**Board B452**

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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**Board B453**

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 ($195 \pm 17$ vs $125 \pm 14$ 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 ($0.35 \pm 0.03$ vs. Control $0.26 \pm 0.02$, $P < 0.01$). 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.

# 1479-Pos Dissociation of Calcium Decline from Force Decline by Preload in Isolated Rabbit Myocardium

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**Board B455**

It is well known that the rate of intracellular calcium ($[Ca^{2+}]_i$) decline is an important factor governing relaxation in unloaded myocardium. However, it remains unclear to what extent, under near physiological conditions, the intracellular calcium transient amplitude and kinetics contribute to the length dependent increase in force and increase in duration of relaxation. We hypothesize that myofilament properties, rather than calcium transient decline, primarily determines duration of relaxation in adult mammalian myocardium. To test this hypothesis, we simultaneously measured force of contraction and calibrated $[Ca^{2+}]_i$ transients in isolated, thin rabbit trabeculae, at various lengths at 37 °C. Time from peak tension to 50% relaxation ($RT_{50(tension)}$) increases significantly with length (from 49.8 ± 3.4 ms to 83.8 ± 7.4 ms at an $[Ca^{2+}]_o$ of 2.5 mM), while time from peak calcium to 50% decline ($RT_{50(calcium)}$) was not prolonged (from 124.8 ± 5.3 ms to 107.7 ± 11.4 ms at an $[Ca^{2+}]_o$ of 2.5 mM). ANOVA revealed that $RT_{50(tension)}$ is significantly correlated with length ($P < 0.0001$). At optimal length, varying the extracellular calcium concentration increased both developed force and calcium transient amplitude, but $RT_{50(tension)}$ remained unchanged ($P = 0.90$), while intracellular calcium decline actually accelerated ($P < 0.05$). Thus, an increase in muscle length will result in an increase in both force and duration of relaxation, while the latter is not primarily governed by the rate of $[Ca^{2+}]_i$ decline.

# 1480-Pos CD Antibody Microarrays Identify Patients With Acute Coronary Syndrom And Distinguish Between Patients With Stable And Unstable Angina

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**Board B456**

Currently, no blood-based test can rapidly and objectively distinguish between stable angina pectoris (SAP - chest pain when increased myocardial oxygen demand is not satisfied by an appropriate coronary blood flow), and unstable angina pectoris (UAP - where inadequate coronary flow produces pain at rest). In the search for appropriate identifying biomarkers, most methods have focused on serum-based tests. However, since leukocytes play an active role in the progression of coronary artery disease, we hypothesize that these cells can provide novel markers of SAP and UAP and may indeed be able to distinguish between then. Here we use antibody microarrays containing 82 cluster of differentiation (CD) antibodies (plus isotype controls) that selectively immobilize specific types of leukocytes from a suspension of applied peripheral blood mononuclear cells. This differential capture depends on the expression patterns of CD antigens expressed on their surface membranes. We find that the pattern of immobilization of leukocytes from both SAP and UAP patients with acute coronary syndrome (ACS) significantly differs from age- and gender-matched healthy subjects (Australian Red Cross Blood Service blood donors). Within the ACS group, 15 SAP patients exhibited significant ($p < 0.05$) changes in the intensity of 10 of the 82 CD antibody spots in the array compared to 19 healthy blood donors. In the UAP group, the intensity of these 10 changes increased and an additional eight CD antigens differed significantly ($p < 0.05$) between the blood donors and UAP patients. These preliminary data suggest that it is now appropriate to engage a larger clinical trial to test the hypothesis that these antibody arrays can be used to diagnose ACS and can monitor the progression from SAP to UAP.

# 1481-Pos Cell Transplantation in Infarcted Hearts Increases $Ca^{2+}$ Sensitivity In Surviving Myocardium Remote from the Infarct: Morphological Integration of Cell Graft and Altered Phosphorylation of Myofibrillar Proteins

F. Steven Korte, Alicia Moreno Gonzalez, Jin Dai, Kent Chen, Hans Reinecke, Veronica Muskheli, Charles E. Murry, Michael Regnier

