cytes were fixed five days after transfection and stained for alpha-actinin and Krp1. Immunofluorescence revealed that cardiomyocytes with low levels of Krp1 often lacked mature myofibrils as defined by alpha-actinin localization in Z-lines. Instead, cells contained many dots of alpha-actinin, or Z-bodies. Despite the defect in myofibril accumulation, cells were otherwise healthy as indicated by lack of apoptosis, normal spreading over time in culture, and retention of intact organelles assessed by electron microscopy. To quantitate the change in phenotype, cardiomyocytes were categorized according to the dominant pattern of alpha-actinin organization. Almost all cells transfected with control siRNA were filled with well-aligned myofibrils, and only 5% accumulated dots of alpha-actinin. In contrast, 55% of cardiomyocytes transfected with Krp1 siRNA were filled with Z-bodies or narrow Z-lines that were often periodically spaced in series resembling newly forming myofibrils. Confocal microscopy of cells stained for actin, myosin, and myomesin demonstrated that these structures contained sarcomeric proteins with longitudinal periodicities similar to mature myofibrils, and electron microscopy showed normal thick and thin filaments. However, fibrils remained thin and separated. The data indicate that Krp1 is specifically required for lateral fusion of adjacent fibrils into mature myofibrils but is not necessary for periodic longitudinal organization of actin and myosin filaments.

1455-Pos Endothelin-1 and Phenylephrine Mediated Alterations in CapZ Actin Binding Affinity occur via PIP2 and PKC-Mediated Mechanisms

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Board B431

Cardiac myocytes undergo hypertrophy in response to agonist stimulation via cytoskeletal and sarcomeric remodeling. We hypothesized that reduced affinity of the actin capping protein (CapZ) for actin facilitates the hypertrophic response by enhancing sarcomeric addition and remodeling. Therefore, CapZ dynamics were analyzed by fluorescence recovery after photobleaching (FRAP) in neonatal rat ventricular myocytes (NRVM) treated with endothelin-1 (ET) (100nM, 4h treatment) or phenylephrine (PE) (10 μM, 24h treatment). We introduced GFP fusions of either wild-type or one of two actin binding deficient ß1 mutant (L262R or a C-terminal deletion) CapZ constructs via recombinant adenovirus into NRVM. Exogenous protein localized to the Z-disk, and cell surface area increased after ET treatment in cells infected with each of the 3 CapZ vectors, indicating a responsiveness to hypertrophic stimuli. FRAP analysis 30 min postbleach showed wtCapZ infected cells treated with ET recovered more completely than controls (77±9% vs. 50±6%, $p<0.001$, $n=8$). Similar results were found with PE (77±5%, $p<0.05$, $n=8$). Studies of mutant CapZ recovery demonstrated that L262R recovery was similar to untreated cells (55±7%, $n=12$), whereas the C-terminal deletion mutant was similar to that of agonist treated cells (73±12%, $n=5$). We hypothesized the increased CapZ exchange in ET and PE treated cells was PIP2 mediated. PIP2 sequestration with neomycin (500 μM, 4h pretreatment) blocked both ET (43±6%, $p<0.001$, $n=7$) and PE (36±4%, $p<0.001$, $n=11$) mediated recovery. The PKC inhibitor chelerythrine chloride (10 μM, 4h pretreatment) also blocked ET mediated recovery (53±10%, $p<0.001$, $n=6$). Our results suggest that ET and PE alter CapZ actin affinity through PIP2 dependent pathways; ET-1 alteration of affinity is also PKC dependent; and this alteration is mimicked by actin binding mutants of CapZ.

Cardiac Muscle & Regulatory Proteins - II

1456-Pos RhoA/ROCK Signaling in Substrate Stiffness Control of Neonatal Rat Cardiomyocyte Maturation

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Board B432

Cardiac cells mature and adapt to a changing mechanical environment in the first postnatal week. In the absence of differences in adaptation or maturation, we would expect that cells on stiff substrates, which have nearly isometric contractions, would generate more force than cells on softer substrates due to greater actin-myosin overlap throughout the contraction and kinetic effects. However, we have found that the maturation of neonatal rat cardiomyocytes plated on collagen-coated polyacrylamide gels with elastic moduli from 1 to 50 kPa and cultured for 7 days depends on the stiffness of the gel. Cardiomyocytes generated greater mechanical force, had larger calcium transients and sarcoplasmic reticular (SR) stored calcium and had greater expression of some SR components on gels with an elastic modulus similar to the native myocardium, 10kPa, than on stiffer or softer substrates. We also observed stress fiber formation, with reduced sarcomere formation, in myocytes on very stiff surfaces. We hypothesized that the formation of stress fibers may compete with formation of well-defined, aligned sarcomeres on stiff surfaces and that inhibition of stress fiber formation, through inhibition of the RhoA/ROCK pathway might allow more complete cardiomyocyte maturation. To test this hypothesis, we inhibited several components of this pathway and measured functional parameters such as force development and calcium signaling. We found that inhibition of this pathway results in increasing force with increasing stiffness, as initially predicted for identical cells. We conclude that the activation of the ROCK pathway, leading to the formation of stress fibers, results in poor functional maturation, as measured by low force generation and SR calcium, of neonatal rat cardiomyocytes on very stiff substrates.

1457-Pos A Role For Rho-kinase In Ca^{2+}-independent Contraction Induced By Phorbol 12,13-dibutyrate

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Board B433

Phorbol 12,13-dibutyrate (PDBu) is an activator of protein kinase C, and causes contraction not only in a physiological salt solution but also in a Ca^{2+}-depleted solution. We hypothesized that Rho-kinase plays a role in Ca^{2+}-independent contraction induced by PDBu in vascular smooth muscle.

