mutations affect the rate of nucleotide exchange from the nucleotide-binding pocket and show altered ATP-binding and ADP release rates. Homology models of myosin S1, either *D45* or *Mhc5*, suggest a possible mechanism by which the single point mutation can alter the kinetic properties of the myosin head.

2562-Pos Board B532

Comparison Of Mechanical Properties Of Single Intact Fibres From Wild-type And Mlc/migf-1 Mouse Muscle

Barbara Colombini[1], Giulia Benelli[2], Marta Nocella[2], Antonio Musarò[3], Giovanni Cecchi[1], Maria Angela Bagni[1].
[1]Interuniversity Institute of Myology, Dept. Scienze Fisiologiche, University of Florence, Firenze, Italy, [2]University of Florence, Firenze, Italy, [3]Interuniversity Institute of Myology, Dept. Istologia ed Embriologia Medica, University of Rome ''La Sapienza'', Rome, Italy.

In this study we compared the mechanical properties of single intact muscle fibres of wild-type (WT) and MLC/mIgf-1 (TG) mice, in which the localized Igf-1 transgene expression sustains hypertrophy (Musarò et al., *Nat. Genet.* 27, 2001). The study has been focussed on ''static stiffness'' (SS), a non cross-bridge calcium-dependent stiffness previously identified in activated frog muscle fibres (Bagni et al., *Biophys. J.* 82, 2002).

Single intact fibres, dissected from the flexor digitorum brevis muscle, were mounted in an experimental chamber (~23°C) between the lever arms of a force transducer and of an electromagnetic motor to apply fast stretches. Sarcomere length was measured by means of a videocamera and with laser diffraction. Tetanic tension and force-velocity relation in WT and TG mice were not significantly different, however, the maximum shortening velocity (Vmax) was faster than previously reported and comparable with frog muscle. Compared to frog fibres, the plateau of length-tension relation shifted according to the different myofilament lengths. TG fibres exhibited an increase in diameter and maximum force, but specific force was the same as for WT fibres. SS was present either in WT or in TG fibres and its time course, independent from isometric tension, was faster than in frog.

A preliminary analysis suggests that the only significant mechanical difference between WT and TG fibres is in the SS properties. This may be related to a different compliance of the structure responsible of the SS that we speculated could be titin.

2563-Pos Board B533

Reactive Oxygen Species Alter Activation Of Cardiac Myofilaments And Modify Sarcomeric Proteins

Benjamin S. Avner, R. John Solaro.
University of Illinois at Chicago, Chicago, IL, USA.

The generation of reactive oxygen species [ROS] such as hydrogen peroxide [H_2O_2] is elevated in acute and chronic cardiac pathophysiology. Post-translational oxidative modification of sarcomeric proteins important to cardiac function, such as actin and tropomyosin [Tm], represents a possible mechanism by which ROS may induce changes in cardiac function. Here we present data that test the hypothesis that ROS modify the function of ventricular muscle through oxidation of sarcomeric components. We directly exposed cardiac muscle to oxidation by treatment of detergent-extracted myofibril bundles from murine papillary muscles with 2.5 mM H_2O_2. From the same hearts, we prepared homogenates of the ventricular sarcomeric proteins; each sample was divided and processed with and without exposure to H_2O_2. We compared the oxidation state of the proteins, employing electrophoresis to analyze the formation of reduction-sensitive disulfides by oxidized cysteine residues. Compared to untreated fiber bundles, those treated with H_2O_2 showed significantly blunted cooperative activation in response to strong actin-myosin cross-bridge binding, as measured by addition of N-ethylmaleimide modified myosin sub-fragment 1 [NEM-S1]. Cross-bridge dependent effects are important for full activation of the cardiac thin filament and are believed to control the kinetics of ejection and relaxation. Results from ''diagonal'' gels (SDS-PAGE run successively under non-reducing and reducing conditions) revealed reduction-sensitive products which were more abundant in peroxide-treated compared to untreated tissue samples. Western immuno-blot analysis confirmed that these products contained actin and Tm. Overall, our findings represent evidence for the ROS-induced oxidation of myofibrillar proteins along with impairment in cardiac muscle function. Investigation of whether the endogenously generated ROS observed in pathological settings have similar effects *in vivo* will aid in assessing the significance of these modifications, and may suggest a therapeutic target.

