MOLECULAR BASIS OF PHYSIOLOGICAL FUNCTIONS

REDISTRIBUTION OF DNA LOOP DOMAINS DURING TRANSCRIPTION ACTIVATION AND MALIGNANT TRANSFORMATION AS REVEALED BY COMET ASSAY

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Recent studies indicate that DNA loop domains are not only the key element at higher level of chromatin organization but also involved in various functional activities within cell nucleus. In addition to conservative loop domains (e. g., associated with nuclear lamina), many loops may appear as a result of transcription regulation, DNA replication and repair. Therefore, the distribution of the loop domains between different fractions varies in dependence on functional state, phase of the cell cycle and cell type. The aim of this study was to investigate peculiarities of the loop domain organization in lymphocytes at different stages of their activation and in human glioblastoma cells.

Human lymphocytes were isolated by centrifugation in density gradient and transformed with interleukin-2 to obtain lymphoblasts. Human glioblastoma cells (T98G) were grown in DMEM and then synchronized at G1 phase of cell cycle by incubation in serum-free medium for 48 hours. The organization and redistribution of DNA loop domains in these cells were investigated using kinetic approach of single-cell gel electrophoresis (the comet assay). We estimated the DNA amount in the comet tails and contour length of the largest loops in the tails depending on the electrophoresis duration.

We observed significant differences in the kinetics of DNA exit during electrophoresis between all the cell types investigated. First of all, for T98G cells and lymphoblasts at 44th hour after transformation (G2 phase of cell cycle) the decreasing in the maximum amount of DNA that can migrate into the comet tail was observed in comparison with inactive lymphocytes (G0 phase) and lymphoblasts at 24th hour after transformation (G1 phase). This difference was probably due to a decrease in the number of loops with contour length from 50 to 150 kb. Secondly, the amount of small (up to 30 kb) surface loops was almost two-times higher for both types of lymphoblasts in contrast with lymphocytes. Moreover, the correlation between the DNA amount in the comet tails and the tail length implies that the contour length of the loops is distributed exponentially and distribution parameter, the loop density, is dependent on the cell type.

Our findings indicate that the comet assay may be applied to detect some DNA loops redistribution in cells with different transcriptional activity or at various stages of cell cycle.

SIRNA-INDUCED SILENCING OF HYPOXIA-INDUCIBLE FACTOR 3A (HIF3A) INCREASES ENDURANCE CAPACITY IN RATS

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The mechanisms of HIF3α subunit effect on adaptation to physical exercise remain to be uninvestigated.

In our experiments we knocked down Hif3α using siRNA to study rats endurance capacity. Real-time PCR analysis was performed for quantitative evaluation of HIF3α, IGF1, GLUT-4 and PDK-1 in m. gastrocnemius, m. soleus, in the lung and heart tissues. Mitochondrial respiratory function and electron microscopy were performed.

The knockdown of Hif3α using siRNA increases the time of swimming to exhaustion 1.5 times. The level of mitochondrial NAD- and FAD-dependent oxidative pathways is decreased, however efficiency of phosphorylation is increased after HIF3α siRNA treatment. Some destructive changes in muscle tissue were detected in animals with siRNA-inducing silencing of Hif3α.

Optimization of oxidative phosphorylation in mitochondria and increase of HIF dependent gene (Pdk-1) expression explain the effect of Hif3α silencing that led to the significant increase of endurance capacity of rats.

PHAGE-CODED EPS-DEPOLYMERASES DETERMINE THEIR POLYVALENCY

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Bacteriophages are regarded as promising tools for the control of bacterial pathogens. Viruses with broad host range, able to infect not only different strains, but even genera of bacteria are referred as polyvalent. Two bacteriophages of Podoviridae family, E105 and TT10-27, obtained from amylovora-like bacteria *Erwinia "horticola"* and isolated from plant material, affected with fire blight (*Erwinia amylovora*), respectively, are polyvalent. They perform productive infection in 2 different species of bacteria: both phytopathogenic *E. "horticola"* and plant-associated *Pantoea agglomerans*.

Determination of the mechanisms underlying the expansion of host range is a keystone of phage characterization and gives insight into virus/host interaction details. To reveal them, phages' DNA was sequenced and analyzed. Methods of DNA sequencing and Bioinformatics were used.

