ACOUSTICS 2012

Metabolic changes in the tissues of experimental animals in the conditions of high level noise action

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The goal of this paper is to review results of experimental investigations, carried out in the Yerevan state Medical University ndconcerning particularly metabolic changes of lipids of cells biomembranes, the anti- and prooxidants balance in tissues under acoustic stress conditions. The experimental animals (white mongrel rats) underwent 91 dBA noise level influence with maximum energy in the range of medium and high frequency. Intensity of lipid peroxidation and protein oxidation, the changes in the content of alfa-tocopherol, activity of antioxidant enzymes in various tissues has been studied. Simultaneously, structural components of biomembranes, structural and immunological changes in immunogenetic organs were studied as well. Shifts in the thymus, spleen and lymphatic nodes after the 6 hours influence of noise were revealed, in our opinion, are transient, since the whole syndrome corresponds to a relatively late stage of the stress syndrome tension phase. The data obtained revealed significant changes in the intensity of lipid and protein oxidation, alfa-tocopherol content in studied tissues, in the content of phospholipid glycerides in the brain mitochondrial and erythrocyte membranes, depending on both the sex of animals and the duration of noise action.

1 Introduction

Among hazardous factors of the environment extremely dangerous is the high level of industrial, transport and community noise which drastically decreased the resistibility of an organism to different diseases development [4, 30, 33]. As pointed out by Dr Bern O. Knudsen, cofounder of USA Acoustical Society (ASA), the noise, like smog, serves as a tool of slow death. Nowadays it is reviewed as a form of pollution of the environment. In this connection it is difficult to overestimate the degree of the disastrous influence of noise on the living organisms. The history of the development of mankind has been characterized by adaptation to various influences of the environment among which the noise factor hadn't been of paramount importance. However, in the modern environment in economically developed countries, noise has become one of the permanent components of our surroundings, which is not prevented even by sleep [16]. Results of numerous epidemiological, laboratory, occupational and environmental studies are evidence of high rate of blood pressure, hypertension and ischemic heart disease, including myocardial infarction, neurosis, different level loss of hearing, ulcer, diabetes, sexual as well as reproductive function disorders in workers of the enterprises with high level of industrial noise and community noise and are summarised in monographs [2, 30] and scientific papers [5, 7, 14, 17, 21,33, 35].

Epidemiological research provides the possibility of an integral risk estimation of community noise based directly on empirical data gained under genuine conditions of exposure, taking into account any factors which may amplify or attenuate the noise effects.

Nowadays necessity of study of disorders pathogenetic mechanisms development under the noise action becomes obvious.

Considering all the above mentioned it is necessary to study effects related only to the noise influence in experiments, eliminating possibility of side influences.

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, main structural components of which are complex lipids and proteins.

However, scientific literature lacks certain facts referring to the metabolism of the membranes in general and lipid components, in particular, under conditions of high level noise action. The data existed, concerning the changes of separate representatives of lipids in the plasma and blood serum are uncoordinated and are not united by one conceptual approach.

Now it is well-known that under the influence of different agents, stress reaction is developed, simultaneously the intensity of other nonspecific process, namely free radical oxidation activation is changed, leading to the activation of lipid peroxidation processes. As result components of tissues, particularly lipids and proteins undergo oxidation. A great amount of facts concerning the influence of oxygen active forms on the functional state of cells and their role in the pathogenesis of many diseases has been accumulated. The recent studies show that oxygen metabolism products damage the protein strictures first of all.

Taking into consideration the important role of the processes of free radical oxidation and lipid peroxidation (LPO) in the pathogenesis of the diseases listed above [22], we have studied the LPO intensity and the state of the antioxidant activity in tissues to elucidate possible biochemical mechanisms of hazardous effects of noise. The main aim of this investigation is to show the role of changes in lipid composition of membranes, particularly phospholipids-glycerydes (PL-G), fatty acids composition of lipids, α-tocopherol (α-T) content due to LPO intensity in common mechanisms of destructive action of noise in living organisms as well as to suggest possible preventive ways.

