Truncation of the Mobile Domain of Cardiac Troponin I Results in Biphasic Calcium-Dependent Thin Filament Activation

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We focused on the computational modeling of the effects in hiPSC-CMs of the LQT1 syndrome, which affects the slow delayed rectifying potassium current (IKs). To this aim, we fitted our recently published hiPSC-CM model on the IKs data in control and LQT1 hiPSC-CM published by Moretti et al.(2010).
Our results show that the LQT1-induced IKs impairment, quantified as an IKs maximum conductance (GKs) reduction of 75%, caused a marked prolongation (+28%) of the action potential (AP). Experiments and simulations suggest that IKs plays a more important role in the AP repolarization in hiPSC-CMs than in adult cardiomyocytes. In fact, the same GKs reduction simulated in the O'Hara-Rudy (ORd) model of adult human cardiomyocyte resulted in a prolongation of the AP of 4% only. We ascribe the longer hiPSC-CM AP prolongation to a smaller, or not fully developed, repolarization reserve. To test further this hypothesis, we simulated the same GKs reduction also in a hybrid model, built by replacing into the hiPSC-CM model the adult ORd IKr and IK1. Also this simulation resulted in an AP prolongation of 4%.
Our results suggest that the adult IKr and IK1 can compensate the effect of the IKs impairment and furthermore, that if hiPSC-CMs have fully developed IK1 and IKr, they would respond similarly to adult CMs. Finally, in spite of the hiPSC-CM potential as disease in-vitro models, they still show differences from adult cardiomyocytes: computational models can help in comparing data from these two classes of cells.

3656-Pos Board B384
How "Funny" is the Cardiac Pacemaking? a Quantitative Analysis Based on Dynamic Clamp Recordings
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A typical feature of sinoatrial (SA) node pacemaker cells is the presence of an ionic current that activates upon hyperpolarization. The quantitative role of this hyperpolarization-activated current known as the 'funny current', If, in the spontaneous pacemaker activity of SA nodal cells remains a matter of intense debate.
We aimed to characterize the impact of If as implemented in recent conflicting mathematical models of rabbit SA node pacemaker cells by using the Dynamic Clamp technique.
Experiments were performed using the Real-Time eXperiment Interface (RTXI) which is a real-time biological experimentation system based on Real-Time Linux. The time course of If was dynamically reconstructed from the experimentally recorded action potentials of a single isolated rabbit SA nodal pacemaker cell according to the mathematical models of Maltsev and Lakatta(2009) and Severi et al.(2012). Two different protocols were implemented: i) selective If block by Ivabradine (3μM) and substitution with the "syntethic" If ii) comparison of the effects of a virtual Isoprenaline(ISO)-induced shift (7.5 mV) of If activation with the real effects of 1μM ISO.
In relation to the substitution of the Ivabradine-sensitive current, the model of Severi et al. (Sev) allowed to restore the control pacemaking rate (Ctrl [Hz]: 2.77 ± 0.13 Vs Sev: 2.70 ± 0.09, N.S.), whereas Maltsev and Lakatta's (ML) induced only a smaller recovery from current-blocking conditions (Control [Hz]: 2.77 ± 0.13 vs ML: 2.39 ± 0.10, $p<0.05$). Preliminary results on the virtual ISO-induced shift produced a rate increase comparable to the real drug effect ($+42 \pm 9$ % vs $+58 \pm 15$ %, N.S.), suggesting that If accounts for most of the ISO-induced rate increase.
Dynamic Clamp results are in agreement with the quantitative description of the If impact on the modulation of cardiac pacemaking provided by our recent computational sinoatrial model (Severi et al. 2012).