2564-Pos Board B534

Effects Of Blebbistatin And BDM (2,3-Butanedione Monoxime) On The Short-range Mechanical Properties Of Murine Diaphragm Muscle Fibers

Mihail I. Mitov, Kenneth S. Campbell.
Department of Physiology, University of Kentucky, Lexington, KY, USA.

Blebbistatin (BLEB) and 2,3-butanedione monoxime (BDM) are well-know inhibitors of myofilament force production and useful tools in structural and functional studies of cell motility and muscle contraction. In this study, we investigated the effects of BLEB and BDM on the short-range mechanical properties of single chemically permeabilized murine diaphragm fibers. BLEB and BDM were used in separate sets of experiments to reduce isometric force in saturating Ca2+ solution to approximately 50% of the control value. Muscle fibers were subjected to repeated triangular length changes (paired ramp stretches/releases, 0.04 l_0, 0.33 l_0 s^{-1}) imposed under fiber length control in solutions with free Ca^{2+} concentrations ranging from pCa 9.0 to pCa 4.5. Short-range stiffness values were calculated from the slopes of regression lines fitted to the first 15 ms of XY plots of force against muscle length for each stretch response and expressed as Young's Moduli. Analysis of results obtained in control Ca2+ solutions (without BLEB or BDM) showed that short-range stiffness increased proportionately with the level of isometric force. Experiments performed with BLEB showed that short-range stiffness declined in proportion with the reduction in isometric force (no change in the stiffness/force ratio). In contrast, BDM produced a disproportionately large decrease in isometric force (that is, the stiffness/force ratio increased in the presence of BDM, ANACOVA test, $p<0.001$). These results support the hypothesis that BLEB and BDM reduce isometric force in skeletal muscles by different mechanisms. BLEB seems to prevent myosin heads from attaching to the thin filament while BDM probably reduces force by decreasing the rate at which myosin heads undergo tension-generating biochemical transitions.

Cardiac Muscle I

2565-Pos Board B535

Stretching Cardiac Trabeculae Increases the Force by Decreasing the Cross-bridge Weakening Rate in a Velocity Dependent Manner

Moran Yadid, **Amir Landesberg**.
Technion - Israel Institute of Technology, Haifa, Israel.

Background: Stretch increases the force and decreases energy consumption in skeletal muscle. However, the underlying mechanisms and the effects of stretching cardiac muscle remain elusive. We hypothesized that stretch increases the force by modulating the cross-bridge (XB) cycling rates. **Methods:** Trabeculae (n=*6*) were isolated from rat right ventricles. Sarcomere length was measured by laser diffraction and controlled by a fast servomotor. The number of strong XBs (N_{XB}) was quantified by measuring the dynamic stiffness. Ramp stretches (n=42) at different velocities and onset times were imposed on sarcomere isometric twitches. Normalized stress (stiffness) enhancement, σ_E (K_E), was defined as the increase in the stress (stiffness) during stretch normalized by the instantaneous isometric stress (stiffness). **Results:** Stretches yielded identical increases in σ_E and K_E, implying that the stretch increases force by increasing N_{XB}. A unique linear relationship was observed between the instantaneous normalized stress and stiffness, for all the stretch velocities (1.03 ± 0.078, $R^2=0.99 \pm 0.026$), suggesting that the unitary force per XB is constant for all stretch velocities (in contrast, a velocity dependent decrease in the force per XB was obtained during sarcomere shortening, in congruent with previous publication). The rate of σ_E development depended linearly on the stretch velocity (7.35 ± 1.07 [1/μm]). Interestingly, the rate of σ_E development was independent of the stretch onset time, indicating that it is not dominated by changes in XB recruitment, but is an inherent property of the strong XB, since the population of available XB varies during the twitch. **Conclusions:** Constant force per XB, independence on the recruitment rate, and the linear dependence of σ_E on the stretch velocity, strongly suggest that stretch decreases the rate of XB turnover from strong to weak conformation in a velocity dependent manner.

2566-Pos Board B536

Microfabricated Post Array Detectors to assess cardiomyocyte forces induced on their environment via focal adhesions

Anthony G. Rodriguez, Sangyoon Han, Michael Regnier, Nathan Sniadecki.
U. of Washington, Seattle, WA, USA.

