Research Article

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Alpha2- Adrenergic blockers restore noise-induced biochemical and cognitive disorders

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Abstract

Noise-induced stress causes central nervous system dysfunction and neurotransmission impairment in the brain and changes hormone levels which potentially result in psychological and behavioral problems. Considering the significance of the potential negative outcome of the chronic stress, the aim of our investigation was to study the level of inositol triphosphate (IP3) in the mitochondrial fraction of the brain (MFB), behavioral characteristics and the effects of the α2-adrenoblockers beditin and mesedin to reveal their antioxidative effect during noise exposure. The open field tests were used in order to evaluate the behavioral states of the rats. Investigations were carried out on albino rats divided into 4 groups. The 1st group of rats served as a control. The 2nd, 3rd and 4th groups were exposed to 91 dBA of noise; the duration of exposure was 8 hours per day for 60 days. The 3rd group was injected with beditin and the 4th group with mesedin, both intraperitoneally and repeatedly. According to our results, the chronic exposure to high-level noise leads to the decreased level of IP3 resulting development of oxidative stress and metabolism imbalance. The open field test revealed that chronic noise exposure caused noise duration-dependent delay in the locomotor and behavioral activity. Intensity of these changes were dependent on the duration of noise exposure. The administration of α2-adrenoblockers to the noise-exposed animals had a regulatory effect on the studied parameters under the conditions of chronic acoustic stress.

Keywords: Noise, α2-adrenoblockers, mitochondrial fraction of the brain, inositol triphosphate, anxiety.

Background

Noise impact has been demonstrated to induce a variety of physiological effects in biological systems [1]. Noise-induced stress promotes enhancement of the free oxygen radical's intensity because of high respiratory oxygen intake and metabolic turnover. Cells continuously produce free radicals and reactive oxygen species (ROS) as a result of their metabolic processes [2,3]. Lipid peroxidation is a free-radical-mediated chain of reactions that, once initiated, results in oxidative damage of polyun¬saturated lipids. So, the most targets for this kind of action are becoming the components of biolo¬gical membranes. These reactions can be initiated or enhanced by a number of toxic products, including endoperoxides and aldehydes [4,5]. It was asserted that noise exposition in early stages produces long-term cognitive impairment and damage to the hippocampal neurons [6]. At present it is highly actual to find the ways of protection from the stress impact, considering its deteriorative action on the organism's different organs and systems. In a search for new alternatives for α2-adrenergic blockers, 1,4-benzodioxane derivatives mesedin (2-(2-methylamino-4-thiozolyl) -1,4-benzodioxan hydrochloride) and beditin (2-amino-4-thiozolyl -1,4-benzodioxan), have been chosen for the further studies because of being selective and stable blockers of the peripheral α2-adrenoreceptors and less toxic.

Taking into account the important regulatory function of phosphoinositids (PI) in the signaling system and ensuring membrane functions, we studied the content of IP3 as a minor fraction of membrane phospholipids (PL) in the experimental animals' brain mitochondria and behavioral activity ie. anxiety.

Material and Methods

Animals

Investigations have been carried out on albino male rats aged 5-6 weeks, kept in ordinary vivarium conditions, maintained on a 12 h light/dark cycle with food and water and libitum in accordance with the European Communities Council Directive (86/609/EEC) on care and use of animals for experimental procedures. The following specific in vivo conditions were kept for each experiment: 200C temperature and 52% humidity. The animals were accommodated to the laboratory conditions for 7 days prior to the experiment. The rats were grouped into 4 groups (6 per group). The 1st group was a control; the 2nd group (noise) was just exposed to noise, the 3rd

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and 4th groups - to 91 dBA by a white-noise generator (10-20 kHz) of a frequency range of 10000-16000Hz. The duration of noise effect was 7, 30 and 60 days with an 8 h window of noise exposure daily (from 1:00 to 9:00) and was uniform inside the cage, as monitored with a sound meter (ST 11 D). The animals of the 3rd and 4th groups were injected with beditin (beditin - (2-amino-4-thiozolyl -1,4-benzodioxan) (2mg/kg) and mesedin (2-(2-methylamino-4-thiozolyl) -1,4-benzodioxan hydrochloride)) (10mg/kg) intraperitoneally, correspondingly. The first injection was carried out 12 h prior to the exposure. Injections were repeatedly done each 24 h.

