acids 1-843) was expressed with C-terminal Avi and FLAG tags in C2C12 cells. The ATPase activity ($k_{cat} = 8.1 \pm 1.7\ s^{-1}$ and $K_{ATPase} = 58.3 \pm 17.7\ \mu M$) and in vitro motility ($V_{avg} = 2060 \pm 132$ nm/s) of purified wild-type M2β-S1, containing endogenous murine skeletal muscle light chains, was similar to our previous results. In complementary structural work, F-actin-tropomyosin filaments were saturated with the human cardiac S1 in the presence and absence of ADP and then cryogenically preserved. Cryo-EM images of the complexes, collected at the Midwest Consortium for High Resolution Cryo-EM, yielded 3.3-3.4 Å resolution reconstructions, with even higher resolution at the actin-myosin interface, thus increasing the interaction details from what had been previously achieved. Beyond visualization of the ADP and a corresponding ~9° change in lever-arm orientation in the nucleotide-containing complex, only modest structural distinctions were noted between the ADP-saturated and ADP-free myosin at the actin interface. Both complexes show that the CM loop and HLH motif of myosin make extensive, well-known contacts with the actin surface while interaction between myosin loop 2 and actin subdomain 1 is partially resolved. Moreover, in both cases, myosin-head loop 4 penetrates between actin and tropomyosin to interact with charged residues of the tropomyosin coiled coil and thus is likely to modulate tropomyosin position on thin filaments.

515-Pos

Cardiac myosin mechanochemistry underlies left ventricular dysfunction in human ischemic heart failure

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Prolonged occlusion of the coronary arteries can lead to ischemic heart failure (IHF). Molecular changes within the myocardium during IHF are not fully understood. Accordingly, we aimed to characterize the biophysical properties of myocardial contractile proteins in IHF. Ten age-matched human males with similar BMI were included in the study. Cardiac biopsies of left ventricular free wall were taken from patients with ischemic heart failure (IHF, n=5) at the time of heart transplant or from organ donors with no previous history of ischemic heart failure (controls, n=5). Clinical measures prior to retrieval of cardiac samples demonstrated significant differences in controls vs. IHF in measures of ejection fraction and left ventricular internal diameter (LVIDd and LVIDs). Myofibrils and myosin were extracted from left ventricular free wall cardiac samples. Myofibrillar ATPase activity was depressed significantly in the IHF group across all calcium concentrations pCa9 - pCa3 with no change in calcium sensitivity. Isolated myosin was utilized in unloaded *in vitro* motility assays, in which the IHF group propelled actin significantly slower relative to the controls. Mass spectrometry analysis of the extracted myosin demonstrated a similar myosin heavy chain isoform distribution between groups. Synthetic thick filaments (STFs) were formed by decreasing ionic strength of the myosin-containing buffer. Myosin ATPase in the STFs was similar in the absence of actin but depressed in IHF at 20μM actin. Collectively, our data indicates that myosin dysfunction underlies the decreased myofibrillar ATPase previously reported in IHF, suggesting that targeting the myosin motor may be an effective pharmacologic intervention in patients with ischemic heart failure. Future studies in our lab will aim to characterize specific myosin post-translational modifications in heart failure as well as the effect of pharmacologic agents in recovering depressed contractile function.

516-Pos

The synthetic nanomachine powered by bovine cardiac myosin demostrates that force and power of the atrial myosin isoform are less then those of the ventricular isoform

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The functional differences of the α- and β-cardiac myosin that make up the atrial and ventricular chambers of the human heart have been described in detail with solution kinetic analysis, showing that α-cardiac myosin has faster ATP hydrolysis and faster ADP release so that the ATPase cycle time is ten times shorter, but the duty ratio (*r*) is similar to that of β-cardiac myosin. Instead, the isoform-dependence of mechanical performances remains elusive as the force of the β-cardiac myosin was found either twice higher (attributed to a larger *r*) with *in vitro* mechanics or similar in Ca^{2+}-activated skinned myocytes. Here, we use our synthetic nanomachine (Pertici et al., *Nat Commun* 9:3532, 2018) to determine the performance of an array of ~15 heavy-meromyosin fragments (HMM) purified from the bovine atria and ventricle pulling on the actin filament in either isometric or isotonic condition. We find that α-HMM has an isometric force (6.6 ± 0.6 pN) ~3-fold lower than the β-HMM (23.7 ± 3.9 pN), and an unloaded shortening-velocity ~3-fold higher (1.4 ± 0.2 and $0.5 \pm 0.1\ \mu m\ s^{-1}$). These mechanical parameters underpin a maximum power, an essential parameter for cardiac function, twofold lower for α- (0.7 ± 0.1 aW) compared to β-HMM (1.4 ± 0.1 aW). This quantitative description of the performances of cardiac myosin isoforms opens up the potential of the nanomachine for characterizing cardiomyopathy-causing myosin mutations and drugs currently developed for different therapeutic indications.