In Ca^{2+}-free solution substituted with 2mM EGTA, PDBu induced contraction and myosin light chain (MLC_{20}) phosphorylation, the magnitude of which were about 40% of those in a normal Krebs' solution. H1152, an inhibitor of Rho-kinase, but not ML7, an inhibitor of MLCK, inhibited Ca^{2+}-independent contraction induced by PDBu.

In Ca^{2+}-free solution, PDBu increased phosphorylation of MYPT1 and CPI-17, which were inhibited by H1152 as well as Ro31-8220, a PKC inhibitor. In conclusion, Rho-kinase plays an important role in Ca^{2+}-independent contraction induced by PDBu in vascular smooth muscle.

These results suggest that PDBu induced Ca^{2+}-independent contraction by inhibition of MLCP through phosphorylation of MYPT1 and CPI-17.

1458-Pos Cardiac TnI And Phospholamban Are Not Major Targets For NO-mediated Attenuation Of Beta-adrenergic Inotropic Effect

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Board B434

In cardiac muscle, nitric oxide (NO) modulates the β-adrenergic response in normal and pathological conditions. We investigated the role of NO on the regulation of isometric force in response to increasing doses (0.01–1μM) of isoproterenol (ISO) in papillary muscles isolated from mouse hearts that:

1. express normal levels of phospholamban (PLB) and either express cardiac troponin I (PLB/cTnI) or the slow skeletal isoform of TnI (PLB/ssTnI) or
2. do not express PLB and either express cTnI (PLBKO/cTnI) or ssTnI (PLBKO/ssTnI).

All four groups showed a positive inotropic response to ISO, but this response was reduced in PLBKO/ssTnI and increased in PLBKO/cTnI compared to other groups. The NO donor, sperminеNONOate (300μM), was added at the steady state response of force to 1μM ISO. NO induced a small but significant decrease in developed force in PLB/cTnI (10.7±2.2%, n=10), PLB/ssTnI (5.3±1.0%, n=8), PLBKO/cTnI (8.9±3.6%, n=6) and PLBKO/ssTnI (9.5±1.4%, n=10) muscles. NO did not alter the kinetics of contraction or relaxation in any group. The TnI phosphorylation was evaluated using isoeletric-focusing gels. In control conditions the level of TnI phosphorylation was 76.5±4.1% in PLB/cTnI (n=5) and 63.1±6.6% in PLBKO/cTnI (n=4) muscles. In the presence of 1μM of ISO phosphorylation increased to 87.6±4.3% in PLB/cTnI (n=5) and 76.7±4.6% in PLBKO/cTnI (n=5). In the presence of ISO and NO the TnI phosphorylation was 87.1±3.7% (n=5) in PLB/cTnI and 87.8±6.4% (n=5) in PLBKO/cTnI muscles. Our data show that NO attenuates the positive inotropic response to β-adrenergic agonist independent of the presence of PLB or expression of cTnI, suggesting that this effect is not solely due to the phosphorylation of PLB or PKA-dependent cTnI phosphorylation. We propose that other proteins or forms of post-translational modifications are affected by NO in inotropic regulation.

1459-Pos Acute Effects Of Nicotine On Two Transgenic Mouse Models Of Familial Hypertrophic Cardiomyopathy

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Board B435

Familial Hypertrophic Cardiomyopathies (FHCs) are autosomal genetic disorders of the sarcomere that normally result in cardiac hypertrophy, diastolic dysfunction and possibly heart failure. Nicotine's (NIC) deleterious effects on the heart are well-established, yet there is a paucity of information on its effects in FHC patients. Thus, the purpose of this study is to determine if acutely delivered NIC exacerbates cardiac function in two transgenic (TG) mouse models of FHC, α-TM D175N TG and TnT R92Q. *In vivo* pressure recordings from the left ventricle of non-TG (NTG) and TG mice were used to measure indices of cardiac performance in the absence and presence of NIC. In the absence of NIC, both FHC models showed no significant differences for either heart rate or mean left ventricular pressure when comparing NTG and TG mice. The maximal rate of relaxation ($-dP/dt$) was slightly depressed in both FHC models compared to NTGs and the maximal rate of contraction ($+dP/dt$) was slightly elevated in α-TM D175N TGs. Following i.v. injection of NIC (2.5, 5.0 and 10.0 ng/g b.w.*min, which is equivalent to human plasma NIC levels after 1, 2 and 3 cigarettes, respectively), HR and mean left ventricular pressure again showed no significant changes between NTG and both FHC models. However, $-dP/dt$ became increasingly depressed in both FHC models while $+dP/dt$ became increasingly elevated in α-TM D175N TGs only. In conclusion, our data suggest that NIC acutely exacerbates baseline diastolic dysfunction in two forms of FHC and increases contractility in α-TM D175N hearts. One may surmise that more severe forms of FHC or chronic NIC treatment could produce greater alterations in cardiac function that might lead to arrhythmias and even cardiac failure.

1460-Pos Studies of Transgenic Mice Expressing the E361G Mutation in Cardiac Muscle Actin that causes Dilated Cardiomyopathy

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Board B436

We generated transgenic mice expressing the ACTC E361G mutation at 50% of actin in the heart. Transgenic mice exhibited no phenotype in basal conditions. Heart mass, ejection fraction and shortening velocity were the same in E361G and NTG mice (measured by MRI); in isolated cells shortening amplitude, shortening speed and relaxation rate were also unchanged, both in basal conditions and when stimulated at higher frequencies or with added isopreteronol. We isolated actin from mouse hearts. Monomeric E361G actin was unstable but native thin filaments contained 50% mutant actin. Mutant actin, derived from native thin filaments moved 16% slower than NTG actin. In synthetic thin filaments containing human cardiac tropomyosin and rabbit fast skeletal troponin E361G actin had a 1.9-fold reduced Ca2+-senstivity and 8% slower sliding speed at pCa5 as previously found with DCM mutations in troponin T, and C or tropomyosin. In thin filaments containing non-failing human cardiac troponin (TnI 2.1 molsPi/mol, TnT 3.1 molsPi/mol) there was no difference in Ca2+ sensitivity or sliding speed but when the troponin was dephosphorylated by acid phosphatase the Ca2+-sensitivity of NTG actin-containing filaments increased by 3.1x whilst the E361G mutant thin filaments did not change. Thus the main effect of the DCM-causing mutation is that thin filaments maintain a Ca2+-sensitivity characteristic of phosphorylated troponin and do not respond to changes in PKA-dependent troponin I phosphorylation. Thus it appears that the main effect of this mutation is to blunt the inotropic response. Based on these in vitro studies we predict that the mice may have reduced cardiac reserve and would not show a DCM phenotype until they were subjected to chronic stress.

