

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

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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

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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 provoked 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 Mg^{2+} 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

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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

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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 O_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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