mRNA was significantly (P<.05) reduced in group C in comparison with in group A, B or D on day 6. Oral administration of Mao-to starting on day 2 is beneficial for improvement of mortality resulting from acute viral myocarditis in mice with reduced expression of cardiac TNF. These findings suggest crucial implication for starting time of herbal medicine in a murine model of viral myocarditis.

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MicroRNA Function During Cardiac Hypertrophy

Eva van Rooij, Lillian B Sutherland, Ning Liu, Robert D Gerard, Eric N Olson; UT Southwestern Med Ctr at Dallas, Dallas, TX

Diverse forms of cardiac injury evoke a hypertrophic response characterized by an increase in cardiomyocyte cell volume, enhanced protein synthesis, assembly of sarcomeres and activation of a fetal cardiac gene program. Pathological hypertrophy is a major predictor of heart failure and cardiac sudden death. MicroRNAs (miRNAs), which target specific mRNA transcripts for degradation and translational repression, have emerged as key regulators of cell growth, differentiation and death. We have explored the possible involvement of miRNAs in cardiomyocyte hypertrophy and heart failure. Microarray analysis revealed over a dozen miRNAs that were significantly induced or repressed in cardiac tissue from mice that were either exposed to transverse aortic constriction or cardiac-specific over-expression of activated calcineurin, stimuli that induce cardiac hypertrophic remodeling. The changes in expression of these miRNAs were recapitulated in failing human hearts. The potential functions of these hypertrophic miRNAs have been investigated by their cardiac over-expression in vivo and in vitro. Selected cardiac miRNAs have also been inactivated in mice by gene targeting. The functional significance of discreet miRNAs as modulators of the cardiac hypertrophic response will be presented.

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Effect of Erythropoietin on Neointima Formation in Rat Carotid Artery Model of Vascular Injury

Maram K Reddy, Vinod Labhasetwar; College of Pharmacy, Univ of Nebraska Med Ctr, Omaha, NE

Effect of Erythropoietin on Neointima Formation in Rat Carotid Artery Model of Vascular Injury **Maram K Reddy and Vinod Labhasetwar Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198** Various cytotoxic or cytostatic gene and drug therapies targeting vascular smooth muscle cells (VSMCs) have demonstrated some efficacy in inhibiting VSMCs proliferation and neointima formation following angioplasty and/or stent implantation; however, re-endothelialization of the injured artery is critical to its long-term patency. Previous studies have shown that the bone marrow (BM)-derived endothelial progenitor cells (EPCs) are recruited at the vascular injury site and contribute to endothelial recovery. Recombinant human erythropoietin (EPO) has been shown to induce proliferation and mobilization of EPCs and also possesses anti-inflammatory and anti-apoptotic properties. Therefore, we hypothesized that the exogenous administration of EPO would mediate the process of vascular repair by facilitating re-endothelialization and thus could inhibit neointima formation. To test our hypothesis, animals were injected EPO intraperitoneally (5,000 IU/Kg) 6 hr prior to vascular injury and then on every alternate day for one week in a rat carotid artery injury model and morphometric analysis of arteries was carried out at three weeks. Although the EPO treated animals demonstrated nearly complete and continuous arterial re-endothelialization (90% endothelial cell coverage vs. < 20% in saline control), the treatment also resulted in excessive neointima formation (Intima/Media ratio 2.1 ± 0.09 vs. 1.6 ± 0.02, n=5, $p<0.001$), resulting in significant reduction in lumen area (0.16 mm^2 ± 0.01 vs 0.3 mm^2 ± 0.02, n=5, $p<0.001$). The mechanism of excessive neointima formulation in the EPO treated group was shown to be due to excessive neoangiogenic response in the injured artery as represented by CD31-positive structures (103 ± 10/mm^2 vs. 35 ± 5.2/mm^2 saline control, $p<0.001$). Nonetheless, our results explained a common occurrence of vascular access stenosis in patients on EPO treatment during hemodialysis and suggest the cautious use of EPO in patients who are at a risk of vascular injury.