Sequence analysis revealed that E105 belongs to genus of phiKMV-like phages. Its DNA composition is highly unique and shares high percentage of similarity only with 1 genome in GenBank, of *Pantoea* phage LIMElight (NC_019454.1). E105 DNA of 43 856 bp with direct exact terminal repeats is comprised of 55 ORFs, 54 of which are protein coding sequences (CDS) and 1 is a tRNA gene. They are placed on plus-strand and can be divided into early/middle and late genes regions. Phage DNA possesses high GC% content – 54,46%.

Phage TT10-27 is a representative of N4-like phages; its close relatives are *Erwinia amylovora* phages Frozen (NC_031062.1) and Ea9-2 (NC_023579.1). Its DNA is larger (74 143 bp), features direct various repeats, contains 90 ORFs, including 4 tRNA genes and at least 1 gene of bacterial origin; 86 CDS are transcribed from plus-strand (early and middle genes) and minus-strand (late genes). Phage DNA codes heterodymeric T3/T7-like RNA polymerase, as well as virion RNAP, ORF68, huge protein of 3491 aa that is packed into virion along with DNA. GC % content of DNA is 46.8%.

Though phages' E105 and TT10-27 DNA sequences reveal no significant similarity to each other, they both code for a protein, responsible for interaction with host cell surface that possesses depolymerase activity. ORF47 (E105) of 857 aa and ORF83 (TT10-27) of 881 aa reveal homology to EPS-depo (extracellular polymeric substances/exopolysaccharide depolymerases) of various *Erwinia* phages of Podo-, Myo- and Siphoviridae families. E105/TT10-27 EPS-depo protein sequences align in C-terminal region (responsible for interaction with host), and share no similarity in N-terminus, that is responsible for protein attachment to phage virion. Predicted tertiary structure of C-termini of both proteins coinscides with crystal structures of hydrolases (galacturonidases).

Broad host range of viruses E105 and TT10-27 is determined by their attachment apparatus, mainly its EPS-depolymerase activity. Thus, phages of different genera, with different morphology, strategies of transcription/replication share similarly organized EPS-depo protein that allows for their polyvalent feature. Further study of these phage-coded depolymerases can provide the background for antibacterial tools construction against phytopathogens.

CARDIOPROTECTIVE EFFECTS OF PAG ADMINISTRATION

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Hydrogen sulphide (H_2S) is gaseous transmitter that causes many effects in organism including reduction of infarct zone of myocardium and improving vasorelaxation. Exogenously applied H_2S donors protect the heart against ischemia-reperfusion (I/R). In cardiovascular system H_2S is produced from amino acid L-cysteine mainly by cystathionine-gamma-lyase (CSE). CSE can be inhibited with DL-propargylglycine (PAG). Earlier we demonstrated that L-cysteine alone had no significant effect on cardiac function, however, PAG increased resistance of myocardium to ischemia. Pre-treatment of PAG and L-cysteine manifested in strong cardioprotective effects. We decided to investigate how biochemical indexes changed in heart tissue. Six month Wistar rats were divided into four groups: 1 – control, 2 – hearts after 20 min of ischemia and 10 min of reperfusion (I/R 20/10), 3 – PAG (11.3 mg/kg 10 min) + L-cysteine (121 mg/kg 30 min) without I/R and 4 – PAG+L-cysteine after I/R 20/10. The hearts were isolated by Langendorff preparation. The heart tissue was examined for superoxide radical, hydroxyl radical, peroxide, peroxide radical and diene conjugates content, nitrate, nitrite and H_2S levels, iNOS, cNOS and H_2S-synthesizing enzyme (CSE+CBS) activity. I/R caused oxidative stress in terms of increased ROS production. The 2.4-fold increase in diene conjugates indicated intensified lipid peroxidation in I/R cardiac samples probably due to the significant increase in hydroxyl radical ($^{\cdot}OH^{-}$) generation rate. That was absent in I/R group pretreated with PAG+L-cysteine. Lower activity of H_2S producing enzymes and H_2S level was observed in group 4 vs 3. The activity of constitutive synthesis of NO decreased 5.5 times in 2 group and was 2-fold renewed in 4 vs 2. NO production changed in the same manner. There was neither increase in iNOS activity nor NO_3-levels elevation in group 4. PAG+L-cysteine significantly decreased reactive oxygen species production and diene conjugates. Thus, pre-treatment with PAG and L-cysteine showed strong protection against oxidative stress and reperfusion injury induced by ischemia.