1461-Pos Is Fast Cross-Bridge Detachment a Common Feature to *MYH7* and *MYBP3* FHC-Associated Mutations in Human Cardiac Myofibrils?

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Board B437

The R403Q mutation in the β-myosin heavy chain gene (*MYH7*), responsible for familial hypertrophic cardiomyopathy (FHC), leads to significant acceleration of force activation (k_{ACT} and k_{TR}) and relaxation (slow k_{REL} and fast k_{REL}) kinetics and a diminution of maximal isometric tension (P_0) of isolated human ventricular myofibrils (Belus et al., *Biophys. J.*, 2007(suppl) Plat842, 181a). We suggested that the mutation accelerates the apparent rate with which cross-bridges leave their force generating states. Again, using fast solution switching techniques, we report here on the functional behaviour of left ventricular myofibrils isolated from 3 additional FHC patients undergoing septal myectomy: two carrying mutations in *MYH7* (R694C or R442C) and one carrying the Y340X mutation in the cardiac myosin binding protein C gene (*MYBPC3*). Those carrying *MYH7* mutations (R403Q, R692C, R442C) showed qualitatively similar changes: acceleration of relaxation kinetics (>2 times), decreased P_0, marked (R403Q and R442C) or mild (R692C) acceleration of force activation kinetics, and higher Ca^{2+}-sensitivity (ca. 0.3 pCa unit). In contrast, in the patient carrying the *MYBPC3* mutation force activation kinetics are markedly slowed while P_0 (lower) and relaxation kinetics (ca. 2 times faster) behaved like in the *MYH7* mutations (Ca^{2+}-sensitivity is under investigation). This suggests that faster cross-bridge detachment under isometric conditions and greater energy cost of tension generation may be common features to FHC-associated mutations independently of the exact mutation and sarcomeric protein involved.

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1462-Pos The Restrictive Cardiomyopathy (RCM) Troponin I mutation R145W causes an increase in Ca^{2+} sensitivity of force and maximal Ca^{2+} activated force

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Board B438

An arginine (R) to a tryptophan (W) mutation at position 145 in the highly conserved inhibitory domain of human cardiac troponin I (HcTnI) has been associated with RCM, a disease characterized by diastolic dysfunction with normal left ventricular size. In this study, the functional consequences of the HcTnI R145W mutation in transgenic mice were investigated. Simultaneous measurements of the ATPase and force in transgenic skinned papillary fibers from Tg-R145W mice *versus* control mice showed that there was a ∼17 to ∼19 percent increase in the maximal Ca^{2+} activated force and ATPase activity. The rate of dissociation of force generating cross-bridges (ATPase/ Force) was the same in all groups of fibers. These results suggest that the increase in force and ATPase activity was associated with an increase in the number of force generating cross-bridges attached at all activation levels. There was an increase in the Ca^{2+} sensitivity of force and ATPase. In intact fibers, the mutation caused prolonged force and intracellular [Ca^{2+}] transients, as expected due to the increased Ca^{2+} sensitivity (slower dissociation of Ca^{2+} from CTnc). The resistance to stretch in intact Tg-R145W fibers was much greater than resistance in fibers from the control mice, suggesting incomplete inhibition of cross-bridge cycling.

These results indicate that there would be resistance to ventricular filling during diastole. The data shows that there would be an increase in force during systole which could maintain stroke volume despite the compromised diastolic filling.

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1463-Pos The Troponin T Isoform Shift Characteristic of Hypertrophic Cardiomyopathy is Potentially a Cardio-Protective Mechanism

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Board B439

In the human heart, four cardiac Troponin T (HCTnT) isoforms have been observed that are generated by combinatorial alternative splicing of two exons. In adult heart failure, secondary to hypertrophic cardiomyopathy (HCM), there is a re-expression of the fetal isoform of HCTnT, HCTnT4. It is hypothesized that the presence of this isoform in the adult heart may affect Ca^{2+} sensitivity of myocardial contraction given the regulatory importance of TnT in the thin filament. Herein, the functional consequences of this TnT isoform shift in cardiac muscle are studied using actin-TM-activated myosin ATPase activity and skinned muscle fiber functional assays. Ratios of the adult isoform HCTnT3 and the fetal isoform HCTnT4 as well as HCTn complexes made thereof, were chosen to model the functional consequences of the TnT isoform shift occurring in diseased cardiac muscle. Our findings demonstrate that replacement of endogenous CTnT with decreasing ratios of HCTnT3:HCTnT4 (increased relative HCTnT4 content) resulted in a decreased Ca^{2+} sensitivity of force development and an increased level of ATPase activity. We propose that these effects constitute a cardio-protective mechanism in HCM by improving systolic and diastolic function and countering the increase in Ca^{2+}sensitivity seen with most sarcomeric HCM mutations.

