The effects of high level noise and α–adrenoblocker on the oxidation intensity in white rats blood

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1. Introduction

The problem of acoustic stress under modern conditions of economically developed countries has become extremely topical, as more and more people live and work in high environmental noise: long term transportation noise from road, rail or air traffic, noise of equipment, facilities, widely used in the home and workplace, excessively loud music, a dramatic change in sound level of commercials that, eventually, depending on the duration of exposure, level and other characteristics of the noise not only leads to impairment of the auditory organ, but also to an increase in morbidity, development of a number of pathologies, including hypertension, atherosclerosis, ischemic heart disease, myocardial infarction, neurosis, ulcer, diabetes, sexual as well as reproductive function disorders, impairs cognitive functions [1-12].

Nowadays necessity of pathogenetic mechanism of disorder development study under the noise action has become obvious. Noise activates the pituitary-adrenal-cortical axis and the sympathetic-adrenal-medullar axis. Changes in stress hormones including epinephrine, norepinephrine and cortisol are frequently found in acute and chronic noise experiments. [13,14,15]. Results of numerous studies prove the leading role of long term increase of adrenaline on the activation of lipid peroxidation processes in the tissues.

On the basis of the existing data analysis we supposed that in the mechanisms of noise destructive action the leading role belongs to the damage of biomembranes, the main structural components of which are complex lipids and proteins.

Our recent investigations have revealed pronounced shifts in pro-antioxidant system in different tissues of the experimental animals under the noise action, the changes in the structure and functional activity of biological membranes, particularly mitochondrial and erythrocytes, the development of oxidative stress (OS) in which the steady-state balance is...
disturbed and prooxidant processes are dominated creating preconditions of organic lesions [17,18].

It was shown that noise action leads to α-tocopherol (α-T, vitamin E) content exhaustion in tissues due to lipid peroxidation (LPO) processes activation. The intensity and direction of the observed changes of LPO processes, α-T content, phospholipids and fatty acids compositions depend on the duration of noise action, as well as animal gender and noise level [17, 18]. The comparative study of the noise action of different level and duration on organism revealed the development of α-T deficiency in white rats tissues, more expressed in male- rats than in females, as well as prevalence of duration effects on the noise level, which was chosen based on the ISO R1999 recommendations. The results obtained in the textile mill female workers’ blood demonstrate pronounced changes in the lipid components and antioxidant status, similar to the observed ones in the experimental animals. The usage of α-T has had a considerable expressed regulatory and preventive effect both on the experimental animals and people under long term exposure to noise [19,20]. The recent studies show that oxygen metabolism products damage the protein structures as well [21-23].

Therefore the organism antioxidant status maintenance due to antioxidant intake can considerably promote its resistibility. Requirement of healthy man in antioxidants is variable and is in a great deal determined by the intensity of physical work, psychical processes tension degree. Living organisms’ requirement in antioxidants can considerably grow under conditions of many unfavourable physical and chemical factors [24-26].

All this testifies to the necessity of regulation processes, leading to the disorders in pro-antioxidant status of organisms, based on the mechanisms of noise action realization. In our opinion there are two main ways of organism protection from oldest damage the protein structures as well [21-23].

α-T content in the studied samples [32]. The oxidative level of blood serum proteins was investigated by the method based on interaction of 2,4-dinitrophenylhydrazine (DNPH) with the oxidized amino acid residues of proteins, which results in dinitrophenylhydrasone derivatives formation (the interaction products of protein oxidized aminoacid residuals with 2,4-dinitrophenylhydrasine). 1 ml of 10 mM 2.4-DNPH with 2 N HCl was added to 0.1 ml plasma (or serum). The samples were incubated within an hour, vortexing every 15 min. Protein. was precipitated with 1 ml of 20% trichloroacetic acid, followed by centrifugation at 3000 g for 20 minutes. The pellets were washed to remove excess DNHP and lipids with mixture of ethanol – ethyl acetate (1:1). After drying, 3 ml urea was added to samples and boiled in a water bath for 10 minutes. The absorbance of dinitrophenylhydrasone derivatives was registered at a wave length 370 nm by spectrophotometer “Specord-M40”. The content of carbonyl derivatives was calculated by using a molar extinction coefficient 22000M⁻¹ cm⁻¹ [33].

The content of thiobarbituric reactive substances (TBARS), presumably malondialdehyde (MDA), main product of lipid peroxidation processes (LPO) and LPO marker, were determined in plasma and EM. Malondialdehyde condenses with two equivalents of thiobarbituric acid to give a fluorescent red derivative which was assayed spectrophotometrically. Content of malondialdehyde (MDA) determined by the method described in [34]. 0.3 ml of fresh blood serum or plasma without hemolysis was taken for analysis. 1 ml 1% phosphoric acid (pH is important to check, the density of the solution corresponds to 1.004 g/ml), 0.6 ml 1% TBA (stored in a dark bank with moderate heating to dissolve). Test tubes were placed in a boiling water bath for 1 hour. Then, the tubes were cooled with cold water and after adding of 4 ml butanol, were carefully mixed and centrifuged 10 min at 3000 rev/min. Measure the absorbance of the upper phase conducted at a wavelength of 535 nm against butanol. Calculation of the products content that react with TBARS was performed taking into account the molar extinction coefficient of MDA 1.56·10⁵ mol · cm⁻¹. Protein was determined by Lowry [35]. Statistical analysis was made using Graph Pad InStat. Significance of means’ difference was evaluated using paired Student Newman – Keuls test (Anova).

