Urocortin 2 Protects Against Pacing-Induced Alternans via Phosphorylation of Phospholamban in Cardiac Myocytes from Normal and Failing Hearts

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Cardiac alternans is a high risk indicator for cardiac arrhythmias, stroke and sudden cardiac death. The cardioactive peptide Urocortin 2 (Ucn2) exhibits beneficial effects in normal and failing hearts, and elicits PKA-dependent positive inotropic and lusitropic effects in normal myocytes. Thus, we investigated if Ucn2 protects against pacing-induced alternans and elucidated the underlying mechanism.

Experiments were performed on single rabbit atrial and ventricular myocytes from normal and failing hearts. Chronic heart failure was induced by combined pressure and volume overload through aortic constriction in rabbits. Ucn2 was administered to increase the pacing frequency until stable Ca alternans occurred at room temperature. Global Ca transients were measured with the fluorescent Ca indicators Fluo-4 or Indo-1 and monitored simultaneously with mechanical alternans (sarcomere length). In some experiments, cytosolic Ca alternans and intra-SR Ca alternans were simultaneously recorded with the Ca indicators Rhod-2 and Fluo-5N, respectively.

The average alternans ratio (AR = 1-(small-amplitude/large-amplitude)) in atrial myocytes was 0.79, and in normal and failing ventricular myocytes the ARs were 0.69 and 0.64, respectively. Ucn2 (100 nM) completely abolished Ca and mechanical alternans (within 2-3 min) in atrial and ventricular myocytes from normal and failing hearts. An increased sarcoplasmic reticulum (SR) Ca content, together with an enhanced SR Ca release flux, suggested that Ucn2 normalized alternans through effects on SR Ca ATPase (SERCA). Ucn2 increased significantly the level of cyclic adenosine monophosphate (cAMP) in normal cells (~12-fold), and enhanced phosphorylation of phospholamban (PLB) at Ser16 in normal myocytes (~10-fold) and to a lesser extent (~5-fold) in failing myocytes. These data demonstrate that Ucn2 rescues alternans presumably via increased SERCA activity in atrial and ventricular myocytes and thus protects normal and failing hearts from proarhythmic alternans.

NOS1AP Gene Expression is Up-Regulated in Dystrophic Cardiomyopathy

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NOS1AP gene (nitric oxide synthase 1-adaptor protein) is strongly associated with abnormalities in the QT interval of the electrocardiogram and with sudden cardiac death. To determine the role of NOS1AP in the physiology of the cardiac myocyte, we assessed the impact of silencing NOS1AP, using siRNA, on Ca^2+ transients in neonatal cardiomyocytes. In addition, we examined the co-localization of NOS1AP with cardiac ion channels, and finally, evaluated the expression of NOS1AP in a mouse model of dystrophic cardiomyopathy.

Using siRNA, NOS1AP levels were reduced to ~30% of the control levels (p<0.05). NOS1AP silencing in cardiac myocytes reduced significantly the amplitude of electrically evoked calcium transients (p<0.05) and the degree of S-nitrosylation of the cells (p<0.05). Using confocal microscopy, we evaluated NOS1AP subcellular location and interactions with other proteins by co-localization analysis. NOS1AP showed a high degree of co-localization with the L-type calcium channel and the inwardly rectifying potassium channel Kir3.1, a low degree of co-localization with the ryanodine receptor (RyR2) and alpha-sarcromeric actin and no co-localization with connxin 43, suggesting functionally relevant interactions with the ion channels that regulate the action potential duration.

Finally, using immunofluorescence and Western blotting, we observed that in mice with dystrophic cardiomyopathy, NOS1AP was significantly up-regulated (p<0.05). These results suggest for a role of NOS1AP on cardiac arrhythmias, acting on the L-type calcium channel, and potassium channels, probably through S-nitrosylation.

Optimal Reticulated Coverage of the Sarcoplasmic Reticulum

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During every heartbeat, the Ca^2+ that is released must be reabsorbed into the sarcoplasmic reticulum (SR) sufficiently rapidly and the free energy of ATP hydrolysis, to our knowledge state-based models that account for SERCA’s interaction with the endogenous phospholamban inhibitor are less well-developed. We thus propose a SERCA Ca^2+ uptake model that is based on a sequence of crystallographically-determined conformational states. These distinct conformations have been shown to give rise to the cooperativity of cytosolic Ca^2+ binding, and the kinetics of which are altered by phospholamban binding.

