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THE EFFECTS OF SELECTIVE ALPHA-ADRENOBLOCKER BEDITIN ON THE INTENSITY OF LIPID PEROXIDATION AND MEMBRANE PHOSPHOINOSITIDES CONTENT IN ACOUSTIC STRESS CONDITIONS

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Abstract

Our recent investigations have revealed the significant role of alteration in anti- and prooxidant balance, changes in the spectrum and level of membrane phospholipids in tissues in acoustic stress conditions (noise action) with oxidative stress development and efficiency of applying antioxidants of different origin and nature as a preventive measure.

Nowadays, the close attention is given to the phosphoinositides (PIs), which support both a structural and signaling role. Therefore, it is of great importance to learn more about the changes in the content of membrane PI in conditions of acoustic stress, as well as dependence on LPO intensity.

The aim of the current study was to investigate the effects of newly synthesized α_2-adrenoblocker Beditin on the intensity of lipid peroxidation processes in brain mitochondria and blood of white rats , the content of representatives of PI in brain mitochondrial membranes under the acoustic stress conditions and preliminarily injection of Beditin.

Investigations were performed in white inbred male rats weighing 150-200 *g* and kept on a general feeding ration in vivarium. The animals underwent noise influence during 2 hours (*hrs*) at 91 *dBA* with energy maximum in the region of average and high frequency. Beditin was administered at dose levels of 2.0 *mg/kg* and 20 *mg/kg*. The intensity of lipid peroxidation in white rats brain mitochondria (BMs) and erythrocyte membranes (EMs), concentration of α-tocopherol (α-T), total peroxidizing activity (TPA) and the level of malone dialdehyde (MDA) in blood plasma, as well as the fraction PI in BM were studied.

The intensification of lipid peroxidation processes in both BM and blood (EM and plasma) and a decrease in the content of all the fractions of PI of BM under the acoustic stress conditions were registered. Beditin application at the initial dose of 20 *mg/kg* led to the pronounced changes, which testified to infringements in anti-prooxidant status of studied tissues, structures of biomembranes. Beditin in dose 2.0 mg/kg had no significant influence on studied parameters by itself, being administered to intact animals, nevertheless in mentioned dosage it prevents developing of changes in lipid peroxidation intensity, the α-T content and revealed some regulatory effect on the BM PI content, especially in case of PI(3,4,5)P_3 under conditions of acute acoustic stress.

Keywords: acoustic stress, lipid peroxidation, phosphoinositides, α_2-adrenoblocker Beditin

INTRODUCTION

The problem of acoustic stress is very actual in modern life due to drastic increase of noise level in the environment because of transport, technical equipment, household devices noise factor at working places, etc. The noise produced is affecting people at various levels, causing high level of morbidity, especially in hypertonic disease and atherosclerosis. Our recent investigations have revealed the significant role of alteration in anti- and prooxidant balance in tissues under acoustic stress conditions [*Melkonyan M., 1988; Hunanyan L., et al., 2010 a;b*]. Oxidative stress is the condition

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that occurs when the steady-state balance of prooxidants to antioxidants is shifted in the direction of the former, creating the potential for organic damage. According to modern images the oxidative stress (OS) is a non-specific though certain component of pathogenesis at numerous pathologic states of an organism. Organisms are constantly exposed to many different forms of reactive oxygen species (ROSs), which damage proteins, nucleic acids, and lipids, leading to loss of biological functions. The possibility that reactive oxygen-mediated protein damage contributes to the aging processes is supported by results of many studies showing that aging is associated with the accumulation of such protein damage [Stadtman E., *2004*]. It is known that metal-induced generation of ROS results in an attack not only towards DNA, but also to other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation. Once formed, peroxyl radicals (ROO•) can be rearranged *via* a cyclisation reaction to endoperoxides (precursors of malone dialdehyde) with malone dialdehyde (MDA) being the final product of peroxidation process. MDA is mutagenic in bacterial and mammalian cells and carcinogenic in rats [*Burlakova E., Khrapova N., 1985; Skwarek L. and Boulianne G., 2009*].

Nowadays ROSs are well recognized for a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems. ROSs are normally generated by tightly regulated enzymes. Overproduction of ROSs (arising either from mitochondrial electron transport chain or excessive stimulation of NAD(P)H) results in the oxidative stress [*Kovacic P. et al., 2005; Valko M. et.al., 2006*]. This occurs in biological systems when there is an overproduction of ROSs on the one hand and a deficiency of enzymatic and non-enzymatic antioxidants on the other hand. In other words, oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of prooxidant/antioxidant reactions in living organisms.

Beneficial effects of ROSs occur at low/moderate concentrations and involve physiological roles in cellular responses to anoxia, as for example in defence against infectious agents and in the function of a number of cellular signalling systems. The induction of a mitogenic response is another beneficial example of ROSs at low/moderate concentrations [*Kovacic P. and Jacintho J. D., 2001*]. The dual, "twofaced", character of ROSs is clearly substantiated. For example, a growing body of evidence shows that ROSs within cells act as secondary messengers in intracellular signalling cascades, which induce and maintain the oncogenic phenotype of cancer cells, however, ROSs can also induce cellular senescence and apoptosis and can therefore function as anti-tumorigenic species [*Dubinina E., 2006; Valko M., et al., 2007*].

