complex defects in the brain and may have high control over nerve terminal function and dysfunction in the brain.

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S7/2 Modulation of glucose consuming pathways by nitric oxide in neurons: Impact on survival

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Besides its essential role at regulating neural functions through cyclic GMP, nitric oxide is emerging as an endogenous physiological modulator of energy conservation for the brain. Thus, nitric oxide inhibits cytochrome *c* oxidase activity in neurones and glia, resulting in down-regulation of mitochondrial energy production. The subsequent increase in AMP facilitates the activation of 5′-AMP-dependent protein kinase, which rapidly triggers the activation of 6-phosphofructo-1-kinase–the master regulator of the glycolytic pathway–and Glut1 and Glut3–the main glucose transporters in the brain. In addition, nitric oxide activates glucose-6-phosphate dehydrogenase, the first and rate-limiting step of the pentose-phosphate pathway. Here, we review recent evidences suggesting that nitric oxide exerts a fine control of neuronal energy metabolism by tuning the balance of glucose-6-phosphate consumption between glycolysis and pentose-phosphate pathway. This may have important implications for our understanding of the mechanisms controlling neuronal survival during oxidative stress and bioenergetic crisis.

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S7/3 Oxidative stress and mitochondrial dysfunction

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Increased generation of reactive oxygen/nitrogen species and subsequent impairment of the mitochondrial electron transport chain is implicated in the neurodegenerative process. Within the brain, neuronal and astroglial cells may display differential susceptibility to oxidant exposure. Thus, astrocytes can up regulate glutathione availability and, in response to mitochondrial damage, glycolytic flux. While neuronal cells do not appear to possess such mechanisms, neuronal glutathione status may be enhanced due to the trafficking of glutathione precursors from the astrocyte. However, when antioxidant reserves are not sufficient or the degree of oxidative stress is particularly great, mitochondrial damage occurs, particularly at the level of complex IV (cytochrome oxidase). Although the exact mechanism for the loss of activity of this enzyme complex is not known, it is possible that loss and/or oxidative modification of the phospholipid, cardiolipin (CL) is a critical factor. CL is documented to be essential for maximal complex IV activity and is reported to be susceptible to oxidative modification. In order to investigate this suggestion further we have evaluated the effects of peroxynitrite exposure on tissue CL status. Preliminary data revealed loss of functional CL and increased formation of oxidised CL. Furthermore, this oxidation of CL is prevented in the presence of antioxidant molecules such as reduced glutathione and the vitamin E analogue, trolox. Supported by SPARKS (UK).

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S7/4 The effect of calcium on the generation of reactive oxygen species in brain mitochondria

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Calcium as well as reactive oxygen species (ROS) is a key factor in the pathogenesis of several neurodegenerative diseases but it is unclear how calcium regulates the production of ROS in mitochondria. We present evidence that calcium in >1 μM concentration significantly decreases the rate of production of hydrogen peroxide in brain mitochondria supported by succinate or glutamate plus malate in the absence of adenine nucleotides or inhibitors of the respiratory chain. The effect of calcium on the hydrogen peroxide generation was more robust in mitochondria respiring on succinate. The reduced rate of hydrogen peroxide production paralleled a calcium-induced sustained depolarization, loss of NAD(P)H fluorescence and decreased calcein fluorescence signal indicating an increased permeability of mitochondria. In the presence of ADP the calcium-induced NAD(P)H loss and swelling were prevented, hydrogen peroxide generation was decreased but not reduced further by calcium. With glutamate plus malate as substrate, but not with succinate, β-OH-butyrate, or malate alone, calcium, in the presence of ADP induced an increase in the NAD(P)H level and in the membrane potential. It is suggested that an increased permeability of the mitochondrial membrane induced by calcium is a crucial factor in the decreased ROS generation induced by calcium due to depolarization and loss of pyridine nucleotides.

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S7/5 Loss of NAD(H) limits mitochondrial respiration after neonatal cerebral hypoxic ischemia

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The aim of this study was to determine if loss of mitochondrial NAD(H) is responsible for impaired respiration and Ca^{2+} uptake capacity observed with mitochondria isolated after neonatal cerebral hypoxic ischemia (H/I). Postnatal day 7 rats were placed under 7% O_2 for 75 min 24 h after surgical occlusion of the right carotid artery. Animals were sacrificed 20 min after transfer to 21% O_2 and mitochondria were isolated from the ipsilateral (H/I) and contralateral (H) hemispheres. Respiration was reduced by 30% and Ca^{2+} uptake by over 50% in HI compared to H mitochondria, using malate plus glutamate but not succinate (+rotenone) as oxidizable substrates. Addition of 2 mM NAD^+ to the medium resulted in complete reversal of respiratory inhibition and impaired Ca^{2+} uptake for HI mitochondria without any effect on succinate-dependent respiration and either respiration or Ca^{2+} uptake by H mitochondria. Measurements of NAD(H) present in mitochondrial extracts indicated a 30% reduction in HI mitochondria. These and other results suggest that mitochondrial NAD(H) is lost during H/I through transient opening of the permeability transition pore. Reversal of respiratory inhibition by added

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