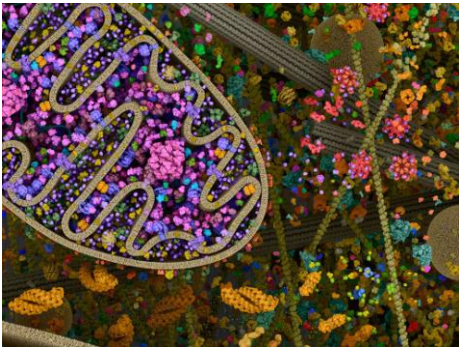
INSTITUTE OF THEORETICAL AND EXPERIMENTAL BIOPHYSICS OF RUSSIAN ACADEMY OF SCIENCES

LABORATORY OF PHARMACOLOGICAL REGULATION OF CELLULAR RESISTANCE

INTERNATIONAL CONFERENCE OF YOUNG SCIENTISTS

MITOCHONDRIAL PORES AND CHANNELS AS PHARMACOLOGICAL TARGETS



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ABSTRACT BOOK AND PROGRAM

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International conference of young scientists «MITOCHONDRIAL PORES AND CHANNELS AS PHARMACOLOGICAL TARGETS» was scheduled from October, 29 to October, 30, 2014 in Pushchino at the Institute of Theoretical And Experimental Biophysics, Russian Academy of Sciences.

The conference highlighted the current understanding of the role of mitochondria in the regulation of intracellular processes and the development of pathologies. The conference was organized in accordance with the implementation of the project «The development of the drugs of target influence on mitochondrial pores and channels for heart and hepar treatment and cancer therapy», supported by a grant from the Government of the Russian Federation (contract № 14.Z50.31.0028) in the framework of the decree of the Government of the Russian Federation from April 9, 2010 № 220 "on measures to attract leading scientists in the Russian educational institution of higher professional education and scientific institutions of the state academies of Sciences and state research centers of the Russian Federation".

The conference included lectures by leading Russian and foreign scientists, plenary meetings, poster sessions.

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1. LECTURES OF LEADING SCIENTISTS

REGULATION OF HEPATIC MITOCHONDRIAL METABOLISM BY ETHANOL

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Liver pathology in alcoholic liver disease (ALD), non-alcoholic steatohepatitis (NASH) and forms of toxicant-associated steatohepatitis (TASH) is indistinguishable. ALD pathogenesis may be related to generation of toxic acetaldehyde, whereas NASH and TASH are associated with generation of malondialdehyde, 4-hydroxynonenal, chloracetaldehyde and other aldehydes by lipid peroxidation and/or toxicant metabolism. With the major exceptions of oxygen and short chain fatty acids, all mitochondrial metabolites cross mitochondrial outer membranes (MOM) via open voltage dependent anion channels (VDAC). Here, I discuss data supporting the hypothesis that ethanol and aldehydes close VDAC, decrease permeability of MOM and suppress normal mitochondrial function, leading to inhibition of energy-consuming metabolism like ureagenesis. Such VDAC closure allows the selective and more rapid mitochondrial oxidation of toxic aldehydes, which permeate mitochondria freely. To further promote ethanol and AcAld metabolism, mitochondria of hepatocytes become uncoupled and depolarized *in vivo* in an ethanol- and AcAld-dependent fashion. Although VDAC closure and ethanol-induced mitochondrial depolarization is adaptive in

promoting more rapid aldehyde detoxification, these mitochondrial metabolic adaptations may also promote hepatic steatosis and lipotoxicity in ALD. The work was supported by grant No. 14.Z50.31.0028.

MITOCHONDRIAL IRON UPTAKE THROUGH MITOFERRIN

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Iron is essential for biosynthesis in mitochondria of heme and iron-sulfur clusters that are essential prosthetic groups for numerous mitochondrial and non-mitochondria proteins. However, iron can also catalyze formation of toxic and reactive hydroxyl radicals ($OH^{\bullet}$). Indeed, mitochondrial accumulation of iron is critical in promoting oxidative stress and cell death as an important contributor to many diseases. The molecular mechanisms how mitochondria take up iron is still unclear. Recently, a six transmembrane domain mitochondrial protein, mitoferrin (Mfrn), was identified that mediates iron transport across the mitochondrial inner membrane and which is required for heme formation and other biosynthetic activities. Mfrn has two isoforms. Mfrn1 (SLC25A37) is a 38-kDa protein that is highly expressed in erythroid cells and in low levels in other tissues, whereas Mfrn2 (SLC25A28), a 39-kDa protein, is expressed in non-erythroid tissues. Lysosomal luminal alkalinization by inhibition of the proton-pumping vacuolar ATPase causes release of lysosomal chelatable iron into the cytosol. Here, I discuss data showing that this lysosomal iron is taken up into mitochondria *via* Mfrn2. High Mfrn2-expressing cells show higher rates of mitochondrial Fe^{2+} uptake and more sensitivity to oxidative stress than low Mfrn2-expressing cells. High Mfrn2-expressing cells also show higher rates of

Ca^{2+} uptake. Moreover, knockdown of Mfrn2 decreases rates of both mitochondrial Fe^{2+} and Ca^{2+} uptake. Mfrn2 physically interacts with the mitochondrial calcium uniporter protein (MCU), as shown by immunoprecipitation and DuoLink assay. Thus, Mfrn2 appears to be a component/regulator of the MCU complex. The data suggest that mitochondrial uptake of Ca^{2+} and Fe^{2+} are closely intertwined and may involve identical or at least shared components (Grant 14.Z50.31.0028).

URIDINE AS A POTENTIAL MEDICINE FOR OXIDATIVE STRESS. THE STUDY OF THE MECHANISM OF ITS ACTION

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Uridine is a pyrimidine nucleoside which increases the concentration of glycogen and UTP in tissues (Haugaard et al. PNAS (1977) 74, 2339). We studied the effect of uridine on animals under oxidative stress. 3 experimental models of oxidative stress were used: 1 - acute inflammation in mice induced by toxins from Gram-negative bacteria (E.coli) lipopolysaccharides (LPS); 2 - acute myocardial ischemia of the rat left ventricle (namely, 60 min occlusion of the left coronary artery (LCA) without reperfusion); 3 - animal endurance under physical load, which was measured by recording the time during which the male Wistar rats swam with a load of 20% of body weight in water at 32^{o}C to exhaustion. It was found that uridine prevented the pathological disturbances in tissues of animals. The endotoxin injection increased the concentrations of several pro-inflammatory cytokines in blood and significantly enhanced the expression level of Hsp72 and NF-κB signaling pathway (phNF-kB and IKK) proteins in spleen lymphocytes and uridine pretreatment decreased it. In case of experimental acute myocardial infarction uridine possesed the anti-ischemic and anti-arrtythmic actions and normalized the energy and oxidize metabolisms in myocardium. In animal with endurance under physical load, uridine in 2 times increased the time of the rats swelling. Mechanisms of its action attributed to the formation in tissues of UDP - activator of mitochondrial ATP-dependent

potassium channel (mitoK_{ATP}) and UTP activating glycogen synthesis. In models when the effects of the uridine are eliminated the specific inhibitors mitoK_{ATP} channel (myocardial infarction and enhanced physical exercise) we assumed that the channel is a significant and perhaps the defining element in the defense against oxidative stress which observed in this pathologies states. In case the bacterial intoxication, when the inhibitor of mitoK_{ATP} does not remove the positive effect of uridine, we can suggest that the effect of uridine is associated with improvement in energy metabolism (increased glycogen synthesis). Thus, 3 experimental models demonstrated that uridine can be used as an effective defense against oxidative stress. The work was supported by Government of RF (No. 14.Z50.31.0028).

MULTIDRUG RESISTANCE OF TUMOR CELLS AND MITOCHONDRIA PORE AND CHANNELS

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Multidrug resistance of tumor cells remains to be one of the key problems for antitumor therapy. It is now clear that tumor cells have a variety of ways to protect themselves from the damaging effects. For example the mechanisms of multidrug resistance can be caused by activation of the transport of xenobiotics from cells, or by changes in pathways of cell death. It is known acquisition of multidrug resistance of tumor cells in multicellular structures (confluent cultures, spheroids, multicellular aggregates), which apparently can be determined by various mechanisms. The well-known role of mitochondria in the initiation of cell death through the release of pro-apoptotic proteins (Cyt C, AIF, Endo G, Smac / Diablo) in cytosol suggests some relationship of drug resistance of tumor cells to states of mitochondrial pores and channels. Our knowledge in this field is not sufficient to answer the question of whether it is possible to suppress the multidrug resistance of tumor cells by modulating the permeability of the mitochondrial pores and channels without damaging the normal cells in the body. This question appears to be quite difficult taking into account variety of different mechanisms of the multidrug resistance in tumor. Search for an answer to this question is crucial for biology and practical medicine. The work was supported by grant from Russian Government №14.Z50.31.0028.

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REGULATION OF CYCLOSPORIN A-SENSITIVE CALCIUM-INDEPENDENT FREE OXIDATION IN LIVER MITOCHONDRIA INDUCED BY FATTY ACIDS. ROLE OF MALONATE

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We studied the effects of long-chain free fatty acids (monocarboxylic palmitic (Palm) and dicarboxylic α,ω-tetradecanedioic (TDA)) on liver mitochondria incubated in the sucrose medium in the presence of succinate and rotenone. It is established that carboxyatractylate (Catr), glutamate, and 10 μM cyclosporin A (CsA) additively and independently inhibit the respiration of liver mitochondria, stimulated by 30 μM Palm (2,5 times). Under these conditions, ΔΨ decreased by Palm increases by Catr and glutamate, while CsA is not effective. Malonate at a concentration of 0.2 mM which inhibits maximum respiration rate in the presence of 50 mM 2,4-dinitrophenol by 45%, inhibits respiration by 12% in the presence of Palm, does not affect the effects of glutamate and Catr but substantially reduces the effect of cyclosporin A. TDA at a concentration of 400 μM stimulates liver mitochondrial respiration by 2 times without influence on ΔΨ. This effect of TDA was completely inhibited by 10 μM CsA and reduced by 0.2 mM malonate, while Catr and glutamate was not effective. It is concluded that CsA-sensitive stimulation of liver mitochondrial respiration by TDA and Palm without changing the ΔΨ may be caused by the switching of part of the respiratory chain complexes to the maximum electron-transfer rate mode without vector displacement of H^+ from

the matrix to the intermembrane space. Malonate is considered as one of the regulators of fatty acid-induced cyclosporin A-sensitive calcium-independent free oxidation in liver mitochondria. This work was supported by the Ministry of Education and Science of the Russian Federation (№ 1365) and by the Russian Foundation for Basic Research (project № 14-04-00688).

