Excitation-Contraction Coupling: Cardiac

2632-Pos Board B602

SEA0400 Fails To Alter The Magnitude Of Intracellular Ca^{2+} Transients And Contractions In Guinea Pig Heart

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SEA0400 is a recently developed inhibitor of the Na^+/Ca^{2+} exchanger (NCX). It suppresses both forward and reverse mode operation of NCX. In our experiment the effects of partial blockade of NCX on Ca^{2+} handling and contractility were studied. The experiments were carried out on Langendorff-perfused guinea pig hearts loaded with the fluorescent Ca^{2+}-sensitive dye Fura-2. Left ventricular pressure and intracellular calcium concentration ($[Ca^{2+}]_i$) were synchronously recorded before and after cumulative superfusion with 0.3 μM and 1 μM SEA0400. Neither systolic nor diastolic values of left ventricular pressure were changed on the effect of SEA0400. Accordingly, the pulse pressure and the kinetic parameter of pulses - time to peak values of pressure, half relaxation time - also remained unchanged in the presence of SEA0400. Although the SEA0400 did not alter the amplitude and the time required to reach peak values of $[Ca^{2+}]_i$, SEA0400 significantly increased the decay time constant of $[Ca^{2+}]_i$ transients. The descending limb of $[Ca^{2+}]_i$ transients were fitted by monoexponencial between 30% and 90 % of relaxation. The obtained the decay time constants are 127 ± 7 ms, 165 ± 7 and 177 ± 14 ms in control and in the presence of 0.3 and 1 μM SEA0400, respectively ($P<0.05$, n=6). The lack of effect of SEA0400 on $[Ca^{2+}]_i$ and contractility in guinea pig heart is consistent with a limited forward mode inhibitory effect of SEA0400 on NCX, which can easily be balanced by the concomitant reduction in Ca^{2+} influx due to the SEA0400-induced suppression of L-type Ca^{2+} current and the reverse mode operation of NCX

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Protein Kinase D Regulates L-type Ca^{2+} Current In Cardiac Ventricular Myocytes

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Cardiac excitation-contraction (EC) coupling is dynamically regulated. through an integration of kinase-phosphatase activities that alter the phosphorylation state of key Ca^{2+} cycling proteins, such as the L-type Ca^{2+} Channel (LTCC). This dynamic balance allows for active modulation of Ca^{2+} influx (I_{Ca}) through the LTCC. This is crucial for normal myocyte function and contractility, and is dysregulated in the failing human heart. Previous studies from our group show that treatment of ventricular myocytes with the phosphatase inhibitor calyculin A (caly A) increased contractility by augmenting I_{Ca} in the absence of humoral stimulation (steady-state). These results provided preliminary evidence that endogenous, LTCC-directed protein kinase activity is responsible for whole-cell I_{Ca} regulation in the steady-state. We explore the hypothesis that protein kinase D (PKD) plays a role in modulating the LTCC in the steady-state. This investigation focuses on the effect of the expression of genetically modified PKD on contractility, and I_{Ca} in cultured adult rat ventricular myocytes. Whole-cell I_{Ca} was recorded from myocytes infected with control adenovirus, constitutive active PKD, and dominant negative PKD. In addition, we studied the effect of caly A on these three different groups. In the constitutively active PKD myocytes, there is an increase in the peak I_{Ca} prior to the administration of caly A in comparison to the control adenovirus myocytes. The increase in peak I_{Ca} in the constitutively active group provide increasing evidence that PKD is required for modulating steady-state I_{Ca}.

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NAADP Dependent Calcium Signalling in Guinea-pig Atrial Myocytes

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Nicotinic acid adenine dinucleotide phosphate (NAADP) was first reported to be a calcium mobilizing messenger in sea urchin eggs, but is now believed to regulate calcium release in several mammalian cell types, including guinea-pig ventricular myocytes (Macgregor et al. 2007). The aim of the present study was to investigate whether NAADP regulates calcium signaling in guinea-pig atrial myocytes.