THE ROLE OF C-DI-GMP IN THE *PSEUDOMONAS AERUGINOSA* RHAMNOLIPIDS BIOSYNTHESIS

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Bis-(3-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) is in the spotlight of the scientists as the result of last achievement of microbial genomics and great interests in microbial communities. Cytoplasmic c-di-GMP is a bacterial secondary messenger, that regulates numerous of physiological processes: cell-to-cell communication, biofilm formation, motility, virulence, etc. Depends on concentration of this regulator bacteria shifts its life-form from motile to sessile (biofilm formation). It is found that c-di-GMP affects all stages of the biofilm formation process in *Pseudomonas aeruginosa* from the beginning of adhesion to biofilm decay. This compound regulates biosynthesis of matrix components, quorum sensing signal molecules, biosurfactants. That fact, that the direction of many processes change in bacterial cells depends on c-di-GMP, led to hypo- and hyperproduction strains construction. Their use allows extending knowledge in the role of this compound in many bacterial cells processes and the possibility to use this molecule as an instrument for biofilm formation control.

The aim of this work was to study the ability to produce rhamnolipids by *P. aeruginosa* strains with different levels of c-di-GMP synthesis. This was used mutant strains: PA01 pJN2133 with very low biosynthesis of c-di-GMP and PA01 wsp F1 with overproduction of this second messenger. For comparison, using wild-type strain of *P. aeruginosa* PA01. The incubation was carried out in LB medium at 37 °C with shaking at 150 rpm/min.

The results show that during the first five days of incubation rhamnolipids content in the environment was maintained at a level that was reached after 24 hours. This pattern of accumulation of surfactant was inherent in all three strains. But for further incubation (the seventh day) the picture changed. When strains PA01 and PA01 pJN2133 rhamnolipids content increased significantly compared to the previous study period (5 days), 3 and 4 times, respectively. The level of surfactant biosynthesis strain PA01 wsp F1 has not changed. It should also be noted that the strain with low level of c-di-GMP in all study periods produces more rhamnolipids than *P. aeruginosa* PA01. On the seventh day the difference between them was 60%.

Previously we found that *P. aeruginosa* PA01 pJN2133 cell is more mobile in comparison to *P. aeruginosa* PA01 cell and have low adhesion and biofilm formation abilities. We assumed that these features of the strain PA01 pJN2133 are due to its ability to synthesize large amounts of rhmnolipids. This was confirmed in this study.

P. aeruginosa strains with reduced biosynthesis c-di-GMP are potential candidates for the biosurfactant production.

HYPERTHERMIA PREVENTS CARDIAC REPERFUSION INJURY PROBABLY VIA INHIBITION OF MITOCHONDRIAL PERMEABILITY TRANSITION AT LANGENDORFF RAT HEART MODEL

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Hyperthermia might induce damage as well as increase of cardiac tolerance to ischemia. Such divergence obviously depends on the degree of temperature increase. Hyperthermia-induced cardiac tolerance is greatly determined by ROS and heat shock proteins implications. On the other hand, one of the main targets for cardioprotection is mitochondrial permeability transition pore (MPTP), which is not described in hyperthermia. We studied two regimes of hyperthermia in order to estimate the dependence between hyperthermia effects at heart function and MPTP opening.

We used Wistar male rats aged 6 months. Isolated hearts were perfused by Langendorff preparation with on-line registration of left ventricular developed pressure (LVDP), coronary flow and evaluation of oxygen utilization by the myocardium. Hyperthermia was modeled as the increase of perfusion solution temperature from 36.5 to 39.5 °C or 41.5 °C during 15 min before 20 min total ischemia and 40 min of reperfusion (I/R). "Opened/closed" state of MPTP was evaluated by UV-measuring of the levels of mitochondrial factor (MF) which released from the inner mitochondrial space into solutes outflow from the coronary vessels of the isolated heart.

In our experiments I/R strongly depressed cardiac contractile activity of isolated rat hearts (n = 5). At the 40th min of reperfusion the average values of LVDP, dP/dtmax and coronary flow recovered only to 30, 35 and 75%, respectively from the initial values. However, the contractile activity of hearts which underwent 15 min of 41.5 °C hyperthermia was not renewed at all (n = 4). On the contrary, hearts which underwent 39.5 °C hyperthermia (n = 6) showed 68%, 71%, 90% recovery of LVDP, dP/dtmax and coronary flow, respectively. At the 5th min of reperfusion oxygen cost of myocardial work was increased 11.3 times compared to 3.2 times in 39.5 °C hyperthermia pretreated hearts. The MF level in outflow solutions collected at the 1st min of reperfusion was significantly lower compared to non-hyperthermia hearts.