1464-Pos The Physiologic Consequences Of Double Heterozygous Cardiomyopathy Mutations In Thin Filament Regulatory Proteins

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Board B440

Inherited cardiomyopathy represents a genetically complex and clinically diverse group of cardiac muscle diseases that can be classified into three subtypes: hypertrophic (HCM), dilated (DCM), and restrictive (RCM). Within a subtype, diagnosed patients present with a broad spectrum of symptoms ranging from benign to malignant outcomes. Contributing to this complexity is the emergence of HCM patients that are double heterozygotes. Generally these patients have more severe clinical outcomes than single mutant allele patients. Acute adenoviral-mediated dual gene transfer to isolated adult rat cardiac myocytes was used to elucidate the primary physiologic basis of double cardiomyopathy alleles at the cell-molecular level. Co-expression of an HCM mutant A63V in a-tropomyosin (Tm) and RCM mutant R193H cardiac troponin (cTnI) were shown to additively slow myocyte relaxation. R193H cTnI had a dominant effect to reduce resting sarcomere lengths while diastolic Ca2+ remained unchanged in all experimental groups. Dual gene transfer of DCM mutant G159D cardiac troponin C (cTnC) with R193H cTnI produced an intermediate phenotype with partially corrected diastolic dysfunction and diastolic tone characteristic of R193H cTnI alone. Co-expression of two potent Ca2+ sensitizing molecules, R193H cTnI and M47A cTnC, was used to maximize the threshold of Ca2+ sensitivity and relaxation deficits in isolated adult cardiac myocytes. Together, M47A cTnC and R193H cTnI additively slowed myocyte relaxation beyond that of the severe cellular diastolic dysfunction caused by the independent expression of either M47A or R193H. In addition, M47A+R193H myocytes had reduced contractility and heightened diastolic tone, which are phenotypes unique to either M47A cTnC or R193H cTnI alone. This study demonstrates that double mutant thin filament proteins can collude to directly alter myocyte diastolic function highlighting a molecular basis for increased disease severity in double heterozyogous CM patients.

1465-Pos Importance Of Myosin Heavy Chain Isoform Expression As A Determinant Of Contraction Kinetics In Pig Myocardium

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Board B441

The ventricles of large mammals including humans express ~10% α myosin heavy chain (MHC) on a predominately β MHC background. In failing human ventricles this distribution changes to 100% β MHC leading to the hypothesis that small amounts of α MHC on a predominately β MHC background can yield significantly higher rates of rise of force in ventricular myocardium. We tested this hypothesis by determining the fundamental rate constants of cross-bridge attachment (f_{app}) and detachment (g_{app}) in pig myocardial preparations expressing purely (100%) α or β MHC. Right atrial (100% α MHC) and left ventricular (100% β MHC) tissue was used to measure ATPase activity, isometric force, and the apparent rate constant of force redevelopment (k_{tr}). The rate of ATP utilization was 8.5-fold higher in α MHC compared to β MHC, while k_{tr} was ~9-fold faster in α MHC myocardium. In addition, tension cost was ~8-fold greater in α MHC. From these values, we calculated f_{app} to be 10-fold higher in α than β MHC, and g_{app} to be 8-fold higher in α MHC. Mathematical modeling of an isometric twitch predicted that the expression of 10% α MHC increased the rate of pressure development (dF/dt_{max}) by 92% compared to expression of 0% α MHC, while expression of 20% and 30% α

MHC increased dF/dt_{max} by 186% and 280%, respectively. These results suggest that expression of even low-levels of α MHC may have a profound effect on systolic ejection.

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1466-Pos Macroscopic Characteristics Of Spatio-temporal Heterogeneity Of Normal Human Left Ventricular Contraction

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Board B442

Experimental evidence on heterogeneity of regional mechanical activity of left ventricular (LV) wall emerges at present. These data are essential for insights to physiological significance of myocardial heterogeneity, as for validating 3D mathematical models of LV that account for accurate morphology and mechanisms of excitation - contraction coupling.

To characterize heterogeneity of LV regional wall motion we developed a computer program for frame-by-frame analysis of 2D-ultrasound LV images in apical two and four chamber views. The analysis was based on conventional radial and centerline methods for calculation of the segmental areas between superimposed end diastolic LV contour and each of the subsequent contours through the cardiac cycle.

Studying group of healthy people (n=22) we found an "M-shaped" distribution of mean regional ejection fraction (REF, assessed as the segmental area attained to LV end systole) along the LV contour. Minimum REF was observed in the apical region. An opposite «W-shaped» distribution was obtained for the time to peak inward regional motion (TPRM). A negative correlation (p=0.002) between TPRM and REF was found.

Pairwise REF analysis revealed a nearly three-diagonal structure of the matrix composed of significant correlation coefficients, where most of the i-th REF strongly correlated only with neighbouring (i−1)-th and (i+1)-th REF. The REF correlation matrix embodies a new quantitative characteristic of LV mechanical function, reflecting the pattern of spatial coordination of regional motion.

The observed features of the LV motion heterogeneity were effective for distinguishing wall motion patterns in patients with ischemic heart disease (n=52) from those in healthy subjects.

The data we obtained characterize the spatio-temporal heterogeneity of LV contraction and illustrate the fine coordination between activation sequence, duration of active state and local contractile activity of ventricular regions that may significantly change in cardiac pathology.

