



INFLUENCE OF CATAICINE ON LIPID PEROXIDATION PROCESSES OF RAT ORGANS IN THE DYNAMICS OF STRESS DEVELOPMENT

G. R. Abdullaev*, B. A. Niyazmetov and K. T. Almatov

Namangan State University, Physical Education Department, Namangan, Uzbekistan.
National University of Uzbekistan named after M. Ulugbek, Tashkent, Uzbekistan.

*** Corresponding Author: G. R. Abdullaev**

Namangan State University, Physical Education Department, Namangan, Uzbekistan.

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ABSTRACT

The emotional-pain stress increases lipid peroxidation in tissues of different organs of rats, the most significant increase in lipid peroxidation observed in the alarm stage. It should be noted, that under these conditions of stress lipid peroxidation increase particularly noticeably occurs in the thymus tissue, then the adrenal, brain and liver.

KEYWORDS: Cataicine, lipid peroxidation, stress, free radicals, mitochondria.

1. INTRODUCTION

At the present time, it is still actual the problem of stress and its effects on various functional systems of the body for