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**Board B457**

Cell transplantation studies on infarcted myocardium have shown improved cardiac function, however, little is known about the underlying cellular/molecular mechanisms that lead to these improvements. We have shown that 8–10 week old allographic neonatal cardiomyocyte grafts in infarcted myocardium have increased $Ca^{2+}$ sensitivity of force as compared to control myocardium. Importantly, this also leads to an increase in the $Ca^{2+}$ sensitivity of force of myocardium remote from the infarct and graft region. We tested the protein isoform composition of thin and thick filament proteins using western blot analysis, and the phosphorylation status of TnI, TnT, tropomyosin (Tm), myosin binding protein-C (MyBP-C), and myosin light chain (MLC) using Pro-Q Diamond staining. Interestingly, graft tissue exhibits primarily adult protein phenotypic characteristics. Adult cardiac (c)TnI was expressed in grafted,

remote, and control myocardium, while embryonic slow skeletal (ss)TnI was undetectable. Similarly, there was no difference in TnT isoform content between control, grafted, or remote myocardium. Electron microscopy showed graft tissue was morphologically mature with well-developed sarcomeres and dyads of T-tubules and sarcoplasmic reticulum. Importantly, there was significantly reduced phosphate incorporation in TnT, Tm, and MLC-2 in remote myocardium from engrafted hearts, and reduced phosphate incorporation across the board in the graft itself, as compared to control or remote myocardium from non-grafted hearts. This suggests reduced phosphorylation of these key proteins which could account, at least in part, for the increased $Ca^{2+}$ sensitivity seen in graft and remote myocardium from grafted hearts. Whether these differences are due to increased phosphatase activity and/or decreased protein kinase activity warrants further investigation.

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## 1482-Pos Larger but Slower Cardiomyocyte Contractions and Ca2+ Transients in Mice with Chronic Heart Failure Following Myocardial Infarction

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**Board B458**

Human heart failure is a chronic progressive disease that can be ascribed to smaller and slower contractions and Ca2+ transients due to depressed sarcoplasmic reticulum (SR) function. We have previously reported that mice with congestive heart failure (CHF) exhibit increased function in viable myocardium in the early stages following myocardial infarction (MI). In the present study we investigated the progression of chronic CHF in this model. MI was induced by ligating the left coronary artery and CHF was confirmed at 1-week post-MI by echocardiography. SHAM operated animals served as controls. Altogether 40% of CHF animals died between 1 week and 10 weeks post-MI. Echocardiograpic measurements showed reduced global cardiac function in CHF at both time points. However, function in the non-infarcted myocardium gradually deteriorated during this time period, as manifested by decreased posterior wall shortening velocity at 10 weeks but not 1-week CHF ($P<0.05$). Single cardiomyocytes isolated from the septum in 10-week CHF were hypertrophied (cardiomyocyte area =130±9% SHAM values, $P<0.05$). Surprisingly, contraction measurements (1 Hz) still showed increased amplitude in CHF (198±18% of SHAM values, $P<0.05$) and unchanged relaxation time. However, time to peak contraction was increased by 15±6% ($P<0.05$) in CHF. Similar changes were observed during fluo-4 monitoring of Ca2+ transients; transient amplitude was increased by 123±24%, and time to peak was 57±11% longer in CHF compared to SHAM ($P<0.05$). In addition, SR Ca2+ load was increased by 42±8% ($P<0.05$) in CHF. Thus, while chronic CHF differs in mice and humans, these data show that slowing of Ca2+ release and contraction may promote CHF progression even when SR stores and the magnitude of Ca2+ release are not reduced.

## 1483-Pos I40A Mutation Increases Phospholamban Pentamer Subunit Exchange and Reduces Oligomerization Affinity

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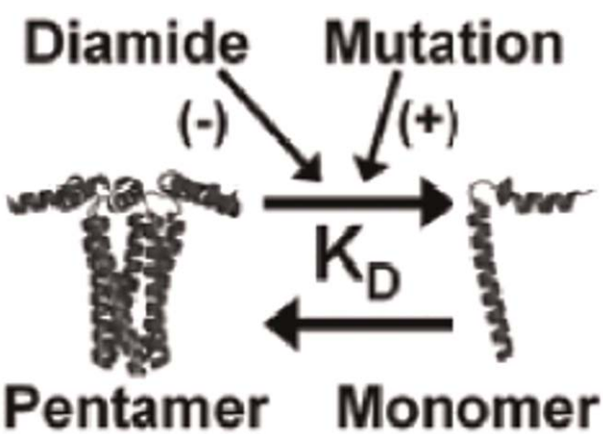
**Board B459**

