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encoding soluble epoxide hydrolase (sEH), attenuated the development of hyperglycemia in response to the pancreatic toxin, streptozotocin. Immunoblots of five week old Akita heart homogenates showed a 30% increase in expression of sEH ($P<0.01$). By twelve weeks, cardiac sEH increased 145% +/- 20% compared to control littermates ($P<0.001$) but no differences were found in hearts from 3 or 4 week old mice. qPCR results suggest that these changes are driven largely by transcriptional regulation with no differences in EPHX2 gene expression at 3 and 4 weeks and a 50% and 100% increase at 5 and 12 weeks respectively. In addition, immunoblots indicate an approximate 100% increase in sEH in gastrocnemius tissue of 12 week old Akita mice ($P<0.01$). Our results suggest that an increase in sEH is a key factor in the development of diabetic cardiomyopathy. Furthermore, the increased presence of this protein in multiple tissues suggests that it may be useful as a diagnostic target.

1601-Pos Board B493

Dyscholesterolemia Alters L-Type Calcium Current Which Protects against Ischemia-Induced Ventricular Tachycardia and Ventricular Fibrillation

Antonius Baartscheer, Vincent Wekker, Cees A. Schumacher, Arie O. Verkerk, Marieke W. Veldkamp, Ruben Coronel.
Academic Medical Center, Amsterdam, Netherlands.

Background: Hypercholesterolemia is associated with alteration of the lipid composition of the sarcolemma which may cause augmentation of the L-type calcium current (*ICaL*) and appears to be protective against ventricular fibrillation in patients with myocardial infarction.

Hypothesis: Hypercholesterolemia increases *ICaL* resulting in action potential (AP) prolongation which protects against ischemia induced arrhythmias.

Methods: ECG was measured in LDL-receptor knockout (LDLr-/-) mice with elevated LDL cholesterol and wild type mice (WT). AP, *ICaL* and calcium handling were determined in left ventricular myocytes. In perfused hearts the left anterior descending artery was ligated. Arrhythmia inducibility was tested every 30s by three premature stimuli with a coupling interval of 10ms longer than the refractory period followed by a 500ms pause. Area at risk (AAR) was determined with Evans Blue.

Results: Cholesterol concentration in left ventricular myocytes was higher in LDLr-/- mice than WT (34.4 ± 2.8 vs 25.5 ± 0.4 μmol/gr protein) resulting in AP and QTc prolongation (APD90 102 ± 4 vs 84.4 ± 3.1, QTc 50.9 ± 1.3 vs 43.8 ± 1.18ms and increased *ICaL* (12.1 ± 1.1, and 9.4 ± 0.7 pA/pF) and calcium transient amplitude (74 ± 4 vs 35 ± 3 nM). The incidence of induced premature beats was not significantly different. In 7/7 LDLr-/- and 8/9 WT hearts arrhythmias could be provoked. In LDLr-/- hearts, only 5/412 induction attempts resulted in VT/VF whereas in WT hearts 30/477 induction attempts resulted in VT/VF. ($p<0.05$) The AAR was not different between LDLr-/- and WT mice.

Conclusion: Hypercholesterolemia protects against the occurrence of reentrant arrhythmias during myocardial ischemia by QTc and AP prolongation due to increased *ICaL*.

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Fatty Acid Overload to Mitochondria Promotes Myocardial Insulin Resistance in Differentiated H9C2 Myocytes

Masao Saotome, Mamoru Nobuhara, Tomoyuki Watanabe, Hiroshi Satoh, Hideharu Hayashi.
Hamamatsu University Schl. of Med., Hamamatsu, Japan.

OBJECTIVE: In chronic heart failure, it is well known that an increase in serum fatty acid (FA) due to adrenergic stimulation induces myocardial insulin-resistance, which further deteriorates myocardial function. Since precise mechanisms of pathogenesis in FA-induced myocardial insulin-resistance is still elusive, we in this study investigated the relationship between FA-induced myocardial insulin-resistance and mitochondrial dysfunction, using an ex vivo insulin-resistant myocytes model.

METHODS and RESULTS: The differentiated H9c2 myocytes were treated with saturated FA (palmitate; 0.2 mM) for 24 hours to produce the ex vivo insulin-resistant myocytes. The palmitate-treated myocytes exhibited an impaired insulin (100 nM)-mediated 2-deoxy-D-glucose (2DG) uptake (1.0 ± 0.1 fold increase from without insulin vs. 1.7 ± 0.1 of control, $P< 0.01$) and attenuated phosphorylation of insulin signaling molecules (IRS-1 and AKT), indicating insulin-resistance. When myocytes were pretreated with perhexiline (2 μM; an inhibitor of mitochondrial FA uptake) the reduced 2-DG uptakes (1.2 ± 0.1 fold increase from without insulin vs. palmitate-treated myocytes, $P< 0.01$) and attenuated phosphorylation of insulin-signaling were restored. The palmitate-treated myocytes revealed the intracellular ATP reduction (74 ± 6 % decrease from control, $P< 0.01$; luciferase assay), mitochondrial membrane potential depolarization (JC-1 ratio of 590/528 nm: 2.9 ± 0.1 vs. 5.3 ± 0.2 of control, $P< 0.01$), indicating mitochondrial dysfunction. When the myocytes were pretreated with perhexiline, the reduced intracellular ATP levels (32 ± 14 % increase from palmitate-treated myocytes, $P< 0.01$) and depolarized mitochondrial membrane potential (JC-1 ratio: 3.5 ± 0.1 vs. palmitate-treated myocytes, $P< 0.01$) by palmitate were restored, indicating the improvement in mitochondrial function.