The delicate balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms.

Having close relation to the structural organization and functional metabolic peculiarities of the surfaces for cell and subcellular formations division, the state of separate phospholipids (PLs) fractions in normally functioning biological systems is characterized by status of phylogenetically strongly programmed constancy [*Hanahan D., 1997*] supplying on the whole the physiological level of the cell vital activity. In this respect the main issue for membrane PLs is based on their maintaining the necessary level of either viscosity, liquidity, fluidity of biological membranes formed due to the complex variety of PL\PL ratio, formation of numerous complexes creating unique character for lipids surrounding membrane proteins, necessary level of hydrophobicity for the given biological system as an element for its functional activity regulation. It concerns more certainly the membrane-bound, lipid-dependent enzymes catalyzing reactions of transmembrane transfer of substances, transduction of outer signal, as well as the constant maintaining of physiological level for ligand – receptor interrelations, and normal stereotype of cell functioning on the whole [*Gardocki M. et. al., 2005*]. The functional activity of PLs, as regulators of microenvironment for membrane proteins, receptory proteins, and numerous membrane-bound lipid-dependent enzymes, is in a close dependence on the qualitative contents of their fatty acids (FAs) spectrum and on percent ratio of saturated and unsaturated representatives of these compounds. Free-radical attack by reactive oxygen species is associated with intensification of lipid peroxidation (LP) that influences the spectrum and level of membrane PLs and conformational transitions in membrane proteins resulting in direct chemical modification of membrane-bound proteins, including enzymes and receptors *via* changes in their lipid microenvironment [*Vladimirov Y., Archakov A., 1972*]. Our recent investigations had also revealed the significant changes in the spectrum and level of membrane phospholipids and their fatty acid composition in different tissues, which were accompanied with the changes in LPO, development of disbalance in pro-antioxidative state under the acoustic stress conditions.

Nowadays, a close attention is given to the phosphoinositides (PIs), which have both a structural and signaling role *via* their recruitment of proteins that contain phosphoinositide-binding domains. The special role of PIs, and at the same time their impaired metabolism associated with disorders such as cancer, cardiovascular disease and immune dysfunction [Rusten T., Stenmark H., *2006*], in pathogenesis of which significant role belongs to the OS, are the evidence of connection between mentioned processes, PIs and their metabolic enzymes. PIs also have a role of precursors of several types of second messengers for certain intracellular signaling pathways.

It is now well appreciated that derivatives of phosphatidylinositol are key regulators of many cellular processes in eukaryotes [*Michell R., 2008*]. PIs located on the cytoplasmic surface of cellular membranes are of particular interest. Therefore, it is of great importance to learn more about the metabolic changes in the content of PIs of membranes under the conditions of acoustic stress, as well as to study their dependence on LPO intensity.

Another significant problem is to elaborate preventive measures against hazardous effects of exposure to noise. There are two main ways of protecting people from the destroying effect of noise: mechanical and biological, which is based on the biochemical mechanisms of acoustic stress development. In accordance with this latter high efficiency of using antioxidants of different origin and nature as preventive measures was shown [*Melkonyan M., 1988; Melkonyan M., Melik-Agaeva E., 1993*]. Nevertheless, we continue the study on compounds, which can be used under conditions of acoustic stress, due to the preventive influence exerted particularly to the LPO processes activated upon similar conditions. Taking into account the vasodilatative, antihypoxic, antiaggregative, antistressor, antidote, radioprotective effects of Beditin (derivative of 1,4-benzodioxane), which are strictly connected with the free radical oxidation processes, Beditin was chosen as an object for research [*Martirosyan O., 1990*]. The mechanism of its action is connected with changes of the reactions mediated by vascular α_2-adrenoreceptors, the transfer of calcium in a cells and the metabolism of arachidonic acid [*Shirinyan E. et al., 2004*]. In particular, it is established that Beditin, depending on concentration, considerably suppresses biosynthesis of some products of lipoxigenase mediated transformations, not influencing the cyclooxygenase. Beditin also shows strongly pronounced calcium antagonistic properties [*Panosyan A. et al., 1989; Grigoryan G. et al.,1991*]. The high efficiency of Beditin blocking action on α_2-adrenoreceptors was also revealed in the carried out research. Thus, unlike well-known α_2-adrenoblocker idazoxan, Beditin, does not influence central α_2 - and vascular α_1- adrenoreceptors specifying its precise selective α_2-adrenoblocker action at the vascular level [*Shirinyan E., 1998*].

The aim of the current study was to investigate the effects of α_2–adrenoblocker Beditin synthesized at the Institute of Fine Organic Chemistry NAS RA, on the intensity of the LPO in white rats brain mitochondria and blood, *to determine the* content of PI representatives in brain mitochondrial membranes in the acoustic stress conditions. Taking into account that malone dialdehyde (MDA) is one of the important markers of LPO and determination of MDA levels is the most practical and confident method usually used for detecting and screening OS and might serve as a marker of treatment prognosis and success, we decided to study the content of MDA in mentioned samples.