2', 3'-CYCLIC NUCLEOTIDE 3'-PHOSPHODIESTERASE MIGHT BE POTENTIAL REGULATOR OF MITOCHONDRIAL MEMBRANE PERMEABILITY

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The 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNP, EC3.1.4.37) catalyzes the hydrolysis of 2', 3'-cyclic nucleotides to form the corresponding 2'-monophosphates. Though CNP was shown to be an integral myelin protein, there is evidence for the presence of this enzyme at lower levels in a variety of other cell types, including amoeboid microglia, lymphocytes, and retinal cells

Recently, we clearly established that CNP is a mitochondrial protein in brain, heart and liver tissues and thus confirmed previous reports from the literature of possible mitochondrial localization of CNP.

Although the exact function of CNP in mitochondria is still unclear, its participation in regulation of calcium-induced permeability transition pore has been reported. Investigating the functional role of CNP in mitochondria, we found that CNP was involved in the regulation of the mitochondrial permeability transition pore (mPTP) opening which occurred under calcium overloading or in response to oxidative stress in mitochondria and can be considered to be an initial stage of apoptosis This was shown for both RBM and rat liver mitochondria. The 2',3'-cAMP- and 2',3'-cNADP-induced acceleration of Ca^{2+} efflux from mitochondria and collapse of the membrane potential as well as Ca^{2+}-stimulated swelling in liver and brain mitochondria were cyclosporine A (CsA)-sensitive, confirming the

involvement of mPTP in this processes.

Within mitochondria CNP was found in the mitoplast and outer mitochondrial membrane of RBM. Here, we showed for the first time that CNP co-immunoprecipitates in mitochondria with main mPTP regulators such as cyclophilin D, VDAC and ANT. Co-precipitation of CNP with COX IV and α-tubulin has also been found.

Finding, that CNP is co-precipitated with CyP- D, ANT and VDAC as well as with α-tubulin in Ca^{2+}-loaded and control mitochondria indicates possible physical association between these proteins in mitochondria. In particular, CNP resided on the outer membrane could tightly interact with VDAC, which is the major protein of the outer membrane, determining permeability of the outer mitochondrial membrane. VDAC channels can be in opened or closed states. In the VDAC closed state its channels are more permeable for calcium that might lead to acceleration of mPTP opening. Both VDAC and CNP bind to α-tubulin. Binding of α-tubulin to VDAC promotes its closure. Thus, CNP might regulate VDAC conductance directly or through α-tubulin binding with following modulation of the permeability of the outer membrane in mitochondria. Co-localization of CNP with cyclophilin D and ANT supported CNP participation in regulation of permeability of the inner membrane, regulating pore opening. The presence of CNP in the mitoplasts and co-precipitation with COX IV indicate its possible interaction with functional complexes of the respiratory chain in non-synaptic brain mitochondria. That was supported by separation of non-synaptic mitochondria proteins by BNE under different functional states. It was discovered that CNP can be associated with all functional complexes (I-IV) as well as with F_0F_1-ATP synthase (complex V). Association of CNP with complexes I-V of

mitochondria was found not only in brain mitochondria but also in heart and liver mitochondria indicative for a common property for different types of mitochondria. CNP association with complexes (I-V) is altered in the presence of threshold calcium concentration leading to mPTP opening and in the presence of CsA (when pore is closed). In Ca^{2+}-loaded non-synaptic mitochondria, we found that CNP was dissociated from Complexes I, V, III and II, while in the presence of CsA (when the pore was closed), association CNP with complexes was enhanced till control level (Ca^{2+}-free condition) and higher. Thus, process of CNP association/dissociation seems to be Ca^{2+}-dependent and CsA-sensitive and probably related with mPTP phenomenon. Effect of G3139 (which blocks VDAC) on CNP association/dissociation with complexes of the respiratory chain and ATP synthase was also examined. It was found that G3139 was able to modulate the CNP association with functional mitochondrial complexes. Our results indicate that CNP interacting with proteins of the inner and outer membrane might regulate permeability of both mitochondrial membrane. This study was supported by RFBR grants (12-04-00671, 13-04-00935, 14-04-00625) and by grant from Russian Federation Government (№ 14.Z50.31.0028).

Ca^{2+}-DEPENDENT PERMEABILIZATION OF MITOCHONDRIA AND LIPOSOMES BY PALMITIC AND OLEIC ACIDS: A COMPARATIVE STUDY

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In the present work, we examine and compare the effects of saturated (palmitic) and unsaturated (oleic) fatty acids in relation to their ability to cause the Ca^{2+}-dependent membrane permeabilization. The results obtained can be summarized as follows. (1) Oleic acid (OA) permeabilizes liposomal membranes at much higher concentrations of Ca^{2+} than palmitic acid (PA): 1 mM versus 100 μM respectively. (2) The OA/Ca^{2+}-induced permeabilization of liposomes is not accompanied by changes in the phase state of lipid bilayer, in contrast to what is observed with PA and Ca^{2+}. (3) The addition of Ca^{2+} to the PA-containing vesicles does not change their size; in the case of OA, it leads to the appearance of larger and smaller vesicles, with larger vesicles dominating. This can be interpreted as a result of fusion and fission of liposomes. (4) Like PA, OA is able to induce a Ca^{2+}-dependent high-amplitude swelling of mitochondria, yet it requires higher concentrations of Ca^{2+} (30 and 100 μM for PA and OA respectively). (5) In contrast to PA, OA is unable to cause the Ca^{2+}-dependent highamplitude swelling of mitoplasts, suggesting that the cause of OA/Ca^{2+}-induced permeability transition in mitochondria may be the fusion of the inner and outer mitochondrial membranes. (6) The presence of OA enhances PA/Ca^{2+}-induced permeabilization of liposomes and mitochondria. The possible mechanisms of PA/Ca^{2+}-

andOA/Ca^{2+}-induced membrane permeabilization, the probability of these mechanisms to be realized in the cell, and their possible physiological role are discussed. The work was supported by Government of RF (No. 14.Z50.31.0028).

INVOLVEMENT OF PALMITATE/CA^{2+}(SR^{2+})-INDUCED PORE IN THE CYCLING OF IONS ACROSS THE MITOCHONDRIAL MEMBRANE

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The palmitate/Ca^{2+}-induced (Pal/Ca^{2+}) pore, which is formed due to the unique feature of long-chain saturated fatty acids to bind Ca^{2+} with high affinity, has been shown to play an important role in the physiology of mitochondria. The present study demonstrates that the efflux of Ca^{2+} from rat liver mitochondria induced by ruthenium red, an inhibitor of the energy-dependent Ca^{2+} influx, seems to be partly due to the opening of Pal/Ca^{2+} pores. Exogenous Pal stimulates the efflux. Measurements of pH showed that the Ca^{2+}-induced alkalization of the mitochondrial matrix increased in the presence of Pal. The influx of Ca^{2+} (Sr^{2+}) also induced an outflow of K^+ followed by the reuptake of the ion by mitochondria. The outflow was not affected by a K^+/H^+ exchange blocker, and the reuptake was prevented by an ATP-dependent K^+ channel inhibitor. It was also shown that the addition of Sr^{2+} to mitochondria under hypotonic conditions was accompanied by reversible cyclic changes in the membrane potential, the concentrations of Sr^{2+} and K^+ and the respiratory rate. The cyclic changes were effectively suppressed by the inhibitors of Ca^{2+}-dependent phospholipase A_2, and a new Sr^{2+} cycle could only be initiated after the previous cycle was finished, indicating a refractory period in the mitochondrial sensitivity to Sr^{2+}. All of the Ca^{2+}- and Sr^{2+}-induced effects were observed in the presence of cyclosporin A. A possible role of Pal/Ca^{2+} pores

in the maintenance of cell ion homeostasis is discussed. The work was supported by and Government of RF (No. 14.Z50.31.0028) and Scholarship grant of the President of RF (Grant №СП-2697.2013.4).

OXIDATIVE PHOSPHORYLATION AND MITOCHONDRIAL PERMEABILITY TRANSITION PORE FUNCTIONING IN RATS WITH DIFFERENT GENETIC PREDISPOSITION TO AUDIOGENIC EPILEPSY

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The disorders of ion homeostasis and energy balance of cell are key events in the genesis of pathogenic seizures under epilepsy. The molecular mechanisms of the epileptic seizures development are not yet elucidated. In this regard, the aim to study of the energy status and ion homeostasis of tissues under epilepsy is very important. Three groups of animals were under investigation: 1 - Krushinskii-Molodkina (KM) rats, which were highly prone to convulsive seizures in response to sound; 2 - KM rats, which were subjected to sonic stress 2 days before the experiment; 3 – Wistar rats, which were not audiogenic epilepsy prone. The following parameters of brain and liver mitochondria of these animals were studied: respiratory rates, oxidative phosphorylation intensity, Ca^{2+} capacity and the rates of palmitate/Ca^{2+}-induced mitochondrial swelling. It has been found that succinate-dependent respiration rates of brain mitochondria of KM rats is inhibited by 20-25% compared to Wistar rats. The changes in brain mitochondria respiration of KM rats are detected only when complexes II and III of the respiratory chain are involved and only after preliminary sound exposure. Using glutamate/malate and succinate as substrates, the changes described above are observed in liver mitochondria of both KM rats groups - without sound exposure and after acoustic stress. It is also found that time of ADP phosphorylation of liver mitochondria of KM rats is

increased by 26-34%, indicating a decrease of phosphorylation efficiency. The mitochondrial Ca^{2+} capacity, defined as amount of accumulated calcium ions, is reduced in brain and liver mitochondria of KM rats after sound exposure. A possible role of changes of oxidative phosphorylation intensity and mitochondrial permeability transition pore functioning in the epileptic seizures development is discussed. The work was supported by Government of RF (No. 14.Z50.31.0028) and Scholarship grant of the President of RF (Grant №СП-2697.2013.4).

