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.

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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.

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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.

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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