MATERIAL AND METHODS

Investigations were performed in white inbred male rats weighing 150-200 *g* and kept on a general feeding ration in vivarium. The animals were divided into 6 groups: rats of the 1st group served as a control, rats of the 2nd, 4th and 6th groups underwent noise influence (91 dBA) with maximal energy in the region of average and high frequency. The animals of the 3rd-6th groups were intraperitoneally injected Beditin 1 hour prior to the experiment. Beditin was administered at doses 2.0 *mg/kg* (5th and 6th groups) and 20 *mg/kg* (3rd and 4th groups). So, the 4th and 6th groups underwent combined action of noise and Beditin. Preparation of brain mitochondria (BM) [*Prokhorova M., 1982*] and erythrocyte membranes (EM) [*Limber G. et. al., 1970*] were performed by differential centrifugation method; the concentration of protein was determined by D. Lowry [Lowry D. et al., 1951]. We studied the intensity of ascorbate-dependent LPO in white rats BM and EM, concentration of α-tocopherol (α−T) [*Duggan D., 1954*] and the level of malone dialdehyde (MDA) in blood plasma [*Yoshioka T. et. al., 1979*] in all experimental groups. The activity of lipid peroxidation was demonstrated by the output of MDA, which together with thiobarbituric acid results in colour reaction. Its intensity has been registered spectrophotometrically (SF-4A) at the wavelength 535 *nm* [*Vladimirov Yu., Archakov A , 1972*].The level of MDA was expressed in *nM* of MDA per *mg* of protein. Total peroxidizing activity (TPA) was determined by A. Pokrovskij [*Pokrovskij A., 1969*]. PIs were obtained by the selective acidic extraction by L. Bergelson [*Bergelson L. et. al., 1981*]. The residues on the filter paper after extraction of phospholipids (PL) were used for phosphatidylinositols extraction procedures. The precipitates were washed with mixture of chloroform–methanol–concentrated HCl (2:1:0.01). After repeatedly shaking during 37 *min* incubation at room temperature, the mixture was centrifuged at 4000 *g* x 5 *min*. Extraction was repeated twice in the same manner. After the third centrifugation, supernatants from the three extractions were combined. The total extract was washed in a stepwise mode: first with 1N HCl, then with the mixture of chloroform – methanol - 1N HCl (3:48:47) and finally with chloroform – methanol – 0.01 N HCl (3:48:47). After each step, the mixture was centrifuged at 4000 *g* x 5 *min* and the upper layer of supernatants was removed. The resulting acidic extracts were treated with methanol until transparency of solution and pH was brought up to 4.9 with NH_4OH. PI extracts were analyzed using microthin-layer chromatography on silica gel-impregnated plates (sorbent layer thickness = 5-7 *microns*) and saturated with sodium oxalate. Before using, the plates were activated by incubation at 110°C for 15 min. PI extracts were resolved with a mixture containing chloroform – methanol – 4N NH_4OH at the ratio 9:7:2 [*Bergelson L. et al., 1981*].

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

The results were processed by Student's method and SPSS 12.0 program.

RESULTS

Changes of MDA concentration testify to intensification of ascorbate-dependent LPO in both BMs and EMs under conditions of the acoustic stress (AS), which corresponds to the previously reported (Table 1) [*Melkonyan M., 1988*].

According to the previously used and reported dose levels of Beditin, studies were done in two series. The dose of 20 *mg/kg* was considered the most effective preventive measure at several pathologic states [*Shirinyan E., 1998*]. Therefore, in the first series of our experiments the effect of 20 *mg/kg* Beditin intraperitoneal administration on the studied parameters was investigated in intact animals and animals exposed to noise.

In both groups Beditin administration led to an increase of the ascorbate-dependent LPO intensity in both BMs and EMs, so the level of MDA in samples increased. The concentration of α-T in EMs and plasma in animals of these groups decreased. The intensity of changes was more expressed in the EMs, where combined action of Beditin and noise led to the tremendous decrease of α-T content. Data obtained from the investigation of plasma TPA demonstrated significant increase

Table 1.

Changes in the intensity of ascorbate-dependent lipid peroxidation in BM and EM, α-T content in EM and plasma, TPA in plasma under conditions of acoustic stress, administration of 2.0 *mg/kg* and 20 *mg/kg* Beditin and their combination

