NAD^+ but not NADH appears to occur through NAD^+ selective transport. Reversal of metabolic dysfunction by NAD^+ may be a mechanism responsible for neuroprotection observed with exogenous NAD^+.

doi:10.1016/j.bbabio.2008.05.182

(S7) Neuronal mitochondria symposium abstracts (poster and raised abstracts)

S7.6 Glutamate exposure of cortical neurons evokes initial oxidation of NADPH in mitochondria followed by a delayed oxidation of NADH: Delayed redox and calcium deregulation

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Excitotoxic neuronal injury and mitochondrial dysfunction are components of several neurological disorders. We addressed an important bioenergetic parameter, the NAD(P)(H) redox state in glutamate-exposed cultured cortical neurons using NAD(P)H autofluorescence imaging and HPLC. The mitochondrial NADPH pool of the neurons became partially oxidized within the first 60 s of the glutamate stimulation, while the redox state of mitochondrial NAD(H) pool remained unchanged. The initial NADPH oxidation was linked to aminotransferase reaction, and did not correlate with the later fate of the cell. Oxidation of the mitochondrial NADH occurred suddenly after a delay and coincided with the delayed calcium deregulation (DCD). The cytosolic NAD(H) pool was gradually oxidized and became fully oxidized by the time when the DCD occurred. Supplementation of glutamate-stimulated neurons with substrates capable of reducing NAD^+ in the cytosol (lactate and malate) decreased the incidence of DCD, supporting an upstream role of oxidation of the cytosolic NADH in DCD, whereas mitochondrial substrates pyruvate, β-OH-butyrate and acetoacetate were without an effect. We conclude that DCD is preceded and augmented by the "deregulation" (oxidation) of the cytosolic NAD(H) pool.

doi:10.1016/j.bbabio.2008.05.183

S7.7 Cyclophilin D independent swelling of neuronal mitochondria in single cells induced by Ca^{2+} overload

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The aim of the study was to determine whether lack of cyclophilin D, a putative mitochondrial permeability transition pore (mPTP) modulator, protects neuronal mitochondria against Ca^{2+} overload during chemical anoxia combined with glucose deprivation. Mitochondrial swelling is an indicator of the opening of the mPTP and this was measured here with a novel quantitative *in situ* single cell assay in primary cultures of wild type or cyclophilin D knockout neurons. In control conditions no difference was found in the morphology of wild type versus knockout neuronal mitochondria during Ca^{2+} overload induced by addition of Ca^{2+} ionophore (4BrA23187). No mitochondrial swelling was detected during 20 min of ionophore addition in 30% of examined neurons cultured from wild type and in 25% from knockout animals. The rest of the population responded with swelling and fragmentation of mitochondria within 650–800 s after addition of the ionophore in both types of neurons. During chemical anoxia combined with glucose deprivation Ca^{2+} overload evoked an almost immediate swelling of mitochondria both in wild type and knockout neurons. Our results demonstrate, that cyclophilin D is not involved in Ca^{2+} overload induced mitochondrial swelling of neurons either in normal, or in pathological conditions, which presumes the existence of a cyclophilin D independent pathway of the opening of the mPTP.

doi:10.1016/j.bbabio.2008.05.184

S7.8 Partial inhibition of complex I activity causes an increase in release of glutamate from the cytoplasmic pool of synaptosomes

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Dysfunction of the mitochondrial electron transport chain has been established to be a characteristic of numerous chronic neurodegenerative disorders, and recent evidence indicates that a reduction in complex I (NADH quinone oxidoreductase) activity is widespread in the brains of Parkinson's disease patients. This study aims to model the effects such a reduction on glutamate release from the nerve terminal. Using rotenone it was found that inhibition of complex I activity by 40% increased the Ca^{2+}-independent component of glutamate release from depolarised synaptosomes. Highest rates of Ca^{2+}-independent glutamate release were found to occur between 60–90% complex I inhibition. The increase in glutamate release was found to correlate to a decrease in ATP level. Inhibition of complex I activity by 40% was also shown to cause a significant collapse in mitochondrial membrane potential ($\Delta\psi_m$). Hypoglycaemic conditions were modelled by substituting 2-deoxyglucose for glucose, and this potentiated the effects of rotenone on Ca^{2+}-independent glutamate release, ATP and $\Delta\psi_m$. Our results are in accordance with those from studies that show that glutamate release into the moribund substantia nigra is increased in Parkinson's disease, and add to evidence for the involvement of slow excitotoxicity in the pathogenesis of the disease.

doi:10.1016/j.bbabio.2008.05.185

S7.9 Analysis of respiratory responses of neuronal cells to the decrease of extracellular calcium

