Duchenne muscular dystrophy represents a severe inherited disease of striated muscle, caused by a mutation in the dystrophin gene and characterized by progressive loss of skeletal muscle function. Most patients also develop dystrophic cardiomyopathy resulting in dilated hypertrophy and heart failure. On the cellular level, absence of dystrophin affects mechanical membrane stability and intracellular Ca signaling in cardiomyocytes. Cellular mechanisms leading to deterioration of cardiac function remain elusive. We tested whether defective excitation-contraction (EC) coupling contributes to impaired cardiac performance. EC-coupling gain, a measure for the effectiveness to amplify the Ca signal by Ca release from the sarcoplasmic reticulum (SR), was determined from control and dystrophin-deficient *mdx* hearts. Ca currents were measured with the whole-cell patch-clamp technique, while Ca transients were simultaneously recorded with confocal imaging of fluo-3. Initial findings indicated subtle problems of EC-coupling in *mdx* cells despite matched SR Ca loading. However, lowering extracellular Ca, a maneuver used to unmask latent EC-coupling problems, was surprisingly much better tolerated by *mdx* myocytes. Normalized to control conditions, the EC-coupling gain in *mdx* cells reached 112% compared with 31% in control cells, suggesting hypersensitive EC-coupling. Further investigation of this apparent increase in Ca sensitivity by inducing slow elevations of intracellular Ca resulted in Ca oscillations after a much shorter delay in *mdx* cells, consistent with enhanced Ca sensitivity of SR Ca release channels (ryanodine receptors, RyRs). Elevated cellular reactive oxygen species (ROS) generation in dystrophy suggests redox-modifications on the RyR, enhancing its Ca sensitivity. Preincubation of *mdx* cells with a ROS scavenger normalized the EC-coupling hypersensitivity back to control cardiomyocytes. Our data suggest that in dystrophin-deficient cardiomyocytes, EC-coupling mechanisms are altered, partly due to potentially arrhythmogenic changes in Ca sensitivity of redox-modified RyRs.
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54-Plat
The Skeletal L-type Ca^{2+} Current is a Major Contributor to Excitation-Coupled Ca^{2+} Entry (ECCE)
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The term Excitation-Coupled Ca^{2+} Entry (ECCE) designates the entry of extracellular Ca^{2+} into skeletal muscle cells which occurs in response to prolonged depolarization or pulse trains, and which depends on the expression of both the 1,4-dihydropyridine receptor (DHPR) and the type 1 ryanodine receptor (RyR1). The ECCE pathway is blocked by pharmacological agents that also block store-operated Ca^{2+} entry, is relatively insensitive to nifedipine (1 μM), and is permeable to Mn^{2+}. We have examined the effects of these agents on the L-type Ca^{2+} current conducted via the DHPR. We found that the non-specific cation channel antagonists 2-APB, SKF 96356, La^{3+} and Gd^{3+} all inhibited the L-type current. In addition, complete (>97%) block of the L-type current required concentrations of nifedipine >10 μM. Like ECCE, the L-type channel displays permeability to Mn^{2+} in the absence of external Ca^{2+} and produces a Ca^{2+} current that persists during prolonged (~10 s) depolarization. This current appears to contribute to the Ca^{2+} transient observed during prolonged KCl-evoked depolarization of intact myotubes because (*i*) the transients in normal myotubes decayed more rapidly in the absence of external Ca^{2+}, (*ii*) the transients in *dysgenic* myotubes expressing SkEIIIK (a DHPR α_{1S} subunit pore mutant thought to conduct only monovalent cations) had a time course like that of normal myotubes in Ca^{2+}-free solution and was unaffected by Ca^{2+} removal, and (*iii*) after block of SR Ca^{2+} release by ryanodine, normal myotubes still displayed a large Ca^{2+} transient whereas no transient was detectable in SkEIIIK-expressing *dysgenic* myotubes. Altogether, these results indicate that the skeletal muscle L-type channel is a major contributor to the Ca^{2+} entry attributed to ECCE. Supported by NIH NS24444 and AR44750 to K.G.B., and MDA 4155 to R.A.B.

55-Plat
Post-Tetanic Calcium Transients In Adult Skeletal Muscle Fibers Are Frequency-Dependent And Fiber Type Specific
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Isolated adult *Flexor digitorum brevis* fibers from 4-6 weeks-old mice, loaded with Fluo-3 were stimulated with trains of 270, 0.3 ms pulses at different frequencies. We observed a fast calcium tetanus (associated with contraction) and a second, slower signal, similar to those previously described in cultured myotubes. The slow signal (more than the fast one) was inhibited by 25 micro M nifedipine, suggesting a role for DHPR in its onset and by the IP_3R inhibitor Xestospongin-C (5 micro M). The amplitude of post-tetanic calcium transients depended on both stimulus train frequency and duration; a bell shaped curve frequency was obtained with a maximum at 10-20 Hz. Likewise, signal amplitude was proportional to stimulus train duration. Fibers isolated from *soleus* muscle completely lack slow calcium transients. Using immunofluorescence, we have found that all three IP_3R isoforms are present in adult muscle at different levels and that IP_3R-1 is differentially expressed (with a mosaic pattern) in different types of muscle fibers, being higher in a subset of fast-type fibers. ERK 1/2 phosphorylation of adult muscle fibers after tetanic stimulation appears to relate slow calcium signals to transcription-related events. These results support the idea that different calcium kinetics for the slow signals mediated by IP_3R may exist in different types of muscle fibers and participate in the activation of specific transcriptional programs of slow and fast phenotype. FONDAP 15010006, Bicentenario-PSD24, FONDECYT 1080120