A combination of electrophysiology and calcium imaging was used to investigate the actions of NAADP in atrial myocytes stimulated to fire action potentials. Photorelease of NAADP from its caged derivative increased the calcium transient amplitude with little or no change in action potential characteristics. This effect on calcium transients was relatively slow to develop, the peak effect of a 52 ± 11% increase ($P<0.05$, n=7) occurring 2 minutes after photolysis. Photorelease of caged phosphate (as a control) had no effect on calcium transient amplitude or action potential parameters.

In a separate series of voltage clamp experiments to record L-type calcium currents, calcium transients were increased by 55 ± 13 % ($P<0.05$, n=5) 3 minutes after photorelease of NAADP, without significant effect on L-type calcium current. Photorelease of caged phosphate was without effect.

Bafilomycin A1, an inhibitor of the NAADP signaling pathway, reduced calcium transient amplitude by 47 ± 4 % ($P<0.01$, n=6). Pharmacological inhibition of sarcoplasmic reticulum function with a combination of ryanodine and thapsigargin reduced calcium transient amplitude by 74 ± 2 % ($P<0.01$, n=6). In the presence of ryanodine and thapsigargin, bafilomycin was without further effect ($P>0.05$, n=6). Staining of acidic organelles with LysoTracker red produced a punctate pattern of localization that could be prevented by bafilomycin.

These results are consistent with a role for NAADP in modulating calcium release in atrial myocytes.

Macgregor et al. (2007). J Biol Chem 282(20), 15302-11.

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Early Exercise Training After Myocardial Infarction Prevents Contractile, But Not Electrical Remodeling Or Hypertrophy

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Exercise started early after myocardial infarction (MI) improves *in vivo* cardiac function and myofilament Ca response. We investigated whether this represents partial or complete reversal of cellular remodeling. Mice with MI following LAD ligation were given free access to a running wheel (MIexe, N=8) or housed sedentary (MIsed, N=10) for 8 weeks and compared to sedentary sham-operated animals (SH, N=8). Myocytes were enzymatically isolated from the non-infarcted LV, excluding the border zone. Contraction was measured during electrical field stimulation at 1, 2 and 4 Hz; membrane currents and $[Ca^{2+}]$ were measured under whole-cell patch clamp, with Fluo-3 as Ca^{2+} indicator, all at 30°C. Data are shown as mean ± SEM. Myocytes in MI were significantly longer and further hypertrophied after exercise (165 ± 3 μm in MIexe *vs.* 148 ± 3 μm in MIsed and 136 ± 2 μm in SH; $P<0.05$). Cell width was not different. Unloaded cell shortening was significantly reduced in MIsed (at 1 Hz, L/L_0= 4.4 ± 0.3% *vs.* 6.7 ± 0.4% in SH; $P<0.05$, also at 2 and 4 Hz). Exercise restored cell shortening to SH values (MIex at 1 Hz, L/L_0 = 6.4 ± 0.5%). Diastolic Ca^{2+} levels increased at 4 Hz in all groups but to a lesser extent in MIexe and SH ($[Ca^{2+}]_{rest}$ 128 ± 20 nM in MIexe, 135 ± 27 nM in SH, 199 ± 27 in MIsed; $P<0.05$). $[Ca^{2+}]_i$ transient amplitude, I_{CaL} and SR Ca^{2+} content were not different between the 3 groups. I_{to} was significantly reduced in MIsed (27 ± 5 pA/pF vs. 43 ± 7 pA/pF in SH) but was unchanged in MIexe (26 ± 6pA/pF; $P<0.05$). Early exercise training after MI restores cell contraction to normal values without significant changes in $[Ca^{2+}]_i$ consistent with changes at the myofilament level. However, this beneficial effect is not a complete reversal of remodeling as hypertrophy and reduction of I_{to} are not affected.

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Does Deregulation Of Calcium Handling Precede Or Follow Alterations Of Cardiac Function During Progression To Heart Failure?

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In heart failure (HF), abnormalities of in vivo cardiac function are closely correlated with reduced sarcoplasmic reticulum (SR) Ca release and myocyte contractility, supporting the concept that HF is a result of deranged Ca cycling. However, most studies addressing the role and mechanisms of altered Ca handling in HF have been performed at advanced stages of HF providing little information as to whether these deficiencies are causes or consequences of HF. In the present study we compared the time course of development of alterations in myocytes Ca handling, using a canine tachypacing model of chronic HF. In-vivo function of left ventricle was assessed at several time points (1, 4, and >8 mo) with parallel studies in single ventricular myocytes of Ca handling utilizing Ca imaging in intact, permeabilized, and patch-clamped cells.

