modelers have already linked the mechanical and the electrical activity in their formulations and showed how those activities feedback on each other. Feed forward modeling is a step towards reproducing the neurological control and thus, to have a complete description of the heart performance, particularly when changes in frequency are involved. The presumptive sigmoidal dependence of parameters on pacing frequency used here can be refined but is a valid starting point to implement the convergence of cardiac chronotrophy and inotropy.

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TRPC1 and TRPC4 Channels Contribute to Basal Cardiac Calcium Signalling via a Constitutively Active Background Calcium Entry

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Transient receptor potential (TRP) channels have been assigned to a wide array of important physiological functions but in cardiac myocytes TRPC channels are almost exclusively associated with diseases. Using TRPC1/C4 double KO or TRPC1 and TRPC4 single KO mice we investigated a putative physiological role of TRPC1/C4 channels in cardiac myocytes. We have used high-speed confocal microscopy, video-imaging and electrophysiology of single ventricular myocytes to investigate local as well as global calcium handling, contractility and electrical properties of the cells. For TRPC1/C4 dKO mice we found decreased global calcium transients, with both amplitude (20% reduction) and basal, diastolic calcium concentration (around 15%) affected. Cellular contractility was reduced by more than 35%. L-type calcium current density was constant but the calcium content of the sarcoplasmic reticulum (SR) displayed a 20% reduction. Calcium sparks showed an almost 20% reduction in amplitude while other spatiotemporal parameters were unchanged. Both Na/Ca exchanger and SR-calcium pump activity were unchanged. In Mn-quench experiments we found an almost 50% reduction of Mn entry in unstimulated conditions when comparing cells from TRPC1/C4 dKO and wt mice. Using myocytes from TRPC1 or TRPC4 single KO mice we observed a reduction of global calcium handling and SR-calcium content for both genotypes. From these data we concluded that both, TRPC1 and TRPC4 channels, play an important role for basal cardiac calcium handling.

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Relationship Between Ca^{2+} Alternans and T-Wave Alternans: Role of Calsequestrin and Sorcin

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Several pathologies are associated with defects in Ca^{2+} handling; One example is T-wave alternans (TW-Alt). TW-Alt is observed as alternating beat-to-beat changes in the T-wave of the electrocardiogram (ECG) and constitutes an important arrhythmogenic mechanism. The likelihood of TW-Alt increases with tachycardia and is thought to be associated with abnormalities in intracellular Ca^{2+} handling. Thus, the regulation of the Ryanodine Receptor (RyR_2) could be essential in understanding the genesis of Ca^{2+} alternans (Ca-Alt) and its relationship with TW-Alt. To modify Ca^{2+} signaling we knocked out two Ca^{2+} regulatory proteins: Sorcin and Calsequestrin (Csq2). Ca^{2+} transients were measured from the epicardial layer of murine hearts (n=48) using Pulsed Local Field Fluorescence Microscopy. In addition, simultaneous intracellular action potentials (AP) and ECGs were obtained. Ablation of Csq2 or Sorcin alone did not induce any significant changes in the time course of Ca^{2+} transients or APs. Interestingly, when both proteins were knocked out (Csq/Sorcin KO) a significant change in the time to peak of the transients were observed (WT 16.1 ± 2.4 vs. Csq/Sorcin KO 24.5 ± 1.65). The prolongation of the release can be explained by the modification of the RyR2 gating by Csq2 and Sorcin. The restitution of Ca^{2+} transients was not modified in Sorcin KO, however Csq KO or Csq/Sorcin KO displayed dramatic changes in the time course of the restitution. Finally, maximum Ca-Alt at 32 °C was significantly shifted to higher heart rates (from 11.5 Hz to 15 Hz and 14 Hz, respectively) in Csq2 KO and Csq/Sorcin KO but not in Sorcin KO. AP repolarization alternans and TW-Alt were also modified in Csq2 KO and Csq/Sorcin KO. We conclude that Ca^{2+}-binding proteins can regulate the RyR_2 synergistically, modifying bath the frequency dependency of Ca-Alt and electrical alternans as well.