DISCOVERY FOR CHEMICAL COMPOUNDS ABLE TO SENSITIZE TUMOR CELLS TO SELECTIVE ANTICANCER CYTOKINE TRAIL

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Currently applied in clinic anticancer drugs are not sufficiently selective and effective, and there is an urgent need to develop new more efficient and safe treatment options. One of the most significant breakthroughs in this direction was the discovery in the late 90s, human protein TRAIL (Tumor necrosis factor-related apoptosis-inducing ligand). TRAIL ligand induces selectively apoptosis in tumor cells by binding to two death receptors (DR4 and DR5) and become on of the most promising candidate for cancer treatment. Unfortunately some cancer cell types are resistant to TRAIL. Therefore, overcoming this resistance is necessary for effective TRAIL therapy. Solution to the problem would be to use TRAIL in combination with substances acting synergistically and capable to sensitize cancer cells to TRAIL-induced apoptosis. Such an approach allows to significantly improve the overall efficiency of anticancer therapy by overcoming tumor resistance. Search for effective sensitizers and study of the mechanisms of tumor cells resistance to TRAIL-induced apoptosis is a primary goal of current research. This work was supported by grant from Russian Government (№14.Z50.31.0028).

THE EFFECTS OF MALONATE AND INORGANIC PHOSPHATE ON CYCLOSPORINE A - INSENSITIVE CA^{2+}-DEPENDENT NONSPECIFIC PERMEABILITY OF THE INNER MEMBRANE OF LIVER MITOCHONDRIA INDUCED BY A,Ω-DIOIC ACIDS

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Recently we found that the α,ω-dioic acids (among them α,ω-hexadecanedioic acid (HDA) was most effective) are able to induce the opening of the "non-classical" cyclosporin A (CsA)-insensitive pore in the liver mitochondria loaded with Ca^{2+} or Sr^{2+}. In the present work we study the effects of a competitive inhibitor of succinate dehydrogenase - malonate, and one of the inductors of "classical" CsA-sensitive mitochondrial permeability transition- inorganic phosphate (Pi) on the process of pore induction by Ca^{2+} and HDA. It is established that the malonate at concentrations up to 500 μM, added to the liver mitochondria loaded with Ca^{2+}, inhibits Ca^{2+} release from the matrix, the decrease in membrane potential and organelle swelling induced by HDA. Further increase in the concentration of added malonate causes a decrease in mitochondrial membrane potential and completely suppresses pore opening by HDA and Ca^{2+}, which, as shown previously, requires energization of organelles. It is known that one of the triggers of the CsA-sensitive Ca^{2+}-dependent pore - P_i, which promotes rapid and massive accumulation of Ca^{2+} in the mitochondrial matrix, has no effect on the CsA-insensitive swelling of organelles induced by palmitic acid and Ca^{2+}. At the same time, we found that under similar experimental conditions P_i dose-dependently inhibits the HDA/Ca^{2+}-induced pore

opening. Proceeding from the fact that the induction of pore opening by HDA and Ca^{2+} occurs on the matrix side of the inner mitochondrial membrane, we assumed that malonate and P_i can inhibit transport of α,ω-dioic acid into the organelle matrix and thus prevent the induction of permeability.

INTERCELLULAR ADHESION INCREASES DRUG RESISTANCE OF ACUTE MYELOID LEUKEMIA CELLS

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Acute myeloid s leukemia (AML) is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. Therapeutic efficiency in treatment of AML ranges from 20 to 45%. One of the causes is an acquired drug resistance of leukemic cells after drug treatment. Another and more important cause is a de novo or primary resistance of to induction of cellular death. This form of leukemic cells resistance is associated with microenvironment such as other cells and soluble factors (interleukins, growth factors, CSFs).

In multicellular aggregates THP-1 cells was resistant to rh izTRAIL, etoposide and sorafenib. Disruption of cell-to-cell contact decreased the drug resistance. In multicellular aggregates of primary BMMC was resistant to sorafenib, etoposide, and all cells were resistant to izTRAIL. Disruption of cell-to-cell contact decreased the resistance to

sorafenib and etoposide but not to rh izTRAIL. In multicellular aggregates of THP-1 cells and BMMC are no changes in the mitotic activity of cells. Also, the THP-1 cells and BMMC in multicellular aggregates there is an increase the expression of anti-apoptotic protein Bcl-2. We suppose that one of the causes of de novo AML drug resistance can be multicellular aggregations of AML cells in bone marrow. The work was supported by Grant 14.Z50.31.0028 of Government of Russian Federation and grant from the Russian Foundation for Basic Research (RFBR 14-04-32183).

INTERCELLULAR ADHESION-MEDIATED RESISTANCE OF TUMOR CELLS TO DIETHYLDITHIOCARBAMATE (DDC) IS INHIBITED BY VITAMINE B12: A POSSIBLE ROLE OF VDAC

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Search for new approaches to anticancer therapy made it necessary to revise the role and scope of already known drugs. For example, disulfiram, well-known anti-alcoholism drug and antidote for heavy metal poisoning, is studied recently as a potential anticancer agent. Derivative of disulfiram, diethyldithiocarbamate (DDC), an inhibitor of Cu, Zn-superoxide dismutase, also draws attention in this respect. DDC can readily form a complex with copper, which penetrates the cell membrane causing intracellular redistribution of metal. Mitochondria have been shown to be of the main targets of DDC cytotoxic effect.

We investigated if DDC can cause the death of human tumor cell lines HEp-2, A431 and HT1080 in culture. It was found that the DDC cytotoxicity depends on the cell confluence, with 100-fold difference in IC50 for sparse and confluent cultures. We have found that cobalt-containing vitamin B12 (25 uM), which toxicity in combination with thiols NAC and GSH has been shown previously, is also able to increase the cytotoxic effect of DDC and, of especially valuable, to eliminate intercellular adhesion- mediated drug resistance (ICAMDR) of tumor cells to this substance. It has been shown recently that apoptosis inhibitor 4'-

diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) inhibits oligomerization of VDAC (Keinan N et al., 2010). Treatment with DIDS (200 uM, 1 h- preincubation) allowed to partially eliminate the cytotoxic effect of the combination of 1 mM DDC + 25 uM B12 (the number of surviving cells increased from 19 ± 5.4% to 54 ± 5%). Thus, it is possible that VDAC oligomerization plays a role in eliminating the ICAMDR of tumor cells to the cytotoxic effects. This issue needs further investigation. This work is supported by Government of RF (No. 14.Z50.31.0028).

MITOCHONDRIA OF DYING CELLS AS A CENTER OF ASEPTIC CALCINOSIS NUCLEATION IN HEART VALVE AND VESSEL TRANSPLANTS

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It is known that cardiovascular diseases are the major cause of human death throughout the world and responsible for 30% of all deaths. Without progressive development of surgical techniques of reconstruction or replacement of damaged valve or vascular structures this figure will pose a real threat. The development of the efficient substitutes or reconstructive biomaterial to repair function of damaged heart valve or vessels is thus a relevant task. It is also known that the main problem of biological substitutes of heart valve or vessel is a pronounced ability of these biomaterials to undergo calcification in the body of the recipient that lead to the early loss of hemodynamic function and repeated time-consuming operations. In this connection, study of the mechanisms of biomaterial calcification in order to repair functions of heart valve and vessels is a vital task.

In the laboratory of tissue engineering (Pushchino) a hypothesis was suggested that initiation of calcification in

transplants occurred because of calcium and phosphates accumulation in mitochondria of the dying cells of the donor (Akatov, Fesenko etc., 2006, 2010, 2012). According to this hypothesis during cell death (when sampling, slow treatment, transplantation, a period of warm ischemia etc.) calcium accumulates in the mitochondria and as a result of it the primary crystals of hydroxyapatites are formed and further crystals grow (mineralize) by physico-chemical pathways. The evidence obtained suggests that under death of artery cells (for instance in the case of ischemia of lower extremities), when for a certain period of time after cell death the mitochondria remain viable, calcification of the vascular wall may also occur by the mechanism described above (Fadeeva I.S., 2013, 2014)

The results of this work are crucial not only for understanding the mechanism of pathological mineralization of biomaterial for replacement or reconstruction of heart valve and vessels, but also for widening our knowledge about the mechanisms of aseptic (ectopic) calcification of the tissues in the body, in particular at medial calcification of Monckeberg and atherosclerosis of vessels. This work is supported by Government of RF (No. 14.Z50.31.0028), Scholarship grant of the President of the Russian Federation (Grant №СП-6867.2013.4), grant of the Russian Foundation of Basic Research (14-04-32191) and project of Ministry of Education and Science (№768).

FORMATION OF LIPOFUSCIN FROM MITOCHONDRIA DURING HEATING AND LIGHTING

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Formation of lipofuscin (LF) leads to aging and degeneration of cells. LF produces due to spontaneous conjugation of broken proteins and lipid peroxides. Also, a LF analog – melanin – appears in skin and eyes upon sun-light irradiation. In cells of brain, heart and other organs, LF is produced mainly in the aging mitochondria (MT), since they are a source of reactive oxygen species participating in the process.