Sample		MDA (*nM/mg* protein)		α - tocopherol (*µg*)		TPA (λ=600 *nm*)
		BM	EM	EM	Plasma	
Group 1	Control	40.9± 0.3 $p<0.001$	11.11 ± 0.07 p<0.05	3.7 ± 0.08 $p<0.001$	2. 9 ± 0.1 $p<0.05$	0.22 ± 0.01 $p<0.001$
Group 2	Noise action	56.0 ± 0.5 $p<0.001$	12.6 ± 0.2 $p<0.001$	1.75 ± 0.06 $p<0.001$	1.45 ± 0.09 $p<0.001$	0.39 ± 0.03 $p<0.001$
Group 3	Beditin (20.0 *mg/kg)*	54.3 ± 0.5 $p<0.001$	17.6 ± 0.16 p<0.001	1.65 ± 0.07 $p<0.001$	1.65 ± 0.09 $p<0.001$	0.35 ± 0.05 $p>0.05$
Group 4	Beditin (20.0 *mg/kg)* + Noise action	56.8 ± 0.6 $p<0.001$ $p_1>0.05$	14.5 ± 0.1 $p<0.001$ $p_1<0.01$	0.1 ± 0.02 $p<0.001$ $p_1<0.001$	1.3 ± 0.03 $p<0.02$ $p_1>0.05$	0.42 ± 0.02 $p>0.05$ $p_1>0.05$
Group 5	Beditin (2.0 *mg/kg)*	38.6 ± 0.4 $p<0.001$	8.3 ± 0.2 $p<0.001$	3.65 ± 0.12 $p<0.001$	2.5 ± 0.1 $p<0.05$	0.35 ± 0.05 $p<0.05$
Group 6	Beditin (2.0 *mg/kg)* + Noise action	41.3 ± 0.3 $p>0.05$ $p_1<0.001$	11.3 ± 0.9 $p<0.05$ $p_1>0.05$	2.8 ± 0.1 $p<0.05$ $p_1<0.02$	2.3 ± 0.1 $p>0.05$ $p_1>0.05$	0.32 ± 0.05 $p>0.05$ $p_1>0.05$

Note: *p* – compared with control; p_1- compared with noise

in its level, as a side effect of the membrane stability decrease, which might be a result of LPO intensification and phospholipases activation. According to data received, it was concluded that application of Beditin at the initial dose of 20 *mg/kg* led to development of changes in anti-prooxidant status of studied tissues, to the structural and functional disorders, and Beditin was not efficient as a regulator of OS.

Taking into account the results of the investigation and considering possible dose dependence, in the second series of experiments 2.0 *mg/kg* Beditin was used**.** The effect of 2.0 *mg/kg* dose was investigated because the maximum α_2-blocking and vasodilating effect of Beditin was shown at doses of 2-5 *mg/kg* (*i/p and i/v*). At the same doses one of its basic metabolic effects is the blockade of stress-mediated increased trans-membrane transfer of Ca^{2+} ions into the intracellular structures surpassing in its blocking action the effects of a classical Ca^{2+} antagonist Verapamil [*Ghukasyan T. et al., 2005*]. Participation of the secondary messengers, in particular inositol-triphosphates in the processes of regulation of Ca^{2+} ions transfer and close spatial link of α_2-adrenoreceptors and calcium channels was also considered [*Hideaki K. et al., 1997*].

The intensity of peroxidation processes as well as the level of α-T in EM and plasma under conditions of Beditin administration and in the group with combined Beditin administration and noise action slightly decreased (unverified changes) (Table 1). According to the data received, 2.0 *mg/kg* Beditin had no significant influence by itself, being administered to intact animals; nevertheless, at the mentioned dosage it prevented developing changes both in intensity of lipid peroxidation and in α-T content under conditions of the acute acoustic stress.

In our previous investigations significant qualitative and quantitative changes in the phospholipids-glycerols content of biomembranes of cells from different tissues were shown to correlate with changes in anti-prooxidant system representatives; particularly it was shown that LPO increase was accompanied with the increase of lysophosphatidylcholines, markers of membranes disorders. This latter was an evidence of phospholipase A_2 activation under acoustic stress conditions, being a common effect for OS as well [*Melkonyan M., 1988*].

Table 2.

Changes in phosphoinositides (PIs) fraction under conditions of acoustic stress and administration of Beditin

Sample	PI (3)P	$PI(3,5)P_2$	$PI(3,4,5)P_3$
Control	198.4 ±6.0	70.5 ±5.2	48.2 ±4.8
Noise	153.0 ± 21.6 $p>0.05$	26.4 ± 3.3 $p<0.001$	20.0 ±0.7 $p<0.001$
Beditin (2.0 *mg/kg*)	208 ±23 $p>0.05$	66.9 ±4.2 $p>0.05$	43.0 ±1.0 $p>0.05$
Beditin *and* Noise action	132 ±12 $p<0.001$ $p_1<0.001$	40.1 ±4.8 $p<0.01$ $p_1<0.001$	41.8 ±2.1 $p>0.05$ $p_1<0.001$