3657-Pos Board B385
A Mechanistic Analysis of Variability Between Ventricular Cardiomyocytes
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Variability between individuals and samples is an important yet poorly studied phenomenon. One recently developed theoretical approach to the study of variability is the use of "population-based" models, where a large number of related models are studied instead of a single model. In particular, parameter sensitivity analysis can be performed by generating a large population of related models with varied parameters (such as different levels of ion channels), running simulations of those models to calculate physiological outputs (such as action potential duration, APD, and Ca^{2+} transient amplitude, $\Delta[Ca^{2+}]_i$), then performing regression to relate the outputs to the parameters. This produces a matrix of sensitivity coefficients, each of which represents a quantitative and testable prediction of how changes in each parameter will affect each output. We have applied this method to a model of the rat ventricular myocyte that contains separate formulations for epicardial and endocardial cells. The analysis implicated the L-type Ca^{2+} current density (G_{Ca}) as an important determinant of $\Delta[Ca^{2+}]_i$ and APD in both cell types, with a reduction in G_{Ca} causing decreased APD and $\Delta[Ca^{2+}]_i$. A reduction in transient outward K^+ current density (G_{to}) causes an increase in APD in both cell types. Surprisingly, reduction of G_{to} was predicted to increase $\Delta[Ca^{2+}]_i$ in epicardial but not in endocardial cells. Experimental studies in progress confirm the surprising prediction that changes in G_{to} can indeed influence Ca^{2+} transients in rat ventricular myocytes. This work demonstrates how population-based modeling can generate counterintuitive and testable predictions, and also illustrates a quantitative framework to mechanistically relate measured differences in the levels of currents to behavioral variability between isolated cells.

Muscle Regulation

3658-Pos Board B386
Dcm Mutations in Tropomyosin Induce Loss of Thermal Stability When the Mutation is Present in a Single Chain of the Tm Dimer
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Individuals carrying cardiomyopathy mutations normally have the mutation in one of the two genes and therefore both wt and mutant proteins will be present in the cell. In the case of dimeric protein then homo and heterodimers are likely to exist side by side. We have made α-tropomyosin (Tm) in which one or both chains of the dimer carry a single point mutation associated with dilated cardiomyopathy (DCM). The mutations are E40K, E54K and D230N. Protein unfolding was monitored for a 7 μM solution of Tm (0.5 M KCl, 5 mM $MgCl_2$, 1 mM DTT, 20 mM KPi buffer pH 7.0) using the CD signal at 222 nm with a constant heating rate of 1 °C min^{-1}. The unfolding profile was fully reversible and repeated unfolding curves were superimposable. Each Tm unfolded in via 3 unfolding transitions; those for wt Tm occurred at 40, 47 & 53 °C. The presence of a mutation in a single chain caused a loss of thermal stability with significant unfolding at 37 °C. The least stable transition occurred with a mid-point at 3-5 degrees lower temperature for each of the three Tms carrying a single mutation. The other two transitions were similar to wt for all three constructs. When both chains carried the mutation, the all three transitions were similar to those of the wt Tm. Supported by the Wellcome Trust and a University of Kent Studentship.

3659-Pos Board B387
Disease Causing Troponin C Mutations Have Varied Effects on Actin Regulatory States
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Cardiomyopathy associated mutations in troponin often affect the actin-activated ATPase activity of myosin and the response of force and ATPase activity to Ca^{2+}. Three hypertrophic cardiomyopathy TnC mutants A8V, C84Y, and D145E were examined for their effects on the distribution of states of regulated actin in vitro. In the absence of activating Ca^{2+}, regulated actin filaments containing the C84Y mutant produced a 2.5x elevation in ATPase activity of skeletal myosin subfragment 1 compared to wild type; the other mutants had normal activity. We examined acrylodan-tropomyosin fluorescence changes that occur following rapid detachment of S1 from regulated actin in the absence of Ca^{2+}. All troponin mutants gave a fluorescence increase indicative of a normal transition into the inactive state. The A8V mutant differed from wild type and other TnC mutants at saturating Ca^{2+}. Actin filaments containing the A8V TnC mutant produced an ATPase rate in Ca^{2+} that was 1.3x wild type. For comparison, the Δ14TnT mutant that destabilizes the inactive state was 1.7x wild type. Interestingly, the effects of the A8V-TnC and Δ14-TnT mutants were additive leading to a Ca^{2+}-activated rate 2.3x wild type. The A8V TnC mutant appeared to favor the active state relative to wild type but only in the presence of Ca^{2+}. This behavior differed from the Δ14 mutation of TnT where the distribution was also altered at low Ca^{2+} conditions.