We have obtained LF in rat liver mitochondrial suspensions by prolonged heating or by 450-watt xenon lamp irradiation. Quantity of LF was measured by its fluorescence (excitation maximum was 360 nm and emission maximum was 460 nm).

LF formation has been shown to be spontaneous, depending on the temperature and time incubation of MT. Incubation of MT during 24 hours at 37°C has yielded more LF than at 4°C. Incubation of MT for 4 hours at 37 °C was less effective.

Simultaneously with the appearance of LF, two peaks - at 460 nm and 520 nm in aging MT were detected. It is caused,

as was previously shown, by output of endogenous flavins (primarily - FMN) from MT into solution. The release of FMN into solution was accompanied by loss of the NADH-oxidase activity and activation of oxidative processes.

Process of LF formation in MT was strongly activated by a powerful light irradiation. The largest contribution to the LF formation was given by the UV light in the range of 220 - 320 nm. Under irradiation of MT in a glass cuvette (non-transmitting the UV below 320 nm) the yield of LF was less than during irradiation in a quartz cuvette (passing UV until 220 nm). The fluorescence band of LF, arising in the course of intensive irradiation of MT, was characterized by a maximum at 460 nm (emission was 360 nm). The flavin peak at 520 nm was not detected due to photo-degradation of FMN and FAD under the irradiation.

Decrease in the oxygen content (in 1.5-2 times) in the sample of MT leads to significant (too in 1.5-2 times) reduction of the LF quantity during irradiation (oxygen was removed by two ways - by purging a suspension of MT by argon and a 30-minute pre-incubation of the MT sample with 10 mM succinate in a hermetic cuvette).

In control experiments, without MT, the irradiation of lysozyme, bovine serum albumin and other proteins leads to formation of the same LF spectra. This is due to formation of well-known Schiff bases that does not require the presence of any lipids. It is obvious that in the course of photo-oxidation of the proteins, the double bonds are formed in the presence of oxygen/ Depleted of the oxygen environment significantly reduced the amount of LF.

This study was supported by RFBR grant (14-34-50310), by grant from Presidium RUS «Fundamental sciences-medicine», 2014 and by grant of BRL, Taiwan, 2014.

DISRUPTION OF POTASSIUM HOMEOSTASIS AND OXIDATIVE EXCHANGE OF RAT BRAIN AND LIVER MITOCHONDRIA IN EXPERIMENTAL EPILEPSY

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It is known that epilepsy is characterized by a decrease in the membrane potential of the cell and an increase in the extracellular concentration of potassium ions. By increasing the level of extracellular potassium ions, from 2 to 9 mM, cell membrane potential decreases from -63 mV to -52 mV, and depolarization leads to the appearance of seizures. The mechanism of occurrence of epileptic seizures is not yet clear. The investigation of the energy and ion exchange in the tissues in epilepsy is very important.

The research used three groups of animals: 1 - rat Krushinsky Molodkina (KM), which is highly susceptible to convulsive seizures in response to sound; 2 - KM rats that 2 days before the experiment were subjected to acoustic stress; 3 - control rats, which are not prone to audiogenic epilepsy. These animals were studied in order to examine the following parameters, which are important for the function of rat brain and liver mitochondria: respiratory rate and intensity of oxidative phosphorylation, the rate of transport of K^+ ions, potassium capacity, and the concentration of malonic dialdehyde.

Respiration velocity of rat brain mitochondria from KM rats was 20-25% less than control. The changes were found

during the 2^{nd} and 3^{rd} regions of the respiratory chain, and only in KM exposed to acoustic stress prior to measurements. In liver mitochondria changes in respiration were observed on both substrates and both without sound action as well as KM rats exposed to acoustic stress.

The rats of potassium ion uptake in the mitochondria of both brain and liver of all KM rat groups were decreased compared with control animals (average 20-30%). The amount of potassium in brain mitochondria was slightly reduced and statistically increased in brain mitochondria. The amount of hydrogen peroxide, estimated by malonic dialdehyde, was higher in brain and liver mitochondria of KM rats exposed to acoustic stress than KM rats and control.

This work was supported by grants from the Government of the Russian Federation № 14.Z50.31.0028 and DPNNiT № 2014/281/2495.

ADAPTATION OF LEWIS LUNG CARCINOMA CELLS IN CULTURE AND EVALUATION OF THE ACTION OF TARGETED ANTICANCER AGENTS IN VITRO

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Introduction

Selective effect of chemotherapeutic agents for the treatment of tumors is usually limited to the characteristic structural features of the tumor blood vessels (randomness of tumor angiogenesis, blood flow disturbance, and elevated interstitial fluid pressure), creates a barrier to the transport of anticancer drugs, which leads to a decline of the effectiveness of treatment and the development of drug resistance. The molecular mechanisms causing abnormal vascular architecture are not well understood, it is believed that one of the key factors is the imbalance of pro- and antiangiogenic factors.

We adapted cells of LLC (Lewis lung carcinoma) to cell culture for studying the spectrum of the sensitivity of this line to various chemotherapeutic agents to continue to use these data in the co-administration of substances that increase the permeability of the parenchymal tumor tissue. In earlier experiments performed in our laboratory on male mice C57BL/6 has been shown that the tripeptide iRGD improves penetration of the macromolecular structures (Evans blue-labelled albumin) through the layer of vascular endothelial cells into tumor tissue.

Materials and methods

We used the following chemotherapeutic agents: sorafenib, ABT-263, ABT-737 (Selleckchem, USA).

Lung tumors equal to 1 cm in diameter were excised, washed three times in Hanks' balanced salt solution, cut into small pieces and incubated in 30 mL collagenase solution (2 μg/mL in Hanks' medium). Then tumor tissue was disrupted by passage through a 14 gauge blunt needle, the resulting suspension was filtered through 70 micron filter.

Isolated cells were cultured in Petri dishes (100 mm in diameter) at 37ºC and 5% CO2 in DMEM supplemented with 10% FBS, 100 U/mL of penicillin and 100 μg/mL of streptomycin. The media was changed every 3-4 days (1:10). Cells from growing cultures were seeded into 96-well culture plates for the cytotoxicity assay. Cytotoxicity was evaluated by the ratio of the number of living cells in the test and the control (untreated toxic agents) cultures after 24 hours of the agents exposition. To determine the number of living cells culture was stained with crystal violet followed by photometry. Cell viability was also assessed by trypan blue staining.

Results

Sorafenib has been found to have a selective toxic effect on LLC cells at a concentration of 5 μM/mL. ABT-263 has a similar toxic effect on LLC cells at a concentration of 20 μM/mL. It was found that ABT-737 was the least effective among all three chemotherapeutic agents, since the concentration of 20 μM/mL survived 20% of tumor cells.

In the future we plan to investigate the co-administration of these cytotoxic compounds with substances causing increasing the permeability of the tumor parenchyma, such as tripeptide iRGD. Research funded by Ministry of Education and Science of the Russian Federation

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MELATONIN EFFECT IN MITOCHONDRIA MIGHT BE EXERTED VIA 2',3'-CYCLE NUCLEOTIDE-3'-PHOSPHODIASTARASE

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It is known that melatonin is a hormone of pineal gland, which regulates major physiological processes including the sleep wake cycle, pubertal development and seasonal adaptation. In addition to its relevant antioxidant activity, melatonin exerts many of its physiological actions by interacting with membrane MT1 and MT2 receptors and intracellular proteins such as quinone reductase 2, calmodulin, calreticulin and tubulin. Melatonin MT1 and MT2 receptors are G protein coupled receptors which are expressed in various parts of the CNS .Additionally, melatonin has been known as a radical scavenger with the ability to remove all typies of ROS.

Melatonin's ability to counteract ROS has special relevance as it crosses all morphophysiological barriers and it is widely distributed in tissues, cells and subcellular compartments, because of its distinct physical and chemical properties. Mitochondria are considered as the main target of melatonin. Both in vivo and in vitro experiments have demonstrated that melatonin protects mitochondria against oxidative damage and improves mitochondrial function. Melatonin stabilizes the mitochondrial inner membrane, increases the activity of mitochondrial electron transport chain complexes and improves mitochondrial respiration and ATP production as well as regulates mitochondrial gene expression. Finally, melatonin was shown to inhibit the

function of permeability transition pore which forms in response to calcium overload or oxidative stress. It should be noted that at the moment composition of mPTP and mechanism of its regulation are not established. In this connection, it is worth to note that recently we identified 2',3'-cyclic nucleotide-3'-phosphodiesterase (CNP) in rat brain mitochondria (RBM) which was able to prevent mPTP opening, while its substrate were able to stimulate mPTP. CNP was found to be localized in the outer and inner membranes of mitochondria. Expression of CNP was revealed to be reduced in rat brain mitochondria in aging. Also, melatonin secretion is known to diminish in aging. Our results demonstrate that after purification of RBM, isolated from rats of different ages under chronic administration of melatonin, content of CNP was increased in the myelin fraction and non-synaptic mitochondria. Along with pro-apoptotic proteins such as cytochrome c and apoptosis-inducing factor, CNPase releases from mitochondria. In melatonin-treated RBM mitochondria with chronic administration, CNP release reduced in mitochondria isolated from both young and old rats. Taking into consideration that C-terminal of CNP contains G-protein like domain, and ability of CNP to bind tubulin, calmodulin and RNA as well as our results, we suggested that melatonin action in mitochondria might be exerted via CNP. This work was supported by mega-grants of the Government of the Russian Federation № 14.Z50.31.0028 and grants of RFBR №№12-04-00671, 13-04-00935, 14-04-00625.