The energetics and dynamics of phospholamban (PLB) oligomeric interactions were investigated by measuring FRET between CFP- and YFP-PLB in AAV-293 cells. I40A mutation of PLB reduced, but did not abolish PLB-PLB FRET. A survey of a range of protein concentrations indicated that reduced FRET was not due to structural differences between I40A- and WT-PLB oligomers. Rather, the dissociation constant ($K_D$) increased 4-fold with mutation. Furthermore, we observed a greatly increased rate of PLB oligomer subunit exchange for I40A-PLB, as quantified by the Förster Transfer Recovery (FTR) technique. This suggests the I40A mutant's increased $K_D$ arises from a faster off-rate ($k_{off}$). Specifically, WT oligomers were temporally stable, and showed no FTR over 80s, but I40A-PLB complexes showed rapid subunit exchange ($\tau$=8.4s). I40A-PLB oligomer stability was restored by crosslinking of transmembrane cysteines with diamide. We conclude that subunit exchange from WT-PLB pentamers is slow, and does not occur on the time scale of the cardiac cycle. I40A mutation partially destabilizes the pentamer, increasing the $K_D$ and subunit exchange rate. These methods may be generally useful for measuring the dynamics and relative affinities of membrane protein complexes at equilibrium in live cells.



# 1484-Pos Co-expression Of SR-targeted AIP Improves SR $Ca^{2+}$ Handling In CaMKII$\delta_c$ Overexpressing Mice, But Cardiac Remodeling Is Accelerated

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**Board B460**

Cardiac myocyte overexpression of CaMKII$\delta_c$ leads to cardiac hypertrophy and heart failure (HF), possibly due to altered myocyte Ca handling. A central defect might be an increased diastolic sarcoplasmic reticulum (SR) Ca leak that decreases SR Ca load and Ca transient amplitude. We hypothesized that CaMKII inhibition at the SR membrane would decrease the leak, improve Ca handling and prevent HF. Our approach was to crossbreed CaMKII$\delta_c$ overexpressing mice (CaMK) with mice expressing the CaMKII-inhibitor AIP targeted to the SR via a modified phospholamban (PLB)-transmembrane-domain (SR-AIP). SR-AIP expression was confirmed via Western Blot and did not affect CaMKII$\delta_C$ expression level in double-transgenic mice (CaMK/SR-AIP). SERCA and RyR expression levels were unaltered, but PLB expression was slightly increased in CaMK/SR-AIP compared to CaMK. As hypothesized, the shift in diastolic Ca upon application of Tetracaine (diastolic SR Ca leak) in isolated cardiomyocytes was reduced in CaMK/SR-AIP (8.7±1.1 nM; n=20) vs CaMK (19.0±1.8 nM; n=17). Consistent with these observations, Ca spark width and duration in permeabilized myocytes from CaMK/SR-AIP were reduced (vs. CaMK), while frequency and amplitude were unchanged (integrated spark signal was reduced by ~26%). In intact myocytes co-expression of SR-AIP enhanced twitch amplitude and SR Ca load vs. CaMK (SERCA and NCX function were unchanged). However, despite the improved Ca handling, cardiac remodeling was accelerated (ventricular weight/body weight ratio 10.2±0.8 mg/g in CaMK vs. 13.5±0.9 in CaMK/SR-AIP at 90 days of age) and cardiac function worsened (lower fractional shortening (%FS) in CaMK/SR-AIP (6.4±1.3%) vs CaMK (14.3±1.4%)). We conclude that inhibition of SR CaMKII in CaMK mice improves Ca handling, but does not rescue the HF phenotype. This implies that CaMKII$\delta_c$ exerts SR-independent effects that lead to hypertrophy and HF.