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A profound decrease in extracellular Ca^{2+} ($_eCa^{2+}$) occurs during neuronal activity or ischemia, while Ca^{2+}-free conditions are commonly used in biological experiments. In this study we examined the dynamics of respiration of neurosecretory PC12 cells and cerebellar granular neurons upon sequestration of $_eCa^{2+}$. By monitoring intracellular oxygen ($_iO_2$) by means of dedicated $_iO_2$-sensing probe and time-resolved fluorescent detection, we observed a marked transient activation of respiration in response to chelation of $_eCa^{2+}$. Subsequent depolarization of the plasma membrane with

high $_eK^+$ (1–12 min after treatment with EGTA) had an additive effect on cellular O_2 consumption, reducing $_iO_2$ to 60% of air saturation. The respiratory response was accompanied by Na^+ influx, a decrease in cytosolic and mitochondrial Ca^{2+} and partial depolarization of plasma membrane, while the mitochondrial membrane potential (MMP) and cellular ATP remained unchanged. The effect of EGTA was down-regulated by the depletion of Ca^{2+} stores and dissipation of proton gradient across the mitochondrial membrane, up-regulated by mitochondria uncoupling and was independent on MMP. The respiratory effect was largely reduced by the inhibition of mitochondrial Na^+/Ca^{2+} exchanger ($_mNCX$) and Na^+/H^+ exchangers. We suggest that such respiratory response is driven by a non-selective Na^+ influx, activation of $_mNCX$ and increased mitochondrial Na^+/H^+ exchange. This leads to the acidification of matrix, loss of $_mCa^{2+}$ and acceleration of mitochondrial proton pumps to restore proton gradient.

doi:10.1016/j.bbabio.2008.05.186

S7.10 Bioenergetics and mitochondrial transport in hippocampal neurons

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Impaired transport of mitochondria in neurons and bioenergetic deficit are increasingly recognized to be of pathological importance in neurodegenerative diseases. To study the relationship between transport and bioenergetics we have developed a novel image processing technique to quantify organelle velocity in cultured cells. This combines measurement of motion and bioenergetic parameters while minimizing photodynamic oxidative artifacts evoked by fluorescence excitation. To describe populations of mitochondria in resting cultured hippocampal neurons in addition to motion analysis, measurements of mitochondrial thiol redox status by mitochondrially-targeted redox-sensitive GFP and mitochondrial membrane potential by TMRM were performed. Mitochondria with more oxidized thiol redox status had lower membrane potentials and were smaller in size. These mitochondria were more motile than the average, however mitochondrial motility was only slightly dependent on the observed bioenergetic parameters, and correlated the best to their size. Mean velocities of mitochondria were unaltered by glycolytic inhibition and decreased by inhibition of oxidative phosphorylation. To stop motion cessation of both ATP sources was required. Depolarization of mitochondria when the ATP-synthase was inhibited did not further decrease the mean velocity and affect the directionality of the motion. It is concluded that mitochondrial motors respond to the global ATP level, which is mainly determined by the oxidative phosphorylation. The mitochondrial membrane potential does not regulate mitochondrial transport in hippocampal neurons.

doi:10.1016/j.bbabio.2008.05.187

S7.11 Metabolic control analysis of bioenergetic function in synaptosomes

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The aim of this study was to use metabolic control analysis (MCA) to examine the spread of control amongst the electron transport chain (ETC) complexes over the process of mitochondrial oxidative phosphorylation in rat brain synaptosomes. Oxygen consumption and ETC activities were titrated with appropriate inhibitors to determine the flux control coefficients and the energy threshold levels. The flux control coefficients for complex I, complex II/III and complex IV were found to be 0.30, 0.20 and 0.19, respectively and the energy thresholds for complex I, complex II/III and complex IV were determined to be ~15%, ~35 and ~30%, respectively. These results indicate that complex I exerts a high level of control over synaptosomal bioenergetics, suggesting that complex I deficiencies in neurodegenerative disorders, such as PD, may compromise mitochondrial oxygen consumption in the nerve terminal, possibly leading to neuronal dysfunction. In addition, the effect of coenzyme Q on the flux control coefficient and energy threshold effect of complex I was examined. No statistically significant difference in flux control coefficients and energy thresholds for complex I was found, however, during titration of complex I activity with rotenone the presence of coenzyme Q decreased the rate of inhibition of oxygen respiration. These results suggest that complex I in the nerve terminal possess sensitive control over mitochondrial respiration rates and may be a therapeutic target for neurodegenerative conditions in which complex I activities are decreased.

doi:10.1016/j.bbabio.2008.05.188

(S8) Mitochondria and cell physiology symposium lecture abstracts

S8/1 Iodothyronines and mitochondria

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Studies of the effects of iodothyronines on mitochondria have been focused on T3, but recently other iodothyronines such 3,5-diiodothyronine (T2), have been identified as possible peripheral mediators of the effect of thyroid hormones on cell respiration. In this context, we have shown that T2 powerfully reduce adiposity in high-fat-fed rats by increasing the burning of fats. Now we report that T2 in a short term is able i) to affect mitochondrial fatty acid oxidation rate in skeletal muscle ii) to activate the AMPK-(ACC)-malonyl CoA signalling pathway iii) to affect mitochondrial thermogenesis. The administration of T2 to hypothyroid rats induced an increase in mitochondrial oxidation when palmitoyl-CoA (+104% vs. hypo), palmitoyl-carnitine (+80% vs. hypo) and succinate (+30% vs. hypo) were used as substrates. These results suggest that T2 stimulates mitochondrial fatty acid oxidation by activating more metabolic pathways: -import of fatty acid into the mitochondria–beta oxidation cycle–$FADH_2$ linked respiratory pathways. Indeed, T2 is able to activate the AMPK signalling pathway known to direct lipid partition towards oxidation and to induce the activation of mitochondrial fatty acid import. T2 also enhanced skeletal muscle mitochondrial thermogenesis by activating pathways involved in the dissipation of proton motive force not associated to ATP synthesis ("proton leak"), the effect being