calcium concentration. In the cardiac myocyte, CALM binds to several important components involved in excitation, in particular L-type Calcium Channels (LTCC), Cardiac Ryanodine Receptors (RYR2), and Calcium/Calmodulin-dependent protein Kinase II (CaMKII). CALM plays a role in LTCC and RYR2 channel dynamics through direct binding interactions and through CaMKII-dependent phosphorylation. Recent studies into the impact of specific single nucleotide polymorphisms (SNPs) in CALM have been linked to specific changes in the behavior of LTCC's and RyR2's and also to various cardiac disease states (e.g. Long QT Syndrome and Catecholaminergic Polymorphic Ventricular Tachycardia). In addition, additional CALM SNPs have been observed in publicly available genomic databases based upon genomic sequencing of the general human population and these SNPs that have not yet been functionally classified. Using a computational mutagenesis approach based on Delaunay tessellation and statistical geometry, the impact of specific SNPs on the structural stability of CALM-binding interactions has been predicted. These predictions indicate the likelihood that a specific SNP mutation alters CALM structure and thereby its function. Results indicate certain SNP mutations have a differential impact on CALM binding stability to its various targets. Deeper analysis of CALM structure suggests the possible underlying mechanism by which these mutations can cause cardiac disease and may explain why certain mutations correlate with a particular disease state.

**1342-Pos Board B293**

**The Functional Complex Composed of the Sodium/Bicarbonate Cotransporter and the Soluble Adenylate Cyclase (sAC) Modulates Basal Cardiac Contractility**

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In addition to the adenylate cyclase (AC) associated to the plasma membrane, a new source of cyclic AMP (cAMP) was identified, the soluble AC (sAC). However, the physiological function of sAC in the heart is unknown. The cardiac $Na^+/HCO_3^-$ cotransporter (NBC) promotes the cellular co-influx of $HCO_3^-$ and $Na^+$. Since sAC is a $HCO_3^-$-dependent enzyme, we aimed to investigate the potential impact of the relationship between the activity of NBC and sAC on cAMP-dependent cardiac contractility. Rat ventricular myocytes were loaded with Fura-2 or Fluo-3 in order to measure $Ca^{2+}$ transient amplitude (CaT) by epifluorescence or $Ca^{2+}$ sparks frequency (CaSF) by confocal microscopy, respectively. Sarcomere shortening as contractility index was measured simultaneously with epifluorescence. The NBC blocker S0859 (10μM) induced a negative inotropic effect (NIE) in the presence of $HCO_3^-$ (Control: 19.1 ± 3.2% vs. S0859: 14.6 ± 2.6%; n=9, P<0.05) which was associated with a decrease of 18.5 ± 2.6% in CaT. S0859 failed to induce a NIE in the absence of $HCO_3^-$. The selective inhibitor of sAC, KH7 (1μM) decreased contractility (Control: 15.7 ± 0.7% vs. KH7: 11.3 ± 0.9%, n=5, P<0.05) and CaT (15.7 ± 4.9%) only in $HCO_3^-$. KH7 also prevented the NIE of S0859 (KH7+S0859: 11.1 ± 0.9%, n=5). Since cAMP activates the kinase PKA, which in turn increases $Ca^{2+}$ release through sarcoplasmic reticulum RyR channels, CaSF was measured as an index of RyR open probability. The increase in CaSF observed when field stimulation frequency was increased from 0.5 to 3 Hz (Control variation ratio: 1.23 ± 0.1) was reversed in the presence of S0859 (0.62 ± 0.2, n=5, P<0.05) only when $HCO_3^-$ was present in the extracellular medium. In summary, these results demonstrate for the first time that the complex NBC-sAC plays a relevant role in $Ca^{2+}$ management and basal cardiac contractility.

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**DMSO Protects against Stress-Induced Sealing of Cardiac T-Tubules**

