for ATP (ATeam), respectively.**Results:**Overexpressed RyR1 was partially localized in mitochondria, but the another RyR isoform RyR2 did not show any co-localization with mitochondria. Over-expression of RyR1 but not MCU and RyR2 showed the mitochondrial fragmentation. Fragmented mitochondria showed higher $[Ca^{2+}]_m$ at peak and sustained Ca^{2+} transients compared to elongated mitochondria. RyR1 overexpressed cells had higher $[ATP]_m$ at the basal condition and showed more ATP production in response to $[Ca^{2+}]_c$ elevation compared to non-transfected cells.**Conclusion:**Mitochondrial RyR1 strongly modulates mitochondrial morphology, Ca^{2+} influx and Ca^{2+}-induced ATP production in cardiac cells.

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Intracellular Na^+ Overload of Cardiomyocytes Causes ROS Induced CaMKII Activation, Leading to RYR Dysfunction and Diastolic Ca^{2+} Mishandling

Serge Viatchenko-Karpinski[1], Dmytro Kornyeyev[1], Peidong Fan[1], Zhan Jiang[1], John Shryock[1], Mark E. Anderson[2], Luiz Belardinelli[1], Lina Yao[1].
[1]Gilead Sciences, Inc, Fremont, CA, USA, [2]The University of Iowa, Iowa City, IA, USA.

Introduction: Intracellular sodium (Na^+_i) overload of cardiomyocytes is associated with abnormal calcium (Ca^{2+}_i) homeostasis and cell dysfunction. We hypothesized that the impaired Ca^{2+} handling is triggered by reactive oxygen species (ROS) -activated Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII) which in turn on modulation of ryanodine receptor (RyR) activity, leading to Ca^{2+} leak from the sarcoplasmic reticulum (SR).
Methods: We used confocal fluorescent imaging of intact and membrane-permeabilized rabbit ventricular cardiomyocytes to study Na^+_i overload, Ca^{2+}_i transients, and ROS production. Na^+_i overload in intact cardiomyocytes was induced by anemone toxin-II (ATX-II), a late Na^+ current (I_{NaL}) enhancer. CaMKII oxidation and phosphorylation were measured using Western Blot analysis.
Results: Rabbit ventricular myocytes treated with ATX-II (5 nM) exhibited Na^+_i overload, aberrant Ca^{2+}_i transients, and enhanced ROS production, accompanied by CaMKII oxidation and phosphorylation. CaMKII activation was reduced by the I_{NaL} inhibitor ranolazine (10 μM), by antioxidants (5 mM DDT or 10 μM Q10co), or by the CaMKII inhibitor KN93 (10 μM). These findings indicate a pathogenic cascade of events from Na^+_i overload to ROS production and to CaMKII activation. To determine how ROS and CaMKII contribute to the Ca^{2+}_i mishandling caused by Na^+_i overload, we studied spontaneous Ca^{2+} waves in membrane-permeabilized myocytes. Superfusion with high Na^+ solutions elevated diastolic Ca^{2+}_i and accelerated Ca^{2+} waves frequency. These effects indicated Ca^{2+} leak through RyRs. These Ca^{2+} changes were dependent from ROS and CaMKII inhibition.
Conclusions: The data show that Na^+_i overload causes Ca^{2+}_i mishandling through increased ROS production and CaMKII activation. Na^+_i overload enhances ROS production, which activates CaMKII, leading to the phosphorylation of RyRs, resulting in SR Ca^{2+} leak and increase in diastolic Ca^{2+}.

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The Nature of Ca^{2+}-Controlled Gating in the Human Cardiac Ryanodine Receptor (hRyR2): A Zero Ca^{2+} Approach

Saptarshi Mukherjee, Nia Lowri Thomas, Alan J. Williams.
Cardiff University, Cardiff, United Kingdom.

RyR2-mediated intracellular Ca^{2+} release is pivotal in maintaining the rhythmic contraction of cardiac myocytes. The precise mechanism by which binding of cytosolic Ca^{2+} triggers gating is unknown. Functional studies of single RyR2s in lipid bilayers provide insights into gating behaviour as detailed structural information regarding the nature of the Ca^{2+}-dependent gate or Ca^{2+} binding site(s) is as yet unavailable.
This study aims to uncover the properties of the Ca^{2+}-controlled 'gate' by examining changes in single channel gating in the virtual absence of Ca^{2+}. To visualize these changes, ryanodine was employed to keep the channel open in a subconductance state in the presence and absence of Ca^{2+}, while K^+ was used as the permeant ion for better resolution of single channel currents. On reducing the Ca^{2+} from contaminant levels to nominally zero on both cytosolic and luminal sides of the ryanodine-modified hRyR2 (using 3.5mM EGTA), a significant decrease in channel Po is seen at positive (current direction: cytosolic-luminal) holding potentials but not in the physiological direction of current flow (0.99 ± 0.002 to 0.75 ± 0.05 at +40mV ($p<0.0005$) and 0.99 to 0.98 ± 0.009 at −40mV; n=9). Analysis of dwell times demonstrates that Po decreases by increasing the frequency of closings (~170 fold increase at +40mV, ~8-fold at −40mV). Further examination of the voltage sensitivity of the modified channel reveals a sharp dependence of activity on potential. High cytosolic Ba^{2+} had a direct inhibitory effect on the gating kinetics induced by the removal of Ca^{2+}. This study demonstrates that ryanodine modifies RyR2 by acting on a Ca^{2+}-controlled gate and gating of the modified channel is voltage-sensitive. HMM-based modeling of this data provides further insights into this fundamental process of Ca^{2+}-induced channel opening. This study is supported by the BHF.

