817-Pos Board B603
Biophysical Properties of UCP1
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Brown adipose tissue (BAT) is a specialized mammalian organ that coverts body fat reserves into heat. This conversion is mediated by BAT mitochondria and is important for maintaining body temperature and reducing fat depositions. Burning fat in BAT mitochondria does not result in ATP synthesis due to unusually high conductance of the inner mitochondrial membrane (IMM). This BAT-specific conductance dissipates the mitochondrial electrochemical gradient used in other tissues for ATP production and converts the energy of fatty acid oxidation into heat. The molecule responsible for the high conductance of the IMM and uncoupling of oxidative phosphorylation in BAT mitochondria is uncoupling protein 1 (UCP1). UCP1 is a BAT-specific protein that belongs to the superfamily of mitochondrial solute carriers (SLC25). UCP1-mediated uncoupling is activated by fatty acids and inhibited by purine nucleotides. In spite of the fact that UCP1 was identified decades ago, the mechanism of UCP1 operation is unknown due to lack of direct methods to study its activity. Here, we resolve the problem by directly recording UCP1 currents across the IMM of BAT mitochondria using the patch-clamp technique. To identify UCP1 current, we compare currents recorded from the wild-type and UCP1 (-/-) mitochondria. The detailed electrophysiological analysis demonstrated that UCP1 translocates fatty acid anion in symport with H^+ , while the fatty acid hydrophobic tail prevents dissociation of fatty acid from UCP1, causing UCP1 to operate as H^+ carrier. This work establishes the mechanism of fatty acid dependent mitochondrial uncoupling which is implicated in metabolic and age-related diseases.

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Role of Polyhydroxybutyrate in Mitochondrial Calcium Transport
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Polyhydroxybutyrate (PHB) is a biological polymer which belongs to polyesters class and ubiquitously present in all living organisms. It has been demonstrated that mammalian mitochondrial membranes contain PHB polymer consisting of up to 120 butyrate residues. We found before that mitochondrial PHB complexed with Ca^{2+} and inorganic polyphosphate forms channels with properties similar to mitochondrial permeability transition pore. It is also experimentally proven that PHB in its free form possesses ionophoretic properties and mediates Ca^{2+} transport through the model phospholipid membranes. We hypothesized that PHB might significantly contribute to the process of mitochondrial Ca^{2+} transport. To test this idea we investigated Ca^{2+} transport in mitochondria with decreased levels of PHB. Mitochondrial PHB was reduced enzymatically by targeted expression of specific bacterial PHB hydrolyzing enzyme (PhaZ7) in mitochondria of mammalian cultured cells. Experiments were performed using transiently transfected cultured cells: HepG2, U87 and HeLa. PHB deficiency induced changes in mitochondrial metabolism resulting in decreased mitochondrial membrane potential (measured by TMRM) in HepG2 but not in U87 and HeLa cells. Kinetics of mitochondrial Ca^{2+} transport was measured using mitochondrial Ca^{2+} sensitive fluorescent probe - x-Rhod-1 and confocal microscopy. Mitochondrial Ca^{2+} signal was stimulated either by addition of ATP or Histamine in experiments with intact cells or by addition of increased concentrations of calcium to the recording solution in digitonin permeabilized cells. We found that overexpression of mitochondrially targeted PHB depolymeraze in mammalian cultured cells dramatically inhibits their Ca^{2+} uptake. Ca^{2+} uptake in these cells was restored by addition of mimic of calcium uniporter with electrogenic Ca^{2+} ionophore properties - ferutinin. Our data suggest that PHB is previously unrecognized essential component of mitochondrial Ca^{2+} uptake system.

