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.

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(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

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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.

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

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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 capacity 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.

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

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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).

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S12.10 Role of mitochondrial DNA mutations in periodontitis

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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