Thus, 41.5 °C hyperthermia depresses the heart function and aggravates reperfusion injury of isolated rat heart, whereas 39.5 °C hyperthermia reveals cardioprotective effect in terms of contractile activity restoration and optimization of oxygen utilization by ischemized myocardium and greatly decreases MPTP opening at the reperfusion.

THE PROPERTIES OF ION CHANNELS IN THE NUCLEAR ENVELOPE OF CARDIOMYOCYTES

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The nuclear membrane forms a semi-permeable barrier for the movement of molecules and ions. Transport between the cytoplasm and nucleoplasm occurs through a large number of nuclear pores and ion channels with different biophysical properties.

In this work we have studied the biophysical properties of the ion channels in the nuclear membrane of cardiomyocytes. Nuclei from cardiomyocytes were isolated by homogenization. Single ion channels were recorded from nucleus-attached and excised patches of the nuclear membrane in the voltage-clamp mode of the patch-clamp technique.

We have registered many ion channels with different properties in the nuclear membrane of cardiomyocytes, including LCC-channels, and inositol-1,4,5-trisphosphate receptors. Our results indicate that LCC-channels were selective for monovalent (K^+ and Na^+) cations and demonstrated voltage dependence. At positive potentials, the activity of these channels is significantly more intense than at negative potentials. In symmetrical KCl solution, the slope conductance of the LCC-channels in the nuclear membrane of cardiomyocytes was 209 ± 13 pS (n = 44). Considering biophysical properties such as conductance, kinetics and voltage dependence, we conclude that in the nuclear membrane of cardiomyocytes are expressed similar or identical LCC-channels, which we had identified and described earlier in the nuclear membrane of neurons.

We have also first registered IP3Rs in the native nuclear membrane of cardiomyocytes, which differ in their properties from of all other isoforms of IP3Rs. The slope conductance of the IP3Rs in the nuclear membrane of cardiomyocytes in symmetrical KCl solution was 384 ± 5 pS (n = 4). All known types of IP3Rs are blocked by Ca^{2+} in concentrations less than 1 μM, however, our experiments showed that IP3Rs of cardiomyocytes were not blocked by high concentration of Ca^{2+} (up to 100 μM) in solution. It is known that IP3R2 prevail in the nuclear membrane of cardiomyocytes, therefore we assume that we recorded receptors of type 2 in this membrane.

Therefore, we have found in the native nuclear membrane of cardiomyocytes LCC-channels. We are the first to register the inositol 1,4,5-triphosphate receptors and to show that they are functional. Also, we found many types of ion channels with different conductivity, which have yet to be investigated.

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THE EFFECT OF BUTHIONINE SULFOXIMINE ON REALIZATION OF CARDIOPROTECTIVE EFFECT OF ISCHEMIC PRECONDITIONING

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The phenomenon of ischemic preconditioning (IPre) is a known powerful protective strategy that is implemented in many experimental models *in vitro*, in situ or *in vivo*. It is well established that the main target of carioprotective effect of IPre is mitochondrial permeability transition pore (MPTP) that is opened under reperfusion. Since IPre demonstrates antioxidant effects, we hypothesized that IPre might be realized via activation of glutathione system. Thus, the aim was to evaluate the effect of glutathione synthesis inhibitor - buthionine sulfoximine – BSO at protective action of ischemic preconditioning in isolated rat heart model.

We used 6 month Wistar male rats. BSO (22 mg/kg, Sigma) was injected intraperitoneally (for 40 min). We registered cardiodynamic parameters (left ventricular pressure (LVP), dP/dt, heart rate, coronary flow) and oxygen utilization by Langendorff isolated hearts. IPre (3 episodes of 5 min ischemia and reperfusion) was provided before 20 min of ischemia and 40 min of reperfusion. The levels of mitochondrial factor (as an indicator of MPTP opening) were measured as increased UV-optical density (OD) of coronary solutions outflow the isolated hearts before and after ischemia.

