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Mechanisms of Abnormal Ca^{2+} Transients in Pathophysiological Ventricular Muscles Determined by Ca^{2+} and Membrane Potential Imaging

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Abnormal Ca^{2+} signals, including delayed/desynchronized onset of Ca^{2+} transients, occasional missing Ca^{2+} transients and Ca^{2+} transient alternans, are often observed in cardiac muscles under pathophysiological conditions. To investigate how these abnormal Ca^{2+} responses can be generated, we monitored membrane potential and Ca^{2+} signals using a fluorescent membrane potential indicator and a Ca^{2+} indicator in the same preparation. Papillary muscles were dissected from guinea pig ventricles and loaded with di-4-ANEPPS and rhod-2 AM. Mono-wavelength Ca^{2+} signals and ratiometric action potential signals were sequentially obtained using the Nipkow-disc confocal microscope and W-view system. Control signals were obtained from cardiac muscles paced in a normal Krebs solution, whereas abnormal Ca^{2+} signals were induced by pacing them in a non-flowing Krebs solution. There were two types of causes for the failed and alternating Ca^{2+} transient generation, i.e., failed or alternating immature action potential generation and abnormal EC coupling with relatively constant action potentials. In cells showing delayed initiation of Ca^{2+} transients, action potential onset was also delayed and the rate of rise was slower than that in healthy cells. Effects of an inhibitor of gap junction channels and a Na^{+} channel blocker suggest that the delayed onset of action potentials can be explained primarily by impaired gap junctions and partly by Na^{+} channel inactivation.

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Competitive Regulation of Calcium and Zinc Ions in Cardiomyocyte Contraction-Relaxation Function

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Zinc (Zn^{2+}) and calcium (Ca^{2+}) ions are divalent cations having common chemical properties leading to their competing for the same regulatory channels and pumps in the intact cardiomyocyte. Diastolic dysfunction may be due in part to elevated diastolic Ca^{2+} concentration ($[Ca^{2+}]$). We hypothesized that Zn^{2+} reduces systolic and enhances diastolic function due to its effects on Ca^{2+} regulation. We examined the effects of 32μM extracellular zinc ($[Zn^{2+}]_{ext}$) exposure and intracellular zinc ($[Zn^{2+}]_{int}$) accumulation on rat cardiomyocyte function. We measured sarcomere dynamics, $[Ca^{2+}]_{int}$ by Fura-2FF and $[Zn^{2+}]_{int}$ by FluoZin-3 under three $[Zn^{2+}]$ conditions: no $[Zn^{2+}]_{ext}$ and low $[Zn^{2+}]_{int}$; 32μM $[Zn^{2+}]_{ext}$ and low $[Zn^{2+}]_{int}$; 32μM $[Zn^{2+}]_{ext}$ and high $[Zn^{2+}]_{int}$. Cardiomyocytes were paced at 2Hz, exposed to 2mM $[Ca^{2+}]_{ext}$ at 37°C. After reaching $[Zn^{2+}]_{int}$ steady-state, 10mM caffeine was rapidly applied to measure sarcoplasmic reticulum (SR) Ca^{2+} content and Na^{+}-Ca^{2+} exchanger (NaCaX) efflux rate. Sarcomere shortening velocity and peak shortening were significantly ($P<0.05$) reduced with $[Zn^{2+}]_{ext}$ exposure in either low or high $[Zn^{2+}]_{int}$ conditions. Interestingly, peak shortening was significantly enhanced with high $[Zn^{2+}]_{int}$ compared to low $[Zn^{2+}]_{int}$. Diastolic sarcomere length was significantly increased with high $[Zn^{2+}]_{int}$. Peak $[Ca^{2+}]_{int}$ was significantly reduced under 32μM $[Zn^{2+}]_{ext}$ with high $[Zn^{2+}]_{int}$, which was consistent with lower SR Ca^{2+} content detected by caffeine experiment. SR Ca^{2+} uptake rate by SERCA and NaCaX efflux rate were not affected by $[Zn^{2+}]_{int}$. All the above changes due to Zn^{2+} were not observed in control cardiomyocytes without $[Zn^{2+}]_{ext}$ exposure. These findings suggest that Zn^{2+} competes with Ca^{2+} for calcium channels (L-type and SR release channels) and thereby reduces contractile function without affecting SERCA or NaCaX. Interestingly, high $[Zn^{2+}]_{int}$ causes a slightly increased contractile function despite the reduction in peak $[Ca^{2+}]_{int}$, suggesting that $[Zn^{2+}]_{int}$ enhances myofilament contraction by mechanisms yet to be explained.

