<sup>2+</sup> release channel in the sarcoplasmic reticulum and 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 have been identified in the RyR1 gene of MH and CCD patients. It is widely believed that MH and CCD mutations cause hyperactivation of the Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR), resulting in abnormal Ca<sup>2+</sup> homeostasis in skeletal muscle. CICR shows biphasic Ca<sup>2+</sup> dependence, thus the activity can be determined by three parameters: sensitivity to activating Ca<sup>2+</sup>, sensitivity to inactivating Ca<sup>2+</sup>, and the gain (i.e., peak activity). However, it remains unclear how the disease-associated mutations affects these parameters. In this study, we expressed several RyR1 channels carrying different MH/CCD mutations at amino-terminal region in HEK cells and tested their CICR by live-cell Ca<sup>2+</sup> imaging and [<sup>3</sup>H]ryanodine binding. Our results suggest that the amino-terminal disease-associated mutations divergently affects the parameters of CICR depending on the sites for mutation. The underlying molecular mechanism will be discussed.

#### 579-Pos Board B334

##### Functional Analysis of Ryanodine Receptor Carrying Malignant Hyperthermia Associated Mutations

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Ryanodine receptors, located in the sarcoplasmic/endoplasmic reticulum (SR/ER) membrane, are required for intracellular Ca<sup>2+</sup> release that is involved in a wide range of cellular functions. Malignant hyperthermia (MH) is a pharmacogenetical complication of general anesthesia resulting from abnormal

Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR) via the type 1 ryanodine receptor (RyR1) in skeletal muscles. The typical symptoms include a rapid increase in body temperature and induction of a hypermetabolic state with skeletal muscle rigidity. More than 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 fura-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 Ca<sup>2+</sup> 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 Ryanodine Receptor Calcium Channel Alter Calcium Dependent Inactivation

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Skeletal and cardiac ryanodine receptors (RyRs) are ~65% similar in their primary sequences, though differences in their regulation by physiological molecules have been observed. Skeletal RyR (RyR1) is inhibited by millimolar Ca<sup>2+</sup> with ~10 fold higher affinity than cardiac isoform (RyR2). Using RyR1/RyR2 chimera channels and [<sup>3</sup>H]ryanodine binding measurements, we found that two distinct regions are involved in isoform-specific Ca<sup>2+</sup>-dependent inactivation. One region includes two EF hand Ca<sup>2+</sup> binding motifs (RyR1 amino acids 4081-4092 and 4116-4127) and the other contains the second transmembrane segment (S2). The results suggest a possible cytoplasmic domain interaction between these two regions (or involving the flanking regions of S2). Human disease associated mutations have been identified in S2-S3 cytoplasmic loop of RyR1. We found that G4733E and R4736W malignant hyperthermia associated mutations reduced affinity for Ca<sup>2+</sup> dependent inactivation of the channels by 5-6 fold (IC<sub>50</sub>: 6.7 ± 0.6 mM (G4733E) and 5.5 ± 0.2 mM (R4736W) vs 1.1 ± 0.1 mM (wild type RyR1)), whereas mutations in S4-S5 cytoplasmic loop (T4825I and H4832Y) reduced affinity by 2-3 fold. We also found that the activities of G4733E- and R4736W-RyR1 mutants are suppressed at 10-100 μM Ca<sup>2+</sup>, and the suppressions are relieved by 1 mM Mg<sup>2+</sup>, which was observed in recombinant wild type RyR2 (Chugun et al., (2007) *Am. J. Physiol. Cell Physiol.* 292, C535) but not in wild type RyR1. Taken together, G4733E and R4736W mutations in S2-S3 loop confer RyR2-type Ca<sup>2+</sup> dependent inactivation and Mg<sup>2+</sup> activation on RyR1. The S2-S3 cytoplasmic loop may play a key role for domain interaction involved in isoform-specific Ca<sup>2+</sup>-dependent inactivation of RyRs. Supported by NIH (R03AR061030), AHA (10SDG3500001), and NSF (EPS0903795).

#### 581-Pos Board B336

##### Ryanodine Receptor Interaction with FKBP12 is Modulated by the RyR N-Terminus Repeat Region

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The ryanodine receptor (RyR) is an ion channel involved in the release of Ca<sup>2+</sup> 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 crystallised (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 RyR1 interaction with FKBP12 was tested using [<sup>3</sup>H]ryanodine binding to measure the Po of the channel of solubilised pig RyR preparations and microsomal preparations. A dose response to FKBP12 reduced activity