The goal of this paper is to review some results of investigations performed in our University.

2 Experimental procedure

Investigations were carried out on white rats of both sexes weighing 150-200 g, conventionally fed in a general feeding in vivarium. The animals were divided into 6 groups: rats of the 1-th group serve as a control, rats of the 2, 3, 4, 5, 6-th groups underwent noise influence (91 dBA) with maximal energy in the region of average and high frequency. The noise was obtained by white noise generator joint with attenuator. The acoustic system supplied the reproduction in the range of 63-16000Hz.

The periods of noise influence were consequently 2hr, 8 hr, and 7, 28 and 56 days, each day 8 hr noise exposition. The animals were decapitated under slight ether anesthesia. Erythrocyte membranes (EM) were isolated by Limber

[19]. The content of α-T was determined fluorimetrically by Duggan [12]. The activity of ascorbate-dependent LPO (ADLP-nonenzymathic) and NADPH-dependent LPO (NDLP- enzymathic) systems in membranes was determined by malonyl dialdehyde (MDA) accumulation [34]. The content of MDA in plasma was determined by Yoshioki [36]. The oxidative level of blood serum proteins was investigated by the method based on interaction of 2,4-dinitrofenylhydrazine (DNFH) with the oxidized amino acid residues of proteins [11]. Activity of superoxide dismutase (SOD, µmoles GSH/ml), was determined by Nishikimi [31], glutathione peroxidase activity (GSHPx, unit/mg protein/min) by Pinto [32]. Determination of PL-G was made in a total lipid extract [13.] with the further fractionation by monomer ascending chromatography in a thin-layer silica gel ("Merk", Germany) using standards ("Sigma", USA). PI were obtained by the selective acidic extraction by Bergelson [6]. Protein was determined by Lowry [20].

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).

The results were treated statistically using standard programs.

3 Results and discussion

Taking into account that the character of changes in both, the male and female animals was similar, but the intensity of changes of some studied parameters was more expressed in male rats. In the report mainly are represented data concerning male rats [24, 25, 26]. Erythrocytes are considered as an unique model for assessing the condition of the body, assuming that the level of violations of the EM metabolism largely reflects the depth of the pathological process in general, which is proved by our data also, so this report presumably represented results concerning erythrocytes.

The level of diene conjugates (one of the primary products of LPO) and MDA content (Figure 1,2) in plasma was sharply increased by single action (acute acoustic stress) in 2nd and 3rd groups, but after 8hr-daily action during 7 days practically no verified changes were observed. But the further everyday action led to the increase of the level of these parameters, which is an evidence of LPO processes activation. The observed activation of the processes of LPO is explained by the realization of the stress syndrome with the release of stress hormones, particularly cathecholamines, under conditions of acoustic stress, as it was shown in various studies and is connected with the realization of their lypotropic effect and activation of lipid oxidation [3, 8, 10, 22, 23]. The integral index of the ability of ME to be oxidized, activation of processes in both the inducible enzymathic and non enzymathic LPO processes, was recorded almost in all experimental groups (Figure 3).

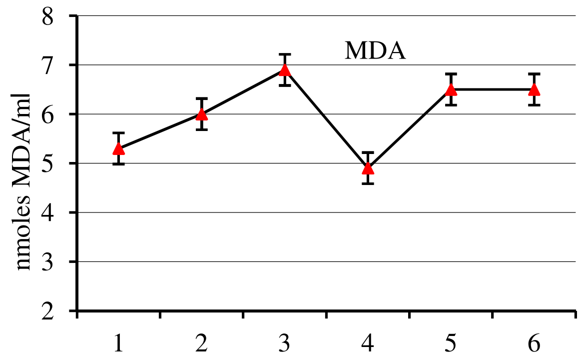

Figure 1: The MDA level in the plasma of white male rats under the noise (91 dBA) action.