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Cellular Mechanisms of Contractile Impairment in Human Chronic Atrial Fibrillation

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Chronic Atrial Fibrillation (cAF) is associated with contractile impairment. Down regulation of L-Type Ca^{2+}current plays a major role in determining contractile dysfunction. However, additional EC-Coupling changes could be involved in human cAF. We dissected atrial trabeculae from left atrial appendages of cAF patients undergoing cardiac surgery and used them for simultaneous force and action potential recordings. Cells isolated from the same samples were used for Ca^{2+}current, Ca^{2+}transients, and reticular Ca^{2+}content(caffeine) measurements. Samples from sinus rhythm (SR) patients were used as controls. Despite 75%reduction in basal force, positive inotropic responses to isoproterenol, stimulation pauses, and high $[Ca^{2+}]_e$ were preserved in cAF. Basal Ca^{2+}current and Ca^{2+}transients were decreased (30%of SR) but showed a greater increase upon inotropic stimuli, reducing the difference with SR. No difference was found in time-course of mechanical restitution, suggesting no major impairment of Ryanodine Receptor function. The finding that sarcoplasmic reticulum Ca^{2+} content was not reduced in cAF suggests that Ca^{2+}release impairment could be due to a change from synchronous EC Coupling to *propagated* Ca^{2+}-induced Ca^{2+}-release (CICR), in which a fast subsarcolemmal Ca^{2+}rise is followed by Ca^{2+}diffusion-mediated signal propagation toward the cell core. The following observations in cAF preaparations supports this idea: 1)Ca^{2+}transients showed a fast monophasic rise(subsarcolemmal Ca^{2+}-release only) but exhibited a biphasic, dome-shaped aspect (peripheral rise followed by inward Ca^{2+}-wave spread) upon inotropic stimulations; 2)Ca^{2+}recirculation fraction decreased, suggesting an increased role of NCX*vs.*SERCA, consistent with a non-propagated peripheral Ca^{2+}rise; 3)twitch force transitorily increased after abrupt reduction of intracellular Ca^{2+} buffering by the SERCA blocker CPA. Reduction in T-tubules density and/or increased cytosolic Ca^{2+}buffering (e.g.increased myofilament Ca^{2+}sensitivity) could be crucial in cAF for transition to *propagated*-CICR and Ca^{2+}-wave spread impairment. Ca^{2+}trigger enhancement or Ca^{2+}diffusion improvement could promote recruitment of deeper myofibril layers and increase twitch force.

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Ionic Cellular Mechanisms for the Brugada Syndrome in Canine Myocytes

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Background: The Brugada syndrome is a right ventricular (RV) arrhythmia that is believed to be responsible for up to 20% of sudden cardiac deaths. The disease is related to mutations of cardiac Na, Ito, or Ca channel genes. In this study we used a combination of dynamic clamp and computational modeling to address two questions; the cellular mechanism of the electrical abnormality in Brugada syndrome and the potential basis of the RV wall contractile abnormality in the syndrome.

Results: Tetrodotoxin (TTX, 1 −3 μM) was used to reduce cardiac INa by ~50-75%, to mimic a Brugada syndrome-like setting in canine ventricular myocytes. Such INa reduction resulted in prolongation of action potential duration (APD) or all-or-none repolarization in RV epicardial myocytes, but not in RV endocardial or LV cells. These repolarization changes were associated with attenuation or blocking of myocyte contraction and peak Ca transient. Dynamic clamp and mathematical modeling were used to examine the interplay of INa and Ito and its influence on AP morphology. Both reduction of INa and increase of Ito have similar bi-phasic effects on APD. Reduction of INa shifts the APD-Ito density curve to the left. As a result, in the presence of a large Ito, INa reduction either prolongs APD or results in collapse of the AP, depending on the exact density of Ito.

Computational modeling showed that these repolarization changes alter myocyte Ca dynamics mainly by reducing Ca influx through the L-type conductance.

Conclusion: INa reduction alters repolarization by shifting the APD-Ito relationship and reducing the threshold for Ito-induced all-or-none repolarization. These cellular electrical changes suppress myocyte EC coupling and mechanics. As such, the contractile abnormality of the RV wall in Brugada syndrome may be secondary to the electrical abnormalities.

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Role of the Transient Outward Current In Regulating Mechanical Properties of Canine Ventricular Myocytes

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Background: The transient outward current (Ito) is a major repolarizing current in the heart. Reduction of Ito density is consistently observed in human heart failure (HF) and animal HF models. It has been proposed that Ito, via its influence on phase 1 repolarization of the action potential, facilitates L-type Ca2+ current activation and sarcoplasmic reticulum Ca2+ release, and that its downregulation may contribute to the impaired contractility in failing heart.

Results: We used the dynamic clamp to examine the influence of Ito on the mechanical properties of canine left ventricular myocytes. In endocardial