517-Pos

Conduction differences between atrial and ventricular working myocardium elucidated through previously unrecognized nanoscale structural organization

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The intercalated disc (ID) is a complex, heterogeneous structure that affords electrical (gap junctions; GJ) and mechanical (adherence junctions [AJ], desmosomes [Des]) coupling between cardiomyocytes. Electrogenic proteins underlying the action potential upstroke (cardiac sodium channels [$Na_V1.5$], inward-rectifier potassium channels [$K_{ir}2.1$] and sodium potassium ATPase [NKA]) enriched within ID nanodomains are emerging as vital machinery for cardiac impulse propagation. ID structure is thus a critical determinant of cardiac conduction. We used indirect correlative light and electron microscopy (*iCLEM*) to assess ID ultrastructure (transmission electron microscopy; TEM) and molecular organization (confocal, STORM superresolution microscopy) in mouse atria and ventricles. TEM uncovered structural differences from the micro- through nano-scales including key factors that may underlie faster atrial conduction: larger interplicate regions and more numerous GJs with associated perinexi. Confocal microscopy revealed ID enrichment of $Na_V1.5$, $K_{ir}2.1$ and NKA with more intense immunosignals in atrial myocytes than ventricular. STORM defined the $Na_V1.5$, $K_{ir}2.1$ and NKA distribution relative to AJ, Des, and GJ: In the ventricle, $Na_V1.5$ associated most closely with GJ (median intercluster distance: 117 nm), $K_{ir}2.1$ with Des (151 nm), and NKA with both GJ (165 nm) and AJ (150 nm). Next, percent of each electrogenic protein localized within 100 nm from ID junctions: 35% of $Na_V1.5$ around GJs, 49% of $K_{ir}2.1$ around Des and 33% and 39% of NKA near GJ and AJ respectively. Protein organization within atria ID had some notable differences: $Na_V1.5$, $K_{ir}2.1$ and NKA was shifted closer to GJs, $Na_V1.5$ to Des, and $K_{ir}2.1$ and NKA to Ncad. These data provide the first ever comprehensive quantitative picture of ID ultrastructure and molecular organization. Functional implications of these nanoscale structural differences will be elucidated by implementation into our recently published 3D finite-element computational model.

518-Pos

Mavacamten depresses human atrial contractility in the same EC50% range as human ventricle

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In obstructive HCM patients, recent phase III placebo-controlled randomized EXPLORERHCM clinical trial demonstrated the efficacy and safety of Mavacamten in reducing left ventricular (LV) outflow tract gradient and ameliorating exercise capacity (Olivotto et al. 2021). Both effects are attributable to a local reduction of LV septal contractility. Although very promising, this class of small molecules can globally depress cardiac muscle contractility with potential detrimental consequences on the overall cardiac output. Here we characterized the effect of Mavacamten on the contractile properties of human atrial myocardium (from surgical samples), expressing different levels of α and β myosin isoforms. In intact trabeculae Mavacamten (0.5-5.0 μM) reduced the amplitude of atrial twitches in a dose dependent manner with a variable effect on twitch duration, ranging from no effect in atrial myocardium expressing >95% α myosin to a mild acceleration of peak and relaxation times in atrial myocardium with a 50:50% α:β myosin ratio. In human atrial myofibrils expressing >95% α myosin (15°C), Mavacamten had a fast, fully reversible, and dose-dependent negative effect on Ca^{2+}-activated isometric force, with an EC50% close to that of human ventricular myofibrils expressing 100% β-myosin. Importantly, at variance with what reported in ventricular myofibrils (Scellini et al 2021), Mavacamten strongly depressed the kinetics of force generation of human α-myosin atrial myofibrils similarly to what had been observed in fast skeletal muscle myofibrils. In summary, Mavacamten reduced atrial twitch amplitude with variable impact on twitch

kinetics, likely related to different α:β myosin ratios and to the different impact of the drug on the fast and slow myosin isoforms. As in obstructive HCM ventricular filling strongly relies on atrial contraction, depressing atrial contractility with Mavacamten could detrimentally reduce ventricular systolic function.

