discrepancies between the measurements of $[Ca^{2+}]_M$ with dyes or targeted proteins are also qualitative in some cases and significant changes in the behaviour or kinetics of $[Ca^{2+}]_M$ appear when comparing measurements obtained with both kinds of methods [3, 4]. We have made here a systematic comparison of the response of two fluorescent dyes, rhod-2 and rhod-FF, and two Ca^{2+}-sensitive proteins, aequorin and pericam. Our results show that measurements obtained with aequorin and pericam are consistent in terms of dynamic Ca^{2+} changes. Instead, fluorescent dyes failed to follow Ca^{2+} changes adequately, especially during repetitive stimulation. In particular, measurements obtained with rhod-2 or rhod-FF evidenced the previously reported Ca^{2+}-dependent inhibition of mitochondrial Ca^{2+} uptake [5], but data obtained with aequorin or pericam under the same conditions did not. The reason for the loss of response of fluorescent dyes is unclear. Loading with these dyes produced changes in mitochondrial morphology and membrane potential, which were small and reversible at low concentrations (1-2 mM), but produced large and prolonged damage at higher concentrations. Our results suggest that $[Ca^{2+}]_M$ data obtained with these dyes should be taken with care and confirmed with other methods.

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15P.16 Inhibition of nitric oxide synthase protects hypercholesterolemic mice mitochondria against permeability transition

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Atherosclerosis is associated with elevated levels of oxidized products derived from nitric oxide (NO) and superoxide radicals indicating nitroxidative stress. We have recently shown that hypercholesterolemic LDL receptor knockout mice (*LDLR*$^{-/-}$) mitochondria release high levels of reactive oxygen species (ROS). The aim of this work was to verify the effect of a nitric oxide synthase (NOS) inhibitor (L-NAME) on the membrane permeabilization and redox state of *LDLR*$^{-/-}$ liver mitochondria. Mitochondrial permeability transition (MPT) (cyclosporine sensitive swelling and calcium release), ROS (H2DCF-DA and Amplex-red) and NO production rates (DAF-FM diacetate), and protein S-nitrosothiol content were determined in *LDLR*$^{-/-}$ and control liver mitochondria before and after administration of L-NAME, *in vitro* (50 μM) and *in vivo* (1 mg/Kg/day, during 14 days). The *LDLR*$^{-/-}$ mitochondria presented higher levels of nitrotyrosine (Western Blot), which was undetectable in control mitochondria. *In vitro* L-NAME protected *LDLR*$^{-/-}$ mitochondria against MPT. However, in control mitochondria, L-NAME favored MPT. These results were also observed after *in vivo* chronic L-NAME treatment. Under all conditions, L-NAME reduced mitochondria ROS and NO production rates. Mitochondrial protein S-nitrosothiol content decreased only in L-NAME treated control but not in *LDLR*$^{-/-}$ mitochondria. These results suggest that *LDLR*$^{-/-}$ mitochondria are under nitroxidative stress which is normalized by L-NAME treatment, thus correcting their higher susceptibility to MPT. On the other hand, inhibiting physiological NO production in control mitochondria promotes MPT which is associated with decreased protein S-nitrosothiol content. Therefore, mitochondrial nitric oxide synthase activity seems to be directly involved in the nitroxidative stress in the atherosclerosis prone *LDLR*$^{-/-}$ mice. These findings might be relevant for the vascular wall cell death that occurs in atherogenesis.

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15P.17 Mitochondria energy metabolism and store-operated calcium entry in *mdx* mouse myoblasts

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Duchene muscular dystrophy (DMD) is a neuromuscular genetic disease leading to progressive damage of muscle and premature death. DMD is caused by mutation in the dystrophin encoding gene leading to lack of dystrophin. Patients with DMD exhibit aberrant calcium homeostasis and altered energy metabolism. As dystrophin seems to appear not before muscle cells differentiation any phenotypic changes in *mdx* myoblasts have been unexpected. In contrary to such assumption, a significant increase in nucleotide-dependent receptors activity in *mdx* myoblasts was described. Here we found that myoblasts derived from *mdx* mouse exhibit significantly decreased oxygen consumption, enhanced mitochondrial membrane potential and ROS production, stimulated lactate synthesis but unchanged ATP content. Interestingly, in *mdx* myoblasts stably transfected with minidystrophin-encoding gene some features of wild phenotype were restored. This latter observation strongly indicates that all changes observed in *mdx* myoblasts in comparison to the wild cells were related to the point mutation in dystrophin gene. Moreover, changes in mitochondrial metabolism correlated with enhanced rate of thapsigargin-induced store-operated Ca^{2+} entry. Although a direct link between these events can not been excluded, changes in SOC activity due to enhanced expression of proteins involved in store-operated Ca^{2+} channel formation and/or activation have to also be considered. In sum, these results confirm our earlier findings indicating that the point mutation in dystrophin-encoding gene may give variety of phenotypic changes at the early stage of muscle cell differentiation.