Biochemical Analysis

Determination of triphosphoinoside (IP3) content

a) Extraction of phospholipids of the mitochondrial fraction of the brain

To determine PL, it was extracted from the mitochondrial fraction of the rat brain using the Bligh and Dyer method modified by Kargapolov [7]. For this purpose, 2-3 ml of a mixture containing chloroform and methanol in a ratio of 1: 2 was added to the samples and filtered. The precipitate formed on the filter was washed three times with a chloroform-methanol mixture (2:1) and once with warm methanol. 2-3 ml of 0.2% CaCl2 solution was added to the filtrate and shaken for 2 min, after which 2-3 ml of chloroform was added. The filtrate was centrifuged for 3-5 min at 3000 rpm, and then the surface water-methanol phase was isolated. The residue was washed once with 2 ml of chloroform solution to remove the remaining lipids. After centrifugation, chloroform extracts were added. The resulting extract was washed three times with a solution of chloroform-methanol-0.02% CaCl2 (3:48:47) with centrifugation each time. By removing the surface water-methanol phase, a chloroform extract of phospholipids was obtained.

b) Acid extraction of phosphoinositide's of the mitochondrial fraction of the brain

To obtain PI, the method of selective acid extraction was used [8]. To isolate PI, we used precipitates on the filter after PL extraction, to which a mixture of chloroform-methanol- HCl concentrated solution (2: 1: 0.01) was added. At the room temperature, shaking time to time, after incubation for 37 minutes the mixture was centrifuged for 5 min at 4000g. The extraction was repeated twice. After the third centrifugation, the supernatants were combined. The total extract was washed sequentially by: 1N HCl, then a mixture of 1N chloroform-methanol -1N HCl (3:48:47) and chloroform-methanol–0.01N HCl (3:48:47). After each single washing, the mixture was centrifuged for 5 min at 4000g. After centrifugation, the upper layer was removed, and methanol was added to the cloudy acidic extracts until the solution became clear, then the pH was adjusted to 4.9 using the NH4OH concentrated solution.

Behavioral tests

An open field [9] is a wooden square-shaped plate (60x60) with the 20cm high walls. The animals' behavior was evaluated within 5 minutes in the middle of the plate. Approximately 3 minutes before each assay, the animal was removed from its home cage and placed in a clean holding cage for transfer. Rats were placed in the center of the open field and allowed to explore the apparatus for 5 minutes. Each trial was recorded for later analysis, using a video camcorder positioned 2.1m above the apparatus. After the 5-minute tests, the rats were taken back to their home cages and the open field cleaned with 70% ethyl alcohol and permitted to dry between tests.

Statistical analysis

All analysis was performed using BIOSTA system. All measurements were represented as mean ± SEM. Significance of means' difference was evaluated using paired Student Newman – Keuls test. Statistical significance, determined by one-way ANOVA, was set at $P<0.05$ (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

Results

Changes in the intensity of lipid peroxidation (IP3) in the MFB under the chronic acoustic stress conditions

Analysis of peroxidation intensity of lipids revealed statistically significant shifts in IP3levels in MFB under the chronic noise influence (Table 1). Our results have shown decreased level of TPI in the 2nd group after 7, 30 and 60 days in the acoustic stress conditions ($t=10,5$, $p<0,001$; $t=9,6$, $p<0,001$; $t=-12,8$, $p<0,001$, respectively). The noise effect has been decreased by influence of α2-adrenoblockers, which was noticeable in higher levels of IP3 in the 3rd ($t=3,5$, $p<0,01$; $t=2,6$, $p<0,05$; $t=2,4$, $p<0,05$, respectively) and the 4th groups ($t=2,5$, $p<0,05$; $t=1,8$, $p=0,1$; $t=0,45$, $p=0,66$, respectively). Moreover, the data of IP3 in the 4th group rats were close to control after 60 days of exposure.

Number of entries to the periphery zone in the open field

(Figure 1) shows the number of entries to the periphery zone in the open field test under the acoustic stress conditions after 7, 30 and 60 days. Rats of the 2nd group have had higher number of entries to the periphery zone compared with the 1st, 3rd and 4th groups. The 3rd group rat's behavior is quite notable, because of their smaller number of entries to the periphery zone after 30- and 60-days' action of noise compared with the control, while that for the 7 days of exposure were close to control. The data of the 4th group rats for the 7, 30and 60 days of exposure were less than the controln (Figure 1).

Discussion and Conclusion

The results obtained indicate onto changes in the studied biochemical parameters and the cognitive function, so testify about the regulative effects of the α2-adrenoblockers under the conditions of the chronic noise.

The stress realization phenomenon is a nonspecific response of the central nervous system (CNS) to a stressor with several morphological and biochemical deviations including those characteristics to the oxidative stress [10].

To date, researchers' attention is focused on IP3, as minor

Table 1: *Content of inositol triphosphate in the of mitochondrial fraction of brain under the chronic noise conditions after 7, 30 and 60 days.*

Groups	7X8	30X8	60X8
Control	46±1,61	46±1,61	46±1,61
Noise	23±1,46***	26±1,31***	19±1,3***
Noise + Beditin	37±1,94**	40±1,67*	41±1,5*
Noise + Mesedin	40±1,77*	42±1,5	45±1,52

The statistical difference is provided between control and experimental groups.
*(*P < .05, **P < .01, ***P < .001).*