There is vast potential in regenerative medicine to improve cardiac muscular dysfunction, but difficulties arise in functionally integrating implanted cells

into host tissue. To address functional integration, the physiological interactions between muscle cells and their microenvironment need to be further elucidated. We are using microfabricated post array detectors (mPADs) as novel functional assays to assess contractile forces transferred through focal adhesions to the extracellular environment from cardiomyocytes. mPADs utilize an array of microscale posts that deflect as cantilever springs in response to forces applied by cells cultured upon them. Contractile forces of neonatal rat cardiomyocytes (NRCs) are calculated by analyzing the deflection of the posts and multiplying by their spring constant. The measured forces are compared to vascular smooth muscle cells (VSMCs), cells with similarly disorganized contractile apparatus, and adult rat cardiomyocytes (ARCs). Using immunofluorescence of vinculin, both NRCs and VSMCs demonstrate the ability to create focal adhesions to the fibronectin coatings on the tips of the posts. Preliminary results indicate that VSMC produced a total force of 570 ± 175 nN/cell which is almost 6-fold greater than NRCs. However, NRCs produce 50% more force per unit area than VSMC. Furthermore, spontaneous beating of NRCs yielded an additional maximal contraction of 55.6 nN per post per beat above the baseline which is about two orders of magnitude lower than reported forces produced by ARCs. The use of mPADs provides a tool to further the understanding of the stress and strains created by cardiomyocytes onto their local environments through their focal adhesions. With this new technique to assess muscle adhesion and contractility, we seek to characterize the physiological interactions that implanted muscle cells must recapitulate to advance new therapies for cardiovascular regenerative medicine. HL61683 & UWRRF

2567-Pos Board B537

Integrin Response To Altered Actin-Myosin Mechanochemistry In Cardiac Myocytes

Timothy J. O'Donnell, Michael S. Carter, Mariam A. Ba, Maria L. Valencik, Josh E. Baker.
University of Nevada Reno, Reno, NV, USA.

Altered contractility in cardiac myocytes associated with motor protein mutations has been implicated in several pathologies such as familial hypertrophic (FHC) and dilated (DCM) cardiomyopathies. The mechanism(s) by which these altered contractile forces result in cell phenotype changes are poorly understood. However, they are generally believed to involve mechanotransduction of force by cell adhesion molecules such as the integrin class of proteins. Here we investigate the use of blebbistatin and other small molecule effectors of actin-myosin mechanochemistry as a chemical model for the reduced contractile force associated with the pathogenesis of DCM. In order to establish intercalated-disk cell associations in vitro that are representative of in vivo conditions, we employ micropatterned cardiac myocyte cell culture, where the extracellular matrix proteins collagen and laminin are printed onto the culture vessel surface in 10μm wide lines. Fluorescent polymer microspheres (0.25μm dia.) are embedded in the collagen layer. As the beads move from myocyte contraction, the movement amplitude is measured by nanometer-resolution position analysis, both in the presence of blebbistatin and in the rescue state where blebbistatin is removed. Relative force generation is calculated from the position analysis data and matrix modulus. Cells are then fixed and prepared with fluorescent antibodies for observation of connexin-43 and β1 integrin expression and localization by confocal microscopy. By employing micropatterned cardiac myocyte cell culture, optical contractile force measurement, and subsequent observation of the expression and localization of connexin-43 and β1 integrin, we assess the changes in integrin activity due to blebbistatin-induced reduction in contractile force.

2568-Pos Board B538

A Direct Method to Measure the Restoring Force and Slack Sarcomere Length of Intact Cardiomyocytes

Nicholas M.P. King[1], Michiel Helmes[2], Henk Granzier[1].
[1]University of Arizona, Tucson, AZ, USA, [2]University of Oxford, Oxford, United Kingdom.

