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 ischaemia 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].

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doi:10.1016/j.bbabio.2014.05.016

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.

doi:10.1016/j.bbabio.2014.05.017

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, 2 h) in a sugar cane 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 6 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.

doi:10.1016/j.bbabio.2014.05.018