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