Platform M: Excitation-Contraction Coupling

1043-Plat

Simultaneous Phosphorylation of RyR2 by PKA and Camkii is Required for Induction of CA-Dependent Arrhythmia Caused by MIR-1 Overexpression

Dmitry Terentyev[1], Andriy E. Belevych[1], Radmila Terentyeva[1], Inna Gyorke[1], Sarah Sansom[1], Mickey M. Martin[1], Jody L. Martin[2], Maha Abdellatif[3], Cynthia A. Carnes[1], Terry S. Elton[1], Sandor Gyorke[1].
[1]The Ohio State University, Columbus, OH, USA, [2]Loyola Univeristy Chicago, Maywood, IL, USA, [3]University of Medicine and Dentistry of New Jersey, Newark, NJ, USA.

We recently showed that disruption of localization of PP2A phosphatase activity to the ryanodine receptor (RyR2) complex by overexpression of the muscle-specific microRNA, miR-1, stimulates excitation-contraction coupling and results in increased arrhythmogenic potential in cardiac myocytes. In the present study, we examined the role of PKA and CAMKII as mediators of the effects miR-1 on Ca signaling and arrhythmogenesis using cellular electrophysiology and Ca imaging complemented with quantitative measurements of RyR2 phosphorylation at PKA site S-2808, and at CAMKII site S-2814. Adenovirally-mediated 2-fold overexpression of miR-1 resulted in 10-fold increase in arrhythmogenic potential measured as a frequency of spontaneous Ca waves and DADs in myocytes exposed to 100 nM isoproterenol (ISO). Quantitative imminoblotting using site-directed phosphospecific-antibodies showed that RyR2 phosphorylation in miR-1 overexpressing cells was low at S-2808 under basal conditions. Exposure of myocytes to ISO maximized phosphorylation at S-2808. Phosphorylation at S-2814 under basal conditions was maximal and did not further increase in the presence of ISO. Additionally, ISO increased SERCa-mediated SR Ca uptake and SR Ca load through phosphorylation of phospholamban (PLB). To define which of these factors (increased CaSR content or increased RyR2 PKA phosphorylation) mediated the increased arrhythmogenic potential we infected myocytes with viral constructs of a dominant-negative PLB mutant that accelerates SERCa-mediated SR Ca uptake by displacing endogenous PLB from SERCa. Myocytes coexpressing miR-1 and dnPLB did not exhibit enhanced predisposition to Ca-dependent arrhythmia in the presence of ISO despite maximal SR Ca load. Importantly treatment of cells with either PKA or CAMK inhibitors completely abolished increased arrhythmogenic activity. We conclude that neither CAMKII nor PKA phosphorylation alone is sufficient to produce the changes in RyR2 activity that underlies the arrhythmogenic disturbances caused by miR-1 overexpression.

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Defective RYR2 Channels Trigger Ventricular Arrhythmias in Duchenne Muscular Dystrophy

Jérémy Fauconnier[1], Jerôme Thireau[1], Steven Reiken[2], Cécile Cassan[1], Sylvain Richard[1], Stefan Matecki[3], Andrew R. Marks[2], Alain Lacampagne[1].
[1]INSERM U637, Montpellier, France, [2]Columbia University College of Physicians & Surgeons, New York city, NY, USA, [3]INSERM ERI25, Montpellier, France.

Duchenne muscular dystrophy (DMD) affects one in 3500 newborn males and usually leads to death from respiratory or cardiac failure by age 30. Interestingly, the severity of cardiomyopathy is not proportional to the severity of skeletal muscle disorder. Among DMD patients, the cardiac phenotype varies with age from no discernable cardiac left ventricular enlargement or dysfunction to early onset of dilated cardiomyopathy (DCM) with heart failure. The incidence of DCM in DMD patients has been estimated to be 25% by 6 years of age, 59% at 10 years of age and ~100% in adults. DMD patients often exhibit electrocardiographic abnormalities and frequent premature ventricular contractions. As the cardiomyopathy progresses, ventricular arrhythmias (VA) increase, often leading to sudden death. Most of the electrical and functional abnormalities have been attributed to cardiac fibrosis. However, electrical abnormalities may occur in the absence of overt cardiac histopathology and ECG changes are similar in patients with DMD regardless of presence of DCM.

Here we show that structural and functional remodeling of the cardiac sarcoplasmic reticulum (SR) Ca2+ release channel/ryanodine receptor (RyR2) occurs in the mdx mouse model of DMD. RyR2 from mdx hearts were S-nitrosylated and depleted of calstabin2 (FKBP12.6) resulting in "leaky" RyR2 channels and diastolic SR Ca2+ leak. Inhibiting the depletion of calstabin2 from the RyR2 complex with the calcium channel stabilizer, S107 ("rycal"), inhibited the SR Ca2+ leak, restored normal Ca2+ transients, inhibited isoproterenol induced aberrant depolarizations in isolated cardiomyocytes and prevented arrhythmias in vivo.

