We found that an age-dependent reduction in the intrinsic HR in vivo (22%) in 24- vs 3-mo mice is accompanied by a 29% reduction in single SANC AP-firing rate. These are associated with a number of changes stemming from compromised SR Ca^{2+}-function, including reduced Ca^{2+}-cycling and diminished response to pharmacologic SR stress. These changes coincide with decreased expression of crucial SR Ca^{2+}-cycling proteins, including SERCA2 and RyR2, and also NCX1. Aged SANC were also found to have reduced SR Ca^{2+}-load compared to young, as well as a reduced size, number and duration of spontaneous LCRs. Finally, the sensitivity to PDE inhibition-associated increase in PLB-phosphorylation, LCR size, amplitude and number were reduced in 24- vs 3-mo SANC. Thus, a deterioration in intrinsic Ca^{2+}-clock kinetics in aged SANC due to deficits in intrinsic SR Ca^{2+}-cycling and its response to a PDE stress appears to be involved in age-associated reduction in intrinsic resting AP-firing rate, and intrinsic HR in vivo, and may also underlie the age-associated reduction in the acceleration of HR in response to stress.

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Contribution of Ca-Regulated Ion Currents to the Action Potential Morphology During Cardiac Alternans

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Cardiac alternans, described as periodic beat-to-beat alternations in contraction, action potential (AP) morphology or cytosolic Ca transient (CaT) amplitude, is recognized as high risk indicator for cardiac arrhythmias, stroke and sudden cardiac death.

We investigated mechanisms of cardiac alternans in single rabbit atrial myocytes. CaTs were monitored simultaneously with membrane currents or APs recorded with the patch clamp technique, and revealed a strong correlation between CaT amplitude and AP duration (APD) during alternans. Investigation of [Ca]i-Vm interactions indicates that disturbances in Ca signaling are the primary events mediating activity of Ca-regulated ion channels and leading to the changes in AP morphology. Voltage-clamp in form of pre-recorded APs (AP clamp) was used to assess the contribution of Ca-regulated membrane currents by measuring the difference between currents recorded during large and small alternans CaTs. Ca-dependent currents exhibited a large outward component (2.9 ± 1.1 pA/pF in amplitude) during AP phases 1 and 2 that was followed by an inward current (0.5 ± 0.1 pA/pF in amplitude; n=7) during AP repolarization. We also investigated contributions of Na/Ca exchange (NCX), L-type Ca, Ca-activated Cl and small conductance Ca-activated K currents to the AP morphology during Ca alternans. ~90 % of the initial outward current was blocked by substitution of Cl ions or application of Cl channel blocker (DIDS) indicating that this current is mainly carried by Ca-activated Cl channels. In summary, during Ca alternans the prominent prolongation of AP at APD10 to APD30 repolarization levels during the small CaT is due to reduced Ca-activated Cl current while the overall effect of other Ca-sensitive currents (NCX, L-type Ca, small conductance K) cause slight shortening of AP in the APD50 to APD90 range.

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A Small Number of Cells is Sufficient to Trigger a Cardiac Arrhythmia: Stochastic Computational Studies

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Cardiovascular disease is the leading cause of death world-wide and this is due in large part to arrhythmias. Here we examine the cellular and subcellular basis of Ca^{2+} dependent arrhythmias. In order to understand how calcium dynamics, plays a role in arrhythmogenesis, we have investigated normal and dysfunctional Ca^{2+} signaling in heart cells at high temporal and spatial resolution. Spontaneous calcium release occurs normally as Ca^{2+} sparks. When RyR2 open probability increases to a high level, then Ca^{2+} sparks can activate Ca^{2+} waves. These propagating elevations of $[Ca^{2+}]i$ can activate inward NCX current (INCX) that may contribute to early afterdepolarization (EADs) and underlie delayed after-depolarization (DADs). However, how cellular currents lead to full depolarizations of the myocardium and how they initiate extra systoles is still not fully understood. Some earlier studies that have investigated this question suggest that as many as about ~700,000 cells must undergo such behavior to initiate a propagating action potential or an arrhythmia. Here we present the results of our study which explores how many cells must be entrained to initiate arrhythmogenic depolarizations in ''realistic'' computational models. The model presented here suggests that only a small number cells must activate in order to trigger an arrhythmogenic propagating action potential. These conditions were examined in 1D, 2D, and 3D taking into account heart geometry. The finding that only a small number of cells is required to trigger an arrhythmia provides a plausible mechanism by which cardiac arrhythmias might occur.

