disorganized T-tubular structure in failing myocytes, which is also known to promote Ca^{2+} release dyssynchrony. Specifically, we observed irregular gaps between adjacent tubules where Ca^{2+} release was markedly delayed, occurring only after Ca^{2+} diffusion from regions where tubules were present. Thus, slowed and dyssynchronous Ca^{2+} release in failing myocytes results from a combination of altered ryanodine receptor function and T-tubule disorganization. We suggest that the sub-population of slow, small amplitude Ca^{2+} sparks in CHF may represent ryanodine receptors which are functionally uncoupled from their neighbours.

555-Pos

Cardiotrophin-1: Another "player" in Cardiac Calcium Handling

Gema Ruiz-Hurtado[1], Nieves Gómez-Hurtado[2], Javier Díez[3], Victoria Cachofeiro[4], Ana Maria Gómez[1], Delgado Carmen[2].
[1]U-637, Inserm, Montpellier, France, [2]Department of Pharmacology, School of Medicine, University of Complutense and CIB (CSIC), Madrid, Spain, [3]Centre of Applied Medical Research, University of Navarra, Pamplona, Spain, [4]Department of Physiology, School of Medicine, University of Complutense, Madrid, Spain.

Cardiotrophin-1 (CT-1) is a cytokine member of the interleukin-6 superfamily produced by cardiomyocytes and fibroblasts in the heart, in situations of haemodynamic overload, or in the presence of humoral factors as aldosterone. CT-1 is able to induce hypertrophic growth and dysfunction of cardiomyocytes *in vitro*. Moreover, plasma levels of CT-1 are elevated in patients with cardiac hypertrophy and heart failure (HF) and correlated with the severity of the disease. On the other hand, it is well established that alterations in calcium handling are involved in cardiac dysfunction during HF. However, it is yet unknown whether CT-1 modulates Ca^{2+}handling in cardiomyocytes. Here we analyzed CT-1 effects on $[Ca^{2+}]_i$ handling in rat single cardiomyocytes. The L-type calcium current (I_{CaL}) was registered using whole-cell patch-clamp technique. Intracellular calcium $[Ca^{2+}]_i$ transients and Ca^{2+} sparks were viewed by confocal miscroscopy in cardiomyocytes loaded with the fluorescence Ca^{2+} indicator Fluo-3 AM. Treatment of cardiomyocytes with 1 nM CT-1 for 30 min induced a significant increase in I_{CaL} density compared to control cells (at -10 mV: -16.0 ± 0.9 vs. 11.9 ± 0.7 pA/pF; $P < 0.01$). The activity of ryanodine receptors (RyRs), estimated by Ca^{2+} spark frequency, was significantly increased in cardiomyocytes treated with CT-1 (Ca^{2+} sparks$\cdot s^{-1}\cdot 100\ \mu m^{-1}$: 2.3 ± 0.3 vs. 4.3 ± 0.5; $P < 0.01$). Moreover, we observed that the increase in the total Ca^{2+} spark frequency produced by CT-1 could be attributable to the increased propensity of some clusters of RyR to release Ca^{2+}repetitively. Thus, we conclude that CT-1 is able to alter Ca^{2+} handling in isolated cardiomyocytes, enhancing the Ca^{2+} influx through L-type Ca^{2+} channel and the Ca^{2+}release from sarcoplasmic reticulum through RyRs.

556-Pos

Occurrence of Spontaneous Sparks in Ventricular Myocytes From Junctional and Non-junctional Ryr Clusters

Natalia S. Torres[1], Alex Rock[1], Eleonora Savio-Galimberti[1], Frank B. Sachse[1,2], John H.B. Bridge[1].
[1]Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, UT, USA, [2]Bioengineering Department, University of Utah, Salt Lake City, UT, USA.

In isolated rabbit ventricular myocytes, we found a significant number of ryanodine receptor (RyR) clusters that are not associated with the sarcolemma (non-junctional RyRs). The contribution of non-junctional RyR clusters to calcium transients is unclear. Here, we investigated if these non-junctional RyRs are able to produce spontaneous local calcium release events (sparks), and compared the probability of non-junctional versus junctional sparks. We imaged spontaneous sparks in cells loaded with fluo-4 and bathed in Tyrode solution with dextran (Molecular Weight: 10 kDa) linked to Texas Red dye. We evoked spontaneous sparks using field stimulation in the presence of 1 μM isoproterenol and 4 mM calcium. After 5 stimuli applied with a frequency of 0.5 Hz, we simultaneously imaged the sarcolemma and spontaneous sparks using a line scan confocal microscope (Biorad MRC-1024). Furthermore, a 3D image stack of the Texas Red associated signal was acquired to identify the sarcolemma including the transverse tubular system. We classified sparks as non-junctional if their distance to the sarcolemma is larger than 1 μm. All other sparks were assumed to be junctional. In measurements on 12 isolated cells, 38 sparks (51%) were identified as non-junctional, 36 (49%) as junctional. Our measurements clearly demonstrate that non-junctional RyR clusters are able to release calcium and produce spontaneous sparks. We expect that our approach for distinguishing between non-junctional and junctional sparks underestimates the number of non-junctional sparks. If this is true, the probabilities of the types of sparks are similar to probabilities of the two types of RyR clusters identified in related immunolabeling and microscopic studies. This would suggests that spark generation probability of RyR clusters does not depend on their type.

