levels of miR-1 and miR-133 were significantly increased in HF myocytes compared to controls (2 and 1.6 fold accordingly). Western blotting showed that PKN1 knockdown (KD) of SCW significantly decreased cathepsin-L (alpha) and catalytic subunits, specific targets of miR-1 and miR-133 validated by luciferase-reporter assay, were decreased in HF cells. Analysis using phospho-specific antibodies confirmed that RyR2 phosphorylation at Ser-2814 was significantly increased in HF myocytes compared to controls. CaMKII inhibitory peptide reduced the frequency of spontaneous Ca waves in paced current-clamped HF myocytes to low control values. These findings suggest that altered levels of major muscle-specific microRNAs contribute to abnormal RyR2 function in HF by depressing localized phosphatase activity to the channel, thus leading to excessive phosphorylation of RyR2s.

3045-Pos Board B150
Voltage-Dependent Anion Channel 2 modulates Resting Calcium Sparks, but Not Action Potential-Induced Global Calcium Signaling in Cardiac Myocytes
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Voltage-dependent anion channels (VDACs) are pore forming proteins predominantly found in the outer mitochondrial membrane and is thought to transport calcium ion (Ca2+). In this study, we have investigated the possible role of type 2 VDAC (VDAC2) in cardiac Ca2+ signaling and Ca2+ sparks using a lentiviral knock-down (KD) technique and two-dimensional confocal Ca2+ imaging in immortalized autorythmic adult atrial cells, HL-1. We confirmed high expression of VDAC2 protein in ventricular, atrial and HL-1 cells using Western blot analysis. Infection of HL-1 cells with VDAC2-targeting lentivirus reduced the level of VDAC2 protein to ~10%. Comparisons of autorhythmic Ca2+ transients between wild type (WT) and VDAC2 KD cells showed no significant change in the magnitude, decay, and beating rate of the Ca2+ transients. Caffeine (10 mM)-induced Ca2+ release, which indicates sarcoplasmic reticulum (SR) Ca2+ content, was not altered by VDAC2 KD. Interestingly, however, the intensity, width, and duration of the individual Ca2+ sparks were significantly increased by VDAC2 KD in resting conditions, with no change in the frequency of sparks. These results suggest that VDAC2 may suppress focal Ca2+ releases through ryanodine receptors in atrial myocytes under resting conditions. The results also indicate that VDAC2 may not regulate action potential-induced global Ca2+ signaling and SR Ca2+ loading.

3046-Pos Board B151
African Trypanosomes Increase Calcium Wave Frequency in Isolated Adult Rat Cardiomyocytes via Secretion of Cathepsin L
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African trypanosomes are blood-borne extracellular parasites which have recently been linked to cardiac dysfunction in ~70% of sleeping sickness patients. Although this may result from an induction of the parasite (e.g. myocarditis), a direct effect of the parasite on the heart has not been investigated. Adult rat cardiomyocytes were incubated with trypanosome growth media containing Trypanosoma brucei Lister427 (30min). A population assay assessed the percentage of cells demonstrating Ca2+ waves within a 1min period. Incubation with live trypanosomes led to a significant increase in the percentage of cells demonstrating Ca2+ waves (54.2 ± 2.8% vs. 79.2 ± 5.1%; media vs. live trypanosomes, P <0.05; n=294 and 3006 cells respectively). This effect was maintained when cells were incubated with supernatant (trypanosomes removed from media by centrifugation) (77.3 ± 2.9%; n=2131 cells). Separate experiments showed the supernatant effect was lost upon boiling (83.7 ± 1.8% vs. 66.3 ± 2.4%; supernatant vs. boiled supernatant, P<0.05; n=527 and 612 cells respectively). Results were confirmed in Fur a2M loaded, field stimulated (1Hz) rat cardiomyocytes perfused with media (37°C). Following 4 min supernatant perfusion, the frequency of Ca2+ waves in the inter-stimuli interval was significantly increased (0.02 ± 0.01 vs. 0.44 ± 0.07 waves/s; media vs. supernatant, P<0.05; n=10). Since the parasite induces a similar phenomenon in brain mono-epithelial cells via cathepsin-L, cytochrome, we examined the role of cathepsin-L in the above effect on cardiomyocytes. In separate experiments, supernatant-tK17777 (specific inhibitor of cathepsin-L) completely abolished the ability of supernatant to increase Ca2+ wave probability (56.3 ± 5.1% vs. 49.1 ± 5.7%; media vs. supernatant-tK17777, P<0.05), whereas CA074 (specific inhibitor of cathepsin-B) had no effect on Ca2+ wave frequency. These data suggest trypanosomes interact with cardiomyocytes leading to increased Ca2+ wave production via cathepsin-L. This may contribute to the cardiac abnormalities observed in patients with trypanosomiasis.

