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

Assegid Garedew, Salvador Moncada
Wolfson Institute for Biomedical Research, Cruciform Building, University College London, Gower Street, London WC1E 6BT, UK
E-mail: a.garedew@ucl.ac.uk

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

Vladimir A. Kosinets
Vitebsk State Medical University, Belarus
E-mail: vkosinets@yandex.ru

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

Alison E. Adams, Richard K. Porter
School of Biochemistry and Immunology, Trinity College Dublin, Ireland
E-mail: adamsae@tcd.ie

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

Gavin P. Davey
School of Biochemistry and Immunology and Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland
E-mail: gdavey@tcd.ie

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