Molecular mechanisms underlying diastolic suction are poorly understood. Several proteins have been implicated to play a role, including extra-cellular proteins, titin, and cytoskeletal proteins. An in vitro measurement of diastolic suction at the cell level is restoring force (or stiffness), a force which is difficult to measure as it requires a cell to be passive below the slack sarcomere length (SL). Previous restoring force studies were made utilizing calcium independent shortening of cardiomyocytes to below their slack length and then upon relaxation measuring the force that developed as the cell is stretched back to its slack length. However, these studies used chemically permeablized cells and, thus, eliminated the membrane and soluble intracellular proteins as possible contributors to restoring force. In the current study we developed a novel method to determine restoring force and the slack sarcomere length of mouse cardiomyocytes that were intact. Intact cardiac myocytes that were below their slack length due to a low level of active force development were attached to flexible carbon fibers. We then added butanedione monoxime (BDM) to inhibit actomyosin interactions and abolish active tension. This led to an increase in the sarcomere length and a negative force. From this new baseline force (-1.34 ± 0.34 mN/mm^2 (mean ± SE)), we stretched the cells across the physiological range from ~1.8-2.2 μm. We determined the sarcomere length at which force is zero (slack SL) to be 1.93 ± 0.019 μm. Plotting the stress-SL relationship we then determined the restoring stiffness from the slope of this plot as 16.1 mN/mm^2/μm. Thus we successfully measured the restoring force - SL relation of intact cardiac myocytes.

2569-Pos Board B539

Titin Isoform Transitions and Passive Stiffness During Skeletal Muscle Development

Coen Ottenheijm, Anna Knottnerus, Danielle Buck, Tiffany Pecor, Xiuju Luo, Henk Granzier.
University of Arizona, Tucson, AZ, USA.

During postnatal striated muscle development, multiple changes in active and passive properties occur, reflecting an altered mechanical demand. For instance, during postnatal cardiac development titin isoform expression switches from large isoforms to small stiffer isoforms, likely affecting diastolic filling behavior. In the present study, we investigated whether titin isoform transitions also take place during *skeletal* muscle development. We used gel-electrophoresis to determine changes in titin isoform size in mice and rabbits of various ages. A titin exon microarray was used to evaluate transcript expression of all of titin's exons. To investigate the mechanical effect of titin isoform transitions, passive properties of neonatal and adult skeletal muscles were determined.

Neonatal mice were found to express large titin isoforms, which are gradually replaced by smaller isoforms during skeletal muscle development. The half transition time of the isoform for tibialis cranialis (TC), soleus, extensor digitorum longus, gastrocnemius and diaphragm were 6, 17, 17, 12, and 10 days, respectively. Essentially similar findings were obtained from NZW rabbits, with the exception that the half-life of the isoform transitions was slightly longer compared to murine skeletal muscles. Titin exon analysis in neonatal murine gastrocnemius muscle revealed increased expression of a large group of exons when compared to adult muscle transcripts, with all upregulated exons coding for exons of the elastic PEVK region of titin. In line with these observations, we found ~50% lower titin-based passive stiffness of murine neonatal soleus and TC when compared to adult muscle.These data demonstrate that during skeletal muscle development titin isoform transitions occur from large compliant isoforms to smaller and stiffer isoforms in adult muscle, likely due to changed expression of PEVK exons.

2570-Pos Board B540

Vinculin Contributes to the Passive Stiffness of Myocardium

Joyce Chuang, Robert Ross, Andrew McCulloch, Jeffrey Omens.
University of California San Diego, La Jolla, CA, USA.

In cardiomyocytes, the costamere links the Z disc to the surrounding extracellular matrix. Proteins at these junctions include integrins, talin, and vinculin (Vin). Vin is also found in the intercalated discs. Cardiac-specific Vin knockout (VinKO) mice have a sudden death phenotype early in life (<12 weeks of age) with a progressive dilated cardiomyopathy leading to 100% mortality by 32 wks. At 7 weeks of age, systolic ventricular function is normal.

We hypothesize that deletion of the costameric protein Vin leads to changes in passive stiffness prior to the onset of systolic dysfunction. Vin deletion may disrupt the normal force transmission pathways from ECM to cytoskeleton through the integrin-based costamere or cell-to-cell force transmission along the myocyte axis, which may manifest in altered passive material properties of the myocardium.

To test the mechanical properties of myocardial tissue, a system was developed in which murine right ventricular papillary muscles could be passively strained in the axial direction while simultaneously measuring force. Papillary muscles from 7 week old VinKO mice and WT controls were isolated and stretched. Stress-strain analysis was used to measure passive stiffness in the direction of myocardial fibers.

Stress-strain curves were significantly different between WT and VinKO papillary muscles ($p<0.05$). The slope of the VinKO curve was less than in the WT curve, indicating that VinKO muscles are more compliant in the fiber