3,5-diiodo-L-thyronine (T2) administration to high fat fed rats, reduces adiposity and body weight gain by stimulating hepatic fatty acid oxidation with a concomitant less efficient utilization of lipid substrates. When injected into hypothyroid rats, T2 is able to increase resting metabolic rate, lipid metabolism and survival to cold. Skeletal muscle, endowed with significant mitochondrial capacity, is an important site for glucose disposal, lipid oxidation and thermogenesis, its mitochondrial proton-leak accounting for a significant fraction of resting metabolism, thus the effects of T2 on mitochondrial skeletal muscle would be of great physiological relevance. To test if T2 could affect mitochondrial skeletal muscle metabolism we injected a single dose of T2 (25 μg/100 g bw) in hypothyroid rats. Within 1 h following T2 injection we detected i) a significant increase in mitochondrial fatty acid oxidation rate (+80% vs hypothyroid rats) and total carnitine palmitoyl-transferase (CPT) activity (+35% vs hypothyroid rats), ii) the activation of AMPK-AcetylCoA carboxylase (ACC)-malonyl CoA signalling pathway, thus suggesting its involvement in the CPT activation by T2 iii) a significant increase (+55% vs hypothyroid rats) of the mitochondrial thioesterase-I activity and a less efficient utilization substrates through an induction of fatty acid-dependent mitochondrial uncoupling. These data give further insight on the effects of T2 highlighting mitochondrial skeletal muscle as one of its targets.

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S8.22 Regulation of mitochondrial complex I by nitric oxide during hypoxia

Alexander S. Galkin, Salvador Moncada
Wolfson Institute for Biomedical Research, UCL, London, UK
E-mail: a.galkin@ucl.ac.uk

Nitric oxide (NO) is known to cause persistent inhibition of mitochondrial respiration as a result of *S*-nitrosation of NADH: ubiquinone oxidoreductase (complex I). Mitochondrial complex I can exist in two interconvertible forms, active (A) and de-active, dormant (D). When the enzyme is idle, the catalytically active A-form is spontaneously converted into the D-form. We have now found in mitochondrial membranes that the conformational state (A or D) of complex I is an important factor for the interaction of the enzyme with nitrosating agents, since only the D-form was susceptible to inhibition by nitrosothiols and peroxynitrite. We also have observed the conversion of the enzyme into the D-form during hypoxic incubation of J774 macrophages, indicating that slowing down of the activity of the mitochondrial respiratory chain in hypoxic conditions leads to spontaneous de-activation of complex I. Treatment of the cells with NO following hypoxic incubation resulted in persistent inhibition of complex I. Thus de-activation of complex I and consequent modification by nitrosating agents prevents transition to the A-form so that the enzyme becomes locked in its D-form. Such locking of complex I in the D-form may have pathophysiological consequences, and may vary depending on the duration of hypoxia, type of tissue and/or the presence of natural effectors of A/D transition such as calcium and free fatty acids.

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S8.23 Oxygen dependency of mitochondrial respiratory chain function in hypoxic pulmonary vasoconstriction

Natascha Sommer, Hossein A. Ghofrani, Ralph T. Schermuly, Werner Seeger, Fritz Grimminger, Norbert Weissmann
Medical Clinic II/V, University of Giessen Lung Center, Germany
E-mail: natascha.sommer@uglc.de