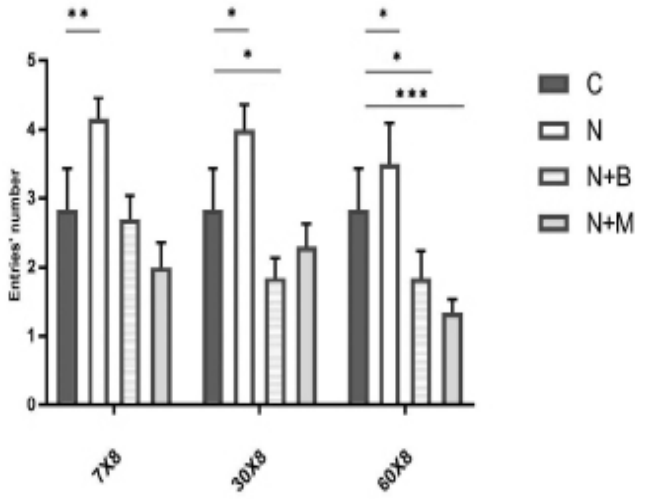

Figure 1: *Number of entries to the periphery zone (each group is of 6 animals, under the 8hr noise /day action). Thus after 7, 30 and 60 days of noise action the rats of the 2nd group have had146,4%, 140,8%, 123,2% number of entries to the periphery zone compared with the1stgroup (in which the activity was considered to be as 100%), respectively, the rats of the 3rd group spent 95%, 64,7%, 64,8%, respectively and the rats of the 4th group spent 70,4%, 80,9%, 47,1%, respectively.*

components of the cell membranes, which play both a structural and a signaling role. Disorders of IP3 metabolism in such pathologies as cancer, cardiovascular diseases, and the immune system dysfunctions, where the acoustic stress is of importance, are the red-ox and the IP3 metabolism imbalance result [11]. IP3 are the precursors of several secondary messengers of various intracellular signaling pathways.

The study of quantitative shifts in the fraction of IP3 of brain mitochondria under the conditions of acoustic stress revealed a decrease in the content of IP3 in the 2nd group compared to other experimental groups, after 7, 30 and 60 days of noise exposure.

Disorders of the metabolism of IP3, associated with the activation of free radical processes have been described in some diseases [12]. The study of quantitative changes in the fraction of IP3 of brain mitochondria under the conditions of chronic acoustic stress revealed a decrease in the content of IP3. As a result, the development of disorders in phosphorylation / dephosphorylation of the inositol ring, responsible for the implementation of regulatory mechanisms of the functional activity of membrane surface proteins and signaling complexes occur [12]. IP3 is also involved in such cellular processes, as cell growth, differentiation and survival, and the signaling regulation. Based on the results of our research, we can state that the impact of noise disrupts the above-mentioned processes leading to pathological events. However, intraperitoneal administration of beditin and mesesdin during exposure to noise has a regulatory effect on the content of IP3 after 7, 30 and 60 days of noise action.

At the same time, the Open field test revealed that noise exposure decreased locomotor activity and increased anxiety level in rats. So, the results presented are evident of noise-induced biochemical and behavioral dysfunction and highlight the regulative effects of the α2-adrenoblockers during chronic noise exposure. Our results showed rats of the noise group were much more anxious than the rats of 3rd and 4th groups. Based on our results, chronic administration of α2-adrenoblockers led to the limitation of stress-related anxiety in rats.

Our results clearly show the harmful effects of noise and the anxiolytic effects of α2-adrenoblockers by a number of entries to the periphery zone, as well. The rats in the OFT tried to stay close to protective walls instead of being exposed to danger in the open area [13]. The noise group rats' higher number of entries to the periphery zone and the less motility in it shows a high level of anxiety of the rats. Significant differences about number of entries to the periphery zone after 7, 30 and 60 days of noise action between the investigated groups demonstrated that the lesser number of entries to the periphery zone of the rats of 3rd and 4th groups was conditioned by the action of α2-adrenoblockers. Anyway, the effect of mesedin became more noticeable after 60 days of the noise impact and the positive manifestation of it was expressed by the lesser number of entries to the periphery zone of the 4th group rats. α2-adrenoblockers decreased a stress effect of the noise more and increased the rats' behavioral activity, correspondingly.

Environmental noise ameliorated conditions causing a noise duration-dependent deficit in regulatory mechanisms of the functional activity of membrane surface proteins and signaling complexes. The latter developed imbalance in the redox status, favoring oxidation of proteins and phospholipids and generating ROS. Moreover, environmental noise decreased locomotive, behavioral activity and increased anxiety.

We can conclude that α2-adrenoblockers, mesedin and beditin, provided a regulatory effect on the investigated values under the chronic acoustic stress, which was mostly expressed by mesedin. Recapitulating our data, it can be suggested, that the α2-adrenoblockers exhibit the antioxidant properties that prevents the intensification of LPO, while maintaining the level IP3 content in the brain mitochondria under the chronic noise conditions.

Declaration of conflict of interest

The authors declare that they have no conflict of interest.

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