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Opposite Changes of Ca^{2+} Wave Threshold and Fractional SR Ca^{2+} Release during SERCA Stimulation in Cardiomyocytes
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In cardiac muscle, PKA-dependent phosphorylation of of the RyRs is proposed to increase their Ca^{2+} sensitivity. This mechanism could be arrhythmogenic via facilitation of spontaneous Ca^{2+} waves. Surprisingly, the level of Ca^{2+} inside the SR ($[Ca^{2+}]_{SR}$) needed to initiate such waves has been reported to increase upon β-adrenergic stimulation, an observation which cannot be easily reconciled with elevated Ca^{2+} sensitivity of the RyRs. We tested the hypothesis that this change of Ca^{2+} wave threshold could occur indirectly, subsequent to SERCA disinhibition. Ca^{2+} currents and transients, or cytosolic and intra-SR Ca^{2+} waves were simultaneously recorded with confocal line-scans in intact and permeabilized mouse cardiomyocytes with rhod-2 and fluo-5-N, respectively. We analyzed changes of several Ca^{2+} signaling parameters during specific SERCA stimulation by ochratoxin A (OTA) and jasmone. SERCA stimulation resulted in a substantial increase of Ca^{2+} wave thresholds (30 ± 5.1%) and reduced fractional Ca^{2+} release. Faster Ca^{2+} wave decay and SR refilling confirmed SERCA acceleration. In patch-clamped myocytes, a decrease of fractional Ca^{2+} release together with a slowing of Ca^{2+} current inactivation and reduced EC-coupling gain was observed. A faster Ca^{2+} transient decay corroborated the pharmacological SERCA stimulation. These results suggest that SERCA stimulation alone can elevate the intra-SR threshold for the generation of Ca^{2+} waves, independently of RyR phosphorylation. Unexpectedly, this occurs without noticeably increasing spontaneous and triggered fractional Ca^{2+} release. This phenomenon could result from an intra-SR mechanism limiting CICR. Supported by SNF.

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Elevated Homocysteine Levels Result in Carbonyl Formation on RyR2 and Enhanced Sensitivity of Sarcoplasmic Reticulum to Activation by Calcium
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Elevated levels in blood serum of homocysteine (>10μmol/L) is strongly correlated with the incidence of heart failure (HF) in humans. We demonstrate that the cyclic thioester, homocysteine thiolactone (HTL), a metabolic product of homocysteine, at a concentration of 100 nM, results in the formation of carbonyls on the ryanodine receptor from sheep cardiac muscle (RyR2), and an enhancement of Ca^{2+} dependent activation of ryanodine binding from 129.7 ± 3.4 nM to 102.0 ± 2.7 nM. While both improper Ca^{2+} handling and elevated homocysteine levels have been considered bio-markers in HF, a direct connection between the two has not previously been made. We propose that HTL reacts with lysine residues on RyR2, generating an Nε-homocysteine-protein, which results in carbonyl formation and an enhancement in the Ca^{2+} sensitivity of RyR2. This is a new molecular mechanism linking elevated levels of Homocysteine, post-translational modification of RyR2, improper Ca^{2+} handling and heart failure. This work was supported by NIH 1 R41 HL105063-01 to J. Abramson and R. Strongin.

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Phosphodiesterase Inhibition Leads to Activation of Epac and Stimulation of Ca^{2+} Release from both the Golgi Apparatus and the SR
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In adult rat ventricular myocytes, local Ca^{2+}-release from the Golgi apparatus (GA) was stimulated by phosphodiesterase-3 (milrinone, 30 μM) or −4 (rolipram, 30 μM) inhibition, while treatment with a high level of isoproterenol alone (1 μM) had comparatively little effect. PDE 3 or 4 inhibition also increased the frequency of SR Ca^{2+} sparks. With rolipram, this occurred without a significant increase in SR Ca^{2+} content suggesting that the increase in spark frequency was primarily mediated by a direct effect on RyR2. The selective Epac activator 8-CPT (30 μM) also stimulated GA Ca^{2+} release events and increased Ca^{2+} spark frequency without increasing the SR Ca^{2+} content. A pull-down assay was used to measure the level of active (GTP-bound) Rap1, providing an index of Epac activation. Basal Rap1 activation was consistently detectable in ventricular myocytes and was significantly ($p<0.05$) reduced (50.2 ± 14%, n=4) following selective inhibition of Epac2 with ESI-05 (25 μM) and increased by treatment with 8-CPT (33.6 ± 12.6 %, n=5) or rolipram (51.2 ± 15.2 % , n=3). These data suggest that the increase in local [cAMP] in response to PDE inhibition can induce activation of Epac, which may in turn stimulate Ca^{2+} release from the GA and the SR. The relative ineffectiveness of ISO at stimulating GA Ca^{2+} release is consistent with compartmentalization of cAMP signalling, which is reduced following inhibition of PDE3 or 4. The financial support from the British heart Foundation and the Wellcome Trust is acknowledged

