S6.5 Nitric oxide and HIF-1α synergise to activate glycolysis during inflammation

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Macrophages play a central role in innate immunity, contributing to both the initiation and resolution of inflammation. Their activation by interferon γ and lipopolysaccharide results in increased production of nitric oxide (NO), stabilization of HIF-1α, loss of mitochondrial function, and a switch to glycolytic metabolism. We have now characterized these changes and their bioenergetic consequences in J774.A1 macrophages. Following activation, there was a stabilization of HIF-1α and a progressive increase in extracellular NO which became detectable after 3 h. The increase in NO was associated with a decrease in mitochondrial- and an increase in non-mitochondrial O_2 consumption. Mitochondrial ATP synthesis decreased while glycolytically-generated ATP increased, more than compensating for the lack of mitochondrial ATP synthesis. Despite this substantial increase in total ATP generation, the steady state cellular ATP content fell due to the increase in ATP demand by the cells, leading to an arrest in cell proliferation and death. The mitochondrial defect in activated macrophages could be prevented by treatment with the NO synthase inhibitor SEITU, which also reduced the stabilization of HIF-1α, partially suppressed glycolysis and restored the cellular ATP. Conversely, knockdown of HIF-1α with siRNA reduced glycolysis and NO production, partially preserved mitochondrial respiration and restored the cellular ATP content. Activation of glycolysis was completely abolished by knockdown of HIF-1α together with SEITU. Thus our results indicate that NO and HIF-1α operate synergistically to activate glycolysis during inflammation.

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S6.6 The influence of Reamberin on mitochondrial functional activity of the intestine muscular layer in acute peritonitis

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In clinical practice Reamberin is used as an antihypoxant–antioxidant–detoxicating solution. It is an isotonic solution which contains sodium succinate 1.5%, sodium chloride 0.6%, potassium chloride 0.03%, magnesium chloride 0.012% and is given by intravenous administration. The aim of the study was to determine functional activity of mitochondria from the intestines muscular layer in acute peritonitis and to assess the influence of Reamberin on their activity. The experiment was performed on 55 rabbits. Peritonitis was induced by intraabdominal injection of polymicrobial suspension of *E. coli* and *B. fragilis*. Reamberin was injected intravenously for 5 days starting 1 h after operation, at a dose of 25 mg/kg (succinate concentration). Mitochondrial oxygen consumption was determined using polarographic method. Succinate was used as a substrate. We demonstrated a significant decrease in mitochondrial function after 6 h of acute peritonitis. ADP/O ratio was significantly ($p<0,05$) reduced by 20±1(5)%. State III and IV respiration were lowered for 49±3(5)% and 14±3(5)% respectively ($p<0,05$), and the respiratory control ratio was reduced by 39±5 (5)% ($p<0,05$). These changes began on day one and waned by the fifth day of postoperative period. Our data show that Reamberin preserves mitochondrial function during the first 24 h after operation and restores it to the fifth day of the postoperative period. Data are presented as mean±sd (n), where n is the number of experiments.

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S6.7 Apoptosis in thymocytes from UCP 1 wild-type and UCP 1 knockout mice

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We analyzed apoptosis by PI (propinium iodide) staining and FACS analysis in thymocytes treated with dexamethasone (0.1 μM), for 2, 4 and 6 h [and controls treated with carrier alone] from wild-type and UCP 1 knockout (KO) mice. We were able to show that there was a ~15% decrease in amount of apoptosis in thymocytes from knock-out mice compared to thymocytes from wild-type animals after treatment with dexamethosone for 4 h ($p=0.02$) and 6 h ($p=0.012$). We also observed a ~5% decrease in the amount of (background) apoptosis in thymocytes from knock-out mice compared to thymocytes from wild-type animals after treatment with carrier for 6 h ($p=0.005$). We conclude that ablation of UCP 1 in thymocytes effects susceptibility of thymocytes to apoptosis.

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(S7) Neuronal mitochondria symposium lecture abstracts

S7/1 Metabolic control analysis of mitochondria in the nerve terminal

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Mitochondrial dysfunction has been associated with neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, as post mortem studies have identified reduced electron transport chain (ETC) activities in mitochondria from various brain regions. In Parkinson's disease complex I is thought to be reduced by up to 40% in certain brain regions and this finding has been used as evidence for the theory that complex I defects are a cause of neurodegeneration due to associated bioenergetic dysfunction. The presence of heterogeneous mitochondria in the brain has complicated matters as isolated nonsynaptic mitochondria have different energy thresholds and flux control coefficients compared to isolated mitochondria of synaptic origin. Complex I is known to exert a high degree of control over oxidative phosphorylation in isolated synaptic mitochondria, and it also contributes to low energy thresholds such that when activities are reduced by 25% and above, respiration rates and ATP levels are subsequently reduced. In comparison to complex III and IV in nerve terminal mitochondria, which possess lower flux control coefficients and energy thresholds of 70 and 60%, respectively, complex I exerts a higher degree of control on oxidative phosphorylation. Intact nerve terminals (synaptosomes) possess even lower thresholds for complex I (15%), that may have important implications for ATP levels and neurotransmitter release. The data suggest that defects in mitochondrial complex I activities are of a higher significance than other ETC

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