519-Pos

Cardiac myosin velocity and force are dramatically improved with an alternative triphosphate substrate

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There is strong interest in enhancing cardiac myosin function in order to identify new potential treatment modalities for heart failure. However, it has been difficult to identify substrates that dramatically enhance the function of cardiac myosin in vitro. Therefore, we assessed the impact of isomers of a novel artificial energy substrate, azobenzene triphosphate, on cardiac myosin function by measuring filament velocity and via single molecule measurement. With the ortho positional isomer of AzoTP cardiac myosin moved actin filaments 62% faster than with ATP ($p<0.001$) using the in vitro motility assay. Subsequent experiments measuring filament velocity vs. substrate concentration suggested that the increase was largely due to an accelerated rate of release of the diphosphate form of the substrate. Consistent with this idea, single molecule measurements using a three-bead laser trap assay demonstrated a reduction in the duration actomyosin bound lifetime with AzoTP compared to. ATP. Myosin also generated 10% more force with AzoTP vs. ATP, using a mini-ensemble of myosin in the three-bead laser trap assay. This was accompanied by a doubling of the frequency of actomyosin binding events, suggesting that AzoTP accelerates myosin's rate of attachment to actin. The meta and para isomers of AzoTP demonstrated reduced function compared to ATP, suggesting myosin is sensitive to the position of the triphosphate on the central benzene ring of the substrate. The enhanced function also appears to be specific for cardiac myosin as the same substrate decreased velocity and force by over 30% in fast skeletal muscle myosin as seen in previous work. Thus these findings demonstrate that the in vitro function of cardiac myosin can be dramatically enhanced by using an abiotic triphosphate substrate.

520-Pos

Deoxy-ATP-mediated improvement of cardiomyocyte energetics and metabolism accompany enhanced contractility

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This study investigates how elevated myosin activator- dATP affects cardiac metabolism. Transgenic mice (Tg) with overexpression of RNRB enzyme (RNR isoform that protects against ubiquitin ligases after cell division) and elevated dATP/ATP (~1%) results in increased cardiac function on multiple biophysical scales and is accompanied by improved cardiomyocyte energetics and metabolism. Isolated cardiomyocytes (CMs) from Tg mice (N=5) had improved basal oxygen consumption rate (OCR) (~2 folds), maximal OCR (~1.8 folds), and spare respiratory capacity (~1.4 folds). Complementary Metabolomic (LC/MS) analysis demonstrated an increase in TCA metabolites and fatty acid oxidation, indicating increased oxidative phosphorylation (OXPHOS) compared to Wt CMs. No morphometric differences in mitochondria were observed (N=3), but an elevated function of Complexes I and II was detected in Tg CMs (N=2), resulting from the augmented OXPHOS. Interestingly, acute treatment with Mavacamten (a selective myosin motor inhibitor) restored basal OCR (N=3) without affecting the maximal OCR in Tg CMs. This suggests that increased basal cellular energy demands may be driven by the destabilization of myosin interacting heads motif (IHM) with dATP, which is recompensed with improved OXPHOS flux. Thus, a small increase in dATP positively affects cellular energetics, possibly via myosin activation. We observed a very similar effect of dATP elevation in CRISPR-Cas 9 edited human induced pluripotent stem cell-derived cardiomyocytes (predominant beta myosin content) overexpressing RNR. Our results suggest that long-term dATP elevation, as a positive regulator of the cardiomyocyte metabolism, may benefit as a therapeutic approach against depressed contractility and energy deficit in the failing heart.