COMPUTATIONAL MODELING ANALYSIS OF MITOCHONDRIAL SUPEROXIDE PRODUCTION

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Excessive generation of reactive oxygen species (ROS) in mitochondria results in oxidative stress and induces cell and tissue injury via the oxidation and impairment of mitochondrial membrane-transport systems. One of the most important targets of oxidative stress is the mitochondrial permeability transition (MPT) pore which is activated by mitochondrial Ca^{2+} synergistically with ROS. In this study, a computational mechanistic model of ROS formation in the mitochondrial electron transport chain (ETC) was developed as a tool to facilitate the quantitative analysis of the factors controlling mitochondrial ROS production to assist in the interpretation of experimental studies. The model was analyzed for different computer-simulated conditions (forward and reverse electron transport, with and without different inhibitors of ETC) to account for a large amount of published experimental data on mitochondrial ROS production and make predictions to be tested experimentally. Computer simulation results confirm that, in addition to ROS formation in Complex III and at the flavin site of Complex I, the quinone binding site of Complex I should be considered as an additional ROS generating site to account for experimental observations on ROS production during reverse electron transfer (RET) in mitochondria.

Computational analysis of ΔpH and ΔΨ dependency of ROS production by ETC accounts for the experimentally observed much stronger sensitivity of the ROS generation rate by Complex I to ΔpH than to ΔΨ.

MITOCHONDRIAL PERMEABILITY TRANSITION PORE IS AS A TARGET OF TSPO LIGANDS INFLUENCE IN CHRONIC MELATONIN ADMINISTRATION.

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Oxidative stress and impairment of Ca^{2+} homeostasis are considered important factors in the development of mitochondrial dysfunction associated in aging. Mitochondrial dysfunction can be considered as the driving force of aging, resulting in programmed cell death is developed, which may be a cause of non-specific increase in the permeability of the inner membrane (permeability transition), caused by the higher level of calcium in the mitochondrial matrix to supra-threshold value or the result of influence free radicals. More attention is paid to research aimed at improving the protective response to oxidative stress by a variety of antioxidants to reduce age-related oxidative damage and mitochondrial dysfunction. Among the effective antioxidants, melatonin should be noted, because it is a natural compound - neuroendocrine hormone produced by the pineal gland (epiphysis). Melatonin is a known antioxidant and protects mitochondrial bioenergetic function effectively.

Translocator protein (TSPO) is one of the component-regulators of the mPTP and consider as a target for neuroprotection, previously denoted as peripheral benzodiazenpine receptor (PBR). TSPO has many important

functions in the cell, regulating cell proliferation, induction of cell differentiation, porphyrin transport and heme biosynthesis, regulation of anion transport, cholesterol transport. TSPO ligands are considered PK11195 and Ro 5-4864 (synthetic, exogenous) and endogenous natural - protoporphyrin IX (PPIX) and diazepam biding protein (DBI). Porphyrins mainly protoporphyrin IX, cause toxic effects due to its ability to generate free radicals, and the melatonin and its metabolites bind free radicals.

In this work, we study the effect of different TSPO ligands on the functional state of mitochondria of rats of different age groups in terms of opening the pores at chronic melatonin administration in order to identify the mechanisms of antioxidant protection of mitochondria during aging. This work was supported mega-grants of the Government of the Russian Federation № 14.Z50.31.0028, RFBR grants №№ 12-04-00671, 13-04-00935, 14-04-00625.

METFORMIN AS A POTENTIAL CELL REGULATOR FOR RESISTANCE TO RADIATION-INDUCED DAMAGE

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Metformin is an activator of cAMP-dependent protein kinase, which is a key cellular energy sensor and regulator of cell division. It is shown that metformin has antiproliferative properties and has the ability to induce a mitochondrial autophagy process through inactivation of the complex mTOR.

The purpose of this work was to investigate the radioprotective properties of metformin.

The study of transrenal DNA (tr-DNA – circulating cell-free DNA, which overcame kidney barrier) in the urine of Wistar rats by real time PCR showed that after taking metformin (60 mg / 100 g) there is a sharp increase in tr-mtDNA to 6:00, while the level of nuclear DNA in the urine remains unchanged.

During X-ray irradiation dose of 5 Gy, the rats taking metformin lead to increased levels of tr-DNA that maintained constant for a week, with a peak at 12 hours. Also, tr-level mtDNA exceeded the norm by more than 6 times, whereas the effect of irradiation showed an increase in mtDNA only 4 times.

Test results on the survival demonstrated that intraperitoneal injection of metformin (60 mg / 100 g) male mice Balb \ c after exposure to X-ray radiation at a lethal dose of 8 Gy increases the percentage of animals surviving up to 18% from 100% of death of the irradiated control animals. The

injection of metformin 0.5 h before the exposure, increased the life expectancy by only a few days and did not affect the survival of the animals.

The obtained results suggest that due to activation of autophagy of damaged mitochondria and temporal control of cell division, metformin assists the recovery of cells from radiation-induced damage, thereby showing radioprotective properties.

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INFLUENCE OF ARYLCYCLOALKYLAMINE DERIVATIVES ON MITOCHONDRIAL FUNCTION

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Mitochondria play important role in the cell metabolic functions, activation of cell death and regulation of calcium homeostasis and so they are an attractive target for primary screening of potential neuroactive compounds allowing to predict as a potential neuroprotective and calcium-regulatory activity, as well as to identify substances with toxicity effect. Most researchers use the swelling of isolated mitochondria and mitochondrial membrane potential assays as primary screening tests. But additional studies often are required.

In series of derivatives of arylcycloalkylamine the compounds which increase the stability of the mitochondria to induction of the mitochondrial permeability transition (MPT), without influencing the membrane potential have been identified, . These data would allow us to suggest its potential neuroprotective effect. But ability to block the MPT by the most active compounds surprisingly correlates with toxicity on primary cultures of rat cortical neurons. One possible reason may be a direct effect on enzyme of the respiratory chains (NADH:ubiquinone reductase). It is shown that the most toxic compounds inhibit the activity of complex 1 of the respiratory chains, but not induce depolarization and in most cases not affects the rotenone-induced depolarization. The degree of suppression of NADH: ubiquinone reductase activity correlates with the

toxicity of a compound. One of the compounds with the significant activity as inhibitor of the complex 1 prevents rotenone-induced depolarization of mitochondria. And this compound has most expressed cytotoxic effect on the neurons culture.

Thus, evaluation of the influence of compounds not only on the mitochondrial transmembrane potential, but also on the activity of the respiratory chain enzymes is a necessary step during the in vitro screening of toxicity of potential drugs for the treatment of neurodegenerative diseases.

CYTOCHROME B5 REDUCTASE 3 (CYB5R3) OR VDAC1 IS THE NADH-DEPENDENT REDUCTASE OF REDOX-CYCLING XENOBIOTICS IN THE EXTERNAL MITOCHONDRIAL COMPARTMENTS?

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It was reported that VDAC1 possesses an NADH oxidoreductase activity and plays an important role in the activation of xenobiotics in the outer mitochondrial membrane. In the presented work, we evaluated the participation of VDAC1 and Cyb5R3 in the NADH-dependent activation of various redox cyclers in mitochondria. It was shown that external NADH oxidoreductase caused the redox cycling of menadione >> lucigenin > nitrofurantoin. Paraquat was predominantly activated by internal mitochondria oxidoreductases. An increase in the ionic strength stimulated and suppressed the redox cycling of negatively and positively charged acceptors, as was expected for the Cyb5R3-mediated reduction. Antibodies against Cyb5R3 but not VDAC substantially inhibited the NADH-related oxidoreductase activities. The specific VDAC blockers G3139 and erastin, separately or in combination, in concentrations sufficient for the inhibition of substrate transport, exhibited minimal effects on the redox cycler-dependent NADH oxidation, ROS generation, and the reduction of exogenous cytochrome c. In contrast, Cyb5R3 inhibitors (6-propyl-2-thiouracil, p-chloromercuriobenzoate, quercetin, mersalyl, and ebselen) showed a similar pattern of inhibition of ROS

generation and cytochrome c reduction. The analysis of the spectra of the endogenous cytochromes b5 and c in the presence of nitrofurantoin and the inhibitors of VDAC and Cyb5R3 demonstrated that the redox cycler can transfer electrons from Cyb5R3 to endogenous cytochrome c. This caused the oxidation of Cyb5OM, which is in the redox balance with Cyb5R3. The data obtained argue against VDAC1 and in favor of Cyb5R3 involvement in the activation of redox cyclers in the OMM. The work was supported by grant from Russian Government (Agreement № 14.Z50.31.0028) and by grant RFFI 14-04-01664 (to Kruglov A.G.).

BENZALKONIUM CHLORIDE: EFFECTS ON BIOENERGETICS OF RAT LIVER MITOCHONDRIA

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Benzalkonium chloride (BAC) is currently the most commonly used preservative in eye drops because of its stability, hypoallergenicity, low cost and pronounced efficiency against bacteria, fungi and protozoa. The cationic nature of BAC (it is a quaternary ammonium) suggests that one of the major target of BAC in the cell may be mitochondria, the only intracellular compartment charged negatively. However, BAC influence on mitochondria has so far been totally ignored. In this report we examined effects of BAC on energy parameters of super-tightly-coupled rat liver mitochondria. BAC was found to be a weak uncoupler, a potent inhibitor of oxidation supported by both succinate and NAD-dependent substrates. Low BAC concentrations did not shunt the electron transport, while diminishing the mitochondrial membrane potential, causing the opening of the Ca2+/Pi-dependent pore, and almost totally blocking ATP synthesis and hydrolysis. BAC promoted hydrogen peroxide production by mitochondria, which was conspicuously diminished by SkQ1, a rechargeable mitochondria-targeted antioxidant. It is conceivable to suggest that BAC may induce mitochondrial dysfunction and, ultimately, energy deficiency also in lacrimal glands, thus promoting the oxidative stress and aggravating the dry eye syndrome and possibly, apoptosis. This suggestion is indirectly supported by findings that high concentrations

BAC cause morphologic disruption of the corneal epithelium and induces apoptosis of Chang's conjunctival cells (Debbasch et al., 2001), damage the human ocular surface and impair the local tolerance to eye drops (Pauly et al., 2009; Brignole-Baudouin et al., 2012). Collectively, these data make it desirable to remove BAC from eye drops by replacing it for another less toxic preservative. Finally, our data anticipate possible beneficial effects of mitochondrial-targeted therapeutic agents for treatment of mitochondrial dysfunction- mediated renal diseases. This work was funded by the research contract with Mitotech LLC, by the Russian Academy of Sciences (program on molecular and cellular biology) and by the Russian Foundation for Basic Research (grant No 13-04-01530).