LV fractional shortening progressively decreased by 50, 65, and 75 % of control values at 1, 4, and >8 month of tachypacing, respectively. The frequency of

Ca sparks and the rate of SR Ca leak increased progressively with the duration of tachypacing, while diastolic [Ca]SR decreased with the duration of tachypacing. Na/Ca exchange activity was significantly augmented at 1 mo, and did not change thereafter. The SERCA-mediated Ca uptake and the density of peak Ca current were not changed up to >8 mo of tachypacing. Progressive decreases in the amplitude of depolarization-induced Ca transients and single-cell contractions were observed only starting at the 4th month of tachypacing. These results suggest that diminished SR Ca release follows rather than precedes deterioration of in vivo cardiac function, thus is not likely to be a cause of HF.

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Ca^{2+} Sensitizing Troponin T Mutations Linked To Hypertrophic Cardiomyopathy Increase Apparent Cytosolic Ca^{2+} Binding

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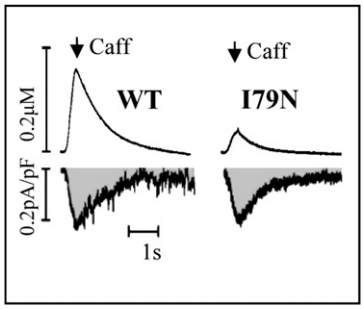
In myocytes, free [Ca^{2+}] is determined by the rate of Ca^{2+} influx and the Ca^{2+} buffering properties of the cytosol. Ca^{2+} binding to myofilaments (primarily Troponin C) represents a major portion of cytosolic Ca^{2+} buffering. To test the hypothesis that increased myofilament Ca^{2+} sensitivity decreases cytosolic free [Ca^{2+}], we studied ventricular myocytes from transgenic mice overexpressing wild-type (WT) and Troponin T mutants (R278C, F110I and I79N). Myofilament Ca^{2+} sensitivity was altered in the following order: R278C < WT < F110I < I79N. Intracellular Ca^{2+} was released by rapid Caffeine application and quantified using the Na-Ca exchanger current intergral. The rise in free cytosolic Ca^{2+} was smaller in myocytes expressing I79N compared to WT despite the fact that the amount of Ca^{2+} released was the same. Both Ca^{2+} sensitizing mutants significantly decreased average K_d, but did not change B_{max}. Independently, we quantified Ca^{2+} influx by integrating L-type Ca^{2+} current (in 0 Na^+ and thapsigargin). Again, the rise in cytosolic free Ca^{2+} was smaller in I79N than WT. Taken together, these data demonstrate that Ca^{2+} sensitizing TnT mutants increase cytosolic free Ca^{2+} binding (by lowering the K_d).



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Sarcomere Shortening Destabilizes the Ca2+ Control System in Ventricular Myocytes: Implications for Understanding Arrhythmias in Familial Hypertrophic Cardiomyopathy

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Familial hypertrophic cardiomyopathy (FHC) results from mutations of contractile proteins. Certain forms of FHC are linked with a surprisingly high incidence of sudden cardiac death. It is a long-standing conundrum how mutations in motor proteins give rise to electrical arrhythmias. Quantitative modeling studies using large-scale simulations reveal an intimate link between the contractile system and the Ca^{2+} control system. This insight may help resolve this conundrum. Our simulations show that a small decrease in sarcomere length (SL) can destabilize the Ca^{2+} control system and increase the probability of spontaneous Ca^{2+} waves. FHC mutations on cardiac troponin T (cTnT) increase myofilament Ca^{2+} sensitivity, which may account for the shortened SL in cardiomyocytes from mice harboring the cTnT mutations. To test the model predictions we conducted experiments using the myofilament Ca^{2+} sensitizer EMD 57033 (Merck) to reduce the resting SL length. EMD in the range of 1-3 μM reduced the resting SL from 1.9 to 1.5 μm without altering sarcoplasmic reticulum Ca^{2+} load, systolic, or diastolic Ca^{2+} levels. Upon cessation of pacing (1 Hz), control myocytes (0 EMD) were quiescent but EMD treated myocytes exhibited spontaneous contractions and Ca^{2+} release. These results are consistent with the model predictions lending support to the idea that FHC mutations destabilize the Ca^{2+} control system, which in turn, become a substrate for arrhythmias.