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Detection and Monitoring of Brain Recovery After Therapeutic Hypothermia in a Post-Cardiac Arrest Rodent Model: A Quantitative EEG Study

Xiaofeng Jia, Matthew A Koenig, Hyun-Chool Shin, Gehua Zhen, Romergryko G Geocadin, Nitish V Thakor; Johns Hopkins Med Inst, Baltimore, MD

Objective: To test the hypothesis that quantitative electroencephalogram (qEEG) can objectively assess functional electrophysiological recovery of brain after hypothermia in an asphyxial cardiac arrest rodent model. Methods: Twenty-eight rats were subjected to 7-minute (n=14) and 9-minute (n=14) asphyxia-cardiac arrest. One half of each group (n=7) was randomly subjected to hypothermia one hour after CPR (T=33ºC for 12 hours) and the other half (n=7) to normothermia (T=37ºC). Continuous monitoring of blood pressure, EEG, and core body temperature and intermittent arterial blood gas (ABG) analysis were undertaken. Neurological recovery after CPR was assessed by serial Neurological Deficit Score (NDS) and qEEG analysis. Information Quantity (IQ), a validated measure of relative EEG entropy, was employed to monitor electrical recovery of the brain. Result: After cardiac arrest, the hypothermia-treated group demonstrated better brain recovery (higher IQ) compared to normothermic controls ($P<0.001$). The 72-hour functional recovery by NDS of the hypothermia group was also significantly better compared to the normothermia group ($P<0.01$). IQ during the hypothermia maintenance period has the highest correlation within the first 24 hours (Pearson correlation 0.746, 2-tailed significance <0.001) with 72-hour functional recovery by NDS. Conclusion: The qEEG IQ measure was able to detect the effects of hypothermia during the first 24 hours which was corroborated by the functional recovery by NDS at 72 hours. These results demonstrate the potential utility of objective measures such as qEEG-IQ to track the response to hypothermia and other potential therapies during the early phase of recovery from cardiac arrest.

P157

Withdrawn

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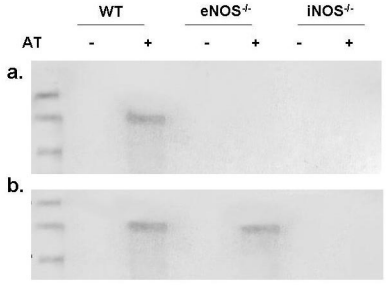
Atorvastatin Activates Cyclooxygenase-2 in the Heart via S-Nitrosylation by Inducible Nitric Oxide Synthase

Yochai Birnbaum, Yumei Ye, Yu Lin, Sheldon Freeberg, Juan Martinez, Jose R Perez-Polo, Barry F Uretsky; Univ of Texas Med Branch, Galveston, TX

Background: Atorvastatin (AT) increases myocardial expression of phophorylated Ser-1777 endothelial nitric oxide synthase (P-eNOS), inducible NOS (iNOS) and Cycloxygenase-2 (COX2). Although AT increases the expression of COX2 in $eNOS^{-/-}$ mice, there is no increase in COX2 activity as occurs in wild-type (WT) mice. In the rat, COX2 activation occurs by iNOS mediated S-nitrosylation. **Aim:** To investigate whether the difference in COX2 activity between the AT-treated WT and $eNOS^{-/-}$ is related to S-nitrosylation. **Methods:** WT, $eNOS^{-/-}$, and $iNOS^{-/-}$ mice received AT 10 mg/kg/d (AT+) or water alone (AT-) for 3 days. Hearts were harvested and subjected to ELISA and immunoblotting. S-nitrosylation of COX2 was assessed by the "Biotin Switch" assay. To verify that the immuno-precipitates contained COX2, membranes were stripped and blots with anti-COX2 antibodies. **Results:** COX2 expression in the AT- groups was very low. AT increased COX2 expression in WT (4,287±104%) and $eNOS^{-/-}$ (4,354±101%), but not $iNOS^{-/-}$ mice (101±3%). However, myocardial 6-keto-$PGF_{1\alpha}$ levels (81.4±0.2 vs. 15.7±0.1 pg/ml; $p<0.001$) were increased by AT only in WT mice, but not in $eNOS^{-/-}$ (16.5±0.1 vs. 15.6±0.1 pg/ml) or $iNOS^{-/-}$ (15.9±0.2 vs. 15.3±0.1 pg/ml) mice. The "Biotin Switch" assay shows that COX2 was S-nitrosylated only in WT mice. Although eNOS is activated by AT in the $iNOS^{-/-}$ mice, there is no S-nitrosylation and activation of COX2. **Conclusions:** COX2 is activated by S-nitrosylation only in WT mice. Although iNOS is intact in $eNOS^{-/-}$ mice, it is not activated and therefore, does not S-nitrosylate the AT-induced upregulated COX2.



a. S-nitrosylation of COX2 in the AT+ WT, but not in the AT- WT mouse, or the $eNOS^{-/-}$ and $iNOS^{-/-}$ mice. b. Immunoblotting with COX2 after stripping the membranes, showing the precipitates in the AT+ WT and $eNOS^{-/-}$, but not the AT+ $iNOS^{-/-}$ mice.

