reactive nitrogen species produced by NO-release immune cells. The aim of this work is to further unravel the mode of action of miconazole on microorganism hemoprotein. Inhibitors that target flavohemoproteins are attractive candidates for antibiotic development. Spectroscopic analysis of the oxidized or reduced flavohemoprotein from *Ralstonia eutrophus* (FHP) in the presence of different antibiotics have been done. Addition of Miconazole and econazole and other antimicrobial substances from plants and algae caused spectroscopical change to FHP indicating heme coordination. To identify protein–drug interactions that contribute to binding specificity and affinity, we performed co-crystallization trials of FHP in the presence of miconazole or econazole. We have obtained crystals of FHP in complex with miconazole and econazole. X-ray diffraction experiments of these crystals is conducted in order to determine the crystallographic structure of the antibiotic–protein complex.

doi:10.1016/j.bbabio.2008.05.235

S10.10 Discrepancy between effects of nitroglycerin and nitric oxide on mitochondrial respiration

P. Dungel, S. Haindl, C. Wagner, M. Jafarmadar, S. Bahrami, H. Redl, A.V. Kozlov
L. Boltzmann Instutute for Traumatology, Vienna, Austria
E-mail: peter.dungel@lbitrauma.org

Nitric oxide (NO) is known to inhibit mitochondrial respiration preferentially by binding to cytochrome oxidase. Such situation is expected in sepsis, which is accompanied by induction of inducible NO-synthase. Nitroglycerin (NG) is a widely used drug, which is believed to exert its biological activity through release of NO. This study aimed at comparison of effects NO and nitroglycerin on mitochondrial respiration and clarifying whether illumination at specific wavelengths recovers mitochondrial respiration inhibited by either NO or NG. NO fully inhibited respiration of liver mitochondria at concentrations occurring under septic shock. The respiration was completely restored by illumination at the wavelength of 430 nm while longer wavelengths were less effective. NG inhibited mitochondrial respiration though the efficiency of GTN was lower compared to NO concentrations observed in sepsis models. However, NG inhibition was absolutely insensitive to illumination regardless of wavelength used. Our data show that visible light of short wavelengths efficiently facilitates the recovery of mitochondria inhibited by NO-gas at the levels generated under septic conditions. The inhibition of mitochondrial respiration by NG is not sensitive to the visible light, suggesting another than NO-gas mediated mechanism of inhibition.

doi:10.1016/j.bbabio.2008.05.236

S10.11 Bioenergetic regulation of nitric oxide production in rat mitochondria

Laura B. Valdez, Tamara Zaobornyj, Silvina Bombicino, Alberto Boveris
Laboratory of Free Radical Biology, School of Pharmacy and Biochemistry, University of Buenos Aires, Argentina
E-mail: lbvaldez@ffyb.uba.ar

Not only heart mitochondrial membranes (2.08 ± 0.08 nmol/min. mg protein), but also heart coupled mitochondria, exhibit an enzymatic production of NO. MtNOS activity is 40% lower in state 3 than in state 4, and shows an exponential dependence on membrane potential. The aim of this work was to further characterize mtNOS activity regulation by the redox state of the respiratory chain and membrane potential. The generation of NO (nmol/min mg protein) by heart submitochondrial particles resulted 0.45 ± 0.02. This value was enhanced up to 0.81 ± 0.09 when mtNOS activity was assessed in the presence of succinate and ATP. The addition of rotenone inhibited by 50% this reversed electron transfer-supported mtNOS activity. Besides, the ability of mtNOS to modulate O_2 uptake and H_2O_2 production, is termed mtNOS functional activity. Supplementation of state 3 mitochondria with L-arginine decreased respiration rates by 15–20%, while addition of L-NAME increased O_2 consumption by 10%. The addition of L-arginine enhanced state 4 H_2O_2 production by 14–21%, whereas supplementation with L-NAME declined H_2O_2 generation by 7–9%. Interestingly, these effects were observed in coupled mitochondria, but not in mitochondrial membranes. We conclude through direct and indirect evidence, that mtNOS activity is regulated by membrane potential; and that respiratory chain electron flow modulates NO production; in agreement with the reported physical interaction of mtNOS and respiratory chain components.

doi:10.1016/j.bbabio.2008.05.237

S10.12 Kinetic model of nitric oxide inhibition of cellular respiration in intact cells

Susana Cadenas[a], Enara Aguirre[a], Félix Rodríguez-Juárez[a], Andrea Bellelli[b], Erich Gnaiger[c]
[a]*Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain*
[b]*University 'La Sapienza', Rome, Italy*
[c]*Medical University of Innsbruck, Austria*
E-mail: scadenas@cnic.es