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Regulation of Wild Type and CPVT-Linked Mutant Cardiac Ryanodine Receptors by Junctin and Calsequestrin

Chloe E. Maxwell, Alan J. Williams, Saptarshi Mukherjee, N. Lowri Thomas.
Cardiff University, Cardiff, United Kingdom.

The human cardiac ryanodine receptor (hRyR2) undergoes luminal regulation by accessory proteins including junctin (JUN) and calsequestrin (CSQ2). Furthermore, it has been suggested that defective luminal Ca^{2+} sensing by RyR2 is a candidate mechanism in the pathogenesis of catecholaminergic polymorphic ventricular tachycardia - an arrhythmogenic disorder caused by mutation of RyR2 or CSQ2. As such, we evaluated the effects of CSQ2 and JUN on mutant channel behaviour. [^{3}H]-ryanodine binding was used to evaluate the cytosolic Ca^{2+} activation of wild-type (WT) or novel CPVT mutant (A4556T, positioned close to a JUN binding site) which were recombinantly (co)expressed in the presence/absence of CSQ2 and/or JUN in HEK293 cells, while store overload-induced Ca^{2+}-release (SOICR) events were monitored to ascertain luminal Ca^{2+} effects. Co-immunoprecipitation experiments demonstrated that WT and mutant proteins showed equal association with both luminal accessory proteins. Co-expression of WT RyR2 with CSQ2 alone did not affect the amplitude, frequency or rate of release of SOICR events while preliminary experiments show that co-expression with JUN (alone and in combination with CSQ2) decreased the amplitude and increased the frequency of events, in line with observations that JUN both inhibits and activates RyR2 depending on SR Ca^{2+} levels. Co-expression of both accessory proteins significantly decreased the rate of release. Activation of WT RyR2 by cytosolic Ca^{2+} was not affected by the presence of either/both accessory proteins. A4556T showed enhanced activation in response to cytosolic Ca^{2+} that decreased to WT levels (at low Ca^{2+} concentrations only) in the presence of both JUN and CSQ2. This indicates that regulation by JUN and CSQ2 to some extent attenuates mutant dysfunction and will be further investigated by monitoring Ca^{2+} release events and single channel analysis. This work is supported by the BHF.

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A Luminal Calcium Sensing Mutation of the Cardiac Ryanodine Receptor Diminishes Calcium Waves and Stress -Induced Ventricular Tachycardias in Calsequestrin Null Mice

Jingqun Zhang[1], Biyi Chen[2], Ang Guo[2], Qiang Zhou[1], Zhen Tan[1], Guogen Wu[1], Long-Sheng Song[2], S.R. Wayne Chen[3].
[1]Rush University Medical Center, Chicago, IL, USA, [2]University of Iowa Carver College of Medicine, Iowa City, IA, USA, [3]the Libin Cardiovascular Institute of Alberta, Calgary, AB, Canada.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disease. Naturally occurring mutations in the cardiac ryanodine receptor (RyR2) and calsequestrin (CASQ2) have been linked to CPVT. The sarcoplasmic reticulum calcium content and the sensitivity of RyR2 to luminal calcium are two important factors determining the propensity for store overload induced spontaneous calcium release, also known as store-overload induced calcium release (SOICR), and SOICR-evoked VTs. We recently found that residue E4872 of RyR2 is critical for luminal calcium sensing. In the present study, we determined whether the RyR2-E4872Q mutation is capable of rescuing the VT phenotype of the CASQ2 null mice. We bred E4872Q+/- mice with CASQ2 null mice to produce CASQ2-/- or CASQ2+/- mice with or without the RyR2-E4872Q mutation. CASQ2-/- and CASQ2+/- mice without the RyR2-E4872Q mutation showed long lasting stress-induced VTs, whereas CASQ2-/- / E4872Q+/- and CASQ2+/- / E4872Q +/- displayed little or no VTs. Thus, the RyR2-E4872Q mutation nearly completely protected the CASQ2 null mice from CPVT. We next studied the impact of the RyR2-E4872Q mutation on calcium handling in intact hearts using linescan confocal calcium imaging. The properties of calcium transients did not differ significantly between CASQ2-/- hearts with or without the RyR2-E4872Q mutation at resting conditions. However, upon elevation of external calcium from 2mM to 7mM or adding 100nM isoproterenol, frequent spontaneous calcium waves occurred in CASQ2-/- hearts without the RyR2-E4872Q mutation, but not in CASQ2-/- / E4872Q+/- hearts. These observations indicate that targeting the luminal calcium sensor of RyR2 represents a new therapeutic strategy for suppressing CPVT and other calcium mediated cardiac arrhythmias. (Support by NIH R01HL75210)