The aim of this study was to correlate the oxygen affinity and redox state of mitochondria of pulmonary arterial smooth muscle cells (PASMC) with hypoxic pulmonary vasoconstriction (HPV) to investigate the role of mitochondria in sensing of acute hypoxia. High resolution respirometry was used for determination of oxygen pressure at half maximal respiration (p50) in PASMCs and remission spectrophotometry for determination of redox state of mitochondrial cytochromes in the isolated lung. Respirometric analysis showed a p50 of 0.11±0.01 kPa (n=7) in PASMCs, which was not significantly different from aortic smooth muscle cells (0.10±0.01% kPa, n=8). Hypoxia induced calcium release in PASMCs was detected at 3% oxygen, which correlates with a 3.6±0.2% inhibition of maximal mitochondrial respiration calculated by the p50 according Michaels-Menten-Kinetics. By remission spectrophotometry we detected a reduction of mitochondrial cytochromes in the isolated blood free perfused and ventilated lung at an oxygen concentration of ≤1% oxygen, whereas HPV starts at an oxygen concentration of 10% oxygen. We therefore conclude that respiration in PASMCs is only slightly inhibited at oxygen concentrations at which acute hypoxic cell response is initiated and that this is not different from non oxygen sensing cells. All data were shown as average±standard error of the mean.

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S8.24 Cell-to-cell cross-talk between mesenchymal stem cells and cardiomyocytes: The role of mitochondria

Tatiana G. Khryapenkova[a], Egor Y. Plotnikov[a], Maria V. Korotetskaya[a], Alena K. Vasileva[a], Maria V. Marey[b], Gennady T. Sukhikh[b], Dmitry B. Zorov[a]
[a]*A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Russian Federation*
[b]*Research Center of Obstetrics, Gynecology and Perinatology, Moscow, Russian Federation*
E-mail: tenella@list.ru

Many studies display efficiency of stem and progenitor cells in injured myocardium function refinement. Integration of transplanted cells into myocardial tissue is of a particular interest. We examined the possibility of cell-to-cell exchange of cytoplasm and organelles between mesenchymal stem cells (MSC) and rat cardiomyocytes. We revealed that after cocultivation cells formed intercellular contacts (through nanotubes or gap junctions) and transient exchange with cytosolic elements could be observed. Confocal and electron microscopy revealed mitochondrial-like structures in the intercellular cytoplasmic nanotubes, indicating a possibility of mitochondrial transport through the nanotubes. We revealed the result of such transport — mitochondria were transferred from MSC to cardiomyocytes. The cytoplasm transport between contacted cells was confirmed by exchange of the fluorescent probe Calcein between cells. Another result of coculturing was the appearance of human cardiac-specific myosin H-chain in MSC. We proposed that penetration of cardiomyocyte's cytoplasm to MSC caused such differentiation by donation of some signaling molecules to ignite MSC differentiation on the cardiac muscular pathway. We can speculate that cardiomyocytes-to-MSC cross-talk caused turning on differentiation of MSC towards contractile cells, and second MSC can donate mitochondria to

cardiomyocytes. Thus potentially transplanted MSC could replace damaged myocardial cells.

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S8.25 Seventeen reasons to believe in parallel activation of ATP supply and demand

Bernard Korzeniewski
Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland
E-mail: benio@mol.uj.edu.pl

Different alternative mechanisms of the regulation of oxidative phosphorylation during rest-to-work or low-to-high-work transition, including the classical negative feedback through an increase in [ADP] and [P_i], have been proposed in the literature. Several dynamic computer models, involving particular mechanisms, have been developed. It is relatively easy, by manipulating with assumptions and free parameter values, to fit different models to one or a few sets of experimental data. Therefore, in order to find out the true mechanism, it is absolutely crucial to test particular mechanisms/models for as broad number of different oxidative phosphorylation properties as possible. Seventeen independent properties of the system on the cellular and physiological level are selected and used as reference points for testing the correctness of particular mechanisms/models. It is demonstrated that only the parallel-activation mechanism (or each-step-activation mechanism), due to which all oxidative phosphorylation complexes are directly activated by some cytosolic factor in parallel with the activation of ATP usage by Ca^{2+}, can explain all seventeen properties, while the remaining mechanisms are able to account for much less than a half of these properties. This conclusion emphasizes the need of experimental identification of the factor/mechanism that directly activates oxidative phosphorylation complexes during elevated work intensity.