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Pioglitazone and Atorvastatin Augment Myocardial Production of 15-Epi-Lipoxin A_4 in the Rat Heart

Yochai Birnbaum, Yumei Ye, Yu Lin, Sheldon Y Freeberg, Shawn P Nishi, Juan D Martinez, Ming-He Huang, Barry F Uretsky, Jose R Perez-Polo; Univ of Texas Med Branch, Galveston, TX

Background: Both statins and thiazolidinediones have anti-inflammatory properties. However, the exact mechanisms underlying these effects are unknown. We investigated whether ATV and PIO increase myocardial content of lipoxin A_4 and 15(R)-epi-lipoxin A_4 (15ELX), both arachidonic acid products with strong anti-inflammatory properties. **Methods and Results:** Experiment 1: Rats received 3-day pretreatment with: 1) water; 2) PIO 10 mg/kg/d; 3) ATV 10 mg/kg/d; or 4) PIO+ATV. Experiment 2: Rats received: 1) water; 2) PIO+ATV; 3) PIP+ATV and valdecoxib, a selective COX2 inhibitor; 4) PIO+ATV and zileuton, a selective 5-lipoxygenase inhibitor; or 5) zileuton alone. There were 4 rats in each group. Heart were harvested and analyzed for myocardial lipoxin A_4 and 15ELX levels, and COX2 and 5-lipoxygenase protein expression. ATV (1.10±0.02 ng/mg; $p<0.001$ vs. sham) and PIO (0.98±0.02 ng/mg; $p<0.001$ vs. sham) significantly increased myocardial 15ELX levels compared to the sham-treated group (0.51±0.02 ng/mg). Myocardial 15ELX were significantly higher in the PIO+ATV group (1.29±0.02 ng/mg; $p<0.001$ vs. each other group). Both valdecoxib and zileuton abrogated the PIO+ATV increase in 15ELX, whereas zileuton alone had no effect. PIO, ATV and their combination resulted in a small increase in myocardial lipoxin A_4 levels, which was not statistically significant. ATV alone, or in combination with PIO markedly augmented COX2 expression. The effect of PIO on COX2 expression was smaller. Myocardial expression of 5-lipoxygenase was not altered by PIO, ATV and their combination. **Conclusions:** Both PIO and ATV increases myocardial levels of 15ELX, an arachidonic acid product with anti-inflammatory properties. This finding may explain the anti-inflammatory properties of both PIO and ATV.

WITHDRAWN **P160**

P161

A Role for Interleukin-Converting Enzyme in Heart Failure

Sabine Merkle, Monika Buitrago, Rudolf Virchow Ctr, Wuerzburg, Germany; Michael P Schoen, Dermatology, Wuerzburg, Germany; Stefan Frantz, Internal Medicine, Wuerzburg, Germany; Robert J A Frost, Rudolf Virchow Ctr, Wuerzburg, Germany; Eva Schmitteckert, Martin J Lohse, Inst of Pharmacology and Toxicology, Wuerzburg, Germany; Stefan Engelhardt; Rudolf Virchow Ctr, Wuerzburg, Germany

Apoptosis of cardiomyocytes is increased in heart failure and has been demonstrated to be a crucial factor for the progression of the disease. For the induction of cardiomyocyte apoptosis