2812-Pos Board B582

Local Control Model Illustrates How Action Potential Morphology Affects Ca^{2+} Release

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The local control theory of excitation-contraction (EC) coupling states that regulation of calcium (Ca^{2+}) release occurs at the nanodomain level, where openings of single L-type Ca^{2+} channels (LCCs) trigger openings of small clusters of ryanodine receptors (RyRs) co-localized within the dyad. This control scheme leads to the EC coupling properties of voltage-dependent gain and graded release. We have formulated a deterministic ''coupled LCC-RyR model'' that captures these properties. The model presented here combines our local control model with a prior model of guinea pig ventricular myocyte electrophysiology, metabolism, and isometric force production. It reconstructs many features of Ca^{2+}-induced Ca^{2+}-release, but the strongest prediction concerns the relationship between action potential (AP) shape and Ca^{2+} release timing. In species expressing the transient outward current (I_{to}), APs exhibit a ''notch'' and Ca^{2+} transients peak soon after the AP upstroke. However guinea pig lacks I_{to}, and its Ca^{2+} transients have been shown to peak much later, aligned with the middle of the plateau phase of the AP. Here the late peak of the Ca^{2+} transient arises from low initial EC coupling gain at the peak of the AP near +50mV. Gain increases as the AP repolarizes during the plateau. Addition of I_{to} and thus the AP notch leads to increased gain early in the AP, resulting in Ca^{2+} transients that peak much earlier. The Ca^{2+} transient time course controls that of contraction. In larger mammalian hearts, expression levels of I_{to} vary with transmural depth. Our model predicts these expression differences will have a major impact on the temporal waveform of Ca^{2+} transients, and therefore timing of contraction. To achieve the most complete understanding of contraction at the whole-heart level, our results indicate that simulations should incorporate both local control and regional expression variability.

2813-Pos Board B583

Adenosine A_{2A} Receptor Activation Decreases Beat-To-Beat Stability of Intracellular Calcium Transients and their Propagation in Atrial Myocytes

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Adenosine A_{2A} receptor ($A_{2A}R$) activation promotes spontaneous calcium release from the sarcoplasmic reticulum (SR), which can potentially destabilize the beat-to-beat response. To test if $A_{2A}R$ activation alters beat-to-beat stability, patch-clamp technique was used in isolated human atrial myocytes to measure beat-to-beat changes in L-type calcium current and the tail current elicited upon repolarization. Calcium imaging was used to measure the calcium transient and its propagation in multicellular atrial HL-1 myocyte preparations. The stimulation frequency was increased stepwise (from 0.2 to 2Hz) and beat-to-beat responses were determined at each frequency, and were classified as uniform alternating or irregular. In human atrial myocytes, 200 nM of the $A_{2A}R$ agonist CGS21680 decreased the fraction of uniform responses at 1 Hz (from 23/36 to 15/36) and reduced the maximal frequency where a uniform response could be maintained (from 1.11 ± 0.10 to 0.80 ± 0.08 Hz, $p<0.05$). The frequency dependent reduction of uniform responses in the presence of CGS21680 was due to the concurrent increase in fraction of irregular responses (from 1/36 to 7/36 at 1 Hz and from 13/36 to 26/36 at 2 Hz). In cultured atrial HL-1 myocytes, CGS21680 also decreased the number of uniform responses from 50/80 to 35/80. Moreover, CGS21680 destabilized the propagation of the calcium transient. Overall, a uniform propagation of 38/80 calcium transients was observed in control conditions and only 22/80 transients showed uniform propagation after exposure to CGS21680. We conclude that stimulation of adenosine A_{2A} receptors promotes the induction of irregular beat-to-beat responses at lower stimulation frequencies and favors a non-uniform propagation of the calcium transient, which may contribute to the generation of atrial arrhythmia.

2814-Pos Board B584

Acute Chemotherapeutic Treatment Induces Chronic Phosphorylation of the Cardiac Ryanodine Receptor

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Doxorubicin is a powerful chemotherapeutic agent used to treat breast cancer. Doxorubicin's use is limited due to the development of cardiotoxic side effects