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Truncation of the Mobile Domain of Cardiac Troponin I Results in Biphasic Calcium-Dependent Thin Filament Activation
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Cardiac troponin I (cTnI) contains a ~40 residue C-terminal mobile domain (cTnI-Md, residues 168-211 in *Rattus norvegicus*) that is known to promote

the blocked state of thin filament regulation. Because it has also been shown to stabilize the closed-state position of tropomyosin, we hypothesized that cTnI-Md is involved in the cooperativity of thin filament activation. To test this hypothesis, we generated the truncation mutant cTnI(1-167) from *R.norvegicus* wherein the entire cTnI-Md had been removed. We used passive exchange to incorporate cTnI(1-167) and FRET labeled cTnC(T13C/N51C)$_{AEDENS-DDPM}$ into left ventricular detergent skinned myocardial fibers. SDS-PAGE and Western blotting demonstrated that cTnC(T13C/N51C)$_{AEDENS-DDPM}$ and cTnI(1-167) were efficiently exchanged into skinned fibers. Intriguingly, incorporation of cTnI(1-167) resulted in biphasic Ca^{2+} dependent thin filament activation as indicated by the tightly coupled force−Ca^{2+} and N-cTnC-opening-Ca^{2+} relationships. Simultaneous force and FRET measurements showed that treatment with 1 mM orthovanadate inhibited force, reduced ensemble-averaged N-cTnC opening, and decreased the Ca^{2+}-sensitivity of activation, but did not affect the cooperativity underlying the biphasic response of N-cTnC opening to increasing Ca^{2+}. Akaike information criteria indicated that a weighted sum of two Hill equations was $>10^9$-fold superior in describing biphasic activation than a single Hill equation. Interestingly, a steady-state cooperativity model based on the concept of tropomyosin being "pinned" by cTnI (J. Mol. Biol., vol. 340, pp. 295-305) was 41-fold superior to the weighted sum of two Hill equations and suggested that blocked-state allosteric communication is severely disrupted by removal of cTnI-Md. We concluded that cTnI-Md helps facilitate allosteric communication between thin filament regulatory units in the blocked state and is therefore essential to achieving a proper contractile response to sarcomeric Ca^{2+} signals during early systole.

3661-Pos Board B389

Phosphorylation of Cardiac Troponin I at Tyrosine 26 Decreases Thin Filament Calcium Sensitivity

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The troponin complex is a critical molecular switch involved in transducing the calcium activating signal into contraction. Troponin I (TnI), the inhibitory subunit of the complex, is phosphorylated as a key regulatory mechanism to alter the calcium regulation of contraction. Altered cardiac contraction is a hallmark of heart failure with several studies demonstrating increased myofibrillar calcium sensitivity. Recent work has identified a novel phosphorylation of TnI at Tyr-26 that is decreased in heart failure with unknown functional effects. Similar to the location of the desensitizing TnI Ser-23/24 phosphorylation, TnI Tyr-26 is located in the unique cardiac TnI N-terminal extension. These data lead us to hypothesize that the N-terminal Tyr-26 phosphorylation of TnI decreases calcium sensitivity of the thin filament, the loss of which may contribute to the altered calcium sensitivity observed in heart failure. To assess the regulatory effects of Tyr-26 phosphorylation, we employed recombinant human cardiac TnI containing phosphate at Tyr-26 induced by treatment with a tyrosine kinase and TnI Tyr-26 phosphomimetic substitutions (Glu or Asp). The effect of TnI Tyr-26 phosphorylation on myofilament calcium sensitivity was assessed by measuring calcium binding to troponin C (TnC) in reconstituted thin filaments. Results demonstrate both Tyr-26 phosphorylation and phosphomimetics decrease calcium binding to TnC compared to filaments reconstituted with non-phosphorylated TnI. To further investigate the effects of TnI Tyr-26 phosphorylation on myofilament deactivation we measured the rate of calcium disassociation from TnC. Results demonstrate filaments containing either Tyr-26 phosphorylated TnI or phosphomimetics increase the rate of calcium dissociation from TnC. Our findings suggest that TnI Tyr-26 phosphorylation functions similarly to Ser-23/24 N-terminal phosphorylation to decrease myofilament calcium sensitivity and increase myofilament relaxation. The loss of TnI Tyr-26 phosphorylation may therefore contribute to altered cardiac contraction in heart failure.

3662-Pos Board B390

Effect of Amino Acid Changes in a Troponin I FHC Hotspot on Protein: Protein Binding and Calcium Sensitivity of Force Development

