activation of caspase 3. Glycine also maintained phosphorylating ability of mitochondria after incubation of rats' brain cortex slices under anoxia for 30 min. Neuronal death during ischemic stroke mediated by glutamate excitotoxicity which results in elevation of intracellular calcium concentration. Elevated concentrations of calcium induce mitochondrial permeability transition pore, which dissipates mitochondrial electrochemical gradient and lead to energy collapse. Therefore we investigated the effect of glycine to influence directly on calcium capacity of isolated mitochondria in conditions close to brain tissue surviving during ischemic stroke. We studied the calcium capacity of isolated brain mitochondria after incubation under anoxia at different temperatures and the effect of glycine on this parameter. Concentration of calcium in the incubation medium and the mitochondrial membrane potential were measured. Incubation of the mitochondria at room temperature (22 °C) under 30 min of anoxia led to a decrease of the calcium capacity of mitochondria by 80–90% compared with intact mitochondria, also significantly decreased sensitivity to cyclosporin A. Calcium capacity at the same conditions and in the presence of glycine 5 mM was reduced only by 50–60%. There was a concentration dependence of this effect and it could be observed under not less than 2 mM glycine. Our data show that glycine prevents decrease of calcium capacity in isolated brain mitochondria during anoxic conditions. These findings suggest a novel mechanism for glycine as a potential stroke therapeutic.

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9P.9 Evaluation of neuroprotective abilities of the novel mitochondria-targeted antioxidants

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Over the world, brain ischemia is one of the most common causes of death and adult disability. Oxidative stress is known to be highly associated with brain ischemia with an important role of mitochondria as a major source of reactive oxygen species. Therefore, therapeutic approaches targeting mitochondrial dysfunction and oxidative damage hold great promise in neurodegenerative diseases. We tested mitochondria-targeted chimeric compounds carrying antioxidant moiety as potential agents to efficiently alleviate the deleterious consequences of ischemic insult. Among all tested compounds the highest efficiency was displayed by SkQR1 consisting of a rhodamine moiety linked to a plastoquinone residue. Brain ischemia in rats was induced by insertion of a silicon-coated thread in the middle cerebral artery (MCA). The volume of brain infarct was determined on the first postoperative day by magnetic resonance imaging. Behavioral test was performed 1 day before the surgery and on the first day after the induction of ischemia. Measuring the proteins content in the brain homogenate tissue was determined by Western blotting. We found that a single intraperitoneal injection of SkQR1 at the concentration of 0.5, 1, 2 mM/kg before and after MCA occlusion significantly diminishes infarct volume and improves performance of a test characterizing neurological deficit of ischemic animals in a dose-dependent manner. An analog of SkQR1 without plastoquinone did not display apparent neuroprotective properties. We also revealed that SkQR1 activates signaling pathways involved in ischemic tolerance induction. We conclude that beneficial effect of rhodamine derivative of mitochondria-targeted compound SkQR1 causing significant improvement of neurological functions and decreased infarct volume may be explained by a direct antioxidative effect of the drug. However, we cannot exclude some other mechanisms of SkQR1 action, in particular, through a mechanism of ischemic tolerance induction.

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9P.10 Oxidative inactivation of mitochondrial creatine kinase: Differential sensitivity of isoforms

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Isoforms of creatine kinase (CK) are key players in energy metabolism of many cells with high and/or fluctuating energy demands by providing energy buffer and energy transfer functions. They are easily inactivated in situations of oxidative stress, which makes them a critical factor for energy failure occurring in many related pathologies. Reactive oxygen and nitrogen species (ROS, RNS) not only induce enzymatic inactivation, which occurs with all CK isoenzymes, but also specific damage to the mitochondrial CK isoforms (MtCKs). This includes impairment of critical MtCK properties like destabilization of the native octameric state or decreased membrane binding capacity [1]. Using purified recombinant proteins, cell homogenates and mitochondria isolated from rat heart and brain, we have compared sarcomeric sMtCK (expressed in heart and skeletal muscle) and ubiquitous uMtCK (expressed in many other tissues) with respect to their sensitivity to oxidative inactivation induced by the drug doxorubicin or occurring spontaneously after extraction under non-reducing condition. Sarcomeric sMtCK showed significantly higher sensitivity to oxidation and was the isoform responsible for the loss of CK activity in heart extracts upon storage under non-reducing conditions. The sMtCK dimer was more easily inactivated as compared to the octamer, and solubilization of sMtCK from membrane (promoting dimerization) made the protein an especially vulnerable substrate for inactivation. This differential susceptibility of the two MtCK isoenzymes has been related to some differences in their molecular structures (e.g. number and surface exposure of cysteine residues). It may contribute to energy deficits that occur in oxidatively stressed heart expressing the sMtCK isoform [2,3].