The data showed that the initial values of heart indexes (LVP, heart rate, coronary flow, dP/dt and oxygen cost) did not differ in control (n = 5) and IPre (n = 7) groups as well as pretreatment with BSO (n = 7). IPre induced a significant restoration of contractile function of isolated rat heart specially in the early postischemic period: at the 10th min of reperfusion LVP was 82.5 ± 8.8% vs 32.7 ± 7.9% in control. In BSO+IPre group the LVP restored to 70.6 ± 7.9%. The relaxation index (dP/dt min) was 78.7 ± 5.8% in IPre, 89.4 ± 16.6% in BSO+IPre vs 28.0 ± 6.7% in control group. Ischemia-reperfusion induced a considerable increase of OD of outflow solutes collected at the 1st min of reperfusion with "peak" of absorbance at 245-250 nm. It was found that IPre strongly prevent appearance of the "peak". Pretreatment with BSO slightly increased OD of coronary effluents at the 1st min of reperfusion, however, it was significantly lower than in control.

Thus, one-time administration of glutathione inhibitor BSO at least in selected dose does not eliminate the protective effect of IPre in terms of heart function restoration. Moreover, the level of mitochondrial factor was not affected by BSO treatment indicating strong realization of IPre-induced protective program.

DIADINOXANTHIN DE-EPOXIDATION KINETICS IN ARSENIC TREATED *PHAEODACTYLUM TRICORNUTUM*

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Phaeodactylum tricornutum is a marine diatom capable of conducting the reactions of the diadinoxanthin cycle. This mechanism is believed to play a crucial role in the photoprotection of algal photosystems and is based on the de-epoxidation of diadinoxanthin (Ddx) with the production of diatoxanthin (Dtx) under the intense light conditions, and the epoxidation of Dtx to Ddx, when amount of light absorbed by diatoms falls to lower level. The aim of this work was to evaluate the influence of arsenic stress on diadinoxanthin cycle. *Phaeodactylum tricornutum* was cultured in the f/2 Guillard's medium. As a source of arsenic, sodium arsenate (Na_3AsO_4) was used. After the growth in media containing different concentrations of sodium arsenate, the cultures were exposed to intensive light and samples of media were collected after 5, 10, 15, 25 and 45 minutes of exposition. Additionally for the analysis of the rate of Dtx epoxidation, the samples after 45 min of strong light illumination were incubated for 4 hours under weak light condition. All the samples were immediately frozen in liquid nitrogen, stored in -80 °C and then the content of Ddx and Dtx was measured by HPLC with photodiode array detector. The ratio of Dtx content to the sum of Dtx and Ddx contents (DES) was determined. The effect of arsenic was observed both on epoxidation and de-epoxidation reaction in *P. tricornutum* cells. Data showed higher de-epoxidation rate, measured as the change of DES value over the course of illumination, in diatoms cultured with the addition of arsenic. Moreover, such treatment, at any tested concentration, caused the increase of DES value of non-illuminated cells, compared to the cells not treated with arsenic.

INHIBITION OF H_2S SYNTHESIS IN MITOCHONDRIA REDUCES CARDIAC CONTRACTILE FUNCTION AND INCREASES MITOCHONDRIAL DYSFUNCTION UNDER Ca^{2+} OVERLOAD IN RAT HEART

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Hydrogen sulfide (H_2S) is one of the three biological gaseous mediators, which takes part in regulation of variety of functions in cardiovascular system. H_2S is produced enzymatically by three different enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST), which works in tandem with cysteine aminotransferase (CAT). Because of the fact that the last two enzymes are located in mitochondria and oxidation of H_2S also takes place in mitochondria, it becomes clear that mitochondrial origin H_2S, plays a great role in the cardiac and vascular functions regulation. However little is known about the impact of mitochondrial origin hydrogen sulfide on heart resistance to calcium overload and the sensitivity of MPTP to this cation.

To investigate the role of mitochondrial origin H_2S in cardiac function under calcium overload and in Ca^{2+}-induced MPTP opening in rat hearts.

In our work we used adult (5-7 months) Wistar rats. Cardiodynamic parameters such as left ventricular pressure (LVP), dP/dt, heart rate, coronary flow and oxygen consumption were registered using Langendorf isolated rat heart. Calcium load was carried by adding of $CaCl_2$ in perfusion solution every 10 min until the concentration of calcium increased from 1.7 to 12.5 mmol/l. Rat heart mitochondria were isolated using differential centrifugation method. MPTP opening was registered spectrophotometrically as mitochondrial swelling.