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Cardiac Alternans Occurs through the Synergy of Voltage- and Calcium-Dependent Mechanisms
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Cardiac alternans is characterized by alternating weak and strong beats of the heart. This signaling at the cellular level may appear as alternating long and short action potentials (APs) that occur in synchrony with alternating large and small calcium transients, respectively. Previous studies have suggested that alternans manifests itself through either a voltage dependent mechanism based upon action potential restitution or as a calcium dependent mechanism based on refractoriness of calcium release. We use a novel model of cardiac excitation-contraction (EC) coupling in the rat ventricular myocyte that includes 20,000 calcium release units (CRU) each with 49 ryanodine receptors and 7 L-type calcium channels that are all stochastically-gated. The model suggests that at the cellular level in the case of alternans produced by rapid pacing, the mechanism requires a synergy of voltage- and calcium-dependent mechanisms. The rapid pacing reduces AP duration and magnitude reducing the number of L-type calcium channels activating individual CRUs during each AP and thus increases the population of CRUs that can be recruited stochastically. Elevated myoplasmic and sarcoplasmic reticulum (SR) calcium, $[Ca^{2+}]_i$ and $[Ca^{2+}]_{SR}$ respectively, increases ryanodine receptor open probability (Po) according to our model used in this simulation and this increased the probability of activating additional CRUs. A CRU that opens in one beat is less likely to open the subsequent beat due to refractoriness caused by incomplete refilling of the junctional sarcoplasmic reticulum (jSR). Furthermore, the model includes estimates of changes in Na^+ fluxes and $[Na^+]_i$ and thus provides insight into how changes in electrical activity, $[Na^+]_i$ and sodium-calcium exchanger activity can modulate alternans. The model thus tracks critical elements that can account for rate-dependent changes in $[Na^+]_i$ and $[Ca^{2+}]_i$ and how they contribute to the generation of Ca^{2+} signaling alternans in heart.

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Development of Ca Alternans in Atrial Myocytes is Modulated by Action Potential Morphology
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Cardiac alternans is defined as cyclic, beat-to-beat variations in contraction force, action potential duration (APD) and cytosolic Ca transient (CaT) amplitude at constant stimulation rate.
The bidirectional coupling of beat-to-beat regulation of cytosolic $[Ca]_i$ and membrane voltage (V_m) is a key causative factor for the development of alternans. The majority of previous studies have emphasized the role of $[Ca]_i \rightarrow V_m$ coupling and disturbances in $[Ca]_i$ regulation as the primary cause of APD alternans. In this study we investigated how alternation in AP morphology feeds back on intracellular Ca handling ($V_m \rightarrow [Ca]_i$ coupling) in single rabbit atrial myocytes. From AP recordings during pacing induced simultaneous CaT and APD alternans two AP waveforms were selected that represent a typical short and long AP of APD alternans. These AP waveforms were used as command voltages in subsequent voltage clamp experiments (AP voltage clamp). At pacing frequencies below alternans induction, using a series of short or long APs voltage clamp pulses, APD affected CaT amplitude where short APs caused slightly smaller amplitude CaTs. At higher frequencies CaT alternans could be induced with AP voltage clamp protocols in the absence of APD alternans, however the pacing frequency threshold where CaT alternans could be induced decreased with increasing APD, i.e. a long APD was capable of triggering CaT alternans at lower frequencies than a short AP. When cells were stimulated with an APD alternans protocol (APs of alternating duration as command voltage) the CaT alternans threshold was significantly lower. The data identify a contribution of $V_m \rightarrow [Ca]_i$ coupling to the development and severity of CaT alternans and demonstrate that $V_m \rightarrow [Ca]_i$ coupling affects Ca handling through changes in diastolic SR Ca load and voltage-dependent regulation of L-type Ca channels and Na/Ca exchange.