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15P.18 Mitochondria of activated macrophages utilize glycolytic ATP to maintain membrane potential and prevent apoptosis

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We have previously investigated the bioenergetic consequences of activating J774.A1 macrophages (MΦ) with interferon (IFN)γ and lipopolysaccharide (LPS) and found that there is a nitric oxide (NO)-

dependent mitochondrial impairment and stabilization of hypoxia-inducible factor (HIF)-1α, which synergize to activate glycolysis and generate large quantities of ATP. The upregulation of glycolysis is completely dependent on NO and HIF-1α. Furthermore, HIF-1α stabilization is biphasic, with a reactive oxygen species-dependent early phase (1-2 h after activation) and later phase associated with the release of NO. We now demonstrate, using TMRM fluorescence and time-lapse confocal microscopy, that activated MΦ maintain a high mitochondrial membrane potential ($\Delta\Psi_m$) despite the complete inhibition of respiration by NO. The high $\Delta\Psi_m$ is maintained by utilization of a significant proportion (approx 30%) of total glycolytically-generated ATP and is achieved by the reverse functioning of F_OF_1-ATP synthase and adenine nucleotide translocase (ANT). Treatment of activated MΦ with inhibitors of either of these enzymes (oligomycin or bongkrekic acid), but not with inhibitors of the respiratory chain complexes, led to a collapse in $\Delta\Psi_m$ and to an immediate increase in intracellular [ATP]. This collapse of $\Delta\Psi_m$ was biphasic, with a rapid initial drop of approx 30% followed by a gradual decline, and was associated with translocation of Bax from cytosol to the mitochondria, release of cytochrome *c* into the cytosol, activation of caspases 3 and 9, and apoptotic cell death. Our results indicate that during inflammatory activation "glycolytically competent cells" such as MΦ utilize significant amounts of the glycolytically-generated ATP to maintain $\Delta\Psi_m$ and thereby prevent apoptosis. It remains to be investigated whether the diversion of energy for this purpose, which is also likely to occur in tissues, favours or is detrimental to the successful outcome of an inflammatory response.

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15P.19 Swapping mutants of voltage dependent anion channel highlight the functional importance of the N-terminal and confers anti-ageing features to the cell

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Voltage-dependent anion channels (VDACs) are the most abundant proteins of the mitochondrial outer membrane that form hydrophilic pore structures. VDAC constitutes the main pathway through which metabolites are exchanged between the cytosol and mitochondria and also serves as a site for the docking of cytosolic proteins, such as hexokinase. The structure of mammalian VDAC1 has been recently solved by NMR and crystallization experiments [1]. It shows a rather compact transmembrane channel, formed by 19 amphipathic β strands connected by short turns and loops, with the striking addition of the N-terminal moiety structured with α-helix segments. The N-terminal sequence is located inside the channel, forming a partial obstruction of the wide pore. CD and NMR spectroscopy showed that the 20 aa N-terminal peptide needs a special environment to form an ordered α-helix secondary structure. In addition it was shown that the deletion of the N-terminal does not change the mitochondrial targeting of the protein [2]. In higher eukaryotes three VDAC isoforms exist, but only VDAC1 and VDAC2 are well characterized. On the contrary VDAC3 has been poorly studied and does not show an evident pore-forming ability [3]. In this work, we provide insight into the function of the isoform 3 by exchanging the N-terminal sequence of the human VDAC3 with the homologous sequences of human VDAC1 and VDAC2. The activity of the wild type and chimeric proteins was monitored in Δ*por1* yeast strain. Results obtained in complementation assay, oxidative stress resistance, chronological aging, ROS production and mitochondrial membrane activity measurements, outline the importance of the N-terminal moiety of VDAC isoforms in the function of the protein. Surprisingly swapping mutants show a doubled lifespan in comparison with wild type yeast strains.

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15P.20 Analysis of cellular function by use of cell-penetrating oxygen sensing probes

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Mitochondria are involved in many metabolic pathways such as production of ATP or the Krebs cycle. Dysfunction of the mitochondria can lead to many disorders such as neurodegenerative diseases or metabolic disorders. Therefore, monitoring of intracellular oxygen (as well as the other parameters such as Ca^{2+} or K^+) can be used to assess mitochondrial function, as most of cellular oxygen is consumed by the mitochondria [1, 2]. Sensing of intracellular oxygen in mammalian cell lines can be performed by use of Pt-porphyrin based probes and phosphorescence quenching method. One of the examples of this approach is the recently constructed phosphorescent probes based on Pt(II)-coproporphyrin I (PtCP) dye. These probes were tailored in a manner to pass cell membrane by conjugation to cell-penetrating peptides. Cationic and hydrophobic nature of these probes eliminates the need for transfection reagents [2]. One such probe is PEPP0 which comprises an uncharged PtCP derivative covalently linked to bactenecin 7 (15-24) peptide. The uptake and toxicity of PEPP0 was studied on PC12, HCT116, SH SY5Y and HeLa cells. Fluorescence microscopy imaging has revealed cytoplasmic localization of this probe with a pattern resembling the mitochondrial one. It was found that probe uptake by HCT116 cells, was largely due to endocytosis mechanisms; whereas it was endocytosis, temperature and ATP independent in PC12 cells. Respirometric experiments with mitochondrial inhibitors and uncouplers were performed on differentiated PC12 cells transfected with PEPP0 probe using time-resolved fluorescence (TR-F) measurements (phosphorescence lifetime based sensing of O_2) and different levels of external (atmospheric) hypoxia. This allowed observation of localized deoxygenation of the cells. This probe chemistry and measurement approach utilizes microplates and standard TR-F plate reader, which allows high throughput analysis. Overall, the new intracellular probes are useful for the monitoring of oxygen in cultures of adherent mammalian cells.

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