

knockdown on respiration and glucose-stimulated insulin secretion in INS-1E insulinoma cells. Furthermore, a model will be proposed that predicts a role for UCP2 in the coordination of the physiological response of beta cells to fluctuating nutrient supply.

doi:10.1016/j.bbabo.2008.05.301

(S12) Mitochondria and disease symposium abstracts (poster and raised abstracts)

S12.7 The use of oxygen and pH-sensitive fluorescent probes for the investigation of perturbed cell metabolism

James Hynes^a, Tomas O'Riordan^a, Dmitri Papkovsky^a, Yvonne Will^b

^aLuxcel Biosciences, Cork, Ireland

^bDrug Safety R&D, Pfizer, Inc., San Diego, CA, USA

E-mail: jhynes@luxcel.com

In standard in vitro systems, the main sources of cellular ATP are glycolysis and oxidative phosphorylation. The balance between these two related energy generating systems can therefore inform on disease states and toxicities where perturbed metabolism is implicated. A highly informative approach to the investigation of such metabolic perturbations is to measure both cellular oxygen consumption and extracellular acidification rate (ECA). Analysis of oxygen consumption gives specific information on OxPhos, while measurement of ECA, under the appropriate conditions, provides information on non-aerobic metabolism. Here we present a new method of assessment of ECA of both adherent and suspension cells, using a long-decay pH-sensitive fluorescent probe and convenient 96-well plate format. As probe emission lifetime is used as the readout, a simple transformation, allows the generation of ECA rates in units of [H⁺] per unit time. We also outline how these probes may be combined with the MitoXpress oxygen consumption assay providing a highly informative dual-parameter metabolic analysis. Such parallel measurements allow inferences to be drawn regarding the site of an observed metabolic insult; thereby allowing altered glycolytic activity to be delineated from direct mitochondrial effects. We examine how such an analytical approach may be deployed for the examination of the perturbed metabolism using compounds of known metabolic impact including antimycin, 2-deoxyglucose, oxamic acid and dichloroacetate as models. This analysis is then extended to more relevant models analyzing the effect of a panel of biguanides on cellular metabolism and relating these observations to their proposed mechanism of action.

doi:10.1016/j.bbabo.2008.05.302

S12.8 Glycine prevents mitochondrial impairment caused by left carotid occlusion

Natalia V. Lobysheva^{a,b}, Olga N. Vorontsova^c, Anton A. Tonshin^a, Aleksei A. Selin^b, Lev S. Yaguzhinsky^a, Yaroslav R. Nartsissov^b

^aBelozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia

^bInstitute of Cytochemistry and Molecular Pharmacology, Moscow, Russia

^cState Zakusov Institute of Pharmacology Russian Academy of Medical Sciences, Moscow, Russia

E-mail: nlobysheva@gmail.com

Mitochondria play a sufficient role in neuronal function. Several cerebral disorders like a stroke results in neuronal degeneration and associated with substantial reduction in mitochondrial functional activity and apoptosis induction. Monitoring of mitochondrial capa-

city for oxidative phosphorylation could be used as an indicator of stroke development. There is evidence from clinical studies that glycine reduced brain damages caused by stroke. However, the mechanism of the protection afforded by glycine is not yet known. In the present study we attempted to elucidate the mechanisms of glycine anti-stroke activity. The left carotid artery occlusion was used as a model of brain ischemia. After occlusion respiratory control index of brain cortex mitochondria was measured. It was reduced from 6.7±0.1 to 4.2±0.1 after 24 h occlusion. The development of apoptosis process was also detected, DNA internucleosomal fragmentation and caspase-3 activation was observed. When animals were treated with glycine *per os* before occlusion the reduction of respiratory ratio and caspase-3 activation were prevented. Glycine allows mitochondria to maintain their respiratory activity in ischemic conditions. Our novel data indicate that anti-stroke glycine activity is associated with its ability to prevent mitochondrial disorder and apoptosis development in brain cortex tissue induced by ischemia.

doi:10.1016/j.bbabo.2008.05.303

S12.9 Dynamic regulation of UCP2 concentration in INS-1E pancreatic beta-cells

Vian Azzu, Charles Affourtit, Eamon P. Breen, Nadeene Parker, Martin D. Brand

MRC Dunn Human Nutrition Unit, Cambridge, CB2 0XY, UK

E-mail: va@mrc-dunn.cam.ac.uk

Uncoupling protein 2 (UCP2) is known to exacerbate the diabetic phenotype by diminishing glucose-stimulated insulin secretion (GSIS). It does this by uncoupling substrate oxidation from ATP synthesis; this uncoupling ultimately attenuates exocytosis of insulin-containing granules. In contrast, UCP2 downregulation improves GSIS and ameliorates diabetes.