A kinetic model of nitric oxide (NO) inhibition of cellular respiration was developed in HEK 293 cells expressing the inducible isoform of the nitric oxide synthase (iNOS). Endogenous NO production (ISO-NOP, WPI), O_2 concentration and O_2 flux (OROBOROS Oxygraph-2k) were simultaneously recorded in an extended range of O_2 concentrations. Both competitive reversible binding of NO to reduced cytochrome *c* oxidase (CCO) and uncompetitive binding to oxidized CCO were taken into account. Data analysis, by means of standard least squares non linear minimization routines (Matlab, the MathWorks inc., South Natick, MA, USA), showed that the best fit to the experimental data requires the affinity of CCO for O_2 to be modulated by NO bound to the enzyme, such that the species with NO bound to the uncompetitive site has higher K_m than uninhibited CCO, consistent with the inhibitory activity of NO. Our scheme implies that the oxidized CCO derivative bearing NO bound to Cu_B consumes oxygen, albeit with poor efficiency, in a cycle that presumably releases nitrite or nitrate. The model has predictive value and integrates the complex chemistry of the enzyme and physiological adaptations of the cell. Interestingly, addition of NO scavengers reveals that NO has an activation effect in cells, which partially compensates for inhibition.

doi:10.1016/j.bbabio.2008.05.238

S10.13 Toxicity of parabens in testis mitochondria; a possible role on male infertility

Fátima Martins[a], Ana Margarida[a], Maria M. Oliveira[b], Paulo J. Oliveira[c], Francisco P. Peixoto[a]
[a]*Department of Chemistry, CECAV, UTAD, 5001-911, Vila Real, Portugal*
[b]*Centro de Química-Vila Real, UTAD, 5001-911, Vila Real, Portugal*
[c]*Center of Neuroscience and Cell Biology of Coimbra, Coimbra, Portugal*
E-mail: fpeixoto@utad.pt

Parabens are widely used as preservatives in many foods, cosmetics, toiletries, and pharmaceuticals due to their relatively

low toxicity profile and to a long history of safe use. Besides known to have a null or very weak estrogenic activity in estrogen receptor assays *in vitro*, parabens were demonstrated to affect testosterone levels and sperm counts in adult rodents and to decrease the number of elongated spermatids. The aim of the present study was to evaluate the effect of methyl-, ethyl-, propyl-, butylparaben and the main metabolite (*p*-hydroxybenzoic acid) on mitochondria isolated from rat testis. The results obtained demonstrate that the metabolite does not affect state 3 and state 4 respiration, although paraben toxicity increases with the length of alkyl grouping from methyl to *n*-butyl. Mitochondrial membrane potential was decreased by propyl and butylparaben but it was not affected by methyl or ethylparaben. We also investigated the ability of parabens to induce the MPT pore, as defined by the massive release of Ca^{2+} by mitochondria in the suspension, following repeated Ca^{2+} pulses. Specifically, we measured the total mitochondrial Ca^{2+}accumulation necessary to open the MPT pore in the presence of different parabens. Increased susceptibility to the mitochondrial permeability transition was correlated with the length of alkyl grouping from methylparaben to *n*-butylparaben. The effect on respiratory complexes II–III, IV and V were also investigated but no significant alterations were observed in the range of concentrations used (0–0.25 mM). From these results, we conclude for the first time that parabens can interfere with testis mitochondrial function. Therefore, inhibition of testis mitochondrial function could interfere negatively with the male reproductive capacity.

doi:10.1016/j.bbabio.2008.05.239

S10.14 Skeletal muscle UCP3 gender dimorphism in high-fat-diet-induced insulin resistance in aged rats

Yolanda Gómez-Pérez, Emilia Amengual-Cladera, Antoni Català-Niell, Elena Thomàs-Moyà, Magdalena Gianotti, Ana M. Proenza, Isabel Lladó
Metabolisme Energètic i Nutrició, Department Biologia Fonamental i Ciències de la Salut and IUNICS, Universitat de les Illes Balears, Palma de Mallorca, Spain
Ciberobn, ISCIII, Spain
E-mail: isabel.llado@uib.es