# 1485-Pos Phospholamban Mutants Reveal the Effect Phosphorylation on Oligmerization and Pentamer Conformation

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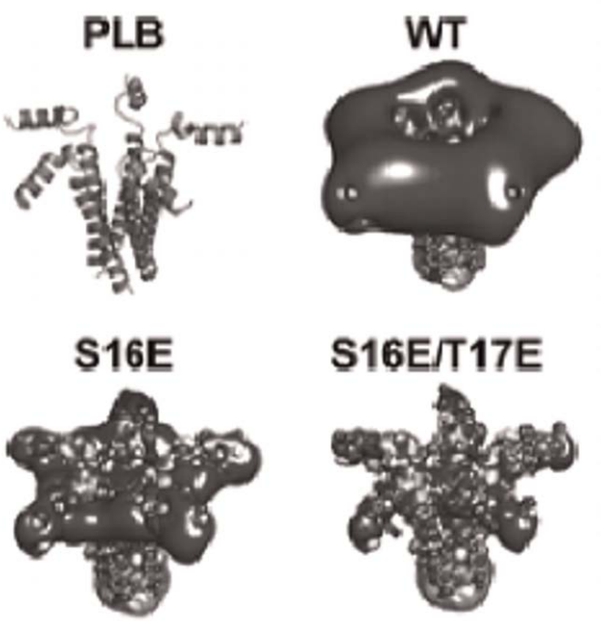
**Board B461**

To investigate how the phosphorylation of PLB perturbs its structure and oligmerization, we measured intrapentameric CFP-YFP FRET of non-phosphorylatable mutant S16A, PKA-phosphorylation mimick S16E, and PKA/CamKII-phosphorylation mimick S16E-T17E. Pseudophosphorylation mutants showed increased FRET. To determine whether this increase was due to greater oligomerization or a conformational change, we examined FRET between CFP and YFP labeled PLB subunits of cells expressing a wide range of protein concentrations. A hyperbolic fit of FRET vs. protein concentration yielded estimates of maximum FRET and relative $K_D$. Probe separation distances, calculated from a computation model of intrapentameric FRET, were on the order of 60 angstroms. We did not observe a resolvable change in the quaternary structure with mutation. Compared with S16A, S16E showed a 57% decrease in $K_D$ and S16E-T17E showed 84% decrease. The data indicate PLB oligomerization is increased by phosphorylation. We attribute this to the reduced electrostatic repulsion between PLB subunits, as mutation significantly neutralizes positive charge on PLB cytoplasmic domains (Figure 1). We conclude that the major effect of phosphorylation is an increase in pentamer-monomer ratio, rather than a change in pentamer structure.




# 1486-Pos Myocardium Viscosity May Contribute To Arrhythmogenesis

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**Board B462**

Cardiomyocyte $Ca^{2+}$-overload often induces arrhythmias. We have earlier shown in a mathematical model that a number of mechanical

factors may contribute to cardiomyocyte rhythm disturbances in the case of $Ca^{2+}$-overload initiated by reduced $Na^{+}$-$K^{+}$ pump (Katsnelson ea. LNCS 2007/*4466*:383–392). In the same model we now studied possible contributions of one more mechanical factor, myocardial tissue viscosity, to arrhythmogenesis in $Ca^{2+}$-overload. Two virtual samples with moderately reduced $Na^{+}$-$K^{+}$ pump were compared. They differ from each other only in coefficients of viscosity. This passive mechanical parameter in the first sample was twice as large as in the second. A result of numerical experiments is that the increase in myocardial viscosity promoted essentially earlier appearance of extrasystoles in the first sample vs. the second during series of contractions initiated by equal regular pacing (e.g. 75 stimuli/min) of both elements preset at the same initial lengths (e.g. 0.88 $L_{max}$). Briefly, the underlying mechanism revealed in the model was based on modulation of CaTnC kinetics by shortening velocity. The higher velocity during initial phase of any contraction led to relatively higher calcium inactivation in the less viscous sample. Hence during the next phase of the contraction, crucial for SR $Ca^{2+}$ accumulation, shortening velocity (and thus CaTnC dissociation rate) turned out to be lower as compared with the more viscous sample. Therefore beat-to-beat $Ca^{2+}$ accumulation in the SR was higher for the latter sample, and a critical level of $Ca^{2+}$-overload was reached in more short time. This produced extrasystoles earlier in the more viscous virtual muscle.