It was shown that the inhibition of mitochondrial H_2S-synthesis enzyme had a negative influence on initial cardiodynamic parameters. In particular, the LVP and the rate of contraction and relaxation of myocardium decreased twice. Coronary blood flow decreased by 1.12 times, while the heart rate was tended to increase. We found that the hearts of experimental animals developed less powerful reaction under the calcium overload that manifested in reduced parameters of LVP, coronary flow and heart work intensity. The inhibition of 3-MST by O-CMH (*in vitro* and *in vivo*) causes significantly dose-dependent increase of Ca^{2+}-induced mitochondrial swelling in adult rat heart. The highest concentration of inhibitor (10^{-3} mol/l) that were used increased this parameter 2.3 times.

H_2S which is synthesized in mitochondria has the great impact on regulation of cardiac functions, in particular on cardiac resistance to Ca^{2+} overload and on calcium-induced MPTP opening in rat heart.

PYRIDOXAL-5-PHOSPHATE RESTORES H_2S SYNTHESIS AND ENDOTHELIUM-DEPENDED RELAXATION OF AORTA SMOOTH MUSCLES IN OLD RATS

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Hydrogen sulfide (H_2S) as well as nitric oxide belong to gas transmitters' family and play an important role in vessels' tone regulation. It was shown that H_2S reveal cardio- and neuroprotective properties preventing extensive ROS generation and apoptosis. H_2S is synthesized from aminoaside L-cysteine by cystathionine-ß-synthase (CBS), cystathionine-γ-lyase (CSE) localized in cytoplasm and 3-mercaptopyruvate sulfurtransferase, mitochondrial enzyme which is coupled with cysteine aminotransferase enzyme (CAT). CBS, CSE and CAT have pyridoxal-5-phosphate (P5P) as co-factor. Earlier we showed decrease of H_2S synthesis with aging, however, role of H_2S in vessels function in aging is still poorly understood. The aim of current work was to study the effect of P5P administration at H_2S synthesis and endothelium-dependent aortic smooth muscles relaxation in old rats.

We used Wistar male rats divided into 3 groups: adult (6 months), old (24 months) and old+P5P. P5P was dissolved in distilled water and administered *per os* in dose of 0.714 mg per kg once a day for 2 weeks. After sacrificing the rats at the end of the treatment, aorta was extracted and muscle contractile activity of aorta rings was measured with tensiometry in a chamber at 37 °C. Norepinephrine was added to induce contraction of aorta smooth muscles, and further acetylcholine perfusion was performed to induce relaxation. Additionally, content of H_2S was measured in aortic tissues.

Our results show that endothelium-dependent relaxation of smooth muscles was greatly impaired in old rats: the index of acetylcholine-induced relaxation was 18.4 ± 4.1% vs 66.5 ± 6.4% in adult rats ($P < 0.001$). After 2 weeks of P5P administration the index of aorta relaxation was 47.7 ± 4.8,% that indicates at least partial renovation of endothelium-dependent relaxation of aortic smooth muscles of old rats treated with P5P. Biochemical measurements showed that H_2S content was 1.6 times lower in old rat aorta compared to adult ones. However, P5P induced a significant 2-fold increase in H_2S content ($P < 0.05$) in aortic tissue.

Thus, we can conclude that endogenous H_2S is greatly engaged in regulation of endothelium-depended relaxation that might be stimulated by pyridoxal-5-phosphate administration in conditions of vessels' tone dysfunction like aging, hypertension etc.

THE EFFECT OF LONG-TERM CONSUMPTION OF LOW DOSES OF Co^{2+} WITH DRINKING WATER ON ERYTHROCYTES HEMOLYSIS

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A number of studies have shown that cobalt ions at the concentrations that exceed 0.6 mg/kg/day (NOAEL – no observed adverse effect level) tend to destabilize red blood cells membranes even under single administration to laboratory animals. Works, that investigate the chronic effect of Co^{2+} ions at much lower concentrations than NOAEL on the structural stability of erythrocytes, are almost absent.

The aim of the present study was to investigate the effect of prolonged action of low doses of Co^{2+} ions on erythrocytes structural resistance to hemolysis.

The effect of intragastric administration of Co^{2+} ions in doses 0.012 and 0.06 mg/kg/day during 15 and 36 days on hemolysis of 3-month old Wistar rats' red blood cells has been studied. The animals were divided into 5 groups. Animals from the group 1 (control group) were receiving clean water during 36 days. Groups 2 and 3 were getting $CoCl_2$ solutions in aforementioned concentrations during 15 days, groups 4 and 5 – during 36 days. Water and salt solutions were administered by intragastric probe daily. Erythrocytes were obtained from the whole blood of rats. Hemolysis was induced by adding HCl to its final concentration of 0.002N. Red blood cells hemolysis kinetics was recorded by changes in the optical density of samples with registration step of 1 s. The time of erythrocytes membranes structural rearrangement before the beginning of their destruction process and the rate of their destruction were selected as the indicators of hemolysis.