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Cardiac t-tubules are critical for efficient excitation-contraction coupling and undergo significant remodeling during various experimental and clinical stress conditions including heart failure. Recently, we have shown that recovery of ventricular myocytes after a brief hyposmotic shock is associated with significant sealing of t-tubules. Dimethyl Sulfoxide (DMSO) is a commonly used solvent and has been reported to have cardioprotective properties although the mechanisms underlying these protective effects remain largely unknown. Therefore, we tested whether the cardioprotective effects of DMSO are mediated by its action on t-tubular remodeling. We found that application of DMSO at the time of resolution of hyposmotic stress, when t-tubule sealing occurs, reduced the amount of fluorescent dextran trapped within sealed t-tubules. The effect of DMSO displayed sharp biphasic concentration dependence with 1% being the most effective dose (~4-fold reduction of dextran trapping; $p<0.01$) while 10% DMSO was ineffective in preventing t-tubule sealing. The data were corroborated by measuring $I_{K1\ tail}$ current which reflects the amount of ttubular membrane. $I_{K1\ tail}$ was recorded in cardiomyocytes that were previously detubulated using the hyposmotic stress protocol. Consistent with reduced dextran trapping in the presence of 1% DMSO, normalized $I_{K1\ tail}$ was significantly larger in cardiomyocytes that had undergone detubulation with 1% DMSO (19.8 ± 2.4 % vs 7.9 ± 2.4 %, $p<0.01$). Image analysis of cardiomyocyte dimensions during the osmotic stress protocol did not reveal any significant differences in cells treated with 1% DMSO upon resolution of hyposmotic stress. In particular, cell dimensions returned fully to pre-stress values in both control and DMSO groups. The data suggest that DMSO prevents t-tubule remodeling independent of its osmotic effects.

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**Dual Role of Functionally Intact Dyadic Junctions in Cardiac Excitation-Contraction Coupling**

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Cardiac excitation-contraction (EC) coupling relies on $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) enabled by an intimate relationship between L-type (CaV1.2) channels and ryanodine receptors (RyRs) at dyadic junctions. How CaV1.2 channels target in proximity to RyRs at dyads is unknown, but likely involves protein interactions mediated by one or more intracellular loops of CaV1.2 pore-forming α1C subunit. We hypothesized that over-expressing α1C intracellular loops that play a critical role in CaV1.2/RyR functional co-localization would disrupt CICR in cardiomyocytes. We over-expressed fluorescent-protein-tagged α1C intracellular loops and termini (NT, I-II, II-III, III-IV, CT) in adult rat cardiomyocytes and assessed their impact on field-stimulation-evoked rhod-2 reported $Ca^{2+}$ transients and other determinants of CICR (ICaL, t-tubule structure, SR $Ca^{2+}$ content, spontaneous $Ca^{2+}$ sparks). Over-expressed I-II and CT uniquely disrupted EC coupling as characterized by two distinct signatures: a sharp augmentation in CICR failure, and an increased propensity for arrhythmic $Ca^{2+}$ transients. Surprisingly, both I-II and CT paradoxically induced a substantial rise in frequency of spontaneous $Ca^{2+}$ sparks. The effects of I-II on CICR and $Ca^{2+}$ sparks were phenocopied by over-expressing the 18-residue AID peptide responsible for auxiliary CaVβ binding to the I-II loop in α1C. Over-expressing a mutated AID (YWI/AAA) that no longer binds CaVβ produced normal CICR and spontaneous $Ca^{2+}$ sparks. The effects of CT on CICR and spontaneous $Ca^{2+}$ sparks were phenocopied by the distal-CT that is unique to α1C, and contains binding sites for several signaling and scaffold proteins. Altogether, the results shed new light on molecular determinants important for CaV1.2 functional targeting in cardiomyocytes, and suggest a new unconventional role for functionally intact dyads– as being necessary to quell spontaneous openings of RyRs.

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**Super-Resolution Analysis of the Distribution of RyR, $Ca_v1.2$ and NCX within the Mammalian Couplon**

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We have developed a dynamically stable 3D dSTORM system, modified to give highly accurate blink positioning deep within a thick sample, to investigate the distribution and localization of the ryanodine receptor (RyR), the L-type calcium channel ($Ca_v1.2$) and the sodium-calcium exchanger (NCX) both on the surface and in the interior of the rat cardiomyocyte. The images have a resolution of 10nm in X and Y and 40 nm in Z, and cover areas of up to 1000 $\mu m^2$ in XY with depths of up to 700nm in Z.

We have been able to identify the position of individual RyR tetramers and have confirmed the recently published tomographic finding that in the resting state most RyR clusters are neither homogenous nor well-ordered. In addition, we found that surface RyR clusters are smaller both in extent and membership than those in the cell interior.

$Ca_v1.2$ was commonly found in small spherical clusters of between 30-100 nm in diameter and were smaller and far denser than their RyR counterparts, with their centers appearing to be tightly packed. $Ca_v1.2$ clusters on the surface of the cell seemed little different from those in the interior, although the surface density was much higher. Super-clusters, made of 3 or more individual clusters were observed throughout the cell.

NCX was more widely distributed and than either RyR or $Ca_v1.2$ and often formed a dense carpet on the cell surface made up of many small spherical