We apply the new SERCA model to a 3-dimensional model of Ca^2+ signaling in a realistic, confocal-microscopy derived cardiac ventricular myocyte, with which we demonstrate the effects of altered Ca^2+ cooperativity on the Ca^2+ transient.
the myofibril being uniformly enshrouded by SR) is between 20-30% even as the heart rate ranged from 1 Hz to 5 Hz. The relatively small amount of SR coverage needed for economic control of Ca\(^{2+}\) is consonant with Berg and Purcell’s (Biophysical Journal 20:193-219) classic finding that, as a consequence of the properties of diffusion, a small fractional covering of absorbers on the cell surface performs almost as well as when the surface is entirely covered by absorbers.

Cardiac, Smooth, and Skeletal Muscle Electrophysiology I

609-Pos Board B364 Species-Specific Comparison of the Cardiac Sodium/Potassium Pump Based on a Minimal Biophysical Model
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The Na\(^+/K\)\(^/-\) ATPase (NKA) plays a critical role in maintaining the concentration gradients, across the plasma membrane, of potassium (which determines the cell’s membrane potential) and sodium, the driving force behind crucial ion-exchange processes, including calcium extraction via the sodium/calcium exchanger. This function has been extensively studied, experimentally and by computational simulations, within the context of the excitation/contraction coupling in cardiac myocytes. An important source of complexity in these strongly coupled systems is the significant species-dependent variability of physiological conditions under which NKA operates, particularly the intracellular sodium concentration [Na\(^+\)]. For example, [Na\(^+\)]\(_i\) ~ 11 mM in rat ventricular myocytes, and ~ 5 mM in guinea pig. An important question is whether (1) NKA is maintained across species and operates in different species-specific regimes; or (2) NKA shows significant species-dependent variations and hence participates directly in defining physiological conditions. Most existing models neglect this fundamental question by assuming a generic NKA formulation derived from disparate experimental sources. To address this problem, we propose a biophysical framework for characterizing NKA function, specifically designed for species-specific parameterization, and produce separate models for rat and guinea pig NKA, each parameterized from fully consistent data sets. We find that the apparent binding affinity for sodium in the rat is lower by a factor of approximately three, whereas the overall pump current magnitude is roughly doubled, relative to guinea pig. These trends mirror those for the [Na\(^+\)]\(_i\) differences, suggesting that NKA kinetics compensates or has adapted to its physiological conditions. Such comparisons allow an analysis of the relative influence of cellular components, ionic conditions, and the action potential on ion transport in cardiac contraction, and ultimately enable the quantification of variations in physiological function of NKA across biological contexts.

610-Pos Board B365 Intermittent Early Afterdepolarizations Caused by Bistability
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Early afterdepolarization (EAD) is one of the main triggers for cardiac arrhythmias. The intracellular sodium concentration ([Na\(^+\)]) is critical in regulating both intracellular calcium (Ca\(^{2+}\)) homeostasis and membrane voltage (V). However, the role of [Na\(^+\)] in the formation of early afterdepolarizations (EADs) is not completely understood. In this study, we applied mathematical modeling to show that slow [Na\(^+\)] accumulation can lead to a novel form of aperiodic voltage dynamics where a sequence of action potentials (APs) with EADs occurs intermittently during regular pacing. We find that these trains of EADs occur quasi-periodically and lead to intermittent EAD propagation and arrhythmias. The mechanism for these intermittent EADs can be traced to the subtle feedback between Na and Ca fluxes (especially via Na/Ca exchange (NCX) and Na/K-ATPase (IPump)) and their effect on the AP duration (APD). We analyzed the whole system by separating the slow [Na\(^+\)] from the fast V-[Ca\(^{2+}\)] subsystem. We found that the fast subsystem exhibits bistability which leads to the observed EADs by forming hysteresis loops as [Na\(^+\)] slowly increases and decreases. That is, [Na\(^+\)] can gradually decline during stable short APDs, but this gradually diminishes outward currents via IPump and INCX, which can result in abrupt APD prolongation with EADs. But that long APD gradually reverses the [Na\(^+\)] decline, and shifts IPump and INCX more inward, which at some point abruptly prevents EADs and reverses APD prolongation. We argue that these intermittent EADs are robust and can occur at physiological [Na\(^+\)]. Our study further provides a possible novel mechanism for the intermittency of cardiac arrhythmias.