According to the obtained results, the rate of the membranes cooperative destruction of the bulk of red blood cells is 1.6 times higher in experimental animals than in control group after 36 days of Co^{2+} administration in both concentrations. Furthermore, the time before the membranes cooperative destruction starts decreases by 16% under Co^{2+} administration during 36 days compared to the control.

Thus cobalt ions during acidic hemolysis enhance the destabilizing effect of HCl even at doses 10 and 50 times lower than NOAEL. The additional destabilizing effect of Co^{2+} ions increases with the dosage and duration of administration.

PROMOTING OLIGODENTROCYTES PRECURSOR CELLS PROLIFERATION AND SURVIVAL IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a neurodegenerative disease at which demyelination of the neurons happens. Microglia, lymphocytes, and macrophages are among the main causes of such effect. Oligodendrocytes Precursor Cells (OPCs) are the main targets of inflammation and immune attacks which cause their death by apoptosis; thus, not only demyelination occurs, but losing the ability of re-myelination is lost as well resulting in MS.

In mice with introduced multiple sclerosis-like disease, a combination of two drugs will be introduced integrating to positively affect OPCs proliferation and survival; therefore, a synergistic effect should be achieved. First, 'WIN55,212-2' chemical compound, with a cannabinoid-like effect, stimulates OPCs proliferation, has neuroprotectant effect and induces oligodentrocytes maturation. Second, Minocycline, lipophylic tetracycline antibiotic, that has anti-apoptotic effect on oligodendrocytes. The drugs will be applied to a non-viral vector, a designed dendrimer will be used. Through stereotactic intra-cranial injection, the dendrimer including the drugs in nano size, will be injected. The experiment can be monitored by using TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick End Labeling) to detect oligodentrocytes cells apoptosis level.

Through the above steps and design, OPCs size and proliferation level should be better, and the reverse on apoptosis level, promoting control of the induced MS case and even an approach to make the case better.

THE IMPACT OF SIRTUIN ACTIVITY MODULATORS ON THE CUMULUS CELLS VIABILITY OF FEMALE MICE IN THE CONDITIONS OF EXPERIMENTAL SYSTEMIC IMMUNE DISORDER

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Resumption of oocyte meiotic maturation and the formation of the first polar body of oocytes is a prerequisite for normal fertilization and further development of the fetus and the ability of the ovary to ovulate; as a result oocyte with haploid sets of chromosomes gets into the fallopian tube for fertilization, which is a prerequisite for female mammals fertility. Systemic immune disorder (SID) is considered to be one of the major factors that may probably affect a female reproductive function. There is an assumption that SID is able to influence both directly, through direct action on oocytes and through indirect mechanisms –the cumulus cells viability, particularly. Thus, above-mentioned pathology is a serious problem for the development of diagnostics and therapy of the female reproductive system pathologies, caused by immune factors. Sirtuins are a class of proteins that regulate a wide range of cellular processes like aging, transcription, apoptosis, and also play an important role in stress resistance by activating oxidant-antioxidant system. As a result, they are able to correct the negative impact of various pathological processes that occur in the body, particularly systemic immune disorder on the process of meiotic maturation of oocytes. Therefore, the assessment of the impact of sirtuin activity modulators on the oocyte meiotic maturation and cumulus cells viability in female mice in the conditions of experimental SID was considered important for the research. To determine the effect of SID on ovarian function we used an experimental model of systemic immune disorder, created by immunizing experimental group of animals (mice) with antigenic suspension. Assessment of apoptotic and necrotic death of immune and cumulus cells performed using the method of *in vivo* dual-color fluorescent dyes of nucleic acids. Investigation of primary DNA damages (the damage index) of immune and cumulus cells was performed using gel electrophoresis of isolated cells method (the comet assay). Our results indicate that experimental systemic immune disorder in mice can cause suppression of cumulus cells viability. It is shown that the number of viable cumulus cells reduced under the experimental systemic immune disorder and the number of cells with morphological signs of apoptosis and necrosis increased, that can probably cause the further decrease of oocytes viability. We have found that sirtuins activation leads to the increase of the percentage of viable cumulus cells – 49.7 ± 0.8% ($P < 0.01$, $n = 7$) versus 39.2 ± 0.8% in the conditions of cultivation without resveratrol. Moreover, we indicated the influence of sirtuin activator – resveratrol (c = 20 μM) and inhibitor – nicotinamide (c = 5 mM) on the cumulus cells viability and it's number of primary DNA damages. We determined the decreasement of cumulus cells DNA damage index in the conditions of cultivation with resveratrol – 3.1 and 3.4 without resveratrol in mice with experimental kidney injury. DNA damage index in the conditions of cultivation with nicotinamide – 3.5. Therefore, our data indicate the role of sirtuin activity modulators on the cumulus cells viability of female mice in the conditions of experimental acute immune kidney injury, thus increasing the likelihood of successful fertilization of the oocyte and passage of further stages of its development.

