and 5 min reperfusion, before generating global normothermic ischemia for 40 min followed by 120 min reperfusion. Samples were processed to extract total and nuclear proteins. In addition, myocardial infarction was studied by staining with triphenyltetrazolium chloride (TTC), and creatine kinase activity in the coronary effluent was measured to determined reperfusion damage. Nrf2 and UCP3 expression levels increased significantly during reperfusion in the nuclear and total tissue extracts, respectively, from both preconditioned and non-preconditioned hearts, suggesting that Nrf2 might regulate UCP3 expression in the heart under oxidative stress, as previously reported in cells. Hearts from UCP3 knockout mice had increased infarct size and creatine kinase activity compared to those from wild-type mice. These results suggest that the Nrf2/UCP3 signalling pathway can be targeted to protect the heart against the damaging effects of IR.

doi:10.1016/j.bbabio.2014.05.349

S3.P9

Hepatic mitochondrial function and efficiency in rats simultaneously exposed to chronic high-fat diet and low doses of persistent organic pollutant

Lillà Lionetti[a], Raffaella Sica[a], Vincenzo Migliaccio[a], Assunta Lombardi[a], Marilena Lepretti[b], Ivana Caputo[b], Rosalba Putti[a]
[a]*Department of Biology, University of Naples Federico II, Italy*
[b]*Department of Chemistry and Biology, University of Salerno, Italy*
E-mail address: lilla.lionetti@unina.it

Introduction: The physiological impact associated with chronic simultaneous exposure to both high-fat diet and low doses of persistent organic pollutants, such as p,p′-diphenyldichloroethene (DDE) (DDT's major metabolite with the highest persistence), is poorly understood. Given that liver is one of the main organs involved in response to both toxic injury and high-fat feeding, the aim of the present work was to investigate the effect of chronic simultaneous exposure to low doses of DDE and high-fat feeding on hepatic mitochondrial function and efficiency, reactive oxygen species (ROS) production and endoplasmic reticulum (ER)-stress in rats. Methods: Three groups of 8 rats were so treated for 4 weeks: 1) standard diet (10% fat J/J) (N rats); 2) high-fat diet (45% fat J/J) (D rats); and 3) high-fat diet plus DDE (10 mg/kg b.w. by gavage) (D + DDE rats). In isolated liver mitochondria, oxygen consumption rates (OCR) in the presence of FADH2-dependent (succinate + rotenone) and lipid (palmitoylcarnitine + malate) substrates were determined polarographically. To test mitochondrial efficiency, we measured the OCR oligomycin/OCR FCCP ratio. ROS and lipid peroxide production was analyzed by determining H2O2 production and TBARs content. Glucose-regulated protein (GRP) 78 expression, as ER-stress marker, was determined in liver homogenates. Results: D rats showed higher fatty acid oxidation rate, ROS production and GRP78 expression vs. N rats. D + DDE rats showed a further increased fatty acid oxidation rate, higher succinate state 4 rates and OCR oligomycin/OCR FCCP ratio suggesting an increased mitochondrial uncoupling compared to D groups. D + DDE groups also showed increases in ROS production and GRP78 expression similar to those found in D rats vs. N. Conclusion: Compared to high-fat diet treatment, the simultaneous chronic exposure to low doses of DDE and high fat diet elicited further increase in lipid utilization that may be useful to cater to energy requests for detoxification processes. In addition, this simultaneous exposure also elicited an increase in the degree of mitochondrial uncoupling that may counteract a possible further increase in ROS production and ER stress caused by toxic injury.

doi:10.1016/j.bbabio.2014.05.350

S3.P10

Activation of UCP and mitoKATP channel efficiently decreases superoxide anion production in insect mitochondria

Malgorzata Slocinska[a], Rosinski[b], Wieslawa Jarmuszkiewicz[a]
[a]*Adam Mickiewicz University Poznań, Poland*
[b]*Grzegorz, Poland*
E-mail address: slocina@amu.edu.pl

Uncoupling proteins (UCPs) and ATP-regulated channels (mitoKATP channels) are proteins located in inner mitochondrial membrane. It was evidenced, that these energy-dissipating systems are present in insect tissues, such as trophic tissue of fat body and leg muscle. In cockroach Gromphadorhina coqereliana, GcUCP4 as well as mitoKATP channel decrease superoxide anion production ($O2^-$) [1,2]. In the present study, we elucidated, whether GcUCP4 and mitoKATP channel collaborate in the modulating of reactive oxygen species (ROS) level in fat body and muscle tissue of cockroach. In isolated mitochondria, UCP was activated by palmitic acid and mitoKATP channel was stimulated by diazoxide or pinacidil and $O2^-$ production was measured by nitroblue tertazolim method (NBT). Simultaneous activation of both proteins resulted in a very efficient decrease of $O2^-$ level, approximately 2.5 times higher than when the proteins were activated separately. Moreover, after the addition of the UCP and mitoKATP channel inhibitors, such as GTP or ATP, the level of $^-$ increased by approximately 25%, but it was still almost twice lower compared to control conditions, where activators and inhibitors were not added. When both UCP and mitoKATP channel were activated in the presence of GTP (an UCP inhibitor), the increase in $O2^-$ production was higher compared to measurements with ATP (a mitoKATP channel inhibitor). These results suggest a bigger amount of UCP protein in insect inner mitochondrial membrane and/or a more significant role of this protein in modulation of mitochondrial ROS formation. The cumulative effects of GcUCP4 and mitoKATP channel activation on mitochondrial $^-$ formation indicate the physiological role of both proteins in ROS formation in fat body and muscle of cockroach. We hypothesize, that GcUCP4 and mitoKATP channel might be implicated in cellular protection against metabolic stress in insect tissues during energy demand events such as molting or flight.

References

[1] M. Slocinska, J. Lubawy, W. Jarmuszkiewicz, G. Rosinski, Evidences for an ATP-sensitive potassium channel in muscle and fat body mitochondria of insect. J Insect Phys. 59 (2013) 1125–1132.
[2] Slocinska M, Antos Krzeminska N, Rosinski G, Jarmuszkiewicz W. Identification and characterization of UCP4 in fat body and muscle mitochondria from the cockroach G. cocquereliana. J Biol Biomembr. 43 (2011) 717–727.
This work is supported by the grant from the PMoES (NN301636440).

doi:10.1016/j.bbabio.2014.05.351