proapoptotic caspases are of central importance. In contrast, the role of *proinflammatory* caspases is unclear. Interleukin converting enzyme (ICE, caspase-1) has been described as a *proinflammatory* caspase that generates active IL-1beta. This study aims to elucidate the cardiac function of ICE. Gene array analysis in a murine heart failure model showed upregulation of myocardial ICE. We confirmed this both in a murine heart failure model as well as in human heart failure (Immunoblot, $+123\pm14\%$, $p<0.0001$). To investigate if the upregulation of ICE is causally involved in heart failure, we generated transgenic mice with cardiomyocyte specific overexpression of ICE. These mice reveal a strong induction of cardiomyocyte apoptosis starting at one month of age (TUNEL, $+407\pm118\%$, $p<0.01$). This is followed by fibrosis and cardiomyocyte hypertrophy (cross-sectional area, $+62\pm4\%$, $p<0.05$). Importantly, cardiomyocyte hypertrophy is not paralleled by an increased ventricular weight. ICE-TG hearts ultimately displayed left ventricular dilatation and wall thinning. Additionally, these mice exhibit a impaired cardiac function as seen in decreased dp/dtmax values (7370 ± 1300 (WT) vs. 4550 ± 1020 mmHg/s (TG)) and increased LVEDP-values ($+763\pm335\%$)). These alterations are not due to increased IL-1beta formation. Adenoviral expression of ICE in primary rat cardiomocytes potently induced both activation of caspase-3 (Immunoblot, $+99\%\pm17\%$, $p=0.001$) and -9 and cardiomyocyte apoptosis (TUNEL, $+776\pm42\%$ vs. LacZ control virus), that could be prevented by an ICE-specific inhibitor. To test the role of endogenous ICE for cardiomyocyte apoptosis we employed ischemia-reperfusion as a strong apoptotic stimulus in ICE-deficient mice. Depletion of endogenous ICE protects from cardiomyocyte apoptosis (3-fold reduction,ICE-KO vs. WT,$p<0.05$). In contrast to previous findings which imply a proinflammatory role of ICE, these data infer a primary proapototic role for ICE in cardiomyocytes. Our findings indicate a functional role for ICE in cardiomyocyte apoptosis.

P162

ANP-cGMP-PKG Signaling Blocks TGF-β1-Induced Myofibroblast Transformation and Phospho-Smad3 Nuclear Translocation, but Not Smad3 Phosphorylation, in Mouse Cardiac Fibroblasts

Yiu-Fai Chen, Dajun Wang, Dongqi Xing, Peng Li, Xu Cao, Suzanne Oparil; Univ of Alabama at Birmingham, Birmingham, AL

Background: We have shown that atrial natriuretic peptide (ANP) modulates the development of pressure overload-induced cardiac hypertrophy; and ANP, via the cGMP-protein kinase G (PKG) signaling, has direct anti-fibrogenic actions on heart by modulating expression of extracellular matrix (ECM) molecules in cardiac fibroblasts (CFs). Transforming growth factor (TGF)-β1, via the Smad signaling, stimulates ECM expression and fibrosis in heart under stress conditions. **Methods:** To test the specific hypothesis that activation of ANP-cGMP-PKG signaling interrupts TGF-β1 signaling by inhibiting TGF-β1-induced phospho-Smad3 (pSmad3) nuclear translocation and myofibroblast (MF) transformation in CFs, quiescent mouse CFs were pre-treated with ANP (1 uM) or 8-Br-cGMP (a cGMP analog, 1 mM) for 30 min, and then exposed to TGF-β1 (1 ng/ml) for 30 min (for Western blot and nuclear translocation) or 24 hrs (for MF transformation). pSmad3 protein levels were assessed by Western analysis. CFs were stained with anti-pSmad3 antibody to assess pSmad3 nuclear translocation by confocal microscopy. Numbers of MFs were assessed using α-smooth muscle actin (α-SMA) staining. Separate groups of CFs were pretreated with KT5823 (a PKG inhibitor, 1 uM) 30 min before exposure to ANP or cGMP. **Results:** ANP and cGMP inhibited TGF-β1-induced nuclear translocation of pSmad3, but did not inhibit TGF-β1-induced phosphorylation of Smad3. Nearly all cells in TGF-β1 treated CFs were α-SMA positive and contained organized α-SMA filaments, indicating complete transformation of CFs to MFs. Pretreatment with cGMP completely inhibited TGF-β1-induced MF transformation (21% of total cells), but did not change basal (23% of total cells) MF numbers. KT5823 blocked the effects of ANP and cGMP. **Conclusion:** These results delineate a specific molecular mechanism by which activation of ANP-cGMP-PKG signaling pathway has direct anti-fibrogenic actions on heart by inhibition of TGF-β1-induced pSmad3 nuclear translocation, MF transformation and expression of ECM molecules in CFs. We hypothesize that interaction between the ANP and TGF-β1 signaling pathways at this level may play an important role in the pathogenesis of cardiac fibrosis and remodeling under stress conditions.

