Excitation-Contraction Coupling: Cardiac

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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