more single-subunit, non-proton-motive NADH dehydrogenases encoded by nuclear DNA. The rotenone-insensitive NADH dehydrogenase from yeast (Ndi1) was successfully used for alleviation of complex I defects in multiple models including nematodes, fruit flies, rats, and human cells. It was also used as a remedy in Parkinson disease models and as an anti-cancer treatment. Many other physiological effects were also discovered in organisms expressing Ndi1, such as extended lifespan and resistance to particular stresses. Recently, an alternative respiratory chain was found in several animal taxa, including tunicates. However, the properties of alternative NADH dehydrogenases from tunicates, which are supposed to be evolutionary closer to humans than fungi, remain uninvestigated. Previously, the alternative oxidase (AOX) from ascidian *Ciona intestinalis* was successfully exploited to by-pass defects in complexes III and IV in fruit fly, mouse and human cell models. The goal of the present work was to express the tunicate alternative NADH dehydrogenase (NDX) in a suitable model organism and investigate its properties. We have found that the gene coding NDX in *C. intestinalis* contains about 4% polymorphisms, which may lead to 1.7–2.1% amino acid changes, thus the cloned gene might code non-functional protein. However, NDX was localized to mitochondria when expressed in *Drosophila* S2 cells, and conferred about 20% rotenone-insensitive respiration for mitochondria of adult fruit flies *Drosophila melanogaster*. The exhibiting of rotenone-insensitive respiration, provided by NDX, required high levels of the expression. NDX conferred also resistance of the fruit flies to 20 mM menadione, heat and cold stresses. However, NDX-expressing flies were more sensitive to the salts of chromium and molybdenum. The resistance of NDX-expressing *D melanogaster* to multiple stresses indicates a possible role of NDX in stress responses of tunicates.

S7.P7

The relationship between cytochrome redox state and oxygen consumption in isolated mouse and beef heart mitochondria during hypoxia

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This study describes a low-noise, rapid spectrophotometric system using visible light (440–605 nm) for the measurement of cytochrome redox state combined with a high-resolution respirometry. The system was tested in an investigation using beef and mouse heart isolated mitochondria (BHImt, MHImt) in order to determine the relationship between respiratory rate and cytochrome redox state at steady-state levels of hypoxia. Monophasic hyperbolic relations were observed between respiratory rate, *j* (with glutamate + malate and saturating ADP concentrations), and oxygen partial pressure, *pO2*, in the range <1.1 kPa for both BHImt and MHImt with *p50,j* (*pO2 at j = 0.5 Jmax*) of 0.015 and 0.021 kPa respectively. The oxidation fractions of cytochromes *a*3, and *c* were biphasic hyperbolic functions of *pO2*. The relationships between cytochrome oxidation states and *j* were more complex with an initial steep decrease in the oxidation fraction of cytochrome *c* to a value of *j* of approximately 0.7 followed by a plateau and a further steep decrease at *j* < 0.2. This relationship was less apparent with cytochrome *b* redox state. Using these functions, it was possible to create a model that successfully described the measured relationship between cytochrome oxidation state and oxygen consumption.

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S7.P8

Combined high-resolution respirometry and fluorometry. Validation of safranin for determination of mitochondrial membrane potential

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Mitochondrial membrane potential (mtMP) is closely intertwined with oxidative phosphorylation (OXPHOS). The exact nature of the interactions of respiration (flux) and mtMP (force) under various physiological and pathological conditions remains unclear, partially due to methodological limitations. We introduce the combination of high-resolution respirometry and fluorometry with the OROBOROS Oxygraph-2 k, using the widely applied mtMP indicator safranin. OXPHOS analysis with mouse brain homogenate revealed that safranin inhibits Complex I linked OXPHOS capacity at commonly applied concentrations and targets primarily the phosphorylation system, without effect on LEAK respiration. Complex II linked OXPHOS capacity was inhibited by ~20% at 2 μM safranin sufficient for mtMP monitoring, mtMP was higher in the LEAK state without adenylates (*ΔN*) than at identical LEAK respiration after ADP stimulation and inhibition by oligomycin (*ΔOmy*). Maximum ETS capacity was reached in uncoupler titrations before mtMP was fully collapsed, whereas respiration was inhibited at increasing uncoupler concentrations and further reduction of mtMP. Examining a pharmacologically induced state of Complex II dysfunction, mtMP was rather insensitive to 50% inhibition of OXPHOS, but responded strongly to addition of inhibitors when respiration was minimized by substrate depletion. The optimum uncoupler concentration supporting maximum ETS capacity varied as a function of pharmacological intervention. Taken together, combined measurement of respiration and mtMP greatly enhances the diagnostic potential of OXPHOS analysis. Respirometric validation of inhibitory and uncoupling effects is mandatory for any fluorophore applied for probing mtMP, in any respiratory state, type of tissue and pathophysiological condition.