UCP2 content in cells appears to be regulated at both the transcriptional and translation levels. We measured UCP2 content by Western blot in INS1E pancreatic beta-cells following addition of cycloheximide to inhibit protein synthesis and observed that UCP2 has a short half-life of about 1 h. To explore this dynamic regulation, we characterised endogenous UCP2 concentrations at the message and protein levels in this pancreatic beta-cell system. We found that over a 24 h period, depending on the concentrations of transcriptional and translational regulators (glucose, serum, glutamine) in the incubation medium, UCP2 protein concentration was 78–432 pg per 100,000 cells (2.6–13.4 ng/mg mitochondrial protein).

doi:10.1016/j.bbabo.2008.05.304

S12.10 Role of mitochondrial DNA mutations in periodontitis

Praturi Gopalakrishna^a, Periyasamy Govindaraj^a, Ayyasamy Vanniarajan^a, Rampalli Viswa Chandra^b, Aileni Amarendra Reddy^b, Lalji Singh^a, Kumarasamy Thangaraj^a

^aCentre for Cellular and Molecular Biology, Hyderabad, India

^bSVS Institute of Dental Sciences, Mahabubnagar, India

E-mail: praturi@ccmb.res.in

Periodontitis is an inflammatory disease affecting the tissues that surround the teeth, as a result of complex interactions between pathogenic bacteria and the host's immune response. There are increasing evidences on the implications of reactive oxygen species (ROS) in the pathogenesis of inflammatory disorders. Therefore, we aimed to analyze the role of genetics, biochemical, membrane potential and evolutionary background in periodontitis. We have

examined a total of 92 patients, of which, 30 (chronic periodontitis [CP] group) were selected for the above analysis. The institutional ethics committee approved this study and informed written consent was obtained from each participant. All clinical parameter scores of the tissue sampling areas in the CP group were significantly higher (<0.001) than in the control group. Complete mtDNA sequencing revealed a total of 264 variations in the patients, including 16 novel mutations, of which 3 were missense mutations (A4234T, A7796G, and G8115R). We also observed significant change in the membrane potential and protein levels of NADH dehydrogenase, Cytochrome c oxidase and HSP 60 in the patients.

doi:10.1016/j.bbabo.2008.05.305

S12.11 Differential inhibition of energy-producing pathways of hepg2 cells by 3-bromopyruvate

Ana Paula P. da Silva, Tatiana El-Bacha, Nattascha Kyaw, Reinaldo S. dos Santos, Wagner S. da Silva, Andrea T. Da Poian, Antonio Galina
Institute of Medical Biochemistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

E-mail: galina@bioqmed.ufrj.br

It was studied the effects of the alkylating agent 3-bromopyruvate (3-BrPA) on ATP producing pathways of HepG2 cells. 3-BrPA decreases HepG2 viability (10% with 10–100 μM ; 25% with 300–1000 μM at 60 min; 60% with 10–100 μM ; 75% with 300–1000 μM at 180 min). 150 μM 3-BrPA did not affect hexokinase-II activity, but incubation with 150 μM for 30 min decreased in 60% lactate production. Cells were incubated with 3-BrPA as above in glucose medium (GM-cells) or glucose-free medium (GFM-cells) and the basal respiration was 10.5 ± 0.62 (GM-cells) or 15.3 ± 0.96 (GFM-cells) $\text{nmol O}_2/5 \times 10^6 \text{ cell} \times \text{min}^{-1}$. A decrease of 22% or 50% of basal respiration by 3-BrPA was detected in GM or GFM cells, respectively. Proton leak was increased only in GM-cells (3.3 ± 0.45 to 5.1 ± 0.5). Maximum respiration was decreased only in GFM-cells (17.3 ± 1.1 to 9.5 ± 1.3). 3-BrPA decreased respiratory control ratio either in GM or GFM cells. In digitonin-permeabilized cells, complex I supported respiration was decreased by 50% in GFM-cells and complex-II supported respiration was inhibited by 50% in both media. Our results suggest that glycolysis and specific sites of mitochondria play a role in 3-BrPA-induced HepG2 death. The toxic effects of 3BrPA depend on the oxidizable substrates supplied to cells.