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Withdrawn

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BRCA1-Associated Protein 2: A Novel Modulator of ERK-MAPK Signaling in Heart Failure

Tim Seidler, Peter Schott, Cornelia Grebe, Manuel Keller, Albrecht Schmidt, Harald Kogler, Gerd Hasenfuss; Univ of Goettingen, Goettingen, Germany

Mitogen activated protein kinase (MAPK) signalling can profoundly modulate hypertrophy and heart failure. BRCA1 Associated Protein 2 (BRAP) was recently identified as an important modulator of Ras dependent MAPK activation in non-cardiac cells. We identified BRAP to be differentially regulated upon load induced heart failure in a proteomics study. Thus, the aim of this study was to analyse the role of BRAP in the myocardium. Adenoviruses for overexpression of BRAP and Raf-BXB, a constitutively active MAPK, were created to study the effects of BRAP in isolated rat cardiac myocytes. Using Real-Time PCR and Western Blot, BRAP expression was analyzed in two rat models (Monocrotaline (MCT)- and pulmonary banding-induced) RV hypertrophy. Further, the expression of BRAP was analysed in non-failing and failing human myocardium. Transgenic mice, overexpressing BRAP cardiac-specifically, were created and analysed. BRAP was found to be upregulated in the hypertrophied RV myocardium of both animal models (BRAP/GAPDH mRNA ratio MCT 0.0051 ± 0.0009 vs. control 0.0031 ± 0.0003, $p<0.002$ ($n=9$); pulmonary banding 0.0047 ± 0.0008 vs. control 0.0032 ± 0.0002, $p<0.02$ ($n=7$)). Western blot results confirmed the mRNA data. Likewise, BRAP was expressed at significantly higher levels in human failing myocardium (DCM). In isolated myocytes, BRAP overexpression profoundly suppressed MEK and ERK MAPK signalling under baseline conditions and following Raf-BXB-stimulation. This suggests an inhibitory effect of BRAP on both adaptive hypertrophy and anti-apoptotic signalling. Three independently created BRAP-transgenic (TG) mouse lines in two different genetic backgrounds exhibited grossly enlarged hearts, compared to wildtype littermates (WT). Echocardiography confirmed dilation (left ventricular end-diastolic diameter 4.57 ± 0.17 vs. $3,57\pm0.04$ mm; $p<0.05$ BRAP vs. WT; $p<0.05$; TG vs WT) and significant reduction of fractional shortening ($14\pm3\%$ vs. $40\pm0.4\%$; BRAP TG vs. WT; $p<0.001$). Kaplan-Maier analysis showed a significantly decreased survival with 50% of mice dying before the age of 24 weeks. These data indicate that BRAP is differentially expressed in heart failure and controls cardiac size and function via inhibition of the ERK MAPK pathway.

P165

Angiotensin II Receptor Imbalance Associated with Neonatal Cardiac Growth Restriction Is a Prelude to Adult Cardiac Hypertrophy

Enzo R Porrello, Univ of Melbourne, Melbourne, Australia; Angelo D'Amore, Baker Heart Rsch Inst, Melbourne, Australia; Claire L Curl, Catherine E Huggins, Stephen B Harrap, Univ of Melbourne, Melbourne, Australia; Walter G Thomas, Baker Heart Rsch Inst, Melbourne, Australia; Lea M Delbridge; Univ of Melbourne, Melbourne, Australia