1467-Pos Transmural Differences in Myosin Heavy Chain Isoform Expression Modulates the Timing of Myocardial Force Generation in Porcine Left Ventricle

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Board B443

Recent studies show that the timing and sequence of mechanical activation varies across the ventricular wall; however, the contributions of myofilament contractile proteins to regional ventricular function are not well understood. To examine transmural differences in mechanical function of porcine ventricles we studied the stretch activation responses of multicellular skinned myocardium isolated from the endocardium and epicardium of the midwall region. We applied a rapid 1% of muscle length stretch to isometrically contracting endocardial and epicardial muscle fibers and found that endocardial fibers exhibited significantly slower overall rates of stretch activation compared with epicardial fibers, specifically, the rate of force decay was 29±4% slower and the rate of delayed force development was 33±5% slower. Rates of force redevelopment (k_{tr}) mirrored stretch activation data, as endocardial fibers exhibited k_{tr} values that were 28±4% slower than epicardial fibers. However, no differences were observed in steady state mechanical properties of endocardial and epicardial fibers as indicated by a lack of difference in minimum resting force, maximum Ca^{2+}-activated force, and Ca^{2+}-sensitivity of force. SDS-PAGE analysis revealed significantly elevated expression of α myosin heavy chain (MHC) isoform in epicardial fibers compared with endocardial fibers (14±3% and 3±2%, respectively, $p<0.05$). Linear regression analysis revealed that the rates of force decay and delayed force development were correlated with MHC isoform expression ($R^2=0.68$ and $R^2=0.74$, respectively, $p<0.05$). No regional differences in the relative abundance or phosphorylation status of other myofilament contractile proteins was detected. These data show that transmural differences in MHC isoform expression contributes to regional differences in the stretch activation response in porcine left ventricle, which presumably modulates the timing of force generation across the ventricular wall to optimize work production during systole.

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1468-Pos Evaluation of Dynamic and Static Mechanical Properties of Guinea Pig Isolated Atrial and Ventricular Cardiomyocytes

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Board B444

The externally homogeneous cardiac pump function requires regionally differing cardiomyocyte properties, including amplitude and dynamics of contraction. For example, developed pressures in atria (A), right ventricle (RV) and left ventricle (LV) differ by approximately an order of magnitude (10^0 vs. 10^1 vs. 10^2 mmHg, respectively). We used the carbon fibre (CF) technique [1] to characterise pre-load effects (stretch up to 30% of slack length, L0) on contractile behaviour of field-stimulated cardiomyocytes, isolated from Guinea pig A, RV, and LV (2 Hz, 37°C). The effects of changes in end-diastolic length (EDL) on active shortening, a cellular expression of the Frank-Starling response, was more pronounced in A cells compared to RV and LV, regardless of whether the pre-load induced increase in force production or in cell shortening ability are concerned. At the same time, passive force rose nearly twice as fast with increased EDL in A cells, compared with both RV and LV ($p<0.05$). The stretch induced gain in the maximal velocities of both shortening and relaxation is also significantly higher in A cells than in LV and RV ($p<0.05$). Also, time to peak of contraction of A cells was significantly lower than in RV or LV cells ($p<0.05$). In addition, A myocytes were significant smaller, both in cross sectional area and resting cell length compared to RV and LV cells ($p<0.05$). In summary, A cardiomyocytes are smaller, stiffer, and show a more pronounced contractile response to changes in EDL. This may be of functional relevance for contractile performance of the relatively thin-walled atrium.

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1469-Pos Spectroscopic, Confocal and 2-Photon Characterisation of Intrinsic Fluorescence in Isolated Heart Cells for Metabolic Studies

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Board B445

Intrinsic fluorescence of cardiomyocytes was examined using a series of approaches. Spectroscopic examination of aggregates of isolated rabbit ventricular myocytes indicated that the major excitation and emission peaks coincided with those of the metabolic coenzymes NADH and FAD. This work indicated that 340nm was optimal for excitation of NADH and 450nm for FAD. Using the 405nm and 490nm wavelengths available on a laser-scanning confocal microscope confirmed fluorescence emission from NAD(P)H and FAD at the single cell level. FCCP (2μM) was used to maximally oxidise NAD(P)H and $FADH_2$ and this was observed as a minimal NAD(P)H and maximal FAD fluorescence. Cyanide (2mM) was used to maximally reduce these metabolites and generated maximal NAD(P)H and minimal FAD fluorescence. From these fluorescence measurements, the mitochondrial redox state of cell aggregates and single cells could be assessed.

Application of this technique on cell aggregates revealed significant changes in NAD(P)H fluorescence, but little change in FAD signal was evident. The average NAD(P)H redox state in cell aggregates containing a mixture of rod-shaped and hyper-contracted cells at room temperature was 0.30±0.06 (n=6). Single rod-shaped myocytes examined with confocal microscopy had an average NAD(P)H redox state of 0.58±0.03 (n=22). FAD fluorescence was clearly modulated using this technique, the average redox state in single cells was 0.17±0.01 (n=22). Similar experiments using 2-photon excitation fluorescence microscopy (exciting at 720 and 750nm) revealed a comparable value for NAD(P)H redox state of 0.57±0.04 (n=20), although FAD fluorescence could not be clearly detected with this method.

In conclusion, the majority of the intrinsic fluorescence of isolated heart cells could be attributed to the metabolic coenzymes NAD(P)H and FAD. Metabolic inhibition enabled modulation of the oxidative status of these enzymes to allow for calculation of relative redox state.

1470-Pos Cardiac Overexpression Of The Creatine Trasnporter Depletes Atp And Adp And Alters Cardiac Function