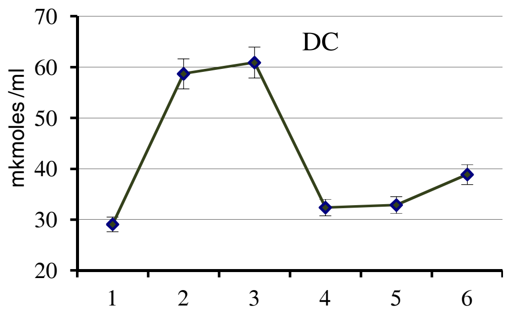

Figure 2: The diene conjugates (DC) level in the plasma of white male rats under the noise (91 dBA) action.

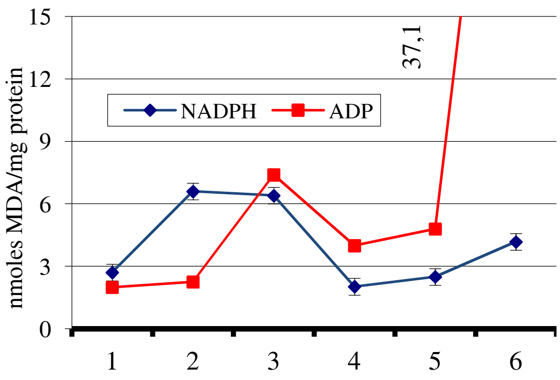

Figure 3:The intensity of Ascorbate- and NADPH-dependent LPO processes in the EM of white male rats under the noise (91 dBA) action.

Table 1. The content of keton dinitrophenyl hydrazones in blood serum under the noise (91 dBA) action (p = * < 0.001)

	356 nm	370 nm	430 nm
Control	29,4 ± 0,9	34,6 ± 0,9	2,2 ± 0,3
Noise, 2hr	63,2 ± 5,1 p<0,001	71,5 ± 5,1 p<0,001	43,1 ± 3,4 p<0,001

The more expressed activation, about 20 times, observed in enzymathic LPO processes after 56 days noise influence.

It was shown also a significant increase of the content of the products of the plasma protein oxidation in acute acoustic stress condition: increase of neutral aliphatic keton dinitrophenyl hydrazones level approximately 2 times, basic – almost 20 times, in comparison with intact group [Table 1]. It is of great importance that the observed sexual differences in the intensity of the process of LPO especially in the myocardial tissue and EM, suggest a more expressed "protection" in females, probably connected with a higher potential of antioxidative activity of tissues. Nowadays it is well known that some estrogen hormones are of a high antioxidative activity which is even higher than the activity of α-T [1,18] The intensity of LPO processes is strongly limited by the existence in tissues of multicomponent antioxidant system comprising a number of enzymes and endogenous antioxidants among which the main place is given to α-T [9]. On each stage of oxygen active form formation the antiradical enzymes (SOD, GSH_Px, catalase) or antioxidants (α-T, vitamin C etc) "break" the free radical formation chains. So, each step of mentioned processes is under the control of antioxidant system.

The intensity of formation of oxygen active forms, their concentration in tissues depend on the antiradical defense enzyme activity, antioxidant content as well as structural components properties.

The main antioxidant of living cells is the lipid soluble compound, well-known as vitamin E, α-T. The study of the α-T level, the main lipid soluble antioxidant and active, obligatory structural component of biomembranes, showed that the changes in blood under acute acoustic stress conditions were negligible, but after 7 everyday action a sharp decrease was recorded in EM in all groups. In plasma, simultaneously, a slight increase (5^{th} group) and, later, after 56 days a verified decrease of α-T content is observed.

