increased propensity for arrhythmic Ca^{2+} transients. The defective EC coupling was not due to a gross misalignment of CaV1.2 and RyR as reported by co-immunolocalization assays. Surprisingly, overexpressed I-II had no impact on CaV1.2 current (ICa,L), whereas CT strongly suppressed ICa,L. The results identify a dominant role for I-II and CT in establishing CaV1.2/RyR functional interrelationship, and suggest a new approach to dissect molecular components and interactions critical for CICR in the heart.

2259-Plat

PIP2 Modulates T-Tubule Remodeling During Heart Failure by Working as a Binding Substrate for BIN1

Neha Singh, Suresh K. Verma, William Marszalec, Gary L. Aistrup, Raj Kishore, J Andrew Wasserstrom.
Northwestern University, Chicago, IL, USA.

The membrane curvature-forming protein BIN1 is known to be involved in T-tubule formation. Phosphatidylinositol-4,5-bisphosphate (PIP2) has been shown to anchor BIN1 and maintain its localization to the sarcolemma. Since heart failure (HF) is characterized by T-tubule remodeling and dyssynchronous Ca^{2+} release, we hypothesized that PIP2-mediated targeting of BIN1 to sarcolemma is compromised during the development of HF and is responsible for T-tubule remodeling. We developed an in-vitro model using HL1 cells that developed tubule-like extensions during BIN1 overexpression. These exogenous BIN1-induced tubules were continuous with the sarcolemma (RH-237 staining) similar to T-tubules in ventricular myocytes. To further understand if the BIN1/PIP2 complex is disrupted in HF, we depleted PIP2 in HL1 cells by inhibition of PI4K or activation of PLCβ1. We treated transfected HL1 cells with wortmannin, endothelin-1 (Et-1), m-3M3FBS and a combination of Et-1 + m-3M3FBS. Treated cells showed significantly more tubule degeneration compared to non-treated cells and membrane PIP2 levels decreased as measured by transfecting HL1 cells with a fluorescent PIP2 reporter (PLCδ1-PH-GFP construct). To further determine whether depletion of PIP2-mediated T-tubules loss is correlated with defects in Ca^{2+} cycling, we isolated adult rat ventricular myocytes, treated them with the same PIP2 depletion agents, then stained them with di-4-ANEPPS in order to assess T-tubule organization. All treated cells showed significantly reduced T-tubule organization than control cells. We also measured Ca^{2+} cycling in these cells during basal (2000ms) and rapid (1000ms) pacing using flou-2AM. Ca^{2+} release was delayed in certain cellular regions in treated myocytes and rise time, time to peak and decay time were significantly increased at both pacing rates. These results show that depletion of PIP2 can lead to T-tubule disruption and suggests that PIP2 is required for T-tubule maintenance and function.

2260-Plat

Spatio-Temporal Relationship Between T-Tubular Electrical Activity and Ca^{2+} Release in Heart Failure

Claudia Crocini[1], Raffaele Coppini[2], Cecilia Ferrantini[2], Ping Yan[3], Leslie M. Loew[3], Chiara Tesi[2], Elisabetta Cerbai[2], Corrado Poggesi[2], Francesco S. Pavone[1], Leonardo Sacconi[1].
[1]University of Florence, Sesto Fiorentino (FI), Italy, [2]University of Florence, Florence, Italy, [3]University of Connecticut Health Center, Farmington, CT, USA.

T-tubules (TT) are invaginations of the surface sarcolemma (SS) that conduct the action potential (AP) to the cardiomyocyte core. Previously, we observed that TT structural remodeling in a rat model of post-ischemic heart failure (HF) was associated with abnormal electrical activity in several t-tubular elements (Sacconi et al. PNAS 2012). To clarify the link between TT abnormalities and Ca^{2+}-dependent arrhythmias, we combined the advantages of an ultrafast random access multi-photon (RAMP) microscope with a double staining approach to optically record t-tubular AP and, simultaneously, the corresponding local Ca^{2+}-transient in different positions across the cardiomyocytes. Despite a uniform AP between SS and TT at steady-state stimulation, in control cardiomyocytes we observed a non-negligible beat-to-beat variability of local Ca^{2+} transient amplitude and kinetics. The beat-to-beat variability was quantitatively similar to the one observed among different tubules during the same beat (spatial variability) and both were significantly reduced by 0.1μM Isoproterenol, which increases the opening probability of Ca^{2+}-release units. As previously demonstrated, some tubular elements fail to propagate AP in rat failing cardiomyocytes. By separately averaging tubules conducting (AP+) or not conducting AP (AP-), we found that Ca^{2+}-transient amplitude was reduced and Ca^{2+}-transient rise was slower in AP- tubules compared to AP+ tubules. Both beat-to-beat and spatial variability of Ca^{2+}-transient kinetics were increased in HF. Finally, TT that did not show AP, occasionally displayed spontaneous depolarizations that were never accompanied by local Ca^{2+}-release in the absence of any pro-arrhythmogenic stimulation. Simultaneous recording of AP and Ca^{2+}-transient allows us to probe the spatio-temporal variability of Ca^{2+}-release, whereas the investigation of Ca^{2+}-transient in HF discloses an unexpected uncoupling between t-tubular depolarization and Ca^{2+}-release in remodeled tubules.