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9P.11 Liver mitochondria and insulin resistance

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According to the research field of scientists, either β-cell, peripheral insulin sensitivity, adipose tissue and related complex signalling environment is believed to be central in the pathology of type-II diabetes. Nevertheless and because of our interest in liver energy metabolism, we incline to consider liver insulin sensitivity and mitochondrial oxidative-phosphorylation relationship to be crucial. In a model of perfused rat liver cells, we have shown that metabolic consequences of uncoupling oxidative phosphorylation depend on the nature of respiratory substrate [1,2]. A progressive decline in respiration, following a transient burst was observed in the presence of carbohydrate or ethanol (cytosolic NADH suppliers) associated with Δp collapse and ATP-to-ADP ratio fall. By contrast, in the presence of octanoate or proline (matricial $FADH_2$ suppliers), a large and sustained increased in respiration was observed while the effect on Δp and ATP/ADP was minimized. This indicates that mitochondrial membrane potential plays a role in determining the nature of oxidized substrate, depending on the pathway of reducing equivalent supply to the chain, i.e. potential-dependent (malate–aspartate shuttle) or independent (quinone pool). Interestingly, it was recently shown that *in vivo* DNP administration to rats exposed to high-fat diet abolished their metabolic abnormalities [3]. We investigated hepatocytes and mitochondrion energy metabolism in rats exposed high-fat diet. We report that the steps involved in β-oxidation pathway were enhanced while the actual rate of mitochondrial oxidation was inhibited. This was accompanied by a higher mitochondrial redox state as well as membrane potential, assessed in situ in intact cells. In addition, a higher rate of reactive oxygen species production was observed with fatty acids as respiratory chain substrates but not with other respiratory-chain substrates. This led us to propose that increased matricial redox state and mitochondrial membrane potential in "insulin-resistant state" leads to decrease β-oxidation. Hence, modulating mitochondrial membrane potential might be attractive to lessen metabolic effects of liver insulin resistance and may represent a valuable therapeutic target because of its capacity to regulate the hierarchy of oxidized substrates.

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9P.12 Tetradecylthioacetic acid influences mitochondrial metabolism and enhances insulin-sensitivity of C2C12 myotubes

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Insulin resistance is a pre-diabetic state preceding diabetes type 2 development. In muscle cells it manifests as an impaired insulin-stimulated glucose uptake due to improper signal transduction from insulin receptor and eventually affected translocation of glucose transporter GLUT-4 into the plasma membrane. It is accepted, that inefficient response to insulin results from abnormally high lipid deposits in muscle cells. Therefore stimulation of fatty acids oxidation might improve insulin sensitivity. This can be achieved by stimulation of mitochondrial oxidative metabolism and/or enhancement of mitochondrial biogenesis. Tetradecylthioacetic acid (TTA) was shown to exert antylipidemic and antiglycemic effect in rats *in vivo*. Its activity was referred to stimulation of fatty acid oxidation probably due to activation of PPARs (Berge RK *et al.*, 2002, *J. Lipid Res.* **43**: 742–750). The aim of our study was to explain biochemical mechanism of TTA action in C2C12 myotubes. We found, that TTA applied at a concentration of 10 μM and 20 μM for 72 h slightly increases oxygen consumption but it doesn't affect ATP level and ROS production. In addition, it doesn't influence total mitochondrial mass measured with NAO probe, but it affects the relative amount of respiratory chain complexes protein. C2C12 myotubes exposed to TTA exhibit increased UCP2 protein content. Moreover, these changes correlate with TTA-evoked enhancement of protein kinase B (Akt kinase) phosphorylation in response to insulin. In conclusion, TTA slightly affects energy cell metabolism and mitochondrial biogenesis. These changes do not compromise cell viability but positively influence the insulin sensitivity of C2C12 myotubes.

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