Note: p – compared with control; p_1- compared with noise

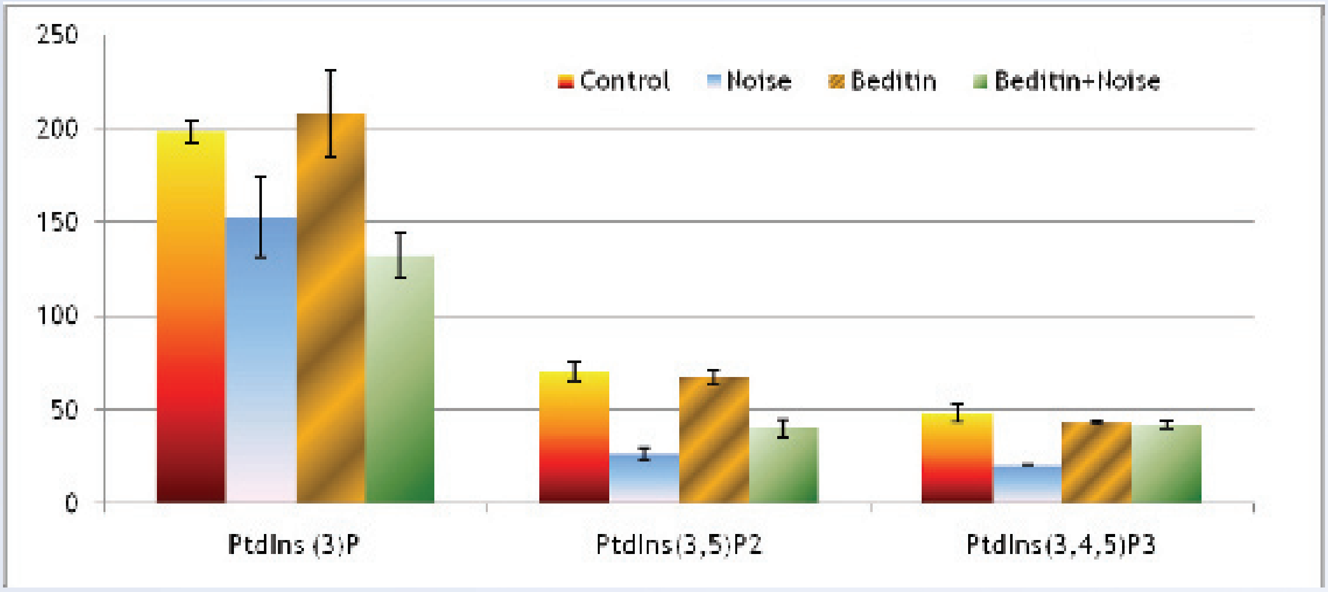

Figure. *Changes in the fraction of phosphoinositides (PIs) under conditions of acoustic stress and administration of Beditin*

Taking into account the importance of Inositol phospholipids in metabolic pathways, their content in BM was studied under the same conditions of an experiment. The study on qualitative and quantitative changes in the fraction of phosphoinositides (PIs) upon the acoustic stress testified to the decrease in content of all the fractions; this latter was significant for $PI(3,5)P_2$ and $PI(3,4,5)P_3$, and insignificant for $PI(3)P_1$.

Administration of 2.0 *mg/kg* Beditin to intact animals did not cause any significant changes in the content of PIs. Nevertheless, preliminary administration of Beditin before noise action exerted the regulatory effect on the content of all the representatives, more expressed in the content of $PI(3,4,5)P_2$(Figure).

DISCUSSION

Data obtained are evidence of structural changes in lipid phase of mitochondrial membrane that might be the result of various processes, among which changes in activity of enzymes involved in metabolism of PIs [*Kuksis A., 2003*] are especially important. Inositol phospholipids represent a minor fraction of membrane phospholipids; yet they play important regulatory functions in signaling pathways and membrane traffic. The phosphorylated inositol ring can act either as a precursor for soluble intracellular messengers or as a binding site for cytosolic or membrane proteins. Hence, phosphorylation-dephosphorylation of phosphoinositides represents a mechanism for regulation of recruitment to the membrane of coat proteins, cytoskeletal scaffolds, or signaling complexes and for the regulation of membrane proteins. It is well-known that metabolism of cells is regulated by numerous systems, among which PIs [*Di Paolo G. and De Camilli P., 2006*] have their own, very important role. The bulk of inositol lipids reside in cell membranes where they are substrates for kinases, phosphatases, and phospholipases. PIs are substrates for phospholipases, thereby generating a number of products that serve as secondary messenger molecules with biological functions on their own. PIs also serve as specific membrane-anchored determinants for the recruitment of a wide range of proteins. Therefore, PIs ideally suit to serve as key regulators of fundamental biological processes in all eukaryotic cells. Resting mammalian cells contain significant levels of PI (3) P, but hardly any of the other 3'- phosphorylated inositides. While the overall levels of PI (3) P almost do not increase upon cell stimulation, the levels of the other 3'-phosphorylated inositides can rise sharply [*Martelli A. et. al., 2007*]. Recent work suggests that phosphoinositide metabolism has an important role in membrane traffic at the synapse. PI(4,5)P2 generation is implicated in the secretion of at least a subset of neurotransmitters. Furthermore, PI(4,5)P2 plays a role in the nucleation of clathrin coats and of an actin-based cytoskeletal scaffold at endocytic zones of synapses, and PI(4,5)P2 dephosphorylation accompanies the release of newly formed vesicles from these interactions. Thus, the reversible phosphorylation of inositol phospholipids may be one of the mechanisms governing the timing and vectorial progression of synaptic vesicle membranes during their exocytic-endocytic cycle [Cremona O., De Camilli P., *2001; Sasaki T. et al., 2009*].

