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# PHYSICAL PARAMETERS OF WHITE RAT ERYTHROCYTE MEMBRANES UNDER CONDITIONS OF ACOUSTIC STRESS

Melkonyan M.M.[1], Sahakyan G.V.[2], Pogosyan G.A.[2], Hunanyan L.S.[1]

[1] Department of Medical Chemistry, Yerevan State Medical University, Yerevan, Armenia
[2] Scientific Research Center, Yerevan State Medical University, Yerevan, Armenia

## ABSTRACT

*The influence of acoustic stress (91 dB, noise action) on the binding parameters of 1- anilinonaphthalin-8-sulphonate (ANS) with ghosts of erythrocytes in vitro and in vivo experiments is investigated for the observation of the changes of lipid-protein intermolecular interactions. Experiments were carried out on white outbreed male rats. Blood was taken by cardiopuncture. During the in vivo experiments the rats preliminary were exposed to 1 hour noise action. For in vitro experiment the erythrocyte ghosts obtained from the blood of intact animals were divided into three groups. One group was the control one. The second one is underwent to noise action during the ANS 20-minute incubation (iv vitro I) and the third group was incubated with ANS during 20 minutes after the 20 minute exposure to noise action of the same parameters (in vitro II).*

*It was shown that high level noise influence leads to the decrease of the speed constant of ANS (Kc) binding with ghosts, parallel to the decrease in the binding center number (N). Thus, under the exposure of the studied factor in vivo experiments Kc and N reduced by 25% and 72.7% correspondingly, in vitro I experiments the same parameters decreased by 50% and 98.75% and in vitro II exposure – by 75% and 98.72%, correspondingly.*

*These results indicate that the influence of high-level noise causes changes in the lipid-protein interaction, which can be the result of molecular reconstruction of erythrocyte membranes as a result of oxidative stress development.*

***Keywords:*** . acoustic stress, erythrocyte membrane, ANS.

## INTRODUCTION

The problem of the acoustic stress in modern conditions of economically developed countries has become extremely topical, as more and more people undergo high level noise environment: the noise of vehicles, equipment, facilities, widely used in 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 the auditory organ impairment in various degree, but also to an increase in morbidity, development of a number of pathologies, including hypertension and atherosclerosis. Previous studies have revealed significant shifts in the balance of pro-antioxidant system in various tissues in experimental animals exposed to noise, the development of oxidative stress (OS) [*Melkonyan M., 1988; Hunanyan L., et. al., 2010*], in which the steady-state balance is disturbed and prooxidant processes dominate creating preconditions of organic lesions.

The activation of lipid peroxidation processes (POL) leads to violations of the lipid component of membranes and membrane structure. According to modern concepts, the OS is nonspecific, but obligatory component in the pathogenesis of many pathological conditions. Organisms are constantly exposed to various reactive oxygen species, leading to damage of proteins, nucleic acids, lipids, resulting in biological function loss.

As one of the main targets of the impact of reactive oxygen species is considered to be unsaturated fatty acids, which are structural components of lipid membranes, their effects leads to qualitative and quantitative changes, in particular, the PL membranes, change in their functional activity. The composition of individual fractions of PL in normally functioning biological systems is char-

*Address for Correspondence:*
Yerevan State Medical University
2 Koryun Street, 0025, Yerevan, Armenia,
Tel.: (374 10) 580343
E-mail: chemistry@ysmu.am

acterized by phylogenetically programmed consistency and makes the close relationship of structure and functions of cellular and subcellular structures, in particular the surface of cell membranes [*Hanahan D.,et.al., 1997*]. In this regard, the main role of membrane PL to maintain the required level of biological membrane viscosity, due to a complex relationship PL / PL, the formation of various lipid complexes with unique properties necessary for normal functioning of the proteins, which they surround, providing 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 normal functioning of cells in general [Di Paolo, et al., 2006].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 POL 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 [*Vladimirov Yu, et al, 1972*].

It is well known that violations of the morpho-functional status of circulating red blood cells in different physiological states of organisms, including stress conditions, are largely determined by the physico-chemical properties of membranes, in particular, the state of the membrane components - lipids and proteins. The physico-chemical properties of lipid bilayer and especially the protein-lipid interactions of erythrocyte membranes can be estimated using the fluorescent probes. Binding parameters of the membrane fluorescent probe 1-8 anilinonaftalin-sulfonates (ANS) with the erythrocyte membrane serves as indicator for the molecular rearrangements in the structure of the membrane, because ANS has a unit negative charge and binds to the protein-lipid contact location in membrane surface layer. The red blood cells providing basic life functions – breathing in the body are the most numerous cellular elements of blood. Erythrocytes are considered as a unique model for assessing the condition of the body, assuming that the level of violations of the erythrocyte structure and metabolism largely reflects the depth of the pathological process in general [*B.V. Novitsky et al, 1999*].