CONCLUSION: Our findings indicated that mitochondrial dysfunction via mitochondrial FA overload may underlie the pathogenesis of myocardial insulin-resistance under HF.

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Oxidative Stress by Hydrogen Peroxide Reduces Cardiac Contractile Protein Performance and Enhances Cardiomyocyte Calcium Release

Ting Yi, Bradley M. Palmer.
Unversity of Vermont, Burlington, VT, USA.

Cardiac oxidative stress correlates with diminished left ventricular function and has emerged as a public health concern. Hydrogen peroxide (H_2O_2) is considered the most relevant reactive oxygen species *in vivo*. Cardiac zinc status appears to protect against the detrimental effects of H_2O_2, although the mechanisms are not known. We examined the role of H_2O_2 in modulating the performance of cardiomyocytes in 36 rats divided into three diet groups: zinc deficient, normal and abundant. Cardiomyocytes were isolated and exposed to 50 μM H_2O_2. within 5 min intracellular zinc ion (Zn^{2+}) rose as observed by FluoZin-3. Confocal microscopy demonstrated that Zn^{2+} diffused away from I-band of the intact cardiomyocyte after H_2O_2. Zinc normal and abundant groups showed significant intracellular Zn^{2+} release (2.4 ± 0.4 nM and 1.9 ± 0.4 nM, respectively) compared to zinc deficient group (0.7 ± 0.2 nM). There were H_2O_2-dependent changes in sarcomere and calcium dynamics in all three zinc diet groups, although no differences in response to H_2O_2 among the groups. Sarcomere peak shortening was significantly increased ($p<0.001$) and diastolic sarcomere length was reduced after H_2O_2 exposure compared to control. Peak systolic calcium was elevated ($p<0.05$) by H_2O_2. We then explored the H_2O_2 effects on cardiac contractile proteins. Skinned rat myocardium displayed shorter myosin cross-bridge time-on and reduced tension after 100 μM H_2O_2 exposure compared to control condition. In sliding filament experiments actin velocity was decreased from 0.74 ± 0.17 μm/s to 0.30 ± 0.1 μm/s after 100 μM H_2O_2 pretreatment of purified rabbit cardiac beta-myosin for 30 min. These data collectively suggest that oxidative stress due to H_2O_2 enhances calcium availability at the cellular level while reducing contractile protein performance at the molecular level and that cardiac zinc status does not protect against changes in cardiopmyocyte performance after H_2O_2 exposure.

1604-Pos Board B496

Hexokinase Isoforms and Glucose Metabolism in Adult and Neonatal Cardiac Myocytes

Guillaume Calmettes, Scott John, James N. Weiss, Bernard Ribalet.
University of California Los Angeles, Los Angeles, CA, USA.

Localization of hexokinase (HK) isoforms to the cytoplasm or mitochondria controls their anabolic (glycogen synthesis) and catabolic (glycolysis) activities. In this study, we compared the effects of HKI and HKII in isolated adult (ARVM) and neonatal rat ventricular myocytes (NRVM) using a set of novel genetically-encoded optical imaging tools to track, in real-time, the subcellular distributions of HKI and HKII, as well as the functional consequences on glucose utilization. We show that HKII, the predominant isoform in ARVM, dynamically distributes between the mitochondria and cytoplasm. Removal of extracellular glucose displaces HKII from mitochondria in ARVM, but not in NRVM, whereas iodoacetate (IAA) displaces HKII in both. HKI, the predominant isoform in NRVM, always remains bound to mitochondria when overexpressed in either AVRM and NRVM, and is not displaced by the above interventions. In ARVM, overexpression of HKI, but not HKII, increased glycolytic activity. In NVRM, knock-down of HKI, but not HKII, decreased glycolytic activity. Swapping the N terminus between HKI and HKII, as well as specific mutations, revealed that the N terminus of HKI is necessary, but not sufficient, for high affinity mitochondrial binding to promote glycolysis. In conclusion, HKI and HKII play major roles in defining the different metabolic profiles of ARVM and NRVM, accounting for the markedly increased glycolytic activity of NRVM.

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Unchanged Mitochondrial Organization and Compartmentation in Creatine Deficient GAMT-/- Mouse Heart

Jelena Branovets[1], Mervi Sepp[1], Svetlana Kotlyarova[1], Natalja Jepihhina[1], Niina Sokolova[1], Dunja Aksentijevic[2], Craig A. Lygate[2], Stefan Neubauer[2], Marko Vendelin[1], Rikke Birkedal[1].
[1]Institute of Cybernetics at TUT, Tallinn, Estonia, [2]University of Oxford, Oxford, United Kingdom.