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S8.26 Mitochondrial bioenergetics of lamprey liver with biliary atresia and steatocholestasis

Margarita V. Savina, Larisa V. Emelyanova, Sergey M. Korotkov, Irina V. Brailovskaya
Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia
E-mail: savina-margarita@mail.ru

The liver of adult lamprey (*Lampetra fluviatilis*) shows total atrophy of the biliary system (the disappearance of canaliculi, ducts and gall bladder) and consequent storage of bile products in the cells. Besides hepatocytes of lampreys, especially of males, in winter period of prespawning migration is filled up by lipid drops. It was discovered that endogenous respiration of such hepatocytes and isolated mitochondria (natural substrate is fatty acids) is sharply suppressed. One of the possible reasons is provocated by long starvation inactivation of the first complex of the respiratory chain, which is involved into the process of β-oxidation. We observed the very low rates of oxidizing the NAD-dependent substrates Pyr+Mal, moreover neither ADP nor DNF affects the rates of oxygen consumption. The enhanced membrane permeability of mitochondria to protons and monovalent cations was discovered, but not to sucrose. The enhanced ion membrane permeability of liver mitochondria is found to be sensitive to EGTA and to cyclosporine A in combination with ADP and Mg2+ and is likely mediated opening the mitochondrial permeability transition pore in its low conductance state.

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S8.27 Keeping it real-time: Cellular bioenergetics in a microplate

David Ferrick
Seahorse Biosciences, North Billerica, Massachusetts, USA
E-mail: david@seahorsebio.com

Understanding mitochondrial function has expanded beyond physiology and obesity/diabetes into areas that include aging, cancer, cardiomyopathy and neurodegeneration. In addition to ATP production, mitochondria are responsible for the β-oxidation of short-, medium- and long-chain fatty acids as well as being central to intermediary metabolism, ROS generation, and apoptosis. The Seahorse Bioscience XF24 Analyzer measures the two major energy producing pathways of the cell, mitochondrial respiration (oxygen consumption) and glycolysis (extracellular acidification), in a sensitive and convenient microplate format. This presentation will focus on how bioenergetic measurements are made using XF and introduce our latest sensor, CO_2, enabling simultaneous measurement of O_2, CO_2 and H^+.

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S8.28 The effect of preserving covalent modification of mitochondria by phosphorylation on oxygen consumption by mitochondria isolated from brown adipose tissue and thymus

Kieran J. Clarke, Richard K. Porter
School of Biochemistry and Immunology, Trinity College Dublin, Dublin 2, Ireland
E-mail: clarkekj@tcd.ie

In this study, we set out to investigate whether covalent modification by any putative phosphorylation of the electron transport resulted in changes in oxygen consumption, by isolating mitochondria from brown adipose tissue and thymus of rats. Any putative phosphorylation was preserved by addition of phosphatase inhibitors KF (10 mM) and Na_2VO_3 (1 mM) in the mitochondrial preparation. Oxygen consumption rates (nmol 0_2/min/mg protein) by mitochondria isolated from brown adipose tissue (state 2, 130±9; state 3 uncoupled, 136±5) from cold adapted rats (4 weeks) in the presence of phosphatase inhibitors were significantly higher (p=0.0372 and p=0.0266, respectively) when compared to oxygen consumption rates by brown adipose tissue mitochondria (state 2, 83±13; state 3 uncoupled, 93±12) isolated from the same pool of animals without phosphatase inhibitors. Oxygen consumption rates by mitochondria isolated from thymus (state 2, 13.0±0.3; state 3 uncoupled, 51.7±0.8) of the 4 week cold adapted group in the presence of phosphatase inhibitors were also significantly higher (p=0.0038 and p=0.0168, respectively) compared to oxygen consumption rates by thymus mitochondria (state 2, 10±1; state 3 uncoupled, 38±6) isolated from the same group, without phosphatase inhibitors. No differences were observed in equivalent comparisons of liver mitochondria. These results suggest that the differences in oxygen consumption rates observed in brown adipose tissue and thymus mitochondria from the cold adapted group are due to phosphorylation of a component(s) of the electron transport chain.

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