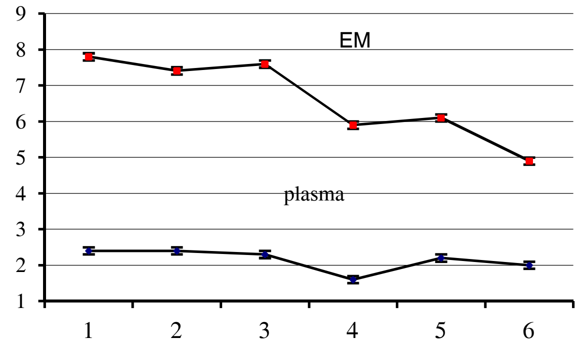

Figure 4: The α– tocopherol content in the plasma (μmoles / dl) and erythrocyte membranes (nmoles / mg protein) of white male rats under the noise (91 dBA) action.

So, we can say that changes have a phase character. The reverse dependence was observed between α-T level and the above mentioned LPO process intensity. In plasma the content of α–T has a tendency to restore to the control level, nevertheless, the verified decrease of level is observed by the end of the experiment. The decrease of α–T content in EM was more expressed. The extension of duration of experiments up to 8 weeks was accompanied with a greater decrease of α-T level in EM, promoting the activation of LPO. Changes of the α-T level in the heart tissue are evidence of higher protection by α-T during first week in male rats and four weeks in female rats (Table 2).

Table 2: The α–tocopherol content in the heart (nmoles/g tissue) of white male and female rats under the noise (91 dBA) action (p = * < 0.001, ** < 0.05).

sex	1	2	3	4	5	6
male	37,1± 0,6	40,0** ± 1,0	40,3** ± 1,2	41,2*± 1,4	30,6** ± 1,9	34,8 ±2,8
female	27,4± 0,7	34,8** ± 1,4	24,4± 1,2	26,0 ±1,4	30,4± 1,8	17,5** ± 1,3

The various dynamics of LPO and α–T content during the experiment predetermined the necessity of study the antiradical protection enzymes activity of tissues under the same conditions.

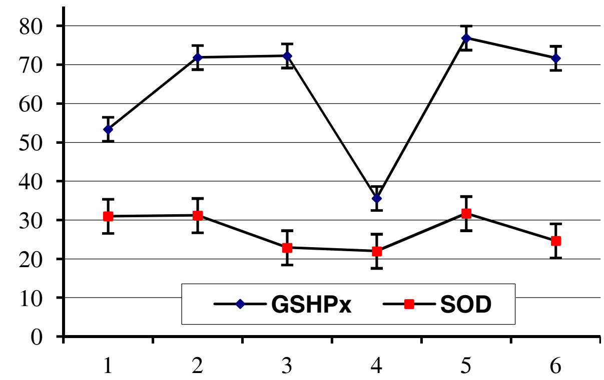

Figure 5: The Superoxide dismutase and glutathione peroxidase activity of the white male rats blood under the noise (91 dBA) action.

The changes in activity of SOD and GSH-Px had opposite directions. GSH-Px activity was higher than the control level in all groups, except seven days of noise influence. The activity of SOD was depressed almost in all experimental groups. Exceptions were after 1hr and 28x8hrs action - the terms which can be characterized as periods of adaptation. However, by the end of the experiments (56 day), the activity of SOD was inhibited. Simultaneously, the GSH-Px activity was higher than the initial level. It must be mentioned that GSH-Px was quite stable, least inclined to oxidation.

Thus, the obtained results predetermined the study of the state of substrates of peroxidation, particularly phospholipids (PL) of membranes. The composition of individual fractions of PL in normally functioning biological systems characterized by phylogenetic programmed consistency and makes the close relationship of structure and functions of cellular and subcellular structures, in particular the surface of cell membranes. In this regard, the main role of membrane PL to maintain the required level of viscosity of biological membranes, due to a complex relationship PL / PL, the formation of various complexes of lipids with unique properties necessary for the normal functioning of the proteins, which they surround, providing the necessary hydrophobicity of the biological system which is a necessary element in the regulation of functional activity of proteins. Much of this applies to both membrane-bound lipid depending enzymes catalyzing the transmembrane transport of metabolites, the external signal transduction and the maintenance of physiological levels of ligand-receptor interactions and the normal functioning of cells in general.