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Cardiac overexpression of the creatine transporter (CrT) in mice leads to heart failure (Wallis et al, Circulation 2005,*112*:3131–9). To characterize the responsible mechanisms, we studied cardiac function and high energy phosphate compounds in transgenic mice expressing FLAG-tagged human CrT at low (LOW) and high levels (HIGH) relative to control, non-transgenic animals (NON). At 4 weeks of age, left ventricular end-diastolic dimensions were increased in HIGH animals (NON = 3.03 ± 0.1 mm, LOW = 2.93 ± 0.06, HIGH = 4.38 ± 0.1*, n=10). Percent fractional shortening also decreased in HIGH but was not changed in LOW mice (NON = 64% ± 1, LOW = 66 ± 3, HIGH = 21 ± 2*). At this time, HPLC analysis of the heart tissue indicated that HIGH had 13-fold higher creatine levels and twice as much phosphocreatine as NON. Furthermore, both ATP (NON = 56 ± 1 micromoles/L, HIGH = 44 ± 2*, n=5) and ADP (NON = 22 ± 1, HIGH = 18 ± 1*) levels were significantly lower in HIGH compared with NON mice. At 8 weeks, ATP and ADP were still significantly lower in the HIGH transgenics. In contrast, although ADP levels were preserved, LOW had reduced ATP levels at 8 weeks but normal cardiac function (ATP NON = 68 ± 4 micromoles/L, LOW = 47 ± 7*, HIGH = 47 ± 4*; ADP NON = 23 ± 1, LOW = 21 ± 3, HIGH = 18 ± 2*, n=10). Overexpression of CrT in the cardiomyocyte depletes ATP. Depletion of both ATP and ADP is associated with LV failure whereas ATP depletion alone does not significantly alter LV function, which likely indicates the importance of ADP as a signaling molecule.

*p<0.05 vs NON, ANOVA, Tukey

1471-Pos Determining the Inotropic Mechanism of Pyruvate

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Pyruvate is a unique inotrope. It does not significantly increase heart rate, myocardial fuel consumption or MVO2. It is a known antioxidant with cardioprotective effects under ischemic conditions. The molecular mechanisms that promote the inotropic effect of pyruvate remain elusive. We hypothesize that the main inotropic effect of pyruvate involves an alteration in myofilament calcium sensitivity. We utilized ultra thin rabbit heart trabeculae, intracellular iontophoretic dye methods and a novel technique involving K^+ contractures to study myofilament calcium sensitivity under near physiologic conditions. After obtaining baseline values pyruvate was infused at 10mM concentration. By utilizing bis-fura-2 to monitor $[Ca^{++}]i$ and under conditions of control versus SR block (ryanodine 1μmol and cyclopiazonic acid 10μmol) we were able to characterize the direct effects of pyruvate on calcium transients and developed force and to isolate them from pyruvate's effects on the SR in normal myocardium. Interestingly under conditions of SR block our preliminary experiments show little to no change in systolic calcium (average increase of 49nM) compared to the relatively large (175%) increase in force development seen with infusion of pyruvate (mean developed force increased from 5.4 to 9.5 mN/mm^2) (n=3). In a separate potassium contracture pilot experiment, where a steady state Force Calcium relationship was obtained at baseline, dip and peak force development, a marked shift to the left from baseline to peak force development (EC50 varying from 433 at baseline to 663nM at peak inotropic effect) developed under conditions of pyruvate infusion indicating an important change in myofilament sensitivity. These experiments suggest that pyruvate's inotropic effect are not explained mainly by $[Ca^{++}]i$ changes but rather by an effect on myofilament calcium sensitivity. Unraveling the underlying mechanisms by which pyruvate imposes its inotropic effect may lead to novel therapeutic strategies in heart failure.

1472-Pos Insulin Stimulates Glucose Uptake Through IP_3-dependent Intracellular Calcium Release In Rat Neonatal Cardiac Myocytes

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Insulin stimulates glucose uptake in muscle cells by translocation of glucose transporter, GLUT4, from intracellular compartments to cell surface. The involvement of Ca^{2+} in insulin-mediated glucose uptake has not been established in cardiac muscles. Our aim was to study the role of Ca^{2+} in insulin dependent glucose incorporation.

Cardiac myocytes were pre-incubated with intracellular Ca^{2+}-sensitive dye FLUO3-AM and Ca^{2+} kinetics were recorded by confocal microscopy. Glucose uptake was evaluated using ^{3}H-2-deoxy glucose (^{3}H-2DG).

Insulin (0.1–100 nM) induced an intracellular calcium ($[Ca^{2+}]_i$) transient with two separable kinetic components. $[Ca^{2+}]_i$ reached maximal relative fluorescence of 224 ± 23 and 219 ± 38 ([ΔF/F]x100) in Ca^{2+}-containing and Ca^{2+}-free external medium respectively. It was completely inhibited by genistein. Nifedipine and ryanodine inhibited the first part (1 s) of the response. Pertussis toxin (PTX), Adβark-ct, LY-294002, U-73122, 2-APB and Xestospongin C inhibited the second component (lasting tens of seconds) leaving fast oscillations. Insulin significantly increased ^{3}H-2DG uptake 5 minutes post addition. ^{3}H-2DG uptake was 4.5 ± 0.2 and 6.8 ± 0.3 fold over control in Ca^{2+}-containg and Ca^{2+}-free medium respectively. Indinavir and ryanodine showed partial inhibition of ^{3}H-2DG uptake, whereas BAPTA-AM, Adβark-ct, LY-294002, Akti1/2, U-73122 and 2-APB completely inhibited ^{3}H-2DG uptake. PTX didn't alter ^{3}H-2DG uptake. In Ca^{2+}-free medium, BAPTA-AM inhibited completely ^{3}H-2DG uptake, whereas indinavir, Adβark-ct, LY-294002, Akti1/2, U-73122 and 2-APB inhibited ^{3}H-2DG uptake only partially.

These results allow us to conclude that IP_3-dependent slow calcium release mediates the increase in glucose uptake induced by insulin in cardiac myocytes.