The phosphatidylinositol monophosphates are present in cells at low levels only, although their levels do not appear to fluctuate greatly. Phosphatidylinositol 3-phosphate has been implicated in membrane trafficking through its interactions with specific proteins in endosomes. Indeed, it is a major determinant of the identity of early endosomes membranes, and participates in most aspects of endosomal function. Phosphatidylinositol 4-phosphate is the precursor for the 4,5-bisphosphate, but it binds to a protein on the cytoskeleton of the cell and has its own characteristic functions. In particular, it is essential for the structure and function of the Golgi complex, where it is required for the recruitment of specific proteins [*Michell R., 2008; Payrastre B., 2004; Skwarek L. and Boulianne G., 2009*]. Phosphatidylinositol 4,5-bisphosphate is especially important as a precursor of further metabolites and because of signaling functions in plasma membrane, where it complexes with and regulates many cytoplasmic and membrane proteins, especially those concerned with ion channels for potassium, calcium, sodium and other ions. In most instances, it increases channel activity, while its hydrolysis by phospholipase C reduces such activity. In particular, it appears to interact with cationic residues of specific proteins in concert with cholesterol to form localized membrane domains that are distinct from the sphingolipid-enriched sites. Moreover, phosphatidylinositol 4,5-bisphosphate and its diacylglycerol metabolites are important for vesicle formation in membranes. For example, the clathrin-coated vesicle pathway is a major pathway for internalization of receptors, such as the transferrin receptor, in cells. Phosphatidylinositol 4,5-bisphosphate is essential for this process as it binds to the "machinery" involved in the membrane, increasing the number of clathrin-coated pits and permitting internalization of transferrin. Phosphatidylinositol 4,5-bisphosphate is intimately involved in the development of the actin cytoskeleton and its attachment to the plasma membrane, thereby controlling cell shape, motility, and many other processes. In the cell nucleus, this lipid is believed to be involved in maintaining chromatin, the complex combination of DNA, RNA, and protein that makes up chromosomes, in a transcriptionally active conformation. It is also an essential cofactor for phospholipase D [*Madesh M. et al., 1997*] and thus affects the cellular production of phosphatidic acid with its specific signalling functions [*Jenkins G., Frohman M., 2005*]. Binding specifically to ceramide kinase, the enzyme responsible for the synthesis of ceramide-1-phosphate, it has an influence on sphingolipid metabolism. Like ceramide-1-phosphate, it binds to and activates the Ca^{2+}-dependent phospholipase A_2, which generates the arachidonate for eicosanoid production. So it is clear that there is a close connection between LPO processes and PI metabolism.

The major functions of phosphatidylinositol 3,5-bisphosphate are realized in membrane and protein trafficking especially in the endosomes. While it is known to be essential to this aspect of cellular metabolism, its precise role is not clear yet. There is recent evidence that it has a role in the responses of mammalian cells to insulin. Phosphatidylinositol 3,4,5-trisphosphate has been implicated in a variety of cellular functions, such as growth, cell survival and differentiation. In particular, it is an important component of a signalling pathway in the cell nucleus. Another important role is the involvement into immune processes. The human immune system utilizes neutrophils, which are highly mobile cells, to eliminate pathogens from the infected tissue. The first step is to track and then pursue molecular signals, such as cytokines, emitted by pathogens. It has now been established that two phospholipids operate in sequence to point the neutrophils in the correct direction and the first one is phosphatidylinositol 3,4,5-trisphosphate, which binds to a specific protein DOCK2 and enables it to translocate to the plasma membrane. At the same time, reciprocal and synergistic links between cAMP and $InsP_3$/calcium paths have long been recognized as the possible leading ways of regulation in case of stress condition. The results of most studies show that increasing cAMP suppresses phospholipase C activation, although a few have reported potentiation. The ability of cAMP to inhibit phospholipase C is thought to occur at the level of phospholipase C-β-phosphorylation [*Rebecchi M.., Pentyala S., 2000; Kim U. et. al., 1989*]. It has long been known that the water-soluble glycerolphosphoinositides, the fully deacylated forms of phosphatidylinositol and the phosphatidylinositol phosphates have key roles in cellular signalling pathways. However, relatively recently it has become apparent that like other lysophospholipids, lysophosphatidylinositol, *i.e.* compounds with a single fatty acid residue only linked to the glycerol moiety, and the polyphospho-analogues may have messenger functions [*Corda D. et. al., 2002*]. They are formed as intermediates in the remodelling of the fatty acid compositions of lipids, and when arachidonic acid is released for eicosanoid biosynthesis. The leading role in this process belongs to phospholipase A_2, activation of which during OS is a generally recognized fact. Thus, the activation of LPO processes serves both as a mechanism of structural remodeling and as a trigger mechanism of many regulatory pathways activation, including PIs.