We investigated whether the gender dimorphism found in mitochondrial function and oxidative stress leads to differences in the development of high-fat-diet-induced insulin resistance in rat skeletal muscle. Fifteen-month-old male and female rats were fed with a high-fat diet (HFD) for 14 weeks. Oral glucose tolerance test was performed. Serum glucose, insulin and adipokine levels were measured. Oxygen consumption, H_2O_2 production and COX, GPx, GRd and Mn-SOD activities were determined in gastrocnemius muscle mitochondria. Catalase activity, TBARS, protein carbonyl groups, UCP3 and GLUT4 levels were measured in muscle homogenate. Control male rats showed a more marked insulin resistance status than females. HFD induced an increase in both muscle mitochondrial H_2O_2 production and in oxidative damage, together with a decrease in the Mn-SOD activity in both genders. However, HFD fed female rats showed a less marked insulin resistance profile than males, and higher mitochondrial oxidative capacity and UCP3 and GLUT4 protein levels. These results point to a gender dimorphism in the insulin resistance status and in the response of skeletal muscle to HFD feeding which could be related to a more detrimental effect of age in male rats.

doi:10.1016/j.bbabio.2008.05.240

S10.15 The high-fat-diet effect on rat liver mitochondrial biogenesis is sex-dependent

Antònia Nadal-Casellas, Emilia Amengual-Cladera, Ana M. Proenza, Isabel Lladó, Magdalena Gianotti
Metabolisme Energètic i Nutrició, Dept. Biologia Fonamental i Ciències de la Salut and IUNICS, Universitat de les Illes Balears, Palma de Mallorca, Spain
Ciberobn, ISCIII. Spain
E-mail: magdalena.gianotti@uib.es

The aim of this study was to investigate the sex-related differences in rat liver mitochondrial biogenesis in response to a high-fat-diet-induced oxidative stress. Ten-week-old male and female Wistar rats were fed with a pelleted standard diet (control group), or with a cafeteria-diet (HFD group) for 26 weeks. HFD rats had free access to a variety of highly palatable foods: cookies, pork liver paté, fresh bacon, chocolate, ensaïmada (a typical Majorcan pastry) and pelleted standard chow. Body weight was assessed once a month; food and energy intake and whole body respirometry were analyzed at the end of the dietary treatment. Mitochondria oxidative capacity, superoxide dismutase and glutathione peroxidase activities, glutathione levels and oxidative damage markers were measured to confirm the high-fat-diet-induced oxidative stress status. Akt and TFAM protein levels, as markers of mitochondrial biogenesis and differentiation, were also analyzed. Liver mitochondria of female rats showed a higher hydrogen peroxide production and an enhanced antioxidant capacity than those of males. The response to the HFD seems to be different between genders. Thus, female rats could counteract better the oxidant effect of the HFD than males, maintaining higher levels of mitochondrial differentiation than males.

doi:10.1016/j.bbabio.2008.05.241

S10.16 Ca^{2+}-induced reactive oxygen species production in alpha-glicerophosphate supported mitochondria

Laszlo Tretter, Zsofia Komary, Vera Adam-Vizi
Department of Medical Biochemistry, Semmelweis University; Neurobiochemical Group, Hungarian Academy of Sciences and Szentagothai Janos Knowledge Center, Budapest, Hungary
E-mail: tretter@puskin.sote.hu

Alpha-glycerophosphate dehydrogenase (α-GPDH) is localized on the outer surface of mitochondrial inner membrane and plays a role in the cytosolic-mitochondrial shuttle of reducing equivalents. Earlier we have shown that oxidation of alpha-glycerophosphate (α-GP) is able to induce reverse electron transport (RET) in brain mitochondria and RET is associated with an accelerated Reactive Oxygen Species (ROS) production. In the present study the effects of Ca^{2+} were studied on the H_2O_2 production in brain mitochondria respiring on α-GP. H_2O_2 formation was measured by the Amplex method. It is shown that in the presence of ADP micromolar concentrations of Ca^{2+} can stimulate α-GPDH-dependent ROS production. ADP prevented opening of mitochondrial permeability transition pore (mPTP) and *via* decreasing the mitochondrial membrane potential ($\Delta\Psi m$) inhibited the RET mediated ROS production. Elevation of calcium concentration up to 5 μM stimulated ROS production and elevated mitochondrial $\Delta\Psi m$. Higher calcium concentrations decreased $\Delta\Psi m$ and also decreased H_2O_2 formation. Blocking the Ca^{2+} uniporter with Ru360 prevented the depolarizing effects of high $[Ca^{2+}]$ and maintained high ROS production. These results show that alpha-glycerophosphate shuttle combined with cytosolic calcium elevation can increase ROS production in brain mitochondria.

doi:10.1016/j.bbabio.2008.05.242