557-Pos

Alteration of Ryanodine Receptor-Mediated Calcium Release in Heart Failure

Aleksey V. Zima[1], Timothy L. Domeier[2], Lothar A. Blatter[2].
[1]Loyola University Chicago, Maywood, IL, USA, [2]Rush University Medical Center, Chicago, IL, USA.

The decrease in contractility in heart failure associates with impaired cellular Ca^{2+} homeostasis that is in part due to altered ryanodine receptor (RyR) function. We studied properties of sarcoplasmic reticulum (SR) Ca^{2+} release in normal and failing rabbit ventricular myocytes using simultaneous measurements of cytosolic ($[Ca^{2+}]_i$) and intra-SR free Ca^{2+} ($[Ca^{2+}]_{SR}$). At a given SR Ca^{2+} content, fractional SR Ca^{2+} release during action potential stimulation was higher in failing than nonfailing myocytes, suggesting increased sensitivity of RyRs in heart failure. In permeabilized myocytes, SR Ca^{2+} content and Ca^{2+} spark frequency were decreased in heart failure, while Ca^{2+} spark amplitude was similar between failing and nonfailing myocytes. To compare these two groups further, SR Ca^{2+} content was experimentally decreased in nonfailing myocytes to the level observed in failing myocytes using SERCA inhibition. When SR Ca^{2+} content was matched, both Ca^{2+} spark frequency and amplitude were markedly increased in failing myocytes, showing that RyRs are more sensitive to release activation. By monitoring $[Ca^{2+}]_{SR}$ during Ca^{2+} sparks, we also observed that the $[Ca^{2+}]_{SR}$ level for spark termination was significantly lower in myocytes from failing hearts. Because Ca^{2+} sparks are a major contributing factor to diastolic SR Ca^{2+} leak, we compared the properties of SR Ca^{2+} leak in normal and failing myocytes. In failing myocytes SR Ca^{2+} leak was significantly faster, particularly at high $[Ca^{2+}]_{SR}$ where Ca^{2+} sparks are the predominant pathway for SR Ca^{2+} leak. These data show that during the progression of heart failure, modifications to RyRs alter both activation and termination of local SR Ca^{2+} release events. At a given SR Ca^{2+} content these effects may increase fractional SR Ca^{2+} release and preserve contractility during systole, however at the cost of increased diastolic SR Ca^{2+} leak and SR depletion.

558-Pos

Abnormal Intra-Store Calcium Handling and Arrhythmogenesis in Heart Failure

Andriy Belevych, Yoshinori Nishijima, Cynthia A. Carnes, Sandor Gyorke.
OSU, Columbus, OH, USA.

Heart failure (HF) patients are known to have increased susceptibility to ventricular arrhythmias. Although abnormal intracellular calcium (Ca) cycling is recognized as an important contributor to the pathogenesis of ventricular arrhythmias, the specific cellular and molecular mechanisms of these arrhythmias remain to be defined. The objective of present study was to investigate the sub-cellular mechanisms of Ca-dependent arrhythmia using time-resolved Ca imaging in the cytosolic and sarcoplasmic reticulum (SR) luminal compartments and the patch-clamp technique in a canine model of tachypacing-induced HF. When rhythmically paced in the presence of the β-adrenergic agonist, isoproterenol, HF myocytes displayed a higher frequency of diastolic Ca waves than control myocytes. In both HF and control myocytes, diastolic Ca waves occurred when [Ca]SR rose above a certain threshold level, which was significantly lower in HF than in control myocytes. Ca signaling refractoriness determined as the time delay between systolic SR Ca depletion and Ca wave initiation was significantly reduced in HF myocytes. Electrical and Ca signaling activities exhibited several distinct potentially arrhythmogenic patterns, including: 1) delayed afterdepolarizations and extrasystolic action potentials (APs) linked to diastolic spontaneous Ca waves; 2) intermittent prolongations of AP duration associated with pre-systolic spontaneous Ca waves and post-systolic triggered Ca waves; and 3) disorganized release uncoupled from myocyte electrical activity. The level of [Ca]SR threshold for spontaneous Ca waves and the time to attain the threshold during the pacing cycle were critical in determining the type of arrhythmogenic abnormality. These experiments suggest a common mechanistic framework for apparently different arrhythmic phenotypes and provide new insights into the relationship between abnormal Ca release and arrhythmogenesis in HF.

559-Pos

Impaired Function of Cardiac Ryanodine Receptors in An Experimental Model of Metabolic Syndrome

Tarin Paulina Barrera-Lechuga, Agustín Guerrero-Hernández, José Antonio Arias-Montaño, **Angélica Rueda**.
Cinvestav-IPN, Mexico, Mexico.

Metabolic syndrome (MS) has become a global epidemic. In Mexico, the prevalence of MS has increased in the last 10 years together with obesity and type-2