REFERENSES

1. *Debbasch, C., Brignole, F., Pisella, P.J., Warnet, J.M., Rat, P., Baudouin, C.* 2001. Invest. Ophthalmol. Vis Sci. 42, 642–652.
2. *Pauly, A., Melon.i, M., Brignole-Baudouin, F., Warnet, J.M., Baudouin, C.* 2009. Invest. Ophthalmol. Vis. Sci. 50, 1644–1652.
3. *Brignole-Baudouin, F., Desbenoit, N., Hamm, G., Liang, H., Both, J.P., Brunelle, A., Fournier, I. Guerineau, V., Legouffe, R., Stauber, J., Touboul, D., Wisztorski, M., Salzet, M., Laprevote, O. Baudouin, C.* 2012. PLoS One. 7, e50180.

ROLE OF CALRETICULIN IN THE FORMATION OF POTASSIUM-TRANSPORTING CHANNELS IN MITOCHONDRIA

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Mitochondrial potassium channels plays the key role in processes of potassium transport in mitochondria, among them mitochondrial ATP-dependent potassium channel (mitoK_{ATP}) is most studied. Using MALDI-TOF-TOF and immunochemical analyses it was detected homology of aminoacid sequences of 57 kDa protein isolated in our laboratory from rat liver mitochondria. As was shown earlier, this protein has properties of mitochondrial ATP-dependent potassium channel during integrating it into a bilayer lipid membrane (BLM) and liposomes. For determined the possible role of kalreticulin in potassium tpansport in mitochondria, polyclonal antibody (AB) on kalreticulin isolated from rat liver microsome were been prepared and purified. The work was carried out on two experimental models: 1) The model of energy-dependent swelling of mitochondria in potassium medium reflecting uptake potassium into the mitochondria, and 2) the model DNP-induced potassium exit from mitochondria showing the operation of the channel in the opposite weld. Carried out inhibitory analysis showed a dose-dependent inhibition of potassium transport in rat liver mitochondria by these

antibodies. The maximum value of inhibition was 55-60%, which is comparable to value of AB-inhibition of mitochondrial potassium transporting protein, obtained earlier by us. At the same time the similar concentration of antibodies had no effect on respiration and oxidative phosphorilation by the same mitochondria. This allows us to conclude that AB directly affects on potassium transpotr in mitochondria but not indirectly via inhibition of respiration. The generality of kalreticulin and mitochondrial potassium-transporting protein indirectly confirmed by recently obtained in our laboratory data of electron microscope, where using specific polyclonal antibodies to mitochondrial potassium-transporting protein m.w. 57 kDa protein. It was shown existence this protein not only in mitochondrial membpane, but in membrane of reticulum. Thus, granules of colloid gold, conjugated with antibody on this protein mainly located in mitochondrial and reticular membranes contact areas. We believe that, as in the case of calcium uptake, uptake potassium into mitochondria occurs in the contact point of both microsomal and mitochondrial membranes. This work was supported by grants from the Government of the Russian Federation №14.Z50.31.0028.

VDAC ISOFORMS 1, 2 AND 3 LACK NADH OXIDOREDUCTASE ACTIVITIES

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VDAC is a most abundant pore-forming protein in the outer mitochondrial membrane (OMM), which provides passage for metabolites to and from the mitochondrial inner compartments. VDAC also participate in the control of permeability transition pore opening (mPTP) and mitochondrial outer membrane permeabilization (MOMP). All VDAC isoforms are capable of binding NAD+ and NADH and possess thiol groups proposed to be catalytically active. Recently several research groups reported that VDAC1 is an oxidoreductase that can reduce ferricyanide and natural acceptors, as well as activate the range of xenobiotics and drugs.

In our preliminary report using inhibitory analysis, immunologic and biochemical approaches we recently demonstrated that Cyb5R3 but not VDAC1 is a reductase of xenobiotics in the OMM.

Our new studies using mouse embryonic fibroblasts and HEK 293 T cell line show that suppression of protein expression of VDAC1, VDAC2 and VDAC3 affected

neither the rate NADH-dependent reduction of ferricyanide nor xenobiotics in both cytosol and the OMM. In contrast, ~90% suppression of Cyb5R3 expression strongly declined NADH-dependent reductase activities in cells and the OMM. Hence, these data further support the thesis that VDAC1 and its other isoforms lack measurable oxidoreductase activities even though are capable of binding NADH.

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PROLONGED CONSUMPTION OF ETHANOL CLOSES PORIN CHANNELS OF THE OUTER MITOCHONDRIAL MEMBRANE AND SUPPRESSES OXIDATIVE PHOSPHORYLATION

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The origination and development of various pathological states is directly related to the disturbance of mitochondrial functions. In the present study, the effect of prolonged consumption of ethanol on the rate of oxygen uptake, the changes of the mitochondrial potential, and the fluorescence of pyridine nucleotides in Wistar rats has been examined. It was found that the prolonged consumption of ethanol considerably affected the respiratory functions of mitochondria, lowering the respiration rate and respiration control. A comparison of the oxidative phosphorylation efficiency with respect to the duration of the redox cycle of pyridine nucleotides of the mitochondrial respiratory chain in the mitochondria of control rats and rats receiving ethanol for 7 months showed that the phosphorylation of one and the same amount of added ADP in the mitochondria of alcoholic rats is greater than in mitochondria of control rats. The ethanol-induced suppression of mitochondrial functions may be related to the closing of voltage-dependent anionic channel (VDAC) in the outer mitochondrial membrane and a decrease in its permeability for water-soluble metabolites. To estimate the involvement of the porin channels of the outer mitochondrial membrane in the inhibition of the oxidative phosphorylation, the effect of erastin, a known

inhibitor of VDAC, on control mitochondria was examined.
It was found that erastin not only considerably increases the time of oxidative phosphorylation but also markedly decreases the rate of phosphorylating and uncoupling respiration. Thus, the erastin-induced inhibition of VDAC changes the functions of mitochondria and makes them similar to alcoholic mitochondria. It may be concluded that the inhibition of ATP synthesis in the oxidation–phosphorylation cycle, observed in mitochondria of chronic alcoholic rats, is caused by the closing of porin channels in the outer mitochondrial membrane. It should be noted that the closing of porin channels in mitochondria of alcoholic rats is incomplete since their incubation with erastin further inhibits the ATP synthesis rate.
These data demonstrate that the metabolism of ethanol slows down the ATP synthesis rate despite the excess of oxygen and the presence of oxidation substrates. The suppression of the ATP synthesis rate is related not to a high NADH/NAD ratio since it also occurs with the use of NAD-independent oxidation substrates but to the closing of porin channels, which is evidenced by the effect of erastin.
This work was supported by a grant from the Government of the Russian Federation p220 (IV), contract №14.Z50.31.0028.

OXIDATIVE STRESS IN THE PATHOGENESIS OF ALCOHOLIC ENCEPHALOPATHY

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The origination and development of various pathological states including alcoholic encephalopathy is directly related to the disturbance of mitochondrial functions. In recent years, a relationship between the metabolic oxidation of ethanol, changes in the permeability of the liver outer mitochondrial membrane, the oxidative stress and the disturbance of brain activity during the development of alcohol encephalopathy has been studied intensively. The data obtained indicate that, during the development of chronic alcohol intoxication, morphofunctional changes occur in the hippocampus, which lead to a decrease in the excitability of this structure and the impairment of the ability to adequately respond to external stimuli. It has been suggested that the development of alcoholic encephalopathy associated with metabolic disorders of mitochondria vital metabolites and increased levels of oxidative stress. In this study, the effect of prolonged consumption of ethanol on the main indicators of oxidative stress in Wistar rats has been examined. It was found that chronic alcohol intoxication leads to increased levels of triglycerides in the liver and in the blood serum of chronic alcoholics. In chronic alcoholic rats, an increase in oxidative stress, a decrease in the level of reduced glutathione, and an enhancement in the intensity of peroxidation processes were observed. In blood plasma, brain and liver tissues of rats consuming of ethanol the concentration of free SH-groups was significantly (almost

twofold) reduced, while the level of MDA and concentration of thiobarbituric acid reactive substances were increased two and four times, respectively. Significant differences were obtained in PMA induced ROS generation in neutrophils and monocytes. Monocytes and neutrophils of chronic alcoholics produced more ROS in response to PMA as compared with the control. It was found that, in addition to an increase in the production of ROS in neutrophils of alcoholic rats, a second chemiluminescence peak appears which may be related to the release of activated myeloperoxidase. Thus oxidative stress is an important mechanism of toxicity of ethanol because alcohol induces lipid peroxidation and membrane protein and nucleic acids oxidation. The ethanol oxidation products and compounds formed in lipid peroxidation processes can induce the closing of porin channels of the outer mitochondrial membrane and the energy dysfunction of mitochondria and the internal oxidative stress. All these processes can induce disorders in inter- and intracellular signaling, synaptic plasticity and an impairment of cognitive functions.

The work was supported by grant from the Government of the Russian Federation p220 (IV) contract №14.Z50.31.0028.