819-Pos Board B605
Increased Activity of Mitochondrial Complex II in Rabbit Heart Failure is Associated with Reactive Oxygen Species Generation and Impaired Excitation-Contraction Coupling
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Background: In heart failure (HF), the increase in cardiac adrenergic tone, while initially beneficial, ultimately contributes to damage to the failing heart. The aim of this study was to evaluate the mechanisms responsible for cell damage during beta-adrenergic stimulation in a rabbit volume- and pressure-overload HF model.
Methods and Results: Field stimulation (1 Hz) of single left ventricular HF myocytes in combination with beta-adrenergic stimulation (isoproterenol, 1 microM) was accompanied by spontaneous pro-arrhythmic Ca^{2+} release (Ca^{2+} waves), contractile dysfunction, and a robust increase in reactive oxygen species (ROS) production, eventually leading to cell death. In HF myocytes FAD/$FADH_2$ levels remained reduced and mitochondrial complex II (succinate dehydrogenase) activity was significantly elevated (by 86%). Increased complex II activity, however did not lead to an increase in ADP-dependent respiration, indicative of an electron leak at complex II. Mitochondrial complex I-mediated state-3 respiration was decreased by 77%, while state-2 respiration remained unchanged. Supplementation of HF myocytes with substrate for complex II (10 mM dimethyl-succinate) caused a dramatic increase in rotenone-sensitive mitochondrial ROS generation compared to control cells and to HF cells treated with complex I substrates. Moreover, dimethyl-succinate itself induced spontaneous Ca^{2+} release in form of Ca^{2+} waves that was further augmented by isoproterenol and prevented by cell pre-treatment with the anti-oxidant Trolox (1 mM). The complex II inhibitor thenoyl-trifluoroacetone (100 microM) significantly decreased mitochondrial ROS generation and normalized isoproterenol-induced Ca^{2+}-transients and cell shortening.
Conclusion: Increased activity of mitochondrial complex II in rabbit HF is a major mediator of oxidative stress leading to impairment of Ca^{2+} handling and contractility.

820-Pos Board B606
Changes in Mitochondrial Calcium and ROS during Ischemia-Reperfusion in Polyphosphate-Depleted Cardiomyocytes
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Loss of mitochondrial function plays a critical role for cardiac cell death during ischemia-reperfusion (I/R) injury. Mitochondrial dysfunction during I/R is caused by a combination of Ca^{2+} overload and increased ROS generation, which lead to permeability transition pore (mPTP) opening and disruption of energy metabolism. We have shown previously that depletion of mitochondrial inorganic polyphosphate (polyP) protected cells from mPTP opening during I/R. The aim of this study was to investigate whether the protective effect of polyP depletion was related to changes in mitochondrial Ca^{2+} and ROS generation. In adult rabbit ventricular myocytes polyP levels were decreased by adenoviral expression of a mitochondrially targeted polyP hydrolyzing enzyme (PPX). I/R was induced by exposing cells to glucose-free Tyrode solution containing 20 mM 2-deoxyglucose and 2 mM NaCN, pH 6.4, followed by superfusion with standard Tyrode solution. In control cells, a significant increase in mitochondrial Ca^{2+} ($[Ca^{2+}]_m$), ROS generation and mPTP opening was observed during ischemia together with a depolarized mitochondrial membrane potential. The increase in $[Ca^{2+}]_m$ was only partially sensitive to Ru360 (Ca^{2+} uniporter blocker), but significantly affected by cyclosporine A (mPTP de-sensitizer) indicating possible Ca^{2+} entry through mPTP. In polyP-depleted cells, however, the opening of the mPTP was significantly diminished despite higher levels of ROS during ischemia. Moreover, in polyP-depleted cells the increase in $[Ca^{2+}]_m$ during ischemia was decreased by Ru360, but was not sensitive to CsA. In both control and polyP-depleted cells ROS generation was attenuated by Ru360. These data indicate that mitochondrial Ca^{2+} uptake during ischemia triggers mPTP opening and ROS generation. The fact that decreased levels of polyP provide protection against mPTP opening despite increased levels of $[Ca^{2+}]_m$ and ROS, support the hypothesis that mitochondrial polyP likely acts as a Ca^{2+} sensor of the mPTP.

821-Pos Board B607
Cardiac Vulnerability to Ischemia/Reperfusion Injury Drastically Increases in Late Pregnancy
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Although the murine late pregnant (LP) heart is speculated to be a better functioning heart during physiological conditions, the susceptibility of LP hearts to I/R injury is still unknown. The aims of this study were to investigate the cardiac vulnerability of LP rodents to ischemia/reperfusion (I/R) injury and to explore the involvement of the mitochondrial function in this vulnerability. In-vivo female rat hearts (non-pregnant (NP) or LP) or Langendorff-perfused

