(endoplasmic reticulum, Golgi complex, mitochondria) was empty. Ca^{2+}_i concentration did not change after treatment of epinephrine and estradiol from November till February. However in October and March estradiol (10^{-5} M) stimulated rapidly increase of $[Ca^{2+}]_i$ (from 60 to 145 nM). Thus in winter during metabolic depression $\Delta\psi_{mit}$ decreased while Ca^{2+}_i concentration increased in lamprey hepatocytes, but in autumn under the epinephrine and cAMP influence energetic suppression was reversible and increase of Ca^{2+}_i after estradiol treatment was observed.

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15P.13 Fluorescent visualization of NAD(P)H oxidoreductase activity in the outer mitochondrial membrane and in cytosol on acute tissue slices

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Free cytosolic and membrane-bound NAD(P)H oxidoreductases play an important role in immune response, detoxication of drugs and xenobiotics, and in signaling. However, the physiological and pathological roles of NAD(P)H oxidoreductases of the outer mitochondrial membrane are not generally recognized even in spite of their capability to produce ROS and regulate the permeability transition pore opening under certain conditions [1]. The main reason for this is the absence of methods for efficient discrimination between cytosolic (microsomal) and outer mitochondrial oxidoreductases. Recently we developed a flow-cytometry-based method for the semiquantitative assessment of the activity of NADH and NADPH oxidoreductases in the outer mitochondrial membrane and cytosol [2]. The method is based on the capability of a range of NAD(P)H oxidoreductases to reduce lucigenin to highly fluorescent water-insoluble dimethylbiacridene by two-electron reduction (DT-diaphorase) or through two consecutive steps of one-electron reduction with an intermediate cation radical (NADH cytochrome b_5 reductase, NADPH cytochrome P450 reductase) [3, 4]. The discrimination of oxidoreductases appeared to be possible due to the fact that oxidoreductases of the outer mitochondrial membrane changed the apparent mechanism of lucigenin reduction (from 1-e to 2-e) as spontaneous oxidation of cation radical by cytochrome *c* oxidase was blocked [2]. The method proposed allowed one to assess and rapidly compare the activity of six groups of NAD(P)H oxidoreductases in different cell lines. However, the method required the use of detached or isolated cells and prohibited a comparison of lines of cells of irregular shape or different size. Here we present a modification of this approach, which allows the assessment, visualization, and discrimination of activities of various NAD(P)H oxidoreductases using acute tissue slices. The approach is suitable for tissues composed of cells of different types, size, and shape (brain, kidney, heart). Costaining with Mito Tracker Red, Hoechst, etc. allows specifying the localization of oxidoreductase activity. The approach can be helpful in studies of the role of NAD(P)H oxidoreductases in the range of physiological and pathological processes.

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15P.14 Upregulation of human selenoprotein H in murine hippocampal neuronal cells promotes mitochondrial functional performance and biogenesis

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Selenoprotein H (SelH) is one of the 25 known selenoproteins. Previous studies have shown that overexpression of SelH in murine hippocampal neuronal HT22 cell line ameliorates neuronal death after UVB irradiation by reducing ROS production and by blocking mitochondrial initiated apoptotic cell death pathway. The objective of this study was to examine the effects of SelH on mitobiogenesis and mitochondrial function. Three experiments were performed. 1) Protein levels of peroxisome proliferator-activated receptor- coactivator (PGC)-1 and -1β (PGC-1 and PGC-1β), nuclear respiratory factor-1 (NRF-1), mitochondrial transcription factor A (mtTFA), and cytochrome *c* were measured using Western blot analyses; mitochondrial respiration and oxygen consumption were measured using oxygraph; and mitochondrial mass was determined using mitotracker coupled with cell imaging. 2) Both SelH- and vector-tranfected HT22 cells (SelH-HT22 and vector-HT22, respectively) were irradiated with 7 J/cm^2 UVB and the above mitochondria-related markers were measured. 3) Selenite was added to the culture media and PGC-1, NRF-1 and mitochondrial respiration were measure in HT22 cells treated with or without UVB irradiation. Our results demonstrated that transfection of human SelH gene into neuronal HT22 cells significantly increased the translational levels of PGC-1 and NRF-1, two key factors that regulate mitochondrial biogenesis. As expected, mitochondrial cytochrome *c* content was elevated, mitochondrial respiration was enhanced and mitochondrial mass was increased in the selH-HT22 compared to vector-HT22 cells. Supplementation of selenite increased the levels of mitobiogenesis regulation factors. We conclude that overexpression of SelH promotes mitobiogenesis and improves mitochondrial functional performance. These effects can also be achieved by supplementation of selenite.

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15P.15 Monitoring mitochondrial $[Ca^{2+}]$ dynamics with fluorescent dyes and targeted proteins

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The dynamics of $[Ca^{2+}]$ in the mitochondrial matrix has received much attention in the last 20 years because of its importance in a large variety of critical cellular processes, from energy production to apoptosis. Measurements of mitochondrial $[Ca^{2+}]$ have been made using two different methods: fluorescent Ca^{2+}-sensitive dyes such as rhod-2 or similar, and fluorescent or luminescent targeted proteins such as aequorin, pericam or camaleons. Unfortunately, data obtained with each of these approaches are very different, both qualitatively and quantitatively, and the reasons for the discrepancies are still unclear. While studies using fluorescent dyes report maximum $[Ca^{2+}]_M$ values of 2-3 mM [1], data obtained with targeted luminescent and fluorescent proteins indicate that $[Ca^{2+}]_M$ can reach much higher values, up to tenths or hundreds of micromolar [2, 3]. Moreover, the

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)-