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## 1487-Pos MEMS Sensors for Arrhythmia Detection and Intervention

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**Board B463**

Despite decades of intensive investigation, sudden death secondary to ventricular fibrillation (VF) remains a leading cause of mortality in the US and other developed countries. Recently, several promising hypotheses regarding the mechanism for VF have been introduced. However, it has not been possible using currently available experimental techniques to determine which theory (or theories) is most applicable to VF. To address this issue, we propose to:

1. construct a cardiac mapping system from nanofabricated components that is capable of assessing cardiac activation and repolarization with high spatial and temporal resolution and with minimal tissue damage;
2. use a novel phase mapping technique to analyze the mapping data, with the objective of identifying the location and number of phase singularities during sinus rhythm, ventricular tachycardia and VF;
3. use the phase singularity data to distinguish between three putative mechanisms for VF - an anchored rotor with fibrillatory conduction, a meandering rotor or multiple rotors.

MEMS technology will be used to construct microscale mechanical needle-like structures with integrated electrodes that are ultrasonically activated, to minimize tissue damage during insertion. The electrode arrays will be used to map activation and repolarization in canine ventricular myocardium in vitro and in normal and acutely ischemic pig hearts in situ during fixed pacing and during VF. The results of this study will lead to significant advances in three key areas: development of devices to map cardiac electrical activity with unprecedented spatial resolution; application of newer and more sophisticated techniques to analyze large mapping data sets; interpretation of high resolution mapping data within the context of novel hypotheses regarding the genesis of ventricular tachycardia and fibrillation.

## 1488-Pos Optical Recording Of Membrane Voltage From Transverse Tubules And Intercalated Discs Using 2-Photon Excitation In Intact Myocardium

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**Board B464**

The aim of this study was to examine the feasibility of using voltage sensitive dyes to measure the sarcolemmal voltage signal within subcellular compartments of cardiomyocytes within the wall of the myocardium using 2-photon excitation of Di-4-ANEPPS. Initial dye characterization was done in dissociated cardiomyocytes stained with Di-4-ANEPPS (10uM). Emission spectral analysis revealed a peak value at 600nm and the excitation spectrum (690–1040nm) indicated a peak at 930nm. Depolarization by extracellular $K^{+}$ revealed a leftward shift in the emission spectrum, but no change in amplitude. Therefore, 930nm excitation provided a ratiometric measurement of membrane voltage as emitted fluorescence was split at 580nm and simultaneously collected by 510–560nm and 590–685nm bands. The voltage-sensitive short/long ratiometric signal was insensitive to changes in dye loading. This optical arrangement was used to measure membrane voltage in a perfused ventricular wedge preparation stimulated on the epicardial surface. Movement was inhibited by blebbistatin (10uM). 2ms linescans along cardiomyocytes were recorded 50–150um from the epicardial surface using a water-dipping 40x objective, (X-Y-Z approximate resolution $1\times1\times2$um). Signals from transverse (T)-tubules and intercalated discs were analyzed separately. At a 350ms pacing cycle length, optical action potential (AP) duration to 75% repolarization ($APD_{75}$) in the T-tubules was 178±11ms (n=7), the 10–90% rise time of the AP (Trise) was 12±2ms and the amplitude was 6±1% (ratio change). The optical signal confined to the intercalated discs showed APs that were insignificantly different from those at the T-tubules ($APD_{75}$ 175±9ms, Trise 11±2ms, and amplitude 7±2%). No voltage signal was detected in non-cardiomyocyte tissue. This work demonstrates that voltage signals can be recorded at microscopic resolution in intact myocardium and that intercalated discs and T-tubules have similar membrane voltage changes.

# 1489-Pos Mechanism of Arrhythmogenesis in DCM Model Mice Associated with Cardiac Troponin T Mutation

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**Board B465**

Dilated cardiomyopathy (DCM) is a disease characterized by weakened and dilated heart which often leads to heart failure and sudden death. Morimoto and his colleagues created a knock-in mouse model of DCM caused by a deletion mutation of K210 (ΔK210) in cardiac troponin T (Circ Res *101*: 185–194, 2007). The mutant mice developed enlarged hearts and often suffered from sudden death with arrhythmia. We studied the mechanisms of the arrhythmogenesis in these mice. Cardiac muscles of left and right ventricles and septum were excised from wild type (WT) and ΔK210 homo mice hearts. Muscles were loaded with rhod-2 or di-4-ANEPPS and Ca2+ signals or membrane potential signals were determined using a laser scanning confocal microscope. Isometric tension developments were separately determined using a force transducer. In ΔK210 mice hearts, spontaneous action potentials and contractions were frequently detected from left ventricle and septum but not from other regions. Action potential durations were prolonged in some regions of those muscles. Effects of various types of channel blockers on the frequency of spontaneous activities were examined. Spontaneous activities were significantly suppressed by a $Na^+$ channel blocker, pilsicainide, but not by $K^+$ channel blocker, nifekarant. A $Ca^{2+}$ channel blocker, nifedipine, did not decrease spontaneous activity at concentrations that suppressed tension developments to 10% of control. These results suggest that the $Ca^{2+}$ overloading, one of causes of ventricular arrhythmias, is not a reason for the enhanced automaticity. Instead, the increased excitability of plasmamembrane may relate to the automaticity. Involvement of various channels and action potential dispersion in the arrhythmogenesis in this DCM-model mice will be discussed.