56-Plat
Negative Relationship Between Fractional SR Ca^{2+} Release and Stimulation Rate
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Typically, contraction amplitude in rodent myocardium is inversely related to stimulation rate. We calculated Ca^{2+} fluxes across the sarcolemma and the sarcoplasmic reticulum (SR) membrane, and SR Ca^{2+} content to estimate the fraction of the SR Ca^{2+} load released at a twitch (FR) during steady-state (SS) stimulation at 0.2-2 Hz in intact rat cardiomyocytes. Cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) was measured with indo-1. SR Ca^{2+} content was determined from the transient in response to 10 mM caffeine in Na^+,Ca^{2+}-free medium. From Ca^{2+} buffer parameters, and $[Ca^{2+}]_i$ values and decline kinetics during transients in which SR Ca^{2+}-ATPase and/or Na^+-Ca^{2+} exchanger (NCX) were inhibited, SR- and NCX-mediated Ca^{2+} fluxes at each rate were estimated and integrated up to attainment of SS diastolic $[Ca^{2+}]_i$. Assuming equal inward and outward Ca^{2+} fluxes at SS, Ca^{2+} influx was considered as the NCX-mediated flux, whereas SR Ca^{2+} release and uptake fluxes were considered equivalent. FR was taken as the ratio of the integrated SR-mediated Ca^{2+} flux and SR Ca^{2+} content. Increasing rate did not affect significantly SR Ca^{2+} content (128 ± 4 and 133 ± 4 μM at 0.2 and 2 Hz, respectively), but decreased ($p<0.01$) both Ca^{2+} influx (16.9 ± 1.4 vs. 6.7 ± 0.3 μM at 0.2 and 2 Hz) and SR-dependent Ca^{2+} flux (93 ± 4 vs. 77 ± 3 μM at 0.2 and 2 Hz). Estimated FR showed a negative relationship with the stimulation rate (0.73 ± 0.03 vs. 0.58 ± 0.02, $p<0.01$), possibly due to decreased Ca^{2+} influx, although action potential duration was increased (APD90= 76 ± 9 vs. 130 ± 11 ms, $p<0.01$). Alternatively, incomplete time-dependent recovery from inactivation of SR Ca^{2+} channels might contribute to depress FR. These results support the proposal that changes in FR underlie the negative force-frequency relationship in rodents. (FAPESP, CNPq, FAEPEX).

57-Plat
Flecainide Inhibits Cardiac Ryanodine Channels And Spontaneous Sarcoplasmic Reticulum Calcium Release In Casq2 Null Myocytes
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Background: Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic syndrome due to cardiac Ca^{2+} release channel (*RYR2*) or cardiac calsequestrin (*CASQ2*) mutations. VT is caused by spontaneous Ca^{2+}-release from the sarcoplasmic reticulum (SR) that generates afterdepolarizations and triggered beats during catecholamine surge. We recently found that flecainide, a class 1c Na^+ channel blocker, suppressed ventricular arrhythmia in *Casq2 null* (*Casq*$^{-/-}$) mice, a model of CPVT. Here, we investigated the effect of flecainide on on cardiac Ca^{2+} handling in *Casq2*$^{-/-}$ myocytes loaded with Fura-2 AM, and on sheep RyR2 RyR2 channels reconstituted in lipid bilayers.
Results: In isoproterenol-stimulated *Casq2*$^{-/-}$ myocytes, flecainide (6 μmol/l) reduced triggered beats by over 70% ($p<0.001$). Unexpected for a Na^+ channel blocker, flecainide also reduced SR Ca^{2+} leak (Ca^{2+} fluorescence ratio: vehicle: 0.12 ± 0.01 vs. flecainide: 0.08 ± 0.01, n=54 per group, p=0.02) and suppressed the rate of spontaneous Ca^{2+} releases (SCRs) from the SR (SCRs/min: vehicle: 48 ± 5 vs. flecainide: 29 ± 5, n=45 per group, p=0.006), suggesting flecainide directly inhibits SR Ca^{2+} release. Lipid bilayer experiments confirmed a direct action of flecainide on RyR2 SR Ca^{2+} release channels: Flecainide induced brief partial closures of channels to a substate with a conductance equal to 20% of the fully open state. On average, flecainide as low as 5 μmol/L caused a 4-fold increase in the frequency of closed events and caused a significant reduction in the open probability from control levels. The effect of flecainide was concentration dependent (IC_{50} ~ 50 μmol/l) and fully-reversible upon washout. Flecainide also inhibited RyR2 channels activated by high luminal Ca^{2+}.
Conclusion: We report a heretofore unrecognized inhibitory action of flecainide on RyR2 channels, which together with flecainide's inhibition of Na^+ channels may explain flecainide's effectiveness in preventing CPVT.
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