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Relative Contribution of Purkinje Fibers to CA^{2+}-Dependent Arrhythmias in a Murine Model of CPVT

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Background. Purkinje fibers (PFs) are critical for coordinated electrical excitation of the ventricles and thought to play a key role in abnormal impulse formation and ventricular arrhythmias. However, the arrhythmogenic properties of PFs as compared to ventricular tissue remain to be elucidated and were the focus of the present study.

Results. To examine the arrhythmic potential of PFs vs ventricular tissue we performed Ca^{2+} and membrane potential (MP) imaging in PFs and ventricular tissue preparations derived from both wild-type (WT) mice and genetically modified mice prone to Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT). PFs and trabeculae dissected from the right ventricles were loaded with the voltage- and Ca^{2+}-sensing dyes RH237 and Rhod-2, respectively. PFs were identified by the characteristic long, narrow shape and lack of T-tubules of Purkinje cells, as evidenced by membrane staining with RH237. In PFs and trabeculae preparations isolated from CPVT but not WT mice, exposure to the beta-adrenergic agonist isoproterenol resulted in frequent diastolic Ca^{2+} releases both spontaneous and triggered, that were associated with corresponding MP signals. Consistent with their ability to lead to triggered events, diastolic spontaneous Ca^{2+} releases showed high synchronicity between adjacent cells in both tissues. Overall spontaneous Ca^{2+} release synchronicity and the rates of occurrence of spontaneous and triggered Ca^{2+} release events were similar between PFs and trabeculae.

Conclusion. These results suggest that the Purkinje system and ventricular tissue possess similar arrhythmic potentials in a setting of CPVT, consistent with a diffuse nature of the genesis of Ca^{2+}-dependent ventricular arrhythmias.

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The Metabolic Modulator Perhexiline Induces Calcium-Cycling Dysfunction and Apoptosis in Cardiomyocyte Syncytia

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Major pathogenic adaptations in chronic heart failure (CHF) include the reversion to glycolytic metabolism and the persistence of an energy deficient state. The use of metabolic modulators to increase the efficiency of glycolytic ATP production has emerged as a new therapeutic approach in CHF. Perhexiline (PHX), an anti-anginal agent, improves myocardial function in CHF patients by a mechanism that is thought to involve the inhibition of carnitine palmitoyl transferase (CPT) which prevents mitochondrial free fatty acid uptake. In silico modelling and cell-based experimental data predict that such alterations to the metabolic steady state would drive other compensatory (mal)adaptations that could negatively impact on cellular phenotype. Here, we investigated the phenotypic consequences of PHX-mediated metabolic modulation on Ca^{2+} cycling and apoptotic susceptibility in HL-1 syncytia. HL-1 cardiomyocytes exhibit a metabolic profile similar to that observed in CHF myocardium (glycolysis >> fatty acid oxidation). Contrary to the expected enhancement of Ca^{2+} cycling, PHX profoundly deranged Ca^{2+} signalling in a time- and concentration-dependent manner. The spatio-temporal organization and intercellular synchronization of Ca^{2+} oscillations was progressively reduced at [PHX] between 0.1 and 2.5μM and contractility was abolished at [PHX] > 5μM. These concentrations are below the levels of drug reported to accumulate in heart tissue following therapeutic dosing. Metabolic activity was compromised in PHX-treated cells and we determined PHX concentration-dependent caspase-linked apoptosis (EC50 ≈ 25μM). Oxfenicine (a potent CPT1 inhibitor) did not recapitulate any of these effects at concentrations up to 100μM. Our data show that, in the context of glycolytic metabolism, PHX provoked dysfunctional Ca^{2+} handling and increased cardiomyocyte susceptibility to apoptosis. These effects appeared to be independent of CPT inhibition.