mouse hearts were subjected to ischemia followed by reperfusion. The infarct size was ~4 fold larger in LP animals compared to NP both in the in-vivo rat model and in the ex-vivo mouse model. The hemodynamic parameters were similar between NP and LP before ischemia. However, after ischemia, the functional recovery was extremely poor in LP mice comparing to NP mice. RPP was reduced from 12818 ± 1485mmHg*beats/min in NP to 1614 ± 438mmHg*beats/min in LP mice. Interestingly, the poor functional recovery and the larger infarct size in LP was partially restored one day post-partum(PP1) and almost fully restored one week post-partum(PP7) to their corresponding levels in NP hearts(e.g. RPP=4716 ± 584mmHg*beats/min in PP1 and 9604 ± 1216mmHg*beats/min in PP7). To explore the role of mithocondiral function in the higher vulnerability of LP hearts to I/R injury, mithocondria was isolated from NP and LP hearts. State 3 oxygen consumption and respiratory control index(RCI) in malate and glutamate energized mitochondria were significantly decreased in LP((93 ± 5 vs. 162 ± 19nAO/min/mg in NP, $P<0.05$; RCI=2.6 ± 0.24 in LP vs. 4.2 ± 0.22 in NP , $P<0.05$). The threshold for opening of the mitochondrial permeability transition pore (mPTP) was much lower in LP hearts (CRC=167 ± 10 vs. 233 ± 18nM/mg protein in NP, $P<0.01$). In conclusion, the higher vulnerability of LP hearts to I/R is associated with increased sensitivity of the mPTP opening to calcium overload and reduced mitochondrial respiration.

822-Pos Board B608

Ischemia Reperfusion Induced Arrhythmia are Prevented by Mitochondrial IK,ATP Opening by Mesenchymal Stem Cell (MSC) Paracrine Factors

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MSC transplantation after I/R injury reduces infarct size and improves cardiac function. There is evidence that this cardioprotective effect is mediated by paracrine signalling, but the mechanism remains yet to be determined. Isolated mouse ventricular myocytes (VMs) were used in an in vitro model of I/R to determine the effect of MSC conditioned tyrode (ConT) on the recovery of VMs from an ischemic challenge (15 min). Measurements of the mitochondrial membrane potential (Ψmito) with TMRM (100 nM), the intracellular Ca concentration (fluo-4/AM) and cell shortening were used as functional readouts. During ischemia VMs exhibit a depolarization of Ψmito and an increase in diastolic calcium concomitant to a decrease in cell contractility. Reperfusion with either Ctrl or ConT resulted in an increase in Ca transient amplitudes. Early After Depolarizations (EADs) frequently observed in Ctrl cells were reduced during ConT reperfusion (EADs: ConT:1% vs. Ctrl: 24%; at 1 min). ConT prolonged VM survival (ConT: 58% vs Ctrl: 33%; at 20 min) and Ca transients returned to pre-ischemic values. After I/R, Ψmito rapidly recovered in Ctrl as well as ConT; however, in Ctrl an exaggerated hyperpolarization of Ψmito (Ctrl: 6 of 9; ConT: 0 of 5 cells) was observed. This hyperpolarization could be prevented by supplementing Ctrl solution after I/R with the ROS scavenger mitoTEMPO (5 μM) or the IK,ATP opener (diazoxide, 200 μM). Enhanced hyperpolarization was induced by supplementing ConT with a blocker of IK,ATP 5-HD (500 μM) or PI3K/Akt inhibitors (LY: 10 μM; Akt iV: 20 μM). In conclusion we could demonstrate that MSC ConT protects VMs from I/R injury by attenuation of arrhythmic Ca release events and by delaying the recovery of Ψmito through PI3K/Akt mediated opening of IK,ATP.

823-Pos Board B609

Reduced Mitochondrial Dynamics in Skeletal Muscle of an Amyotrophyic Lateral Sclerosis Mouse Model

