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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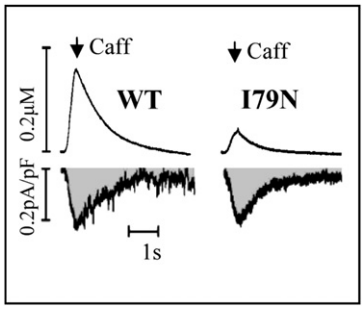
In myocytes, free [Ca^{2+}] is determined by the rate of Ca^{2+} influx and the Ca^{2+} buffering properties of the cytosol. Ca^{2+} binding to myofilaments (primarily Troponin C) represents a major portion of cytosolic Ca^{2+} buffering. To test the hypothesis that increased myofilament Ca^{2+} sensitivity 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.