Taking into account the above mentioned and regarding the erythrocyte ghosts as a suitable model for membrane study we have investigated acute acoustic stress influence on physical parameters of erythrocyte ghost membranes.

The goal of the present study is to investigate the effect of acoustic stress on 1-anilino-8-naphthalene sulfonate (ANS) binding parameters with the membrane which allows us to make a conclusion about lipid-protein intermolecular interactions in the erythrocyte superficial layer in both *in vitro* and *in vivo* experiments.

## MATERIALS AND METHODS

Experiments were carried out on white outbreed male rats (weight 150-220g) in usual conditions of vivarium, with standard nutrition. Experimental animals were divided into 2 groups 21 rats in each. The animals of the 1$^{st}$ group served as control ones (intact group). The animals of the 2$^{nd}$ group underwent 91 dB (high and medium frequency) noise actions during 1 hour. Noise was created by the generator of white noise. Blood was taken by cardiopuncture [*Weis H., 1971*]. Erythrocytes were obtained, membranes isolated and erythrocyte ghosts collected according to Dodge et al. method [*Dodge J. et al., 1980*] with a slight modification: erythrocytes were washed with physiological solution to increase the membrane yield.

Erythrocyte ghost study was carried out by fluorescent *probe* method by means of ANS, which had a single negative charge and was located in the most functionally active superficial layer of the membranes. [*Vladimirov U., Dobretsov G., 1980*]. Probe molecules were irritated in $\lambda$=360nm, fluorescence was registered in $\lambda$=450 nm. Spectrofluorometer Hitachi MPF-4 (Japan) was used in the study.

Fluorescence was measured in constant concentration of membrane protein (0.3 mg/ml, ANS titration – 5-100$\times10^{-3}$M) and ANS constant concentration (5$\times10^{-3}$M) by membrane different concentration (0,1-0,6 mg/ml). The data obtained were expressed in reversed coordinates and the diagrams were built according to Klotz [*Vladimirov Yu. A., Dobrecov G.E., 1980*]. Reaction speed constant (Kc) and the number of *ANS* binding centers (N) were calculated by Sckatchard formula [*Vladimirov Yu. A., Dobrecov G.E., 1980*]. Protein concentration was determined by Lowry [*Lowry O. et al., 1951*]. Erythrocyte ghosts isolated from three

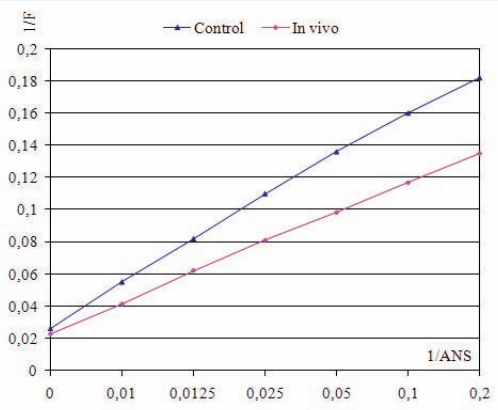


**FIGURE 1.** *Fluorometric curves of erythrocyte ghost titration by ANS probe in reversed coordinates after 1 hour exposure of 91 dB noise on rats ( in vivo).*
*F – fluorescence intensity*
*ANS – probe concentration in ×$10^{-3}$ M,*

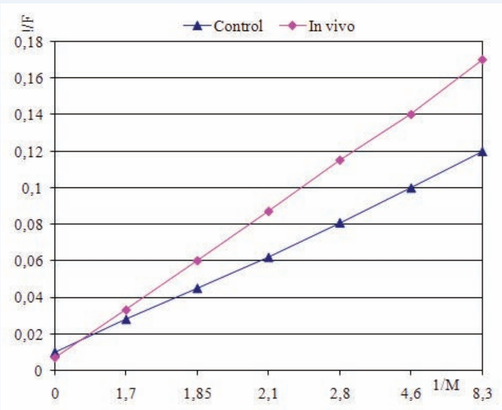


**FIGURE 2.** *Fluorometric curves of ANS titration with erythrocyte ghosts in reversed coordinates after 1 hour exposure of 91 dB noise on rats ( in vivo).*
*F – fluorescence intensity*
*M –protein concentration in erythrocyte ghosts, mg/ml.*

animals were used for each measurement point. Each measurement point was taken as an average of 7 measurements. The data were statistically processed by Student's t-test with the help of statistical program Sigma Plot.

During the *in vivo* experiments erythrocyte ghosts of the rats preliminary were exposed to 1 hour acoustic stress. For *in vitro* experiment the erythrocyte ghosts obtained from the blood of intact animals were divided into three groups. One group was the control one. The second one is undergone to acoustic stress during the ANS 20-minute incubation *(iv vitro I)* and the third group was incubated with ANS during 20 minutes after the 20 minute exposure to acoustic stress of the same parameters *(in vitro II).*

## RESULTS AND DISCUSSION

The figures show the fluorometric curves of erythrocyte ghost titration by ANS probe (Fig.1, 2) and ANS titration with erythrocyte ghosts (Fig. 3, 4) on the reversed coordinates after the acoustic stress.