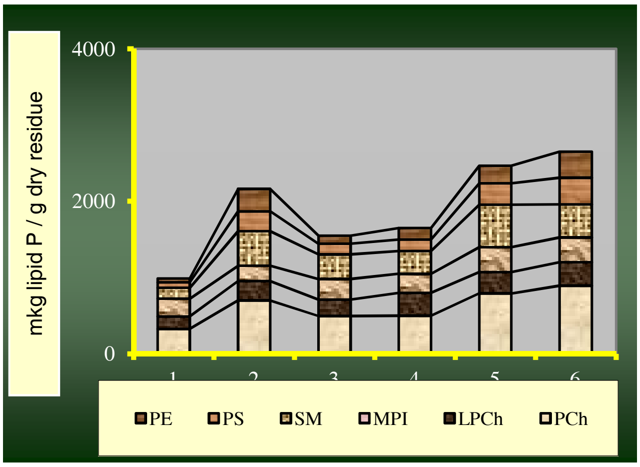

Figure 6: The content of individual phospholipids (PL-G) (PE-Phosphatidyl ethanolamine, PS- Phosphatidyl serine, SM-sphingomyelins, MPI-monophosphoinositides, LPCh-lysophosphatidyl choline, PCh- Phosphatidyl choline) of the EM of white rats under the condition of noise (91 dBA) action.

In turn, the functional activity of the PL, as regulators of the microenvironment of proteins, receptor proteins and many membrane-bound lipid depending enzymes depends on the qualitative composition of fatty acids (FA), the spectrum and the percentages of saturated and unsaturated representatives. Free radical attack of reactive oxygen species leads to an intensification of peroxidation of unsaturated FA, which leads to a change in the level and the PL spectra, conformational changes of membrane proteins, leading to chemical modification of membrane proteins, including enzymes and receptors [33]. The results of our observations testify to expressed increase of SUM PL in animals of both sexes throughout the experiment and the content of lysophosphatydilcholines possessing membranolythic and membranotoxic action. The growth of sphingomyeline and phosphatydilcholine content, referring to hard-oxidizing PHL, testify to the realization of adaptation mechanism, directed to the decrease of membrane oxidation ability. The similar character of changes is observed in the fatty acids composition of EM lipids. These changes growing during the experiment were characterized by increase of content of saturated fatty acids and decrease of content of polyunsaturated fatty acids, particularly arachidonic acid [28]. Taking into account the importance of inositol phospholipids in metabolic pathways, under the conditions of acute acoustic stress their content was studied in brain mitochondrial fraction and decrease in the content of all the fractions under acute acoustic stress conditions was revealed [29]. To estimate physical and chemical properties of biomembranes we studied the binding parameters of 1- anilinonaphthalin-8-sulphonate (ANS) with ghosts of erythrocytes *in vitro* and *in vivo* experiments for the observation of the changes of lipid-protein intermolecular interactions. The results proved that the influence of high level noise causes changes in the lipid-protein interaction due to molecular reorganization of EM as a result of oxidative stress development. The study of organs of immunogenesis revealed structural and immunological shifts in the thymus, spleen and lymphatic nodes under acute acoustic stress conditions, which, in our opinion, are transient, since the whole syndrome corresponds to a relatively late stage of the stress syndrome tension phase [37].

4 Conclusion

The action of noise leads to the structural reorganization and functional change of biomembranes in experimental animals due to LPO process activation and α-T exhaustion in tissues. The intensity and direction of the observed changes of LPO processes, α-T content, PL and FA compositions depend on the duration of noise action.

To protect organisms from negative effects of noise it is necessary to increase antioxidative activity of tissues by usage of antioxidants, particularly, α -T.

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