3047-Pos Board B152
Calcium Handling in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes
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Fibroblasts from human skin biopsies can be reprogrammed into pluripotent stem cells (iPSC), which can then be coaxed to differentiate into myocytes with cardiac-specific properties (iPSC-CMs). The field of iPSCs is still in its infancy but it is increasingly clear that the excitation-contraction-coupling (ECC) machinery of differentiating CMs undergoes proportionally incremental complexity and it remains to be seen whether it reaches complete maturity in cultured cells. We used patch-clamp and confocal Ca2+ imaging for a comparative assessment of ECC in human iPSC-CM and adult cardiomyocytes. In the latter, entry of Ca2+ through the L-type Ca2+ channel (Ld) triggers rapid, uniform release of Ca2+ from the sarcoplasmic reticulum (SR) via CICR. In iPSC-CMs at early stages of differentiation, the current-voltage relationship for Ld is remarkably similar to that of adult cardiomyocytes, indicating that the appearance of a "trigger" for contraction is an early event in the ontogenesis of ECC that doesn't hinder efficient generation of Ca2+ signals. However, primitive iPSC-CMs commonly exhibit a poorly developed SR, as assessed by their variegated response to caffeine and their great dependence on extracellular Ca2+ for contraction. Cells are mostly rounded and t-tubules are absent. As a result, [Ca2+]i transient waveforms appear non-uniform and start at the periphery of the cell, as is expected of a Ca2+ front with focal initiation that propagates later to the interior of the cell. At more advanced stages of differentiation, iPSC-CMs display fairly uniform Ca2+ fronts, suggesting fast propagation of external Ca2+ signals to the interior of the cell. Thus, by this coarse functional estimate, it is expected that iPSC-CMs become accurate models of cardiomyopathies at late stages of differentiation, but the developmental characteristics of ECC is unclear and warrants a systematic approach, which we are currently performing.

3048-Pos Board B153
Impaired Calcium Signaling Refractoriness Contributes to Increased Rate of Diastolic Calcium Waves in Myocytes from Post-Myocardial Infarction Hearts
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Spontaneous Ca2+ waves (SCWs) are recognized as important contributors to triggered arrhythmia. SCWs are thought to arise when [Ca2+]i reaches a certain threshold level, which might be reduced in cardiac disease as a consequence of sensitization of ryanodine receptors (RyR2s) to luminal Ca2+. We investigated the mechanisms of SCW generation by simultaneous measurements of cytosolic and luminal Ca2+ in myocytes from normal and diseased hearts using a canine model of post-myocardial infarction (MI) tachyarrhythmia. The frequency of SCW, recorded during periodic pacing in the presence of 0.8 μM of β-adrenergic receptor agonist isoproterenol, was significantly higher in MI myocardium in control. Rather than occurring at once upon reaching a final [Ca2+]i, transient waveforms appear non-uniform and start at the periphery of the cell, as is expected of a Ca2+ front with focal initiation that propagates later to the interior of the cell. At more advanced stages of differentiation, iPSC-CMs display fairly uniform Ca2+ fronts, suggesting fast propagation of external Ca2+ signals to the interior of the cell. Thus, by this coarse functional estimate, it is expected that iPSC-CMs become accurate models of cardiomyopathies at late stages of differentiation, but the developmental characteristics of ECC is unclear and warrants a systematic approach, which we are currently performing.

3049-Pos Board B154
Inositol 1,4,5 Triphosphate (IP3) Receptors Activate Type 1 ryanodine Receptors to Mediate Ca2+ Sparks Signaling in Adult Mammalian Skeletal Muscle
Andoria Tjondrokoesoemo, Na Li, Noah Weisleder, Jianjia Ma
Ca2+ sparks are the elemental event of Ca2+ release (CICR) that originate from clustered ryanodine receptor Ca2+ release channels (RYR1) in mammalian striated muscles. Previously we found that application of transient osmotic stress to the intact skeletal muscle leads to a robust Ca2+ spark response that is restricted to the periphery of sarcolemmal membrane. Here we...