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1473-Pos A Novel Calcium-Sensitive Fluorescent Probe For Measurement Of Mitochondrial Calcium In Adult Cardiac Myocytes

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Previously small molecule Ca^{2+} indicators such as Indo-1 and Rhod-2 have been loaded into mitochondria via their membrane-permeable AM ester form, however these dyes accumulate in other subcellular organelles and the cytosol. We have utilised a genetically encoded Ca^{2+}-sensitive fluorescent protein based on an inverse-pericam that can be targeted to mitochondria of primary mammalian cells using an adenoviral-mediated expression system (AdMityCam) with an affinity suitable for measuring Ca^{2+} in the physiological range (K_d 190nM). Expression of MityCam in HeLa cells reveals a characteristically distinct mitochondrial network that can be co-localised with the mitochondrial dye Mito-Tracker Red. Confocal microscopy also reveals a distinct pattern of mitochondrial distribution in adult rabbit cardiomyocytes. Cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) and mitochondrial Ca^{2+} ($[Ca^{2+}]_{mit}$) were monitored simultaneously in cardiomyocytes by loading AdMityCam expressing cardiomyocytes with Fura2-AM. $[Ca^{2+}]_{mit}$ and $[Ca^{2+}]_{cyt}$ were recorded at rest and during voltage clamp stimulation (0.5Hz). Upon stimulation, MityCam fluorescence decreased, indicating an in-

crease in $[Ca^{2+}]_{mit}$ to a steady state value. $[Ca^{2+}]_{mit}$ increased by 32±5% whilst Fura-2 fluorescence rose by 198±9.9nM. Beat-to-beat Ca^{2+} transients were observed in both $[Ca^{2+}]_{mit}$ and $[Ca^{2+}]_{cyt}$, rising by 0.49±0.28% and 11.42±2% respectively in steady state. The kinetics of the transient signals was slower in mitochondria compared to the cytosol, time to peak signal was 616±77ms vs.135±11ms respectively. Blocking the mitochondrial uniporter with 5microM FCCP and 1microM Oligomycin caused decreased fluorescence in both HeLa and cardiomyocytes (54.4±1.0 and 25.0±1.4% respectively) with no significant changes in $[Ca^{2+}]_{cyt}$. Subsequent addition of 100microM histamine in HeLa cells caused no further change in $[Ca^{2+}]_{mit}$. In summary, using the MityCam probe, a large steady state signal and small beat-to-beat changes in $[Ca^{2+}]_{mit}$ were observed. The transient signals had considerably slower kinetics compared to cytoplasmic signals.

1474-Pos Uncoupling of Mitochondrial Respiration by Helium-induced Preconditioning is Abolished in Isolated Cardiac Mitochondria from Zucker Obese Rats

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The protective potency of ischemic preconditioning is abolished in Zucker obese rats. Furthermore, the noble gas helium initiates cardiac preconditioning by prevention of mitochondrial permeability transition pore (mPTP) opening. Alterations in mitochondrial bioenergetics are involved in prevention of mPTP opening. Therefore, we investigated the effects of helium preconditioning on mitochondrial respiration in Zucker lean and obese rats.

Chloralose-anesthetized Zucker rats were mechanically ventilated and assigned to one of four groups. Zucker lean (ZL-Con, n=8) and Zucker obese (ZO-Con, n=7) control animals were not further treated. The preconditioning groups (ZL-He-PC and ZO-He-PC, each n=8) received 70% Helium for 3×5 minutes interspersed with 2×5 minutes and one final 5 minute washout period. Hearts were excised and mitochondria were isolated by differential centrifugation. O_2 consumption was monitored after addition of 200 μM ADP (state 3), and after complete phosphorylation of ADP to ATP (state 4). The respiratory control index (RCI) was calculated as state 3/state 4. Data are mean±SD.

There was no difference in both state 3 and state 4 respiration between ZL and ZO control group, respectively. Helium preconditioning reduced in ZL rats the RCI from 2.5±0.1 (ZL-Con) to 2.3±0.1 (ZL He-PC, $p<0.05$) while in ZO rats, it had no effect (ZO He-PC: 2.5±0.1 vs. ZO-Con: 2.5±0.1, n.s.). The reduction in the RCI in ZL He-PC was caused by an increase in state 4 respiration (ZL He-PC: 155±11 nmol O_2 mg/min vs. ZL-Con: 139±10 nmol O_2 mg/min, $p<0.05$), while state 3 respiration was unaffected.

Helium-induced preconditioning causes mild mitochondrial uncoupling, which is possibly involved in prevention of mPTP opening. The absence of mitochondrial uncoupling in Zucker obese rats may explain at least partially the reduced protective potency of cardioprotective interventions in the prediabetic heart.

1475-Pos Increased Intracellular [dATP] Enhances Cardiac Contraction In Embryonic Chick Cardiomyocytes

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Functional effects of increased intracellular 2'-deoxy-ATP ($[dATP]_i$) on living cardiac cells were examined in contractile monoloayers of embryonic chick cardiomyocytes loaded with exogenous nucleotide at varying dATP/ATP concentrations (constant total nucleotide concentration). While ATP is the normal substrate for cardiac contraction, we have previously found significant enhancement of cardiac contractility in vitro when only 10% of normal ATP substrate is replaced with dATP. To determine the functional effects of increased $[dATP]_i$ in living cardiac cells, contractile monloayers of embryonic chick cardiomyocytes were loaded with exogenous dATP via osmotic shock; the influences of increased $[dATP]_i$ on cell viability, average contractile amplitude, rates of contraction/relaxation, beat frequency, and Ca^{2+} transients were examined. Total $[dATP]_i$ of $\geq$ ~100 μM appeared to induce apoptosis while contractile function ceased at $[dATP]_i$ between ~ 70 μM and ~100 μM. Interestingly, $[dATP]_i$ of ~ 60 μM enhanced both amplitude of contraction and the rates of contraction and relaxation without affecting beat frequency. With total $[dATP]_i$ of ~ 60 μM or less, we found no significant change in Ca^{2+} transients as measured by fluorescent Ca^{2+} indicator Rhod-2. These data suggest that there may exist an "optimal" concentration of exogenously loaded $[dATP]_i$ that can result in enhanced contractility in living cardiomyocytes without affecting beat frequency or Ca^{2+} transients. Our findings are especially attractive because earlier strategies for treating heart failure with Ca^{2+} sensitizing agents were found to enhance contractility at the expense of changes in Ca^{2+} signaling, resulting in increased mortality. dATP may have therapeutic potential as a positive inotrope in human heart failure conditions, therefore controlled methods for elevating intracellular [dATP] in vivo should be explored.