INTERACTIONS OF APOLIPOPROTEIN A-I N-TERMINAL FRAGMENT WITH MODEL LIPID MEMBRANES

Trusova V.[1], Gorbenko G.[1], Girych M.[1], Adachi E.[2], Mizuguchi C.[2], Saito H.[2]

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Apolipoprotein A-I (apoA-I) is a key protein component of high-density lipoproteins. It plays a crucial role in lipid metabolism, delivering cholesterol to steroidogenic tissues and/or shuttling it from the periphery to the liver for catabolism (reverse cholesterol transport). Aberrant fibrillization of apoA-I into the amyloid structures is known to be responsible for the hereditary and systemic amyloidosis. Increasingly growing evidence suggests that disruption of cellular membrane by amyloid aggregates underlies the fibril-induced cellular dysfunctions. To get novel insights into the amyloid cytotoxicity, in the present work we utilized fluorescent probe technique to explore the effect of N-terminal fragments of the wild (1-83) and amyloidogenic variants of apoA-I with different substitution mutations on physicochemical properties of lipid membranes composed of phosphatidylcholine and its mixture with cholesterol. Analysis of pyrene spectra did not reveal any marked influence of apoA-I mutants on the hydrocarbon region of lipid bilayer. In contrast, probing the

membrane effects by Laurdan revealed decrease in the probe Generalized Polarization in the presence of aggregated proteins suggesting that oligomeric and fibrillar apoA-I species induce increase in hydration degree and reduction of lipid packing density in the membrane interfacial region. Cholesterol proved to be capable of preventing the lipid bilayer from destabilizing action of apoA-I mutants. These findings may prove of importance for deeper understanding of membrane-mediated mechanisms of amyloid cytotoxicity.

STUDY OF THE EFFECT OF THE MOTOR PROTEIN VIMINTIN ON RAT LIVER MITOCHONDRIAL RESPIRATION

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The laboratory of Mironova G.D. has shown that Wistar rats under hypoxic conditions produced in a hermetically sealed chamber containing a gas mixture (7% oxygen in nitrogen) changed their position of cardiomyocyte mitochondria and caused them to localize near the cell membrane, which changed its configuration in order to provide a larger surface area. This suggests movement of mitochondria towards the cell membrane. The proteins that take part in this process of mitochondria movement are known as motor proteins, mainly vimentin and tubulin. The work of Rostovtseva et al. found that tubulin added to isolated brain mitochondria decreases ADP availability to ANT, partially restoring the low mitochondrial outer membrane permeability (high apparent K_m for ADP). In our present work, we study the influence of other motor proteins, namely vimentin, on Wistar rat liver mitochondrial respiration. Since this study requires pre-incubation of mitochondria (30 min. at 22°C), we determined the optimal antioxidant (taurine) concentration for prevention of disturbance of mitochondrial function. It was found that this optimal concentration is 20μM. For exact determination of K_m, the concentration of

mitochondria in incubation media was selected to be 0.250 mg/mL. Our preliminary data shows that vimentin does not have a significant influence on mitochondrial respiration rate, but decreases the K_m, as it was found for tubulin. The work was supported by Government of RF (No. 14.Z50.31.0028).

INVESTIGATION OF OSTEOINDUCTIVE ACTIVITY OF MATERIALS CONTAINING RECOMBINANT BONE MORPHOGENIC PROTEIN BMP-2

Vezhnina N.V.[1], Chekanov A.V.1, Fadeeva I.S.[1,2], Solovieva M.E.[1], Fesenko N.[1,2], Gorbachev D.P.[2], Akatov V.S.[1,2]

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Long-term studies of bone morphogenetic proteins BMP family showed BMPs to be the key growth factors in the bone regeneration regulation. In particular, BMP-2 has the most effective osteoinductive properties and initiates new bone formation by directing cell differentiation of mesenchymal cells in active osteoblasts.

The balance between growth factor release and retention could be a critical regulator of the efficacy of growth factor-based treatments for bone regeneration as BMPs have been involved in inflammation, systemic iron balance, antibody formation, deleterious effects on the central and peripheral nervous system, and oncogenesis. Therefore, it will be crucial to understand the fundamental physiochemical properties of growth factors to enhance their safe and effective delivery.

The technology for producing recombinant human BMP-2 has been developed in our laboratory in a bacterial expression system. In addition, the methods of incorporation rhBMP-2 into scaffolds have been developed. To date, we are designing the new materials containing rhBMP-2 as osteoinductive factor.

The investigation of the bone formation process in the

materials developed in heterotopic and orthotropic implantation models for a period of 1.5 months in rats has shown that demineralized bone matrix including rhBMP-2 is highly osteoinductive, as evidenced by the presence of new collagen and by the mineralization increasing of about 15-20 times, which was not observed in similar implants without rhBMP-2.

Another research direction has been the investigation of the effect of chemo-attractant cytokine PDGF and the angiogenesis inducer VEGF incorporated into scaffolds on osteogenesis and osteoinductive properties of rhBMP-2. To examine the rhBMP-2 osteoinductive properties, PDGF and VEGF effect in vitro, we have been investigated the osteogenic differentiation of the mouse mesenchymal cell line C3H10T1/2.

On the whole, our investigation results have allowed us to say that materials designed are highly promising for traumatology, orthopedics, dentistry and maxillofacial surgery.

This work was supported by RFBR Grant (№14-04-32191), project of Ministry education and science RF (№768) and Grant Program of the Presidium of RAS (Agreement № 14.Z50.31.0028).

MITOCHONDRIA-TARGETED ACTION OF THE NEW FLUORIDE-CONTAINING TETRAHYDRO-Γ-CARBOLINES

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The impairment of the mitochondria function, in particular the decrease of their ability to regulate calcium homeostasis in cell and the increase of their vulnerability to mitochondria permeability transition (MPT), is recognized as an important event in the Alzheimer's disease pathogenesis. Therefore, it is very attractive to evaluate new potential neuroprotective and pro-cognitive drugs using isolated mitochondria as a target in the primary screening assays systems.

It was previously demonstrated that the neuroprotective properties of Dimebon (2,3,4,5-tetrahydro-2,8-dimethyl-5-(2-(6-methyl-3-pyridyl)ethyl)-1H-pyrido(4,3-b)indole dihydrochloride) in various toxicity models at least to some extent are accounted for by the increase in mitochondria resistance to calcium-induced MPT. In the study reported here we investigated the effects of the new Dimebon structural analogues on the isolated rat brain mitochondria. All the substances under investigation had tetrahydro-γ-carboline pharmacophoric group and fluoride atom(s) outside the pharmacophore in their structure. It was found that all compounds in the concentration range of up to 100 μM (500 nmol/mg mitochondrial protein) had no effect on the mitochondrial membrane potential when mitochondria were energized with Complex I or Complex II substrates. Screening for the action on the calcium-induced mitochondria swelling revealed active and inactive

substances. The rate of the calcium-induced swelling decreased 2fold in the presence of the most active compounds. Moreover, these new Dimebon analogues were shown to significantly increase mitochondria calcium retention capacity. We have identified substances which increased the mitochondria calcium retention capacity more than Dimebon.

Thus, we have identified compounds among the new fluoride-containing Dimebon analogues that increase mitochondria resistance to MPT more effectively than Dimebon.

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The Conference Program

October 29, 2014

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences

Big Conference Room (Main building, 4th floor)

8.00 - 9.30 Registration of participants

9.30 OPENING CEREMONY. WELCOME SPEECH TO THE PARTICIPANTS OF THE CONFERENCE

10.00 - 12.00 - LECTURES OF LEADING SCIENTISTS

10.00 – 11.00 John J. Lemasters, Professor

REGULATION OF HEPATIC MITOCHONDRIAL METABOLISM BY ETHANOL

Medical University of South Carolina, Charleston, USA

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Russia

11.00 – 12.00 Galina D. Mironova, Professor

URIDINE AS A POTENTIAL MEDICINE FOR OXIDATIVE STRESS. THE STUDY OF THE MECHANISM OF ITS ACTION

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Russia

12.00 – 13.30 Lunch – 1.5 hours

13.30 - 16.00 ORAL REPORTS OF YOUNG SCIENTISTS

13.30 Yulia L. Baburina

2', 3'-CYCLIC NUCLEOTIDE 3'-PHOSPHODIESTERASE MIGHT BE POTENTIAL REGULATOR OF MITOCHONDRIAL MEMBRANE PERMEABILITY.

Baburina Y., Krestinina O., Odinokova I., Azarashvili T.

Institute of Theoretical and Experimental Biophysics RAS, Pushchino, Russia

13.50 Olga V. Krestinina

MELATONIN EFFECT IN MITOCHONDRIA MIGHT BE EXERTED VIA 2',3'-CYCLE NUCLEOTIDE-3'-PHOSPHODIASTARASE.

Krestinina O.V., Odinokova I.V., Baburina Yu.L., Azarashvili T.S.

Institute of Theoretical and Experimental Biophysics RAS, Pushchino, Russia

14.10 Mariya I. Shigaeva

ROLE OF CALRETICULIN IN THE FORMATION OF POTASSIUM-TRANSPORTING CHANNELS IN MITOCHONDRIA

Shigaeva M.I.[1], Talanov E.Y.[1], Murzaeva S.V.[1,2],

Mironova G.D.[1,2]

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14.30 Natalia V. Belosludtseva

INVOLVEMENT OF PALMITATE/Ca^{2+}(Sr^{2+})-INDUCED PORE IN THE CYCLING OF IONS ACROSS THE MITOCHONDRIAL MEMBRANE

Belosludtseva N.V, Agafonov A.V, Belosludtsev K.N., Mironova G.D.

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia

14.50 Alexey V. Chekanov

DISCOVERY FOR CHEMICAL COMPOUNDS ABLE TO SENSITIZE TUMOR CELLS TO SELECTIVE ANTICANCER CYTOKINE TRAIL.