Thus, diastolic SR Ca2+ leak via RyR2 due to S-nitrosylation of the channel and calstabin2 depletion from the channel complex likely triggers cardiac arrhythmias. Prevention of the RyR2-mediated diastolic SR Ca2+ leak may provide a novel cardiac therapeutic approach in DMD.

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Sodium Current-Induced Release of Calcium from the Sarcoplasmic Reticulum in Rabbit Ventricular Myocytes

Natalia S. Torres[1], Robert Larbig[2], Alex Rock[1], Frank B. Sachse[1,3], Kenneth D. Philipson[2], Joshua I. Goldhaber[4], John H.B. Bridge[1].
[1]Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, UT, USA, [2]Department of Physiology and Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA, [3]Bioengineering Department, University of Utah, Salt Lake City, UT, USA, [4]Department of Medicine, Division of Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

The hypothesis that Na current (INa) can induce release of Ca from the sarcoplasmic reticulum (SR) by activating reverse Na-Ca exchange (NCX) has been debated since 1990. We tested this hypothesis with epi-fluorescence imaging of adult rabbit ventricular myocytes loaded with the Ca indicator fluo-4. Ca release was triggered with an action potential clamp with and without an initial voltage ramp from −80 to −40 mV, for a duration of 1.5s. We confirmed that this protocol selectively blocked INa without altering Ca influx through L-type Ca channels (LCCs) and SR Ca load. With 0 mM Na in the pipette (to reduce intracellular Na), inactivating INa reduced SR Ca release flux by 27% ± 4% (n=9). With 5 mM Na in the pipette, the Ca release upon inactivation of INa was reduced by 33% ± 5% (n=4). We suggest that increased activation of reverse NCX by increased intracellular Na concentration mainly produced by INa explains these findings. These conclusions are in agreement with studies on normal and NCX knockout mice, which show that INa affects SR Ca release only in normal, but not in NCX knockout mice. In similar experiments, we applied 100 nM TTX to selectively block brain isoforms of Na channels. In the presence of TTX, the SR Ca release flux was reduced by 35% ± 3% (n=6). This effect of INa on Ca release can be explained by early reverse NCX, activated by TTX sensitive INa, which could prime the dyadic cleft with Ca. Furthermore, the results can be explained if INa activation of NCX, and subsequent priming of the dyadic cleft with Ca, increases the coupling fidelity between LCCs and ryanodine receptors within a couplon. Thus the presence of INa increases the likelihood that couplons are activated.

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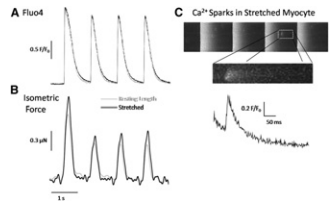
Single Cell Measurements of Isometric Force and Cytosolic Calcium in Intact Mammalian Cardiomyocytes

Benjamin L. Prosser[1], Christopher W. Ward[2], W.J. Lederer[1].
[1]University of Maryland Biotechnology Institute, Baltimore, MD, USA, [2]University of Maryland School of Nursing, Baltimore, MD, USA.

Single cell measurements of contractile force in ventricular cardiomyocytes were pioneered in the early 1990s (Le Guennec et al, 1990) and recently advanced by the Kohl laboratory (Iribe et al, 2006). Here we have modified their technique to directly record isometric force in adult mammalian ventricular myocytes. Using a high sensitivity force transducer, we have simultaneously recorded contractile force with cytosolic Ca2+ transients and sparks in intact cells subject to sarcomeric stretch. Consistent with previous reports and the Frank-Starling law of the heart, stretch led to an increase in active contractile force (Fig. 1B, normalized to passive tension) without significantly altering cytosolic Ca2+ transients (Fig. 1A). Stretch also promoted an increase in Ca2+ spark activity (Fig. 1C), consistent with recent work from our group (Iribe et al, 2009). This technique will be utilized to evaluate the effects of sarcomeric stretch on Ca2+ signals, membrane electrical properties, and contractile force in healthy and diseased or genetically altered heart cells.



1047-Plat

Na/K-ATPase α2-Subunit Preferentially Modulates Ca Transients and SR Ca Release in Cardiac Myocytes

Sanda Despa[1], Yong Wu[2], Jerry B. Lingrel[3], Enrico Stefani[2], Donald M. Bers[1].
[1]University of California Davis, Davis, CA, USA, [2]UCLA, Los Angeles, CA, USA, [3]University of Cincinnati, Cincinnati, OH, USA.

Na/K-ATPase (NKA) is essential in regulating $[Na]_i$, and thus cardiac myocyte Ca and contractility via Na/Ca exchange. Different NKAα-subunit isoforms are present in heart and may differ functionally, depending on differential membrane localization. In smooth muscle and astrocytes, NKA-α2 is located at the junctions with endo(sarco)plasmic reticulum, where they could regulate local Na, and indirectly junctional cleft [Ca]. In contrast, NKA-α1 is ubiquitously