The Hypertrophic Heart Rat (HHR) displays spontaneous cardiomyocyte hypertrophy in association with an apparent reduction in myocyte number in adulthood. This suggests the possibility of reduced hyperplasia or increased apoptosis during early cardiac development. The angiotensin AT_1 and AT_2 receptor subtypes have been implicated in both cellular growth and apoptosis, but the precise mechanisms are unclear. The aim of this study was to determine the relationship between cardiac AngII receptor expression levels and neonatal cardiomyocyte growth and apoptotic responses in the HHR compared with the Normal Heart Rat (NHR) control strain. Cardiac tissues were freshly harvested from male HHR and NHR at several developmental stages (p2 and 4, 6, 8, 12wks). HHR cardiac weight indices were considerably smaller than NHR at day 2 (4.33 ± 0.19 vs 5.01 ± 0.08 mg/g), but 'caught-up' to NHR by 4 weeks (5.10 ± 0.15 vs 5.16 ± 0.11 mg/g). By 12 weeks, HHR hearts were 27% larger than NHR. Tissue AT_{1A} and AT_2 mRNA expression levels were quantified by real-time RT-PCR. Relative to NHR, HHR neonatal hearts exhibited a 4.6-fold higher AT_2/AT_1 mRNA expression ratio. Cultured neonatal cardiomyocytes were infected with AT_{1A} and/or AT_2 receptor-expressing adenoviruses to achieve a physiological level of receptor expression (150 fmol receptor protein/mg total cell protein). In addition, to emulate receptor expression in neonatal HHR hearts, cells were co-infected with AT_{1A} and AT_2 receptors at a 4:1 ratio. Apoptosis incidence was studied by morphological analysis after 72 hours exposure to 0.1 μM AngII. When infected with the AT_{1A} receptor alone, a higher proportion of HHR myocytes appeared apoptotic than NHR ($22.7\pm4.1\%$ vs $1.1\pm0.6\%$, $P<0.001$). This implies that intrinsic differences predispose HHR cells to accentuated AT1-mediated apoptosis. Interestingly, the bax-1/bcl-2 mRNA expression ratio was significantly higher (50%) in HHR neonatal hearts. When cells were co-infected with AT_{1A} and AT_2 receptors, evidence of apoptosis in HHR cells virtually disappeared ($0.4\pm0.1\%$). These findings suggest a novel capacity of AT_2 receptors to counteract accentuated AT_{1A} receptor-induced apoptosis in the HHR in early cardiac growth.

P166

Cardiac Myosin Activator CK-1316719 Increases Myocyte Contractility and Myosin ATPase Activation in a Model of Heart Failure

Robert Anderson, Raja Kawas, Maria Pokrovskii, Guillermo Godinez, Kenneth Lee, John Mak, Bradley Morgan, David Morgans, Jr, Fady Malik, Roman Sakowicz, Kathleen Elias; Cytokinetics, South San Francisco, CA

Improving cardiac contractility by directly activating cardiac myosin may be a preferred treatment for heart failure (HF) over the current inotropic therapies that increase intracellular calcium or act via second messenger activation. Previously we reported that small molecule myosin activators increase cardiac myocyte contractility without increasing intracellular calcium or inhibiting phosphodiesterase in non-failing cells. We now report cellular and biochemical responses to the myosin activator CK-1316719 in a rat model of HF. Heart failure was confirmed *in vivo* by significant decreases in fractional shortening (M-mode echocardiography) in 12-week post myocardial infarction (MI) Sprague Dawley rats compared to sham animals. Myocytes were isolated from the left ventricle and septum for cellular contractility, myofibril ATPase, and mRNA analysis. CK-1316719 (0.4–5 uM) treatment increased cellular contractility equivalently and in a dose dependent manner in both sham and MI myocytes. In contrast, isoproterenol treatment (20 nM) resulted in a truncated response in only MI cells demonstrating β-adrenergic desensitization, a characteristic of HF. Increased ANF mRNA expression in MI cells corroborated HF at the cellular level. In fura-2 loaded myocytes, CK-1316719 increased cellular contractility in both MI and sham cells without increasing the calcium transient, consistent with the proposed mechanism of action. Sham and MI myofibrils displayed similar dose dependent increases in ATP turnover (pCa 6 and 7) with CK-1316719. In summary, treatment with CK-1316719 results in equivalent increases in cardiac myocyte contractility with no change in the calcium transient in both sham and MI cells, and similar ATPase activation increases in sham and MI myofibrils. These results indicate that myosin activators are equally effective in non-HF and HF states and may be useful therapeutics in the treatment of heart failure.

P167

Chronic PDE5A Inhibition Reverses Aging-Associated Loss of AKT Activation in the Heart

David Majure, Thomas Mulhearn, Ryan Tedford, Milena Gebska, Mobusher Mahmud, Hazin El-Haddad, Blake Stevenson, Michael Bridges, Biljana Musicki, Arthur Burnett, Hunter Champion; The Johns Hopkins Med Inst, Baltimore, MD

With advancing age, the cardiovascular system develops decreased endothelial vasoreactivity, prolonged diastolic relaxation, and a reduction in AKT activation. As both increased NOS abundance and diminished nitric oxide signaling have been reported in the aging CV system, the role of NO in the adaptations of the aging CV system remain controversial. Here we tested