2261-Plat

Non-Linear Relationship Between T-Tubule Remodeling and Sr Calcium Release in Failing Rat Ventricle

Jasleen Singh, Nikhil Bassi, Shannon Tai, Shruthi Mothkur, William Marszalec, Neha Singh, Gary L. Aistrup, **J. Andrew Wasserstrom**.
Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA.

T-tubule remodeling in heart failure (HF) may be responsible for abnormal intracellular Ca^{2+} cycling. The goal of this study was to investigate the relationship between T-tubule remodeling and Ca^{2+} release. Ca^{2+} transients and t-tubules were measured in individual myocytes in intact hearts from spontaneously hypertensive rats maintained on the stage of a confocal microscope. T-tubule organization was measured as the organizational index (OI) using a fast Fourier transform. T-tubule remodeling occurred in a progressive manner through stages ranging from a highly organized network to regional loss of t-tubules and development of longitudinal tubules, finally showing gross loss of tubules and occasional bright punctate staining. Ca^{2+} release reflected the loss of T-tubules as an increase in the standard deviation (Heterogeneity Index, HI) in the time to 50% of peak Ca^{2+} (TR50) along the myocyte, indicating that the regional loss of T-tubules is responsible for increased heterogeneity of Ca^{2+} release. Rapid pacing (BCL= 350msec) increased HI at low OI compared to slow basal pacing (700msec), indicating that Ca^{2+} release heterogeneities are exaggerated at higher heart rates. The relationship between OI and TD50 HI was non-linear; there was little effect on TR50 HI until OI fell from a normal above 0.75 to below 0.65, where TD50 HI increased sharply with OI. The relationship between OI and TR50 HI was well-described by an exponential function. Our data show that extensive T-tubule remodeling in HF is required before significant changes occur in Ca^{2+} release, suggesting inherent redundancy in insuring that Ca^{2+} cycling is maintained during early T-tubule loss. T-tubule remodeling causes a non-linear increase in the variability in Ca^{2+} release along the cell length as Ca^{2+}-induced Ca^{2+} release efficiency decreases with distance from trigger Ca^{2+} provided by the closest L-type Ca^{2+} channels.

2262-Plat

Cardiomyocyte Calcium Handling Dysfunction at High Stimulation Frequencies; Increase in Post-Myocardial Infarction Heart Failure and Decrease by Exercise Training

Ole J. Kemi, Alex S. Johnston, Godfrey L. Smith.
Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, United Kingdom.

Ca^{2+} transient alternans, ie incomplete 1:2 Ca^{2+} transients, have been linked to contractile dysfunction at high heart rates in heart failure (HF). We investigated the effect of post-myocardial infarction (MI) HF and the therapeutic effect of exercise training on cardiomyocyte Ca^{2+} handling during increased electrical twitch-stimulation frequencies, in coronary artery-ligated Wistar rats. 4-weeks post-surgery, aerobic exercise training was initiated, with sham-operated (SHAM) and sedentary MI HF rats as controls. MI HF was confirmed by reduced exercise capacity, pathologic cardiomyocyte hypertrophy, and reduced cardiomyocyte contraction and Ca^{2+} transient amplitude (all 20-40%; $p<0.01$). Exercise training partly reversed these effects (all $p<05$ vs MI HF). When increasing twitch-stimulation frequencies 1-4Hz, SHAM cells increased fractional shortening 20% ($p<0.05$), whereas MI HF cells decreased fractional shortening during 1-4Hz 40% ($p<0.01$). Ca^{2+} transient amplitude mirrored these effects. Exercise training restored a positive contraction-frequency relationship ($p<0.05$) and partly reversed Ca^{2+} transient amplitude deterioration during increased twitch-stimulation frequencies ($p<0.05$). Next, MI HF associated with a 4-fold increase in the occurrence of Ca^{2+} transient alternans; 16% of MI HF cardiomyocytes vs 4% of SHAM cardiomyocytes displayed this behavior ($p<0.01$). Exercise training did not alter this (14%; $p>0.05$ vs MI HF), but it shifted the occurrence of Ca^{2+} transient alternans to higher twitch-stimulation frequencies (median frequency SHAM: 4Hz, MI HF 2Hz, MI HF after exercise training 4Hz; $p<0.05$). Finally, Ca^{2+} transient alternans magnitude (difference 1:2 Ca^{2+} transient) increased in MI HF cardiomyocytes (25-50% in MI HF vs 10-20% in SHAM; $p<0.05$); exercise training partly corrected this (20-30%; $p<0.05$ vs MI HF). In conclusion, exercise training partly reversed cardiomyocyte Ca^{2+} handling abnormalities observed during increasing twitch-stimulation frequencies in post-MI HF. This suggests a mechanism for improving reserve capacity and reducing arrhythmia susceptibility.