568-Pos Board B323

Divergent Effects of Disease-Associated Mutations on Type 2 Ryanodine Receptor Channel

Nagomi Kurebayashi[1], Takashi Murayama[1], Akira Uehara[2], Midori Yasukochi[2], Takashi Sakurai[1].
[1]Dept. of Pharmacology, Juntendo University School of Medicine, Tokyo, Japan, [2]Dept. of Cell Biology, Fukuoka University School of Medicine, Fukuoka, Japan.

Type 2 ryanodine receptor (RyR2) is a Ca^{2+} release channel in the sarcoplasmic reticulum and plays a pivotal role in excitation-contraction coupling in heart. RyR2 is the major target for arrhythmogenic diseases, e.g., catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular cardiomyopathy (ARVC). To date, over 150 mutations have been identified in the RyR2 gene of CPVT and ARVC patients. It is widely believed that CPVT and ARVC mutations cause hyperactivation of the Ca^{2+}-induced Ca^{2+} release (CICR), resulting in abnormal Ca^{2+} homeostasis in cardiac muscle. CICR shows biphasic Ca^{2+} dependence against cytoplasmic Ca^{2+}, thus the activity can be determined by three parameters: sensitivity to activating Ca^{2+}, sensitivity to inactivating Ca^{2+}, and the gain (i.e., peak activity). In addition, CICR is also regulated by luminal Ca^{2+}; high luminal Ca^{2+} activates CICR, and vise versa. However, it remains unclear how the disease-associated mutations affect these parameters. In this study, we expressed RyR2 channels carrying several CPVT/ARVC mutations in HEK cells and tested their CICR by live-cell Ca^{2+} imaging and [^{3}H]ryanodine binding. Our results suggest that the disease-associated mutations divergently affects the parameters of CICR depending on the sites for mutation. The underlying molecular mechanism will be discussed.

569-Pos Board B324

Crystal Structure of Cardiac Ryanodine Receptor N-Terminal Region Contains Unique Anion Binding Site Targeted by Disease-Associated Mutations

Lynn Kimlicka[1], Ching-Chieh Tung[1], Anna-Carin C. Carlsson[2], Paolo A. Lobo[1], Zhiguang Yuchi[1], Filip Van Petegem[1].
[1]University of British Columbia, Vancouver, BC, Canada, [2]University of Gothenburg, Gothenburg, Sweden.

Ryanodine receptors (RyRs) are intracellular calcium-release channels of the endo/sarcoplasmic reticulum that are critical to the muscle excitation-contraction coupling. Mutations in the cardiac isoform (RyR2) are linked to life-threatening arrhythmias, such as catecholaminergic polymorphic ventricular tachycardia (CPVT), known for a sudden cardiac arrest. RyR2 is the target for over 150 disease-associated mutations. Here, we present the 2.0Å crystal structure of the N-terminal region of RyR2 (residues 1-547), an area containing 29 distinct disease-associated mutations. The protein folds up in three individual domains similarly to its counterpart in the skeletal muscle, RyR1, but contains a unique central chloride anion that holds together the three domains. The CPVT-associated mutant R420Q targets one of the Arginines coordinating the anion and ablates chloride binding. The crystal structure of the mutant shows reorientations in the first two domains relative to the third, likely destabilizing intersubunit interactions in the context of an intact channel. Chloride anion binding may represent one of the ways RyR2 uses to regulate channel opening.

570-Pos Board B325

Arrhythmogenic Mechanisms in Catecholaminergic Polymorphic Ventricular Tachycardia Linked to RyR2 Loss-of-Function Mutation

Yan-Ting Zhao[1], Carmen R. Valdivia[1], Georgina B. Gurrola[1], Cong Li[1], Patricia P. Powers[2], Héctor H. Valdivia[1].
[1]Internal Medicine, University of Michigan, Ann Arbor, MI, USA, [2]University of Wisconsin-Madison, Madison, WI, USA.

To date, classical mechanisms of arrhythmogenesis in CPVT require spontaneous Ca^{2+} release via *hyperactive* RyR2 channels affected by gain-of-function mutation. Here, we report novel arrhythmia mechanisms in a CPVT-linked RyR2-A4860G mutation that *depresses* channel activity. Murine RyR2-A4860G cDNA was expressed in CHO cells to the same level than wild-type (WT), but recombinant RyR2-A4860G protein displayed dramatically reduced channel activity by [^{3}H]ryanodine binding assay and direct single channel activity recording. Mice heterozygous for RyR2-A4860G mutation (RyR2-A4860G$^{+/-}$) exhibited basal bradycardia without cardiac structural alterations; no homozygotes were detected at birth, indicating the mutation is phenotypically too strong to be harbored in two alleles. Anesthetized RyR2-A4860G$^{+/-}$ mice injected with arrhythmogenic cocktail (120mg/kg caffeine, 2mg/kg epinephrine) displayed ECG alterations of QRT alternans, premature ventricular complexes and bidirectional ventricular tachycardia, while ECGs of WT mice remained uneventful. Simultaneous recording of action potentials (APs) and $[Ca^{2+}]_i$ transients in isoproterenol-stimulated RyR2-A4860G$^{+/-}$ ventricular myocytes showed prolonged APs and interspaced bursts of altered Ca^{2+} release consisting of normal peak followed by a second, prolonged phase of release. Remarkably, early afterdepolarizations (EADs) were observed only during the prolonged phase of Ca^{2+} release, and were abolished by NCX inhibitor CB-DMB or intracellular Ca^{2+} chelator EGTA. SR Ca^{2+} load was higher in RyR2-A4860G$^{+/-}$ cells before EADs but decreased to levels comparable to WT after EADs. Simultaneous recording of $I_{Ca,L}$ and $[Ca^{2+}]_i$ transients showed decreased transient amplitude and prolonged Ca^{2+}-dependent-inactivation of $I_{Ca,L}$ in RyR2-A4860G$^{+/-}$ cells. In conclusion, RyR2-A4860G loss-of-function mutation decreases Ca^{2+} release amplitude and impairs $I_{Ca,L}$ inactivation, both of which gradually overload SR. The resultant SR overload then causes bursts of prolonged Ca^{2+} release, activating electrogenic NCX activity during APs and triggering EADs. The RyR2-A4860G mutation reveals novel pathways by which RyR2 channels engage membrane currents to produce life-threatening arrhythmias.