521-Pos

Effects of mavacamten and sarcomere length on the Ca^{2+}-sensitivity of permeabilized myocardial strips from patients with heart failure

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Heart failure can reflect impaired myofilament-level contraction. In healthy hearts, myofilaments become more sensitive to Ca^{2+} as cells are stretched. This fundamental property of myocardium is termed length-dependent activation and contributes to the Frank-Starling response. Mavacamten is a drug that binds to myosin. Mavacamten is under investigation as a potential therapy for heart disease. To investigate how mavacamten affects molecular-level contraction in failing human myocardium, we analyzed the contractile properties of permeabilized myocardial strips from the left-ventricular free wall of hearts that were explanted during a transplant at the University of Kentucky. All patients gave informed consent (IRB 46103) and had ischemic (that is a prior myocardial infarction, n=6) or non-ischemic heart failure (n=6). Ca^{2+}-activated isometric force was measured in the presence or absence of 0.5 μM mavacamten at 1.9 or 2.3 μm sarcomere length (37°C). In the absence of mavacamten, calcium-sensitivity of contraction (defined as the pCa_{50} value) increased by 0.06 pCa units ($p=0.001$) as ischemic strips were stretched from 1.9 to 2.3 μm. Non-ischemic strips did not show length-dependent activation under these conditions ($\Delta pCa_{50}=0.02$, n.s.) indicating a potential loss of length-dependent activation. Adding 0.5 μM mavacamten reduced maximal force and calcium-sensitivity for both types of failing tissue. Ischemic myocardium continued to exhibit length-dependent activation ($\Delta pCa_{50}=0.04$, $p=0.004$). In non-ischemic tissue, mavacamten increased ΔpCa_{50} to 0.05 ($p=0.006$). Together these data suggest that (1) length-dependent activation is reduced in non-ischemic heart failure, and (2) can be rescued with mavacamten. We conclude that different myofilament-targeting strategies may be required to optimize treatment for patients with ischemic and non-ischemic heart failure.

522-Pos

Thick and thin filament effectors of cardiac myofibril contraction detected in a troponin c-based high-throughput fluorescence lifetime assay

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Cardiac muscle contraction is initiated by Ca^{2+}-induced structural changes of the thin filaments to permit cross-bridge formation. Ca^{2+} binding to cardiac troponin C (TnC) on actin permits myosin binding and ATP binding to myosin allows for cross-bridge cycling during contraction. Site-directed spectroscopic probes attached to TnC at position T53C have been useful to determine structure-function relationships and Ca^{2+} binding kinetics in thin filaments and myofibrils by monitoring fluorescence intensity. This approach would also be useful to screen for drugs that modulate TnC structure in myofibrils to elicit therapeutic functional changes for treating contractile dysfunction in cardiac disease. However, fluorescence intensity measurements in myofibrils exchanged with TnC probes are inherently prone to signal imprecision due to variable pipetting, nonhomogeneous troponin labeling/exchange, and variable sizes of myofibril preparations. In contrast, time-resolved fluorescence (TR-F, i.e., fluorescence lifetime) is largely insensitive to changes in intensity and exhibits ~20-fold improvement in assay precision. Therefore, we have developed a lifetime-based assay using fluorescently-labeled TnC exchanged into porcine cardiac myofibrils to determine the sensitivity and precision of detecting structural changes in TnC due to physiological and therapeutic effectors of thick and thin filament function. First, we confirmed that lifetime was indeed superior to intensity measurements. We then determined the effects of Ca^{2+} to bind TnC and activate the thin filament and ATP to bind myosin and activate the thick filament in mock high-throughput screens. These screening assays were determined to be of excellent quality as indicated by the Z' factor. Finally, we evaluated effects of myosin binding drugs, mavacamten and omecamtiv mecarbil, used to treat cardiac diseases. We conclude that TnC lifetime-based probes allow for precise high-throughput evaluation of thick and thin filament effectors in functioning myofibrils.

523-Pos

The mechanism of cardiac thin filament inhibition: the precision of W7

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The calmodulin antagonist W7 is a direct sarcomere inhibitor that has therapeutic potential for the treatment of hypertrophic and restrictive cardiomyopathy. W7 decreases the calcium sensitivity of force development in cardiac muscle by binding to troponin, the sarcomere thin filament protein that regulates heart muscle contraction. W7 binds to the interface of the regulatory N-terminal domain of cardiac troponin C (cNTnC) and the switch region of troponin I (cTnI), and decreases the binding of cTnI to cNTnC presumably by electrostatic repulsion between W7 and cTnI. We used a series of W7 derivatives to evaluate the importance of precise location of the charged NH_3^+ group of W7 in tuning the cNTnC-cTnI interface. The compounds are W4, W6, W8 and W9, which have three less, one less, one more and two more methylene groups on the tail region than W7, respectively. We constructed a cNTnC-cTnI switch region chimera (cChimera) for small