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Mitochondria are highly dynamic organelles that constantly undergo fusion and fission in order to maintain their normal functionality. Impairment of mitochondrial dynamics is implicated in various neurodegenerative disorders. Amyotrophic lateral sclerosis (ALS) is a fatal disease involving degeneration of motor neurons and atrophy of skeletal muscle. Our recent studies on ALS mouse model G93A have found that its skeletal muscle shows defective mitochondria associated with elevated intracellular Ca^{2+} transients during muscle contraction (*Yi JBC 2011*). It is known that an elevated intracellular [Ca^{2+}] alters mitochondrial mobility in various cell types (*Liu and Hajnóczky Int J Biochem Cell Biol 2009*). Expecting alterations, we evaluated mitochondrial mobility by expressing a photoactivatable green fluorescence protein (PA-GFP) (obtained from Addgene) in G93A skeletal muscle. The PA-GFP was first photo-activated in a small area (~ 10 μm × 10 μm) of a muscle fiber. The time-dependent migration of PA-GFP out of the original area was evaluated in both longitudinal and transversal directions. Migration of PA-GFP over one sarcomere distance (~2.2 μm) was defined as one migration step (*ms*). While mitochondria in normal muscle fibers showed 11.0 ± 1.6 *ms* in 10 min and 18.7 ± 2.3 *ms* in 20 min (n=13), mitochondria in G93A muscle only showed 1.8 ± 0.4 *ms* and 2.0 ± 0.4 *ms* in the respective intervals (n=12). This constitutes a near 10-fold reduction of mitochondrial mobility in ALS muscle. Studies to identify the causes of the reduced mitochondrial dynamics are ongoing. Supported by MDA and NIAMS/NIH.

824-Pos Board B610

Mitochondrial Respiration-Coupled Superoxide Production Underlies Glutamate Excitotoxicity in Motor Neurons

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Glutamate excitotoxicity is responsible for neuron death during both acute neuron injury and chronic neurodegeneration. However, its specific mechanism is not clear. Cytosolic calcium influx and excessive reactive oxygen species generation have been suggested to play important roles. We hypothesized that glutamate-induced neuron death is mediated by cytosolic calcium influx, subsequent mitochondrial permeability transition pore (PTP) openings, and triggered mitochondrial superoxide production. Cultured NSC-34 cells, the motor neuron-like cell line, were differentiated to NSC-34D cells, which express glutamate receptors. Confocal imaging of the NSC-34D cells expressing a newly developed superoxide indicator, mt-cpYFP, revealed a bursting superoxide production event in individual mitochondria, named superoxide flash. The frequency of superoxide flash was positively correlated to mitochondrial respiration. Interestingly, glutamate (1 mM) incubation stimulated superoxide flash activity up to 24 hr, but inhibited it after 48 hr. The glutamate-induced superoxide flash activity was accompanied by a transient cytosolic calcium influx, which was blocked by glutamate receptor inhibitors, MK-801 (10 μM) and NBQX (1 μM), and mitochondrial calcium uniporter inhibition. We previously showed that superoxide flash is a triggered event by PTP openings, which is modulated by calcium. Simultaneous imaging of mitochondrial membrane potential using TMRM and superoxide flash showed that dissipation of membrane potential accompanied each flash in NSC-34D cells. Further, glutamate-induced flash activity was potentiated by atractyloside, a PTP opener, and abolished by cyclosporine A, a PTP blocker. Finally, cell death occurred at 24 hr after glutamate incubation. SOD1 and SOD2 overexpression blocked the glutamate-induced superoxide flash activity and cell death. In summary, we identified a signaling pathway mediating the glutamate excitotoxicity in motor neurons. This pathway includes cytosolic calcium influx-associated mitochondrial calcium uptake, calcium induced PTP opening, and bursting superoxide production coupled to mitochondrial respiration.

Photosynthesis & Photoreceptors

825-Pos Board B611

Correlated AFM-Spectroscopy Imaging of Linear Light Harvesting Protein Aggregates in Bacterial Native Photosynthetic Membrane

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How light energy is harvested in a natural photosynthetic membrane through energy transfer is closely related to the stoichiometry and arrangement of light harvesting antenna proteins in the membrane. The specific photosynthetic architecture facilitates a rapid and efficient energy transfer among the light harvesting proteins (LH2 and LH1) and to the reaction center. Here we report the identification of linear aggregates of light harvesting proteins, LH2, in the photosynthetic membranes under ambient conditions by using atomic force microscopy (AFM) imaging and spectroscopic analysis. Our results suggest that the light harvesting proteins, LH2, can exist as linear aggregates of 2 to 8 proteins in the photosynthetic membranes, and the protein distributions are highly heterogeneous. LH2 antenna proteins are responsible for absorbing most of the light energy for photosynthesis, and efficient intra- and intermolecular energy transfers of LH2 complexes are important for the overall efficiency of the light harvesting mechanism. We combined AFM imaging and spectroscopic analysis with *J* aggregate theoretical calculations to characterize the linear aggregation of LH2. AFM images reveal the linear aggregation of LH2, where the LH2 complexes are tilted to the plane of the photosynthetic membrane. The spectroscopic results support the attribution of LH2