Summarizing the results obtained, we can state that Beditin effects in *vivo* strongly depend on the dose of the compound. Beditin administration to intact animals at the dose level of 20 mg/kg animal body weight, as well as noise influence cause LPO activation in EM and BM membranes and decrease in the level of main endogenous antioxidant α-tocopherol in plasma, EM and BM. Administration of 2.0 *mg/kg* Beditin to intact animals does not cause significant changes in the studied parameters. Moreover, the given preparation shows properties of an antioxidant, preventing intensification of the LPO under conditions of an acute acoustic stress. Thus, Beditin if administered at the dose of 2.0 *mg/kg*, could be considered an efficient antioxidant. The observed data of combined influence of Beditin and noise testify the preventive effect of Beditin (2.0 *mg/kg*) on the intensification of lipid peroxidation in acoustic stress conditions.

Administration of 2.0 *mg/kg* Beditin to intact animals does not cause significant changes in the content of separate representatives of phosphoinositides. However, preliminary intraperitoneal introduction of Beditin before noise influence renders regulatory influence on the content of PIs, mostly expressed in case of PI (3,4,5) P_3

REFERENCES

1. *Bergelson L.D., Dyatlovitskaja E.V., Molotkovsky J.G.* [Preparative Biochemistry of Lipids] [published in Russian]. Moscow. "Nauka (Science)" Publisher. 1981.
2. *Burlakova E.B., Khrapova N.G.* [Lipid peroxidation of membranes and natural antioxidants] [published in Russian]. "Uspekhi khimii" (Successes of chemistry). 1985; 54(9): 1540-1558.
3. *Corda D., Iurisci C. and Berrie C.P.* Biological activities and metabolism of the lysophosphoinositides and glycerophosphoinositols. Biochim. Biophys. 2002; 1582: 52-69.
4. Cremona O., De Camilli P. Phosphoinositides in membrane traffic at the synapse. J. Cell. Sci. 2001; 114(Pt 6): 1041-1052.
5. *Di Paolo G. and De Camilli P.* Phosphoinositides in cell regulation and membrane dynamics. Nature. 2006; 443: 651-657.
6. *Dubinina E.E.* [Products of an oxygen metabolism in functional activity of cells (a life and death, creation and destruction)] [published in Russian]. Physiological and clinical-biochemical aspects. St.-Petersburg. "Medical press" Publishing House. 2006; 276-282.
7. *Duggan D.D.* Spectrofluorometric determination of tocopherols. Arch. Biochem. Biophys. 1954; 84(1): 116-126.
8. *Gardocki M.E., Jani N. and Lopes J.M.* Phosphatidylinositol biosynthesis: biochemistry and regulation. Biochim. Biophys. 2005; 1735: 89-100.
9. *Ghukasyan T.Gh., Harutyunyan S.A., Shirinyan E.A., Garibjanyan B.T., Gevorkyan G.A.* The Ca^{2+}-antagonistic mechanism of action of α_2-adrenoblockers Beditine; Correction of regional hemodynamics at the discirculatory-ischemic states. In: Proceedings of the International Scientific Conference dedicated to the 75th anniversary of YSMU. Yerevan. 2005. P.160-162.
10. *Grigoryan G.H, Panosyan A.G., Oganesyan N.M., Markaryan E.A., Vardanyan S.O., Shirinyan E.A.* [Patent under the demand 4882467/14 (097540)] [published in Russian]. Russia. 1991.
11. *Hanahan D. J.* A Guide to Phospholipid Chemistry. 1997. 224p.
12. *Hideaki K., Hiroshi O., Masatoshi H. et al.* Calcium movements, distribution, and functions in smooth muscle. Pharmacol. Reviews. 1997; 49(2): 158-230.
13. *Hunanyan L. , Sotskij O., Khachatryan L., Manukyan A., Kocharyan K., Shirinyan E.* The effects of high level noise and α−adrenoblocker on the free radical oxidation intensity of white rat serum proteins. In: Abstracts of the International Congress of Young Scientists, YSMU, April 13-14, 2010, Yerevan. The New Armenian Medical Journal. 2010*a*; 4(1): 64-65.
14. *Hunanyan L.S., Sotskij O.P., Khachatryan L.G., Shirinyan E.A., Melkonyan M.M.* [The oxidative modification of white rats serum proteins under the noise and α−adrenoblockers influence] [published in Russian]. Armenian Biological Journal, 2010*b*; 1(62): 79-83.
15. *Jenkins G.M., Frohman M.A.* Phospholipase D: a lipid centric review. Cell Mol. Life. Sci. 2005; 62(19-20): 2305-2316.
16. *Kim U.H., Kim J.W., Rhee S.G.* Phosphorylation of phospholipase C-gamma by cAMP-dependent protein kinase. J. Biol. Chem. 1989; 264(34): 20167-20170.
17. *Kovacic P. and Jacintho J. D.* Mechanisms of carcinogenesis: Focus on oxidative stress and electron transfer. Curr. Med. Chem. 2001; 8: 773-796.
18. *Kovacic P., Pozos R.S., Somanathan R., Shangari N., and O'Brien P.J.* Mechanism of mitochondrial uncouplers, inhibitors, and toxins: Focus on electron transfer, free radicals, and structure–activity relationships. Curr. Med. Chem. 2005; 12: 2601-2623.
19. *Kuksis A.* Inositol Phospholipid Metabolism and Phosphatidyl Inositol Kinases. Laboratory Techniques in Biochemistry and Molecular Biology. Elsevier. Amsterdam. 2003; 30: 970.
20. *Limber G.R., Davie R.F., Baker A.M.S.* Acrylamide gel electrophoresis studies of human erythrocyte membrane. J. Blood. 1970; 36(2): 111-118
21. *Lowry D.H., Rosebrough, N.J., Farr A.L., Randall R.J.* Protein measurement with folin phenol reagent. J. Biol.Chem. 1951; 193(1): 265-269.