Binding parameters of fluorescent probe 1-anilinonaphthalin-8-sulphonate (ANS) with erythrocyte membrane is the indicator for molecular reconstruction evaluation in the membrane structure. [*Vladimirov Yu., Dobretsov G., 1980; Horie T. et al., 1982*]. Taking into account that the ANS is bound in the membrane superficial layer in the protein-lipid contact sites, we could suppose that noted changes are the consequence of the relative location of these molecules.

As it could be concluded from Table 1 noise exposure results in reduction of both binding speed constant (Kc) and ANS binding centre number (N) with

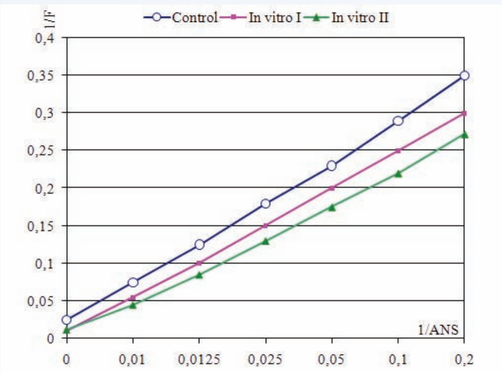


**FIGURE 3.** *Fluorometric curves of erythrocyte ghost titration by ANS probe in reversed coordinates after the 20 minute in vitro exposure of 91 dB noise*
*F – fluorescence intensity*
*ANS – probe concentration in ×$10^{-3}$ M,*

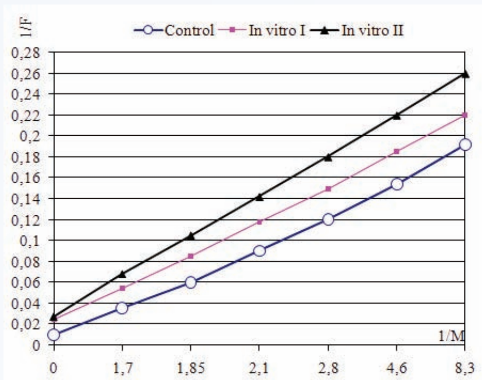


**FIGURE 4.** *Fluorometric curves of ANS titration with erythrocyte ghosts in reversed coordinates after the 20 minute in vitro exposure of 91 dB noise*
*F – fluorescence intensity*
*M –protein concentration in erythrocyte ghosts, mg/ml*

erythrocyte ghosts which is more markedly manifested *in vitro* exposure. Thus, if under the exposure of the studied factor in *vivo* experiments Kc and N reduced by 25% and 72.7% correspondingly, *in vitro* I experiments the same parameters decreased by 50% and 98.75% and in vitro II exposure – by 75% and 98.72%, correspondingly. Maximum effect was observed in noise application during incubation, which proves that noise directly influences ANS binding process with erythrocyte ghosts. It could be explained by both ANS binding mechanisms and protein and lipid component changes of erythrocyte ghosts, which most probably could result in protein-lipid interrelation change in the erythrocyte membrane superficial layer. This suggestion is based on the fact that in vitro II experiments similar changes have been observed. But it should be noted that Kc reduction is more obviously expressed in this case. All the above mentioned allows us to suggest that the observed changes are mainly the consequence of protein-lipid link changes in the erythrocyte membrane superficial layer.

Similar but less expressed changes of the same parameters in vivo experiments could probably be explained by membrane-stabilizing metabolic processes. The changes in protein-lipid interaction in erythrocyte membrane superficial layer observed under noise exposure are probably the consequence of lipid exchange deviation, their peroxidation as well as noise mechanical effect on membrane protein conformation. Though we don't exclude that in insignificant changes of components the change of conformation of protein-lipid binding sites could take place, which is proved by marked changes of ANS binding sites.

Summarizing all the above mentioned we can conclude that noise exposure of the studied parameters results in molecular reconstruction in the structure of erythrocyte membrane superficial layer which is expressed as lipid-protein intermolecular interaction change.

**TABLE 1.**

Binding speed constant (Kc) and the number of ANS binding centres (N) with erythrocyte ghosts after the 1 hour in vivo and 20 min in vitro 91dB acoustic stress

| Parameter | Control | In vivo | In vitro I | In vitro II |
|---|---|---|---|---|
| Kc × $10^{-4}$ $M^{-1}$ | 3,2 ± 0,82<br>n= 21 | 2,4 ±0,59*<br>n= 21 | 1,6 ±0,17*<br>n= 21 | 0,08 ±0,11*<br>n= 21 |
| N× $10^{-9}$ M/mg of protein | 84,06 ±7,37<br>n= 21 | 22,91 ±3,24*<br>n= 21 | 1,05 ±0,21*<br>n= 21 | 1,72 ±0,13*<br>n= 21 |

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