doi:10.1016/j.bbabo.2008.05.306

S12.12 Restriction of glucose metabolism induces a metabolic switch to oxidative metabolism and drastic alteration of gene expression in glioblastoma cell line LN18

Min Wu, Amy Smift, Denise Chen, Orian Shirihai, David Ferrick
Seahorse Bioscience, 16 Esquire Road, North Billerica, MA 01862, USA
E-mail: mwu@seahorsebio.com

The aim of this study was to determine the cellular effect of restricting glucose metabolism using 2-deoxyglucose in LN18 cells on oxygen consumption rate (OCR) and glycolysis rate (ECAR) as well as alterations in gene expression. The cellular OCR and ECAR were determined using XF24 Analyzer which measures the two parameters simultaneously in microplates. Gene expression was analyzed using Affimatrix human genome arrays. The normalized OCR of 2-deoxyglucose treated LN18 cells was increased while ECAR was decreased compared to control after 48 h treatment. This metabolic shift towards OXPHOS was confirmed by an observed increase in the sensitivity of cellular ATP levels to oligomycin as well as increased mitochondrial

respiration capacity. Apoptosis induced by staurosporine was increased in 2-deoxyglucose treated cells. Gene expression analysis revealed a striking alteration in global gene expression as the result of restricted glucose supply. OXPHOS, pentose phosphate pathway, IGF-1 signaling, PI3K/AKT signaling, cell cycle check point, apoptosis signaling and oxidative stress pathways were among 2710 genes showed significantly altered expression. Our data demonstrated that restricting glucose drove LN-18 cells to a more oxidative state accompanied by growth suppression and drastically altered gene expression in pathways that converge at cellular energy metabolism and cell proliferation as well as death.

doi:10.1016/j.bbabo.2008.05.307

S12.13 Hepatitis C virus proteins cause calcium-mediated mitochondrial dysfunction and hif-linked bioenergetic compensatory adaptation

Claudia Piccoli^a, Giovanni Quarato^a, Maria Ripoli^a, Rosella Scrima^a, Annamaria D'Aprile^a, Domenico Boffoli^a, Darius Moradpour^b, Nazzareno Capitanio^a

^aDepartment of Biomedical Sciences, University of Foggia, Foggia, Italy

^bDivision of Gastroenterology and Hepatology, University of Lausanne, Switzerland

E-mail: c.piccoli@unifg.it

Hepatitis C virus (HCV) infection induces a state of oxidative stress that is more pronounced than that in many other inflammatory diseases. In this study we used well-characterized cell lines inducibly expressing the entire HCV open-reading frame to investigate the impact of viral protein on cell bioenergetics. It was shown that HCV protein expression has a profound effect on mitochondrial oxidative metabolism, with specific inhibition of complex I and $F_1F_0F_1F_0$ -ATPase activities, depression of $\text{mt}\Delta\Delta$ and oxidative phosphorylation coupling efficiency, increased production of reactive oxygen and nitrogen species. Importantly, all these effects were causally related to mitochondrial calcium overload, as inhibition of mitochondrial calcium uptake completely reversed the observed bioenergetic alterations. Noteworthy, in spite of the oxidative phosphorylation impairment, survival of HCV proteins-expressing cells was assured by an adaptive up-regulation of glycolytic enzymes. This was linked to normoxic stabilization of the hypoxia-inducible factor (HIF 1 α). Overall, the results presented show that expression of HCV proteins causes deregulation of ER-mitochondrial calcium homeostasis occurring upstream of further mitochondrial dysfunction. The expected bioenergetic unbalance is however compensated by a HIF-dependent transcriptional mechanism. These observations provide new insights into the pathogenesis of hepatitis C and may offer new opportunities for therapeutic intervention.

doi:10.1016/j.bbabo.2008.05.308

S12.14 Novel Mt-DNA missense mutation in ND1 (A3418G \rightarrow N38D) associated with mitochondrial dysfunction in megakaryoblastic leukaemic cells

Maria Ripoli^a, Claudia Piccoli^a, Rosella Scrima^a, Mauro Di Ianni^c, Lorenzo Moretti^c, Massimo Carella^b, Domenico Boffoli^a, Antonio Tabilio^d, Nazzareno Capitanio^a

^aDepartment of Biomedical Science, University of Foggia, Foggia, Italy

^bCSS Hospital, San Giovanni Rotondo, Italy

^cDepartment of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy

^dDepartment of Internal Medicine and Public Health, University of L'Aquila, L'Aquila, Italy.

E-mail: m.ripoli@unifg.it