571-Pos Board B326

Preventing RyR2-S2808 and RyR2-S2814 Phosphorylation does not Alter the β-Adrenergic Response of Mouse Hearts

Emmanuel Camors[1], Randall Loaiza[1], Francisco Alvarado[1], Yanting Zhao[1], Patricia Powers[2], Hector H. Valdivia[1].
[1]Center for Arrhythmia Research, University of Michigan at Ann Arbor, Ann Arbor, MI, USA, [2]Cell and Regenerative Biology, University of Wisconcin at Madison, Madison, WI, USA.

We previously showed that ablation of the cardiac Ca^{2+} release channel/ryanodine receptor (RyR2) phosphorylation at Ser2808 (RyR2-S2808A) has no functional impact on cardiac performance and/or heart failure progression. In this study, we investigated whether this lack of effect may be due to compensatory mechanisms involving altered phosphorylation of the nearby Ser2814 CaMKII phospho-site. We generated a new mouse model where Ser2808 and Ser2814 were genetically replaced by Alanine (RyR2-S2808A/S2814A). Anesthetized RyR2-S2808A/S2814A mice have normal ejection fraction at baseline compared to wild type (WT) mice ($55.1 \pm 2.6\%$ *vs.* $55.9 \pm 2.4\%$, respectively, n=7/group) but exhibit ~20% increase in heart rate (553 ± 33 *vs.* 460 ± 28 bpm, $p<0.05$, n=8/group). Langendorff-perfused hearts from both, RyR2-S2808A/S2814A and WT mice displayed an equally robust increase in the amplitude of isoprenaline (200nM)-stimulated LV contractions (60% and 63% above control in mutant and WT hearts, respectively, n=10/group, $p=0.7$). In agreement with the whole-heart results, isoproterenol (100nM)-stimulated RyR2-S2808A/S2814A myocytes displayed a comparable increase in Ca^{2+} transient amplitude and sarcoplasmic reticulum (SR) Ca^{2+} content than WT cardiomyocytes. Nevertheless, ablation of the S2808/S2814 phosphosites significantly decreased RyR2 refractoriness measured by a 2-pulse protocol (142 ± 7 *vs.* 194 ± 6 ms in the S2808A/S2814A and WT, respectively, n=7, $p<0.01$). This effect was associated with a ~27% increase in SR Ca^{2+} leak (3.22 ± 0.2 *vs.* 2.53 ± 0.2 $\Delta F/F_0.100\mu m^{-1}.s^{-1}$ in the RyR2-S2808A/S2814A and WT myocytes, respectively, n=17/group, $p<0.05$) causing earlier, faster and more frequent spontaneous Ca^{2+} waves. Interestingly, 60% of RyR2-S2808A/S2814A and 40% of the WT Langendorff-perfused hearts (n=10 each) exhibited arrhythmias upon isoprenaline stimulation. In conclusion, our data show that inhibition of S2808 and S2814 phosphorylation leaves intact the β-adrenergic response of the heart but accelerates RyR2 refractoriness, increasing SR Ca^{2+} leak and promoting arrhythmic events.

572-Pos Board B327

Suppressed RyR2 Function Represents a Common Cause of Idiopathic Ventricular Fibrillation and Sudden Cardiac Death

Xiaowei Zhong[1], Yijun Tang[1], Ruiwu Wang[1], Vern Hsen Tan[2], Yingjie Liu[1], Lin Zhang[1], Carlo Napolitano[3], Silvia Priori[3], SuiRong Wayne Chen[1].
[1]University of Calgary, Calgary, AB, Canada, [2]Foothills Hospital, University of Calgary, Calgary, AB, Canada, [3]University of Pavia, Pavia, Italy.

Cardiac Ryanodine Receptor (RyR2) is the major intracellular calcium release channel located in the sarcoplasmic reticulum. A large number of mutations in RyR2 have been linked to inherited cardiac arrhythmias (CPVT, catecholaminergic polymorphic ventricular tachycardia and IVF, idiopathic ventricular fibrillation) and sudden cardiac death. We previously demonstrated that in contrast to CPVT RyR2 mutations, the IVF RyR2 mutation A4860G suppresses RyR2 function. However, A4860G represents the only IVF RyR2 mutation that has been functionally characterized to date. To determine how common suppression-of-function RyR2 mutations are, we systematically characterized 27 RyR2 mutations that are associated with IVF or sudden cardiac death. Each of these mutations was generated by site-directed mutagenesis and expressed in HEK293 cells. Ca release assays revealed that 7 of these mutations suppress or abolish caffeine-induced Ca release. Single cell Ca imaging studies also indicated that these suppression- or loss-of-function RyR2 mutations