22. *Madesh M., Ibrahim S.A. and Balasubramanian K.A.* Phospholipase D Activity in the Intestinal Mitochondria: Activation by Oxygen Free Radicals. Free Radical Biology and Medicine. 1997; 23(2): 271-277.
23. *Martelli A.M.,Evangelist C., Billi A. M., Manzoli L., Papa V.C.L.* Intranuclear 3´-phosphoinositide metabolism and apoptosis protection in PC12 cells. Acta bio-medica. Atenei Parmensis. 2007; 78(Suppl 1): 113-119.
24. *Martirosyan O.M.* [α_2-adrenoreceptors at extreme conditions] [published in Russian] Search of new specific α_2-blockers. PhD Thesis. 1990.
25. *Melkonyan M.M., Melik-Agaeva E.* [Lipid metabolism of blood of the women working in the conditions of influence of noise and its regulation by bioantioxidants] [published in Russian]. In: Abstracts of «Bioantioxidant » - 1992. Moscow. 1993. P. 97.
26. *Melkonyan M.M.* [Lipid peroxidation and application of antioxidants at acoustic stress] [published in Russian]. Author's Abstract of Doctoral Thesis. 1988. 44 p.
27. *Michell R.H.* Inositol derivatives: evolution and functions. Nature. Rev. Mol. Cell Biol. 2008; 9: 151-161.
28. *Panosyan A.G., Grigoryan G.H, Shirinyan E.A., Martirosjyan O.M, Markaryan E.A., Akopov S.O., Gabrielyan E.S.* [Synthesis and research of prostaglandins] [published in Russian]. In: Proceedings of IV conference on prostaglandins. Minsk. 1989. P. 116.
29. *Payrastre B.* Phosphoinositides. In: Bioactive Lipids. pp. 63-84. (edited by A. Nicolaou and G. Kokotos). The Oily Press, Bridgewater. 2004.
30. *Pokrovskij A.A.* [Determination of negligible concentrations of the haemoglobin dissolved in plasma of blood] [published in Russian]. Biochemical methods of research in clinic. Moscow. "Medicina (Medicine)" Publishers. 1969. 349p.
31. *Prokhorova M.I.* [Methods of biochemical researches]. [published in Russian]. Leningrad. 1982; 29- 32.
32. *Rebecchi M.J., Pentyala S.N.* Structure, function, and control of phosphoinositide-specific phospholipase C. Physiol. Rev. 2000; 80(4): 1291-1335.
33. Rusten T.E., Stenmark H. Analyzing phosphoinositides and their interacting proteins. Nat. methods. 2006; 3(4): 251-258.
34. *Sasaki T., Takasuga S., Sasaki J., Kofuji S., Eguchi S., Yamazaki M. and Suzuki A.* Mammalian phosphoinositide kinases and phosphatases. Prog. Lipid Res. 2009; 48: 307-343.
35. *Shirinyan E.A.* Beditine, a new drug development of IFOC. In: Institute of Fine Organic Chemistry NAS RA. Collection of research works. Yerevan. 1998. P. 47-56.
36. *Shirinyan E.A., Harutunyan S.A., Petrosyan A.A., Gevorkian G., Markarian E.A., Vartanian S.O., Medvedev O.S., Garibjanian B.T.* Effect of the post-synaptic α_2 adrenoreceptor antagonist Beditin upon the resistivity of the rats to proconvulsant influences and modifications in the content of Ca^{2+} in the intracellular structures of the brain. Clin. and Exper. Pharmacology. 2004; 67(1): 11-14.
37. *Skwarek L.C. and Boulianne G.L.* Great expectations for PIP: phosphoinositides as regulators of signaling during development and disease. Developmental Cell. 2009; 16: 12-20.
38. Stadtman E.R. Role of oxidant species in aging. Curr. Med. Chem. 2004; 11(9): 1105-1112.
39. *Valko M., Leibfritz D., Moncol J., Cronin M.T.D., Mazur M., Telser J .* Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology. 2007; 39: 44-84.
40. *Valko M., Rhodes C. J., Moncol J., Izakovic M., and Mazur M.* Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 2006; 160: 1-40.
41. *Vladimirov Y.A., Archakov A.I.* [Lipid peroxidation in biological membranes] [published in Russian] Moscow. "Nauka (Science)" Publisher. 1972.
42. *Yoshioka T., Kawada K., Shimada T., Mori M.* Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Amer. J. Obstet. and Gynecol. 1979; 135(3): 372-376.