611-Pos Board B366 Diabetic Hyperglycemia Acutely Affects Action Potentials and Ionic Currents through CaMKII Activation on Rat Ventricular Myocytes
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Diabetes mellitus is a complex disease that involves cardiomyopathy and neuropathy. Ca\(^{2+}\)-calmodulin dependent protein kinase II (CaMKII) is a nodal molecule that participates in many physiological and pathological processes in the heart. Diabetic hyperglycemia has been shown to activate CaMKII through a novel modification of O-linked N-acetylglucosamine (O-GlcNAc) at S279, leading to cardiac arrhythmias at the whole heart/animal level. However, acute hyperglycemia affects specific ion channels and thus action potentials (APs) through O-GlcNAc activated CaMKII are still unclear. To investigate this question, we measured APs and ionic currents on freshly isolated rat ventricular myocytes under acute diabetic hyperglycemia challenge. Glucose (30mM) perfusion significantly reduced the AP amplitude (82.7±5.7% of control, n=8, p=0.011), depolarized the resting membrane potential (~79.8±0.8 mV for control vs. ~73.4±1.8 mV for glucose, n=8, p=0.002), and prolonged the AP duration (117.3±7.1% of control, n=8, p=0.001) on rat myocytes whereas osmolality matched mannitol application did not change any AP parameters. KN-93 (10μM) pre-incubation abolished the glucose effects, indicating that acute glucose application changes cellular electrical activities via CaMKII-dependent manner. Together, these data provide evidence for the arrhythmogenesis of acute hyperglycemia at the cellular level and suggest that CaMKII-modulated ionic currents are responsible for the hyperglycemic effects on APs.

612-Pos Board B367 Transmural Gradient of I\(_{\text{Na}}\) and I\(_{\text{NaK}}\) Profoundly Influence Ventricular Action Potential Duration
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The spatial distribution of transient outward K\(^+\) current (I\(_{\text{to}}\)) and Na/K pump current (I\(_{\text{NaK}}\)) differ in ventricular epicardial, midmyocardial and endocardial cells in a gradually decreasing pattern called transmural gradient. Due to a lack of selective I\(_{\text{to}}\) blockers, it remains unclear whether changes in I\(_{\text{to}}\) alone affect action potential duration (APD) in the ventricle. In this study we use mathematical modeling to address the above question by modifying the kinetics of I\(_{\text{to}}\) and I\(_{\text{NaK}}\) on the framework of Hund-Rudy model to incorporate the transmural gradient. Model simulation results show that I\(_{\text{Na}}\) of physiological values does not affect APD, but artificially increasing I\(_{\text{Na}}\) above its normal value in the epicardiun can prolong APD; further increasing I\(_{\text{Na}}\) beyond a threshold cause collapsing of the AP plateau phase and abrupt shortening of APD. These model simulation results agree with the experimental data from using dynamic-clamp to manipulate I\(_{\text{Na}}\) (Sun and Wang, J Physiol 564:411-419, 2006). Moreover, our simulation results show that the transmural gradient of Na/K pump also affects APD. Together, the I\(_{\text{Na}}\) and Na/K pump affect the intracellular Na\(^+\) and Ca\(^{2+}\) concentrations, which is manifest to influencing the late Na\(^+\) current and Na/Ca\(^{2+}\) exchange current. Due to the interconnectedness of these currents to Na\(^+\) and Ca\(^{2+}\) homeostasis, it is important to incorporate the transmural gradient of ion channels and transporters into mathematical models in order to understand their combined effects on modulating the AP properties across the myocardium. Our modeling platform will help to decipher how the transmural gradients can profoundly affect the cardiac conduction.

613-Pos Board B368 Mechano-Chemotransduction in the Single Cardiac Myocyte Contracting in 3D Elastic Gel
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Objective: Mechanical stress under pathological conditions such as hypertension, infarction and fibrosis can cause heart failure and arrhythmias. However, little is known about the mechanotransduction mechanisms that underlie heart disease development due to previous lack of practical techniques to control the mechanical stress on single myocytes necessary for investigating at cellular and molecular levels. Here we use this system to study the mechanical load effects on modulating myocyte Ca\(^{2+}\) signaling and contraction dynamics. Methods: Recently, we developed a novel Cell-in-Gel system that allows control of mechanical load on single rabbit myocytes during excitation-contraction coupling in a 3D elastic gel matrix composed of polyvinyl alcohol (PVA) and tetravalent boronate-PEG crosslinker. The mechanical load can be controlled.