ALPHA-E-CATENIN INVOLVEMENT IN NEONATAL CARDIOMYOCYTES SIZE REGULATION

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Alpha-E-catenin is a key component of cell-cell adhesion, but recent studies suggest that α-catenins have more complex and diverse functions and involved in some signaling pathways. In this study, we have focused on alpha-E-catenin function in neonatal cardiomyocytes. Cells were isolated from hearts with alpha-E-catenin homozygous and heterozygous knockout and control one, and used for histological and molecular biological analysis. In our previous work, we registered heart and atria enlargement in adult mice with alpha-E-catenin depletion compared to control. In contrast to these data we have found that newborns' cardiomyocytes with full and heterozygous alpha-E-catenin knockout were smaller (length and width) compared to control. Interesting that compared to control cells, the number of binuclear cardiomyocytes was decreased in cells with alpha-E-catenin missing. Additionally, we registered autophagy activation in cardiomyocytes with heterozygous and homozygous alpha-E-catenin deletion. We also registered violation of canonical WNT-signaling in mutant cells.

Collectively our data indicated that alpha-E-catenin has important function in newborns' cardiomyocytes size regulation and survival, probably through regulation of canonical WNT- signaling.

METHODS OF MOLECULAR GENETIC DIAGNOSTICS OF FOOD SAFETY IN UKRAINE

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Standardized methods of diagnosing the safety of food and raw materials are classical methods of food microbiology, which are time-taking, based on the phenotypic characteristics of microorganisms and are not always able to diagnose their toxigenic properties. Analytical information on the inaccuracy of indication of bacillary food poisoning, the need for a preventive analysis of the risks that aerobic and facultative-anaerobic spore-forming microorganisms of the genus *Bacillus* bear, cause the urgency of their detection by accelerated modern methods. Such diagnostics will allow producing new competitive food of guaranteed quality and microbiological safety. The work was aimed at molecular-biological diagnostics of potential causative agents of food poisonings – the contaminants of the genus *Bacillus* – according to the genetic determinants of their toxicity.

Characteristics of 9 morphotypes of contaminants in 117 food samples were studied with standardized classical methods by phenotypic properties. Samples of food for PCR were prepared by the priority method developed by us. PCR was carried out with specific primers to detect toxicity in various kinds of bacilli genes: *nhe*, *hbl*, *cyt K*.

Among the bacillary contaminants of the samples, the *subtilis-licheniformis* group is the most numerous one (20 to 37% of total bacilli count), *Bacillus megaterium* was detected in the amount of 6 to 21%, *B. pumili*s – 4 to 13%, *B. circulans* – 2 to 7%, gas-forming *Paenibacillus polymyxa* and *P. macerans* – the causative agents of bombarding spoilage – 3 to 14% and 2 to 9%, respectively, the microorganisms of the *Bacillus cereus* group (in particular *B. cereus* and *B. thuringiensis*) – 10-31% and 4-13%, respectively. Molecular genetic diagnosis showed the specificity of the contaminants in Ukraine: the presence of the *nhe* gene was detected in 100% of *B. cereus* strains, *hbl* in 60% and *cyt K* in 40% of the strains studied. It should be noted that the presence of the toxicity gene *cyt K* was established for a typical saprophyte – the strain *B. licheniformis*.

Studies of food raw materials and products have confirmed the need to improve microbiological control of product safety by introducing accelerated specific diagnostics of contaminants by molecular genetic methods. Such studies should be continued, which will allow diagnosing both traditional and emergent pathogenic contaminants.