1476-Pos Exercise Training Improves Global And Cellular Contractile Functions In Rats With Post-myocardial Infarction Heart Failure

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In the healthy heart, sub-endocardial cells (ENDO) contract more than sub-epicardial cells (EPI). This gradient of contractility disappears in the failing heart by affecting the ENDO cells. Our study tested the effect of exercise on the global and cellular contractility across the left ventricular (LV) wall in rats with established heart failure following post-myocardial infarction (PMI). Rats were exercised 15 weeks after infarction for 5 weeks on treadmill. Global cardiac function was analyzed by echocardiography. Excitation-contraction coupling (Ca^{2+} transient, shortening) of intact cells isolated from EPI and ENDO LV layers and the stretch-induced sensitisation of Ca^{2+} activation of the myofilaments on skinned cells (Ca^{2+} sensitivity of the contractile machinery at 1.9 and 2.3 μm sarcomere length (SL)) were analyzed.

Echocardiography shows a gradient of shortening velocity from EPI to ENDO altered during pathology and partially restored after exercise. At the cellular level, cell shortening, and Ca^{2+} transient were reduced in PMI in particular in ENDO cells. Ca^{2+} sensitivity of the contractile machinery was reduced only in ENDO PMI at 2.3 μm SL reducing the transmural stretch sensitization. Exercise increased Endo PMI cell shortening by improving both Ca^{2+} transient and Ca^{2+} sensitivity of the myofilaments. Thus exercise performed late after myocardial infarction is able to improve/restore part of the gradient of contractility of the failing heart.

1477-Pos HDAC5 Nuclear Export Is Stimulated By Angiotensin II In Adult Cardiac Myocytes

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We previously showed that there are fundamental differences between endothelin-1 (ET-1) and phenylephrine (PE)-mediated HDAC5 phosphorylation and nuclear export. ET-1-dependent export requires a local IP_3-dependent perinuclear Ca signaling pathway and is mediated by CaMKII and Protein Kinase D (PKD), but not PKC. (*JCI*. 2006;*116*:675–82). However PE, another G_q-coupled pathway, was relatively Ca-independent and required PKC-dependent PKD activity, but not CaMKII or IP_3 (*BJ*. 2007, *92*: 622a). We now examine a parallel hypertrophic pathway activated by angiotensin II (AngII) which may be similar to ET-1 and PE pathways. We infected adult rabbit cardiomyocytes with an adenovirus encoding GFP-HDAC5 fusion protein and tracked levels of nuclear export with confocal microscopy. In quiescent cells, HDAC5 is predominately nuclear and 100nM AngII stimulation resulted in HDAC5 nuclear export (16±6% decline in 60 min vs. 48±9% and 35±4% for PE and ET-1, respectively). Pretreatment with KN93 (a CaMKII inhibitor), Gö6976 (which inhibits PKD) or thapsigargin (depletes Ca stores) blocked nuclear export by nearly half, indicating a role for Ca stores, CaMKII and PKD in the AngII pathway, as is the case for ET-1. Blocking IP_3 receptors with 2-APB almost completely prevented HDAC5 nuclear export, indicating a significant role for IP_3, again similar to the ET-1 pathway (but different from the PE pathway). Unexpectedly, preliminary experiments with BisI (a PKC inhibitor) almost completely blocked AngII-induced HDAC5 nuclear export (more like PE than ET-1). These experiments indicate that AngII induces HDAC5 nuclear export by a pathway similar to ET-1 (involving IP_3-sensitive Ca stores, CaMKII and PKD), but seems to be more sensitive to PKC inhibition (like PE-induced HDAC5 export). Thus these G_q-coupled receptor pathways differ in how they interpret receptor activation with respect to HDAC5 translocation in adult ventricular myocytes.

Cardiac Muscle & Regulatory Proteins - III

1478-Pos S100A2 Gene Transfer Improves The Calcium Cycling And Contractile Properties Of Adult Cardiac Myocytes

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Board B454

Members of the S100 super family are multifunctional signaling proteins that are involved in the regulation of diverse cellular processes. Recently, it has been proposed that S100A1, the most abundant S100 protein in cardiac muscle, plays an important role in the modulation of heart contractile performance. Other members of the S100 family, including S100A2, S100A6 and S100B are expressed in the heart, but their functions are not well defined. The goal of this study was to determine the effects of overexpressing S100A2 on the contractile properties of rat cardiac myocytes. To achieve this goal, we generated adenoviral vectors to express S100A2 in rat adult cardiac myocytes in primary culture. The effects of S100A2 overexpression on Ca^{2+} cycling and contractile properties were determined by simultaneous measuring of unloaded sarcomere shortening and intracellular Ca^{2+} transients. On day 3 after gene transfer, sarcomere-shortening amplitude was significantly increased in S100A2-transduced myocytes compared with control myocytes (195 ± 17 vs 125 ± 14 nm, $P < 0.05$). The rate of relaxation was faster in S100A2 transduced myocytes compared to control myocytes. As well, the intracellular Ca^{2+} transient amplitude was enhanced in S100A2 transduced cardiac myocytes (0.35 ± 0.03 vs. Control 0.26 ± 0.02, $P < 0.01$). This increase in the Ca^{2+} amplitude was accompanied by faster velocities of Ca^{2+} increase and decay. Our data indicate that S100A2 expression improves contractility of rat cardiac myocytes by enhancing the Ca^{2+} cycling properties in myocytes. The gene transfer of S100A2 could be used as a new approach to correct deficient intracellular Ca2+ cycling and contractility in heart disease.