Chekanov A.V.[1]*, Dolgikh N.V.*[1,2]*, Akatov V.S.*[1]

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[2]Pushchino State Institute of Natural Sciences, Pushchino, Russia

15.10 Roman S. Fadeev

INTERCELLULAR ADHESION INCREASES DRUG RESISTANCE OF ACUTE MYELOID LEUKEMIA CELLS

Fadeev R.S.[1], *Solovieva M.E.*[1], *Slaydovskiy D.A.*[2], *Zakharov S.G.*[3], *Senotov A.S.*[4], *Golenkov A.K.*[3], *Akatov V.S.*[1]

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[3]Moscow Regional Clinical and Research Institute; Moscow, Russia

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15.30 Konstantin N. Belosludtsev

Ca^{2+}-DEPENDENT PERMEABILIZATION OF MITOCHONDRIA AND LIPOSOMES BY PALMITIC AND OLEIC ACIDS: A COMPARATIVE STUDY

Belosludtsev K.N.

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia

15.45 Tim Valuev

STUDY OF THE EFFECT OF THE MOTOR PROTEIN VIMINTIN ON RAT LIVER MITOCHONDRIAL RESPIRATION

Valuev T.[1], *Gorbacheva O.S.*[2,3]

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15.50 Maria S. Frolova

FORMATION OF LIPOFUSCIN FROM MITOCHONDRIA DURING HEATING AND LIGHTING

Frolova M.S.[1], Surin A.M.[2], Braslavski A.V.[3], Vekshin N.L.[1]

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16.10 – 16.30 Coffee Break

16.30 - 17.30 Poster session

(Hall of Big Conference Room)

INFLUENCE OF ARYLCYCLOALKYLAMINE DERIVATIVES ON MITOCHONDRIAL FUNCTION

Neganova M.E.[1], Redkozubova O.M.[1], Dubova L.G.[1], Shevtsova E.F.[1], Veselkina O.S.[2]

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INVESTIGATION OF OSTEOINDUCTIVE ACTIVITY OF MATERIALS CONTAINING RECOMBINANT BONE MORPHOGENIC PROTEIN BMP-2

Vezhnina N.V.[1], Chekanov A.V.[1], Fadeeva I.S.[1,2],

Solovieva M.E.[1], Fesenko N.I.[2]., Gorbachev D.P.[2], Akatov V.S.[1,2]

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MITOCHONDRIA-TARGETED ACTION OF THE NEW FLUORIDE-CONTAINING TETRAHYDRO-γ-CARBOLINES.

Vinogradova D.V, Shevtsova E.F.

Institute of the Physiologically Active Compounds RAS, Chernogolovka, Russia

THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND ANTIOXIDANT ACTIVITY OF PHENOLIC COMPOUNDS ON MITOCHONDRIA MODEL

Abdullayev G.T., Abdullajanov N.G., Komilov E.J.

Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Uzbekistan

INTERACTIONS OF APOLIPOPROTEIN A-I N-TERMINAL FRAGMENT WITH MODEL LIPID MEMBRANES

Trusova Valeriya[1], Gorbenko G.[1], Girych M.[1], Adachi E.[2], Mizuguchi C.[2], Saito H.[2]

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OXIDATIVE PHOSPHORYLATION AND MITOCHONDRIAL PERMEABILITY TRANSITION PORE FUNCTIONING IN RATS WITH DIFFERENT GENETIC PREDISPOSITION TO AUDIOGENIC EPILEPSY

Belosludtseva N.V., Venediktova N.I., Mironova G.D.

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia

REGULATION OF CYCLOSPORIN A-SENSITIVE CALCIUM-INDEPENDENT FREE OXIDATION IN LIVER MITOCHONDRIA INDUCED BY FATTY ACIDS. ROLE OF MALONATE.

Adakeeva S.I., Dubinin M.V., Samartsev V.N.

Mari State University, Yoshkar-Ola, Russia

October 30, 2014

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences

Big Conference Room (Main building, 4th floor)

10.00 - 13.00 - LECTURES OF LEADING SCIENTISTS

10.00 – 11.00 Anna-Liisa Nieminen, Professor

MITOCHONDRIAL IRON UPTAKE THROUGH MITOFERRIN

Medical University of South Carolina, Charleston, USA

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Russia

11.00 – 12.00 Vladimir S. Akatov, Professor

MULTIDRUG RESISTANCE OF TUMOR CELLS AND MITOCHONDRIA PORE AND CHANNELS

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Russia

12.00 – 13.30 Lunch – 1.5 hours

13.30 - 15.30 ORAL REPORTS OF YOUNG SCIENTISTS

13.30 Olga S. Gorbacheva

DISRUPTION OF POTASSIUM HOMEOSTASIS AND OXIDATIVE EXCHANGE OF RAT BRAIN AND LIVER MITOCHONDRIA IN EXPERIMENTAL EPILEPSY.

Gorbacheva O.S.[1,2], Shigaeva M.I.[1], Kravchenko S.V.[1], Shchipakina T.G.[1] , Mironova G.D.[1,2].

[1]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia;

[2]Pushchino State Institute of Natural Sciences, Pushchino, Russia

13.50 Alexey G. Kruglov

VDAC ISOFORMS 1, 2 AND 3 LACK NADH OXIDOREDUCTASE ACTIVITIES.

Shmatkova M.L.[1,2], Teplova V.V.[2], Chekanov A.V. [2], Krestinina O.V. [2], Solov'eva M.E. [2], Sheiko T.V. [3], Nikiforova A.B. [2], Kudriavtsev A.A. [2], Craigen W.J.[3], Kruglov A.G. [2]

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[2]Institute of Theoretical and Experimental Biophysics, Pushchino, Russia

[3]Baylor College of Medicine, Department of Molecular and Human Genetics, Baylor, USA

14.10 Anna B. Nikiforova

CYTOCHROME B5 REDUCTASE 3 (CYB5R3) OR VDAC1 IS THE NADH-DEPENDENT REDUCTASE OF REDOX-CYCLING XENOBIOTICS IN THE EXTERNAL MITOCHONDRIAL COMPARTMENTS?

Nikiforova A.B., Kruglov A.G.

Institute of Theoretical and Experimental Biophysics (Russian Academy of Sciences) Pushchino, Russia

14.30 Nikolai I. Markevich

COMPUTATIONAL MODELING ANALYSIS OF MITOCHONDRIAL SUPEROXIDE PRODUCTION

Markevich M.N.[1], *Markevich N.I.*[2,3]

[1]Bauman State Technical University, Moscow, Russia

[2]Thomas Jefferson University, Philadelphia, USA

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14.50 Anton G. Rogov

BENZALKONIUM CHLORIDE: EFFECTS ON BIOENERGETICS OF RAT LIVER MITOCHONDRIA

Rogov A.G., Zvyagilskaya R.A.

A.N. Bach Institute of Biochemistry, Russian academy of Sciences, 119071 Moscow, Leninsky prospekt, 33, Russia

15.10 Irina S. Fadeeva

MITOCHONDRIA OF DYING CELLS AS A CENTER OF ASEPTIC CALCINOSIS NUCLEATION IN HEART VALVE AND VESSEL TRANSPLANTS.

Fadeeva I.S.[1,2], *Fesenko N.I.*[1,2], *Soloviev V.V.*[1,3], *Gorbachev D.P.*[1,2], *Sachkov D.V.*[4], *Akatov V.S.*[1,2]

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[4]A.N. Bakuleva SCCVS RAMS, Moscow, Russia

15.30 – 16.00 Coffee Break

16.00 - 17.00 Poster session

(Hall of Small Conference Room)

MITOCHONDRIAL PERMEABILITY TRANSITION PORE IS AS A TARGET OF TSPO LIGANDS INFLUENCE IN CHRONIC MELATONIN ADMINISTRATION.

Milyaev E.V.[1], Baburina Yu.L. [2], Odinokova I.V.[2], Azarashvili T.S.[2], Krestinina O.V.[2]

[1]Tolstoy State Pedagogical University of Tula, Tula, Russian Federation

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ADAPTATION OF LEWIS LUNG CARCINOMA CELLS IN CULTURE AND EVALUATION OF THE ACTION OF TARGETED ANTICANCER AGENTS IN VITRO

Khairetdinova M.M.[1], Chekanov A.V.[1,2], Fadeev R.S.[1,2], Akatov V.S.[1,2]

[1]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia

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THE EFFECTS OF MALONATE AND INORGANIC PHOSPHATE ON CYCLOSPORINE A - INSENSITIVE Ca^{2+}-DEPENDENT NONSPECIFIC PERMEABILITY OF THE INNER MEMBRANE OF LIVER MITOCHONDRIA INDUCED BY α,ω-DIOIC ACIDS

Dubinin M.V., Khoroshavina E.I., Vedernikov A.A., Adakeeva S.I., Yusupov N.V., Samartsev V.N.

Mari State University, Yoshkar-Ola, Russia

METFORMIN AS A POTENTIAL CELL REGULATOR FOR RESISTANCE TO RADIATION-INDUCED DAMAGE

Minkabirova G.M., Abdullaev S.A.

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia

PROLONGED CONSUMPTION OF ETHANOL CLOSES PORIN CHANNELS OF THE OUTER MITOCHONDRIAL MEMBRANE AND SUPPRESSES OXIDATIVE PHOSPHORYLATION

Teplova V. V., Fedotcheva N. I.

Institute of Theoretical and Experimental Biophysics RAS, Pushchino, Russian Federation

OXIDATIVE STRESS IN THE PATHOGENESIS OF ALCOHOLIC ENCEPHALOPATHY

Teplova V.V., Shubina V.S., Shatalin Yu.V.

Institute of Theoretical and Experimental Biophysics RAS, Pushchino, Russian Federation

INTERCELLULAR ADHESION-MEDIATED RESISTANCE OF TUMOR CELLS TO DIETHYLDITHIOCARBAMATE (DDC) IS INHIBITED BY VITAMINE B12: A POSSIBLE ROLE OF VDAC

Slaydovskiy D.A.[1], *Fadeev R.S.*[2], *Solovyova M.E.*[2]

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Novgorod, Nizhni Novgorod, Russia
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17.00 – 19.00 CLOSING CEREMONY OF THE CONFERENCE

Celebratory banquet

(Banquet Hall, Main building, 1st floor)

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Scientific publication

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