II. Experimental procedure

Investigations were carried out on white male rats weighing 150-200 g kept in ordinary vivarium conditions. They were fed and allowed to drink water ad libitum. The animals were divided into 4 groups: rats of the 1st group serve as a control, animals of the 2nd and 4th groups underwent noise influence (91 dBA) with maximal energy in the region of average and high frequency during 8 hrs; rats of the 3rd and 4th groups were intraperitoneally injected aqueous solution of Mesedin in dose 2mg/kg, 10 hrs prior noise action.

The noise was obtained by white noise generator joint with attenuator. The acoustic system supplied the reproduction in the range of 63-16000Hz. Blood was taken by cardiopuncture, in heparinized syringes [30]. Fibrinogen was precipitated by sodium sulfate.

Erythrocyte membranes (EM) were isolated according to Dodge et al. [31]. Fluorimetric method was used to determine α–T content in the studied samples [32]. The oxidative level of blood serum proteins was investigated by the method based on interaction of 2,4-dinitrophenylhydrazine (DNPH) with the oxidized amino acid residues of proteins, which results in dinitrophenylhydrasone derivatives formation (the interaction products of protein oxidized aminoacid residuals with 2,4-dinitrophenylhydrasine). 1 ml of 10 mM 2.4-DNPH with 2 N HCl was added to 0.1 ml plasma (or serum). The samples were incubated within an hour, vortexing every 15 min. Protein. was precipitated with 1 ml of 20% trichloroacetic acid, followed by centrifugation at 3000 g for 20 minutes. The pellets were washed to remove excess DNHP and lipids with mixture of ethanol – ethyl acetate (1:1). After drying, 3 ml urea was added to samples and boiled in a water bath for 10 minutes. The absorbance of dinitrophenylhydrasone derivatives was registered at a wave length 370 nm by spectrophotometer “Specord-M40”. The content of carbonyl derivatives was calculated by using a molar extinction coefficient 22000M⁻¹ cm⁻¹ [33].

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The research has been approved by Institutional Committee on Bioethics and corresponds to the principles of the Manual of Operation and use of the laboratory animals published by US NIH (№ 85-23, reconsidered in 1985).

III. Results and discussion

The data obtained revealed an increase in concentration of protein oxidation carbonyl products both in plasma and EM proteins under the noise action correspondingly by 18% and 102% (Fig. 1, 2).

Our previous study has revealed a significant increase of white rats plasma proteins and fibrinogen carbonylation under the chronic noise action (40 days), accompanied with the lipid peroxidation intensification in plasma and clotting time increase [37]. Nevertheless in conditions of acute acoustic stress significant changes in carbonylation of fibrinogen were not recorded (Fig.1). Our data obtained have revealed that intensification of proteins carbonylation under acute acoustic stress mainly takes place in EM, increasing about two times as compared with the control group.

Moreover, the study has revealed MDA content increase in EM of the experimental rats, which was significantly higher (15.6 ± 3.8 nmol/mg protein), than that for control rats (7.5 ± 0.9 nmol/mg protein); the shift is more than 108% and significant (<0.001) (Fig.3). However MDA content in plasma decreases in approximately 30% (p<0.05) (Fig.4).

The results obtained prove the oxidizeability increase of EM proteins and lipids under condition of acute acoustic stress and can be directly connected with the effects of stress hormones concentration changes in blood, which give rise to structural transitions in EM proteins, glycoproteins, phospholipids and glycolipids, and as expected to change also the mass transfer rate through the membranes, in particular, the diffusion rate of oxygen, carbon dioxide, H2O, hydrogen ions, etc. ESR spectroscopy studies revealed the presence of adrenaline receptors on the surface of erythrocytes and demonstrated the ability of adrenaline to increase the orderliness of phospholipids. Meanwhile, preincubation of erythrocytes with cytochalasin B inhibits the response to adrenaline. Changes were also revealed in the secondary structure of erythrocyte ghosts proteins under the action of adrenaline and carbachol (an analogue of acetylcholine) [38,39]. Such changes in the secondary structure of membrane proteins can be related with contraction proteins. Cortisol, similar to adrenaline, binds to erythrocyte membranes. The effects produced by the hormones in erythrocyte ghosts are shown to
persist on intact erythrocytes. Thus, stress hormones alter the oxygen transport properties of erythrocytes: hemoglobin and heme indicates a growing affinity for oxygen. These data reflect the deformation of erythrocytes under hormone action [40,41]. It means that these EM structural changes can serve as a background of proteins and phospholipids increase availability for direct action of oxygen active form leading to the oxidation of membrane lipids and proteins.