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Mutations in human cardiac troponin I (cTnI) have been associated with restrictive, dilated and hypertrophic cardiomyopathies. The most commonly occurring residue on cTnI that is associated with familial hypertrophic cardiomyopathy (FHC) is arginine, which is also the most common residue at which multiple mutations occur. Two FHC mutations are known to occur at arginine 204, R204C and R204H, and both are associated with poor clinical prognosis. To determine the effect of these mutations (R204C and R204H), as well as other cTnI mutations, R204P, R204Q, and R204W, calcium-force measurements and cTnI:troponin C (TnC) and cTnI:troponin T (TnT) interactions using the mammalian two-hybrid luciferase assays were utilized. All five mutations showed significant increases in calcium sensitivity of force development ranging from ΔpCa50 0.23 (R204W) to 0.35 (R204P). The mutations associated with FHC, R204C and R204H, had ΔpCa50 values of 0.28 and 0.29 respectively. The cTnI containing the R204P mutation showed the weakest interaction with TnT when compared to wild-type cTnI or the other mutants. The R204H mutation also showed significant impairment in its ability to interact with TnT, while the R204C mutation showed mild impairment when compared to wild-type cTnI. The R204C and R204P mutations showed the greatest impairment in binding to TnC. These results suggest that mutations at the same site on cTnI could affect thin filament interactions differentially, and that significant impairment in the interaction of cTnI with TnT or TnC may be enough to cause significant changes in calcium sensitivity. If the large increase in calcium sensitivity of force development observed with these mutations is associated with the poor prognosis then other R204 mutations are likely to have a poor prognosis. This research was supported by a Hellman Fellowship.

3663-Pos Board B391

In Vivo Analysis of Troponin C Knock-In (A8V) Mice: Evidence that TNNC1 is a Hypertrophic Cardiomyopathy Susceptibility Gene

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Recently, the *TNNC1* gene that encodes cardiac troponin C (cTnC) was found as a target for many hypertrophic cardiomyopathy (HCM) mutations in humans, eliciting alterations in the Ca^{2+} binding properties of the N-domain of cTnC. We genetically engineered knock-in mice containing the HCM-associated A8V mutation in cTnC (heterozygote = KI-TnC-A8V$^{+/-}$; homozygote = KI-TnC-A8V$^{+/+}$) in order to characterize its *in vivo*, molecular and cellular effects. ECHO revealed that at 3 months old (mo) KI-TnC-A8V$^{+/+}$ mice display increased IVRT and E/A ~1 compared to WT, suggesting diastolic dysfunction; whereas KI-TnC-A8V$^{+/-}$ showed signs of cardiac restriction at 14 mo. Histopathology of both genotyped hearts revealed papillary muscle hypertrophy, interstitial fibrosis, and myofibrilar disarray. Real-time PCR analysis at 3 mo demonstrated increases in BNP, α-MHC and β-MHC mRNA levels in the right ventricles of the KI-TnC-A8V mice (only ANP increased in the left ventricle). We identified in intact KI-TnC-A8V$^{+/-}$ and KI-TnC-A8V$^{+/+}$ cardiomyocytes: a significant decrease in the sarcomere length at several stimulation frequencies; prolonged Ca^{2+} and contractile transient kinetics at 4Hz; uncoupling between Ca^{2+} decay (delayed) and contractile (no change) transients at 6Hz; suggesting a mechanical frequency-dependent uncoupling from the Ca^{2+} transient. Furthermore, a decrease in the baseline Ca^{2+} fluorescence and in Ca^{2+} peak percentage was also detected in KI-TnC-A8V$^{+/-}$ and KI-TnC-A8V$^{+/+}$, indicating increased myofilament Ca^{2+} buffering. The calcium sensitivity of contraction in skinned fibers increased in a gene dose fashion: KI-TnC-A8V$^{+/+}$ > KI-TnC-A8V$^{+/-}$ > WT. The rate of relaxation in KI-TnC-A8V$^{+/+}$ cardiac skinned fibers investigated by flash photolysis was found increased, compared to WT. These results suggest that the A8V mutation in cTnC increases Ca^{2+} binding affinity to its N-domain eliciting changes in intracellular Ca^{2+} homeostasis and cellular mechanical function, ultimately leading to diastolic dysfunction.

3664-Pos Board B392

In Vitro and in Situ Structure and Function of the Cardiac Troponin C Familial Hypertrophic Cardiomyopathy-Linked Mutation, L29Q

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Familial Hypertrophic Cardiomyopathy (FHC) is characterized by severe abnormal cardiac muscle growth. The traditional view is that mutations associated with FHC result in an increase in Ca^{2+}-sensitivity of cardiac muscle contraction; however, recent studies indicate that their pathogenesis may stem from a diminished response to troponin I phosphorylation. The mutation L29Q, found in the Ca^{2+}-sensitive muscle regulatory protein, troponin C, has been tenuously linked to cardiac hypertrophy. L29Q is in the regulatory domain