

(endoplasmic reticulum, Golgi complex, mitochondria) was empty.  $\text{Ca}^{2+}_i$  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  $[\text{Ca}^{2+}]_i$  (from 60 to 145 nM). Thus in winter during metabolic depression  $\Delta\psi_{\text{mit}}$  decreased while  $\text{Ca}^{2+}_i$  concentration increased in lamprey hepatocytes, but in autumn under the epinephrine and cAMP influence energetic suppression was reversible and increase of  $\text{Ca}^{2+}_i$  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

Alexey G. Kruglov, Anna B. Nikiforova, Roman S. Fadeev  
Institute of Theoretical and Experimental Biophysics RAS, Russian Federation  
E-mail: krugalex@rambler.ru

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.

### References

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

Natalia Mendeleev, Suresh L. Mehta, Sam Witherspoon, Qingping He, Jonathan Sexton, P. Andy Li  
Department of Pharmaceutical Sciences, BRITE,  
North Carolina Central University, Durham, NC 27707, USA  
E-mail: pli@nccu.edu

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 -1 $\beta$  (PGC-1 and PGC-1 $\beta$ ), 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-transfected HT22 cells (SelH-HT22 and vector-HT22, respectively) were irradiated with 7 J/cm<sup>2</sup> 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 measured 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 $[\text{Ca}^{2+}]$ dynamics with fluorescent dyes and targeted proteins

Rosalba I. Fonteriz, Sergio de la Fuente, Alfredo Moreno, Carmen D. Lobatón, Mayte Montero, Javier Alvarez  
Institute of Biology and Molecular Genetics (IBGM),  
Department of Biochemistry, Molecular Biology and Physiology,  
Faculty of Medicine, University of Valladolid and CSIC,  
Ramón y Cajal, 7, E-47005 Valladolid, Spain  
E-mail: mmontero@ibgm.uva.es

The dynamics of  $[\text{Ca}^{2+}]$  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  $[\text{Ca}^{2+}]$  have been made using two different methods: fluorescent  $\text{Ca}^{2+}$ -sensitive dyes such as rhod-2 or similar, and fluorescent or luminescent targeted proteins such as aequorin, pericam or cameleons. 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  $[\text{Ca}^{2+}]_M$  values of 2-3 mM [1], data obtained with targeted luminescent and fluorescent proteins indicate that  $[\text{Ca}^{2+}]_M$  can reach much higher values, up to tenths or hundreds of micromolar [2, 3]. Moreover, the