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Cellular Mechanism of Ca2+−Dependent Arrhythmogenesis in Failing Myocytes of Aortic Banding Rats

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We have previously shown that myocyte t-tubular system undertakes dramatic remodeling in the failing hearts from rats after long term hypertension (Song et al, PNAS 2006). We further hypothesize that t-tubular remodeling plays an important mechanistic role in unstable Ca2+ homeostasis and therefore Ca2+−dependent arrhythmogenesis during heart failure (HF). To test this idea, we generated a HF model in Sprague-Dawley rats with thoracic-aortic banding (TAB) surgery. TAB rats developed HF in about 12-14 weeks, as confirmed by echocardiography. T-tubular structure and cellular Ca2+ function (Ca2+ sparks, waves, and field stimulated Ca2+ transients) were then examined with laser scanning confocal microscope in single isolated myocytes from TAB and sham operated rats. Confocal imaging of t-tubular system with Di-8-ANEPPS, a fluorescent membrane marker showed remarkable disarray and/or loss of t-tubular system in TAB failing myocytes. Power spectrum analysis indicated that myocytes from failing TAB rats displayed a significant reduction in the power of t-tubular organization (or the regularity of t-tubular distribution), as comparison to sham-operated controls (sham 2.0 vs TAB 1.1, $n > 10$, $p<0.001$). As a result, TAB myocytes exhibited a significantly slower rising phase of Ca2+ transients upon steady state field stimulation (93.5 ± 4.8 ms vs sham control 38.5 ± 2.9 ms at 3 Hz, $p<0.01$). Moreover, TAB myocytes had much higher probability of developing unstable Ca2+ release (Ca2+ waves) during field stimulation (48% vs 3% of control, at 3 Hz). These results further support our previous finding of t-tubular remodeling observed in a hypertensive HF model. In conclusion, t-tubular remodeling is a common structural alteration at the end stage of heart failure, responsible for Ca2+ release instability and Ca2+ dependent arrhythmogenesis during HF.

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Differential Hypertrophic Remodeling Of Cardiomyocytes Determines Distinct Types Of Arrhythmias In The Ischemic Failing Heart: Key Role Of The Ryanodine Receptor

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A better understanding of the mechanisms responsible for sudden cardiac death (SCD) in heart failure (HF) may influence treatment strategies. We investigated whether early and delayed afterdepolarizations (EADs, DADs) coexist in HF, and how they are initiated during disease progression. Cells were isolated from the left ventricle of rats 8 weeks after myocardial infarction (PMI) and from age-matched sham-operated animals, and studied using the whole-cell patch-clamp technique. Cellular arrhythmias were triggered exclusively in PMI cells (40 %) using trains of 5 stimulations at 2.0 Hz. EADs and DADs occurred in distinct cell populations. Cell membrane capacitance measurements showed that EADs occurred in normal-sized PMI cells (<200 pF), whereas DADs occurred in hypertrophic cells (>200 pF). All cells exhibited prolonged action potentials (AP) due to decreased Ito currents. However, additional modifications in Ca^{2+}-dependent ionic currents were observed in hypertrophic cells: a decrease in the inward rectifier K^+ current IK1 and a slowing of L-type Ca^{2+} current (ICaL) inactivation, responsible for the poor adaptation of both ICaL and AP to abrupt changes in the pacing rate. The occurrence of Ca^{2+} sparks, reflecting ryanodine receptor (RyR2) activity, also increased with hypertrophy. Fluorescence measurements using Fluo-4 AM revealed that the amplitudes of [Ca^{2+}]i transients, Ca^{2+} load of the sarcoplasmic reticulum (SR) and Ca^{2+} spark amplitude were inversely correlated with cell size.The trophic status of cardiomyocytes determines the type of arrhythmia triggered in PMI rats, based on differential electrophysiological remodeling reflecting early-mild and late-severe modifications in the function of the ryanodine receptor RyR2.