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S7.P9

Succinate dehydrogenase regulation in normoxic and anoxic conditions in mammal heart and brain

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Succinate dehydrogenase (SDH; complex II) is a pivotal mitochondrial component connecting the TCA cycle and respiratory chain. The enzyme is a subject of feedback control by oxaloacetate (OAA) which is a product of the malate dehydrogenase. It is a competitive inhibitor of SDH with high affinity (Kd ~ 17 nM) and an extremely low dissociation rate (~0.02 min−1) [1,2]. OAA binding depends on the redox state of the enzyme which is fully reduced in the absence of oxygen [2]. The
onset of ischemia leads to significant changes in tricarboxylic acid cycle inter-mediates including OAA in the mitochondrial matrix. Therefore we assessed the effect of ischemia on inhibition of the enzyme by OAA ex vivo. We determined the degree of OAA inhibition by measuring succinate:quinone oxidoreductase and succinate oxidase activity before and after removal of inhibitor. We observed an increase of about 50% in heart and of about 70% in brain upon dissociation of inhibitor. Therefore, SDH was inhibited by OAA in both control and ischaemic samples of mouse heart and brain. We also tested whether or not OAA inhibition is an artefact occurring during membrane isolation [3] or it binds to SDH in situ. OAA was rapidly removed from the isolation media by using glutamate oxaloacetate transaminase and the degree of inhibitor binding was determined in the final preparation. Understanding the process of OAA interaction with SDH in ischaemic conditions in different tissues is of great interest since this enzyme is emerging as another important component in the metabolic cellular response to oxygen deprivation [4].

References

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S7.P10

Plate-based respirometry of intact myotubes: A new system testing physiological and pathophysiological effects of insulin resistance ex vivo
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Mechanistic insights into mitochondrial bioenergetics of muscle are hampered by non-physiological energetic adjustments in cell culture and damage of long-muscle fibers. Assessment of mitochondria is usually performed using permeabilized fibers. Here, we show the isolation of intact muscle tubes from the flexor digitorum brevis in mice that can be used short-term after isolation to test pathological phenotypes and associated dysfunction/adjustments of energy metabolism. Using plate based respirometry (extracellular flux analyzer, Seahorse Bioscience) we first tested the integrity of the myotubal plasma membrane by addition of succinate plus and minus detergent, demonstrating minor flux of succinate to the cell. Then, we verified that resting respiration was minorly impacted by substrate supply using different concentrations of nutrients; but respiration was mainly driven by ATP demand. Notably, the resting respiration of the myotube was very low, as compared to substantial ATP turnover and glycolytic rates in muscle cell culture models that are presumably adaptations to the non-physiological cell culture media. ATP turnover was induced by addition of acetylcholine (Ach) and specificity to the Ach-receptor was verified using the competitive inhibitor tubocurarine. We then tested the effects of insulin on the myotubes, demonstrating increased maximal substrate oxidation and glycolytic rates. Muscle tubes from insulin-sensitive chow-fed vs insulin-resistant high fat diet-fed mice showed differences of maximal substrate oxidation capacity upon insulin treatment and differences of maximal glycolytic flux. We propose that this experimental setup can be used to test various adaptations of muscular energy metabolism in response to physiological and environmental challenge.

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S7.P11

The changes of mitochondrial activity in cells of sugar cane suspension culture at the early stage of cell death, caused by the negative temperature influence
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It is known that biotic or abiotic stressors can increase the level of ROS in plant cells due to violation of mitochondrial metabolism. The changes of mitochondria functional activity at the early stage of cell death activation caused by the action of a negative temperature (−8 °C) in a sugarcane suspension culture (Saccharum officinarum L., grade POJ2878, line resistant to anoxia) have been studied in this work. The short-term treatment of the negative temperature was carried out on the 8th day of cultivation. It has been shown that mitochondrial respiration rate decreased by more than 2 times h after the exposure. This decrease of respiration intensity was caused by reduced alternate cyanide-resistant respiration pathway (AP) contribution. In the control culture of sugarcane AP contribution to the respiration was about 40%, while the AP contribution to the respiration treated with the negative temperature culture decreased to approximately 25%. As AP functioning may regulate reactive oxygen species (ROS) content in a cell, changes of ROS level in the cells of the culture has been studied. It has been stated that the treatment resulted in the significant increase of the ROS content in the sugar cane cells, and it remained for 6 h after the treatment. Along with the increased ROS level, the effect of the negative temperature led to the hyperpolarization of the inner mitochondrial membrane, which gradually decreased during 6 h after the exposure that is in the same period when the respiration rate decreased. Observed changes of the mitochondria functional activity — reducing the intensity of respiration and mitochondrial transmembrane potential could be due to a disturbance of the electron transport along the respiratory chain, caused by the ROS-dependent release of cytochrome c from mitochondria to the cytoplasm, which was also observed after 6 h of temperature treatment. Thus, we can conclude that there are significant disorders of mitochondrial functional activity at the early stage of cell death in the sugar cane culture caused by the action of the negative temperature — reducing the respiration rate and the mitochondrial transmembrane potential, increasing the ROS level in the cells and releasing the cytochrome c from mitochondria. This work was supported by the grant of the Russian Foundation for Basic Research № 14-04-32126.

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