# 1490-Pos Estrogen-induced Upregulation of Proteasome Activity in Mice Heart

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**Board B466**

We hypothesize that estrogen (E2) may protect heart function by promoting proteasome activity to degrade abnormal proteins in cardiomyocytes. Here, we investigated in heart homogenates whether proteasome activity is induced by E2 treatment in ovariectomized (ovx) mice and whether proteasome activity is differentially regulated in males and in females at different estral stages: estrus (under the influence of E2 hours after E2 surge) and diestrus2 (after a prolonged exposure to low E2 levels). Proteasome activity was measured with the fluorescence substrate Suc-LLVY-AMC. The activity was blocked by the proteasome antagonist lactacystin. E2 treatment of ovx mice increased the heart proteasome activity by 1.29±0.04 fold (n=3 control and 4 E2 treated). In the same animals, E2 treatment increased also proteasome activity by 1.7±0.19 fold in liver homogenates, but not in skeletal muscle (1.08±0.05). Proteasome activity was similarly high in conditions with high heart E2 concentration as in males (E2~35 pg/ml) and females at estrus (primed by the preceding proestrus surge of E2~60 pg/ml) when compared with conditions of low E2 (females at diestrus2 after prolonged exposure to low E2 levels, ~15 pg/ml). Values normalized to estrus were 0.92±0.03 for males and 0.76±0.02 for diestrus2 (n=3). Supporting the role of E2, proteasome activity was much higher (1.76±0.12 fold) in myometrium from mice at estrus when compared to diestrus-2. We also investigated E2-induced expression changes in isolated cardiomyocytes from control and E2 treated ovx mice labeled with anti-20S core proteasome complex and -Rpn2 regulatory subunit antibodies. Core and Rpn2 labeling was mainly localized in the nucleus and along the T-tubules. E2 treatment upregulated the core and Rpn2 labeling intensity in the T-tubules. Overall the data is consistent with E2-induced proteasome activity as a mechanism for cardioprotection.

Supported by NIH and AHA.

# 1491-Pos Microarray Analysis of Gene Expression during Adult Ventricular Myocyte Culture

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**Board B467**

Adult cardiac myocytes are routinely maintained in culture for up to 1 week. Changes in cellular morphology, contractile and electrical activity have been documented during this period. In order to understand in more detail the temporal profile and characteristics of changes that occur in culture, we have undertaken a comprehensive analysis of 48 genes encoding receptors, G proteins, ion channels, exchangers, structural and contractile proteins, cytokines and growth factors. Rat ventricular myocytes were maintained in a serum-free culture medium with cytochalasin D[1]. RNA was isolated from cultures after 0, 1, 2, 4, 7 days and the relative content of 48 transcripts determined with RT-PCR on TaqMan arrays. The culture conditions had a marked, time-dependent effect on gene expression: the number of transcripts significantly different vs. day 0 increased throughout the culture period (2 at day 1; 13 at day 2; 20 at day 4; 26 at day 7) ($P<0.05$ RM-ANOVA, n=8 hearts). We used STEM cluster analysis[2] to assign data to temporal expression profile

models to establish whether groups of transcripts for related proteins showed similar patterns of change. Genes whose expression increased progressively throughout the culture period (15/48) included growth factors (Nppa, Nppb, Fgf2, Tgfb1, Tnf), G proteins (Gnai2, Gnaq, Gnas); those whose expression decreased progressively (11/48) included ATP-dependent transporters (Atp1a2, Atp1a3, Atp2a2) and regulatory contractile proteins (Tnni3, Tnnt2). The levels of 9 transcripts, including the caveolar proteins (Cav, Cav2, Cav3), did not change (maximum change < 2- fold). This study shows that even under culture conditions which preserve cellular morphology[1], marked changes in gene expression, many of which are part of "hypertrophy programs", are seen within days.

