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^+.

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(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.

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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.

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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.

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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