The results of our recent study of EM ghosts physical properties, molecular reconstruction in the structure of erythrocyte membrane under the noise action are confirmed by the above mentioned data and indicate that the influence of high level noise causes the changes in the lipid-protein interaction which can be the result of molecular reconstruction in the EM as a result of hormone response, oxidative stress development and direct action of acoustic waves. It was shown that high level noise influence leads to the decrease of the rate constant of ANS binding with ghosts, accompanied with the decrease in the binding centers number, fluidity and polarity changes. The more pronounced changes of oxidation processes in EM compared with plasma also prove that the main mechanism of intense oxidation in EM is connected with structural disorders of membrane proteins and phospholipids due to hormones interaction with membranes. Moreover, adsorption of hormones on hematocytes can proceed either specifically or nonspecifically.

The LPO processes have an important physiological value for the vital functions of cells, they take place in all the tissues with low intensity and are regulated by antioxidant system. The activation of oxidation processes leads to violations of the lipid and protein components of membranes and membrane structure, particularly to intensification of unsaturated fatty acids peroxidation, which leads to the qualitative and quantitative changes in the phospholipids spectra, conformational changes of membrane proteins, chemical modification of nucleic acids, membrane proteins, including lipid-dependent enzymes activity, the transmembrane transport of metabolites, the external signal transfer, ligand-receptor interactions, and the normal functioning of cells in general on the background of antioxidative activity disorders [19,22].

At the same time the results of the main endogenous antioxidant α-T (vitamin E) content study in the blood of animals subjected to noise action reveal a decrease both in plasma (23.6%) and EM (44.2%) (Fig. 5, 6).

α-T is recognized as the most effective endogenous biological antioxidant and adequate level of the α-T is necessary for normal functioning practically for all the types of the body cells. Nowadays α-T deficiency is considered as one of the main causes of the numerous diseases development [22].
The decrease of antioxidant defense leads to intensification of free radicals and oxygen active form formation, which attacks structural components of the tissues [19]. Considering the results of oxidation processes and α-T content under acute stress conditions could be concluded that there is a reverse direction of the shift in both proteins oxidative modification, lipid peroxidation intensity and α-T content, development of pro-antioxidant state disorder in blood. In our opinion, stress hormones effects on structural changes of EM leading to the numerous structural and functional disorders serve as a triggering factor of these processes.

As a protective measure we used α₂-adrenoblocker Mesedin, to elucidate its possible preventive effect under acute acoustic stress conditions. According to the data, peripheral post-synaptic α₂-adrenoceptors promote the formation of adaptive reactions of organisms under acute hypoxia conditions. Mesedin particularly was recorded and proved to be an expressed anti hypoxic effect-possessing compound on different experimental models of hypoxia and in a greater extent and in a wider dosage range compared with known α₂-adrenoblockers-idaoxzan and beditin [27].

Intraperitoneal administration of Mesedin to the intact animals (2nd group) only slightly decreases the content of modified proteins in plasma, at the same time unverified increase of them in EM was observed. The administration of Mesedin to the animals 10 hrs prior the noise action (4th group) leads to the sharp decrease of carbonyl compounds formation (76.5 and 47% correspondingly) in the plasma and EM (Fig.1,2).

The intensity of LPO processes is also decreased in EM of 4th group, compared with the results of animals of the 2nd group (25%), nevertheless the value is significantly higher than the control one. It is worth to note, that changes in the content of TBARS products in plasma in the 2nd group animals were less than in the control group and could be explained by the existence and functioning of the plasma total antioxidant activity high efficiency under conditions of acute stresses in general, and acute acoustic stress in particular (Fig. 3,4) [19, 24].

Nonsignificant changes were registered in the α-T content in plasma of 2nd and 3rd groups of animals, nevertheless in 4th group of rats, subjected to noise action after Mesedin administration a significant decrease in 42% (P<0.001) (0.37±0.02) was registered compared with the control value (0.635±0.24) (Fig.5). The reverse direction of changes was observed in EM: decrease of α-T content in 2nd and 3rd groups of animals (44.2% and 39% respectively) with nonsignificant changes in EM of 4th group, which is the evidence of Mesedin high protective effect on the membranes. The existing approach prove that plasma supplied membranes with α-T in great extent under stress conditions.

It can be considered that Mesedin blocked a peripheral postsynaptic α₂-adrenoceptors in experimental animal blood preventing hormone interaction with the membranes and their effects transmission. The results obtained have proved our expectations according to the development of protein modification, their carbonylation both in plasma and EM due to oxidation intensification under acute acoustic stress conditions. Administration of α₂-adrenoblocker Mesedin to the animals prior to noise action prevents protein oxidation of blood components and reveals noticeable regulatory effect under the acute acoustic stress condition, which is more expressed in EM.

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