Supported by the BHF.

## References

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# 1492-Pos Analysis Of Diffusion Restrictions In Cardiac Muscle Cells

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**Board B468**

Functional interaction between mitochondria and surrounding ATPases has been found from the experiments on permeabilized heart muscle fibers. According to our earlier analysis, such interaction can be induced by relatively local diffusion restrictions for adenine nucleotides. The specific causes of these restrictions are not known but intracellular structures are speculated to act as diffusion barriers. Based on the proximity of sarcoplasmic reticulum (SR) to mitochondria, we hypothesize that SR not only utilizes ATP but may also act as a diffusion barrier leading to functional coupling of ATPases and mitochondria. The diffusion barriers can be enhanced by cytoskeleton proteins localized near SR. With a 3D finite-element model, we attempted to explore, SR as the first candidate for diffusion barrier. The geometry for the mathematical model was constructed using representative mitochondrial and SR structural organization from confocal and electron microscope images. SR and cytoskeleton proteins were assumed to induce the diffusion restrictions around mitochondria and in planes between neighboring mitochondria. Those restrictions were varied as well as a restriction induced by mitochondrial outer membrane to fit the following set of experimental data: mitochondrial respiration rate dependence on exogenous ADP and ATP; effects of pyruvate kinase and phosphoenolpyruvate additions on respiration. According to our simulations, there are many sets of model parameters that were able to reproduce all experiments considered in this work. However, in all the sets, the permeability of SR network and associated cytoskeleton proteins was very low indicating importance of cytoskeleton proteins in formation of diffusion restrictions. Finally, the layer of free water available for diffusion between mitochondria and SR surrounding mitochondria, is expected to be of the order of 50 nm or less.

# 1493-Pos Lineage Tracing of Cardiac Explant-Derived Cells

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**Board B469**

Recent reports suggest that cultured adult cardiac explants produce cells with cardiogenic potential that can form cardiospheres in vitro. The aim of this study was to define the source, morphology and cardiogenic potential of cardiac explant-derived cells using lineage-tracing techniques. The cultured explants produced a heterogeneous population of cells including a distinctive population of small round cardiac-explant derived cells (SEDCs)-fig1.These cells shared some characteristics of cardiac myocytes and survived engraftment in the adult heart. Using MLC2vCRE/ZEG double transgenic, MHCnLAC and Actin-eGFP mice, the SEDCs and other cardiac explant-derived cells from these mice failed to differentiate into cardiac myocytes *in-vivo*, demonstrated by the absence of activation of lineage-restricted reporters and action potential-induced calcium transients. The production of SEDCs was highly dependent on the retention of blood-derived cells or factors in the cultured explant-fig 2. Electron microscopy and immunogold labeling showed that SEDCs were vimentin-positive cells exhibiting phagocytic activity, including uptake of cardiac myocyte sarcoplasmic structures and organelles such as mitochondria-fig3, explaining why they may be positive for cardiac markers on immunohistochemistry. Using lineage tracing, cardiac explant-derived cells are not cardiac progenitors and may acquire the imnunohistochemistical phenotypes of cardiomyocytes by phagocytosis.

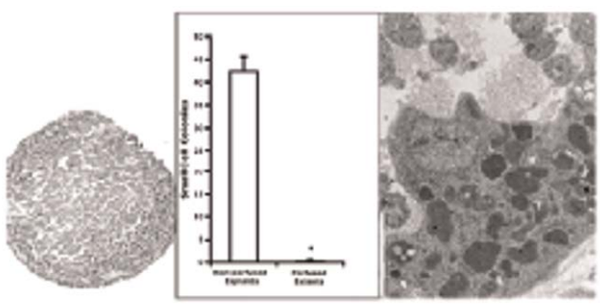

Figure 1 Figure 2 Figure 3

**Excitation-Contraction Coupling-I**

# 1494-Pos Resting Concentration of Ca2+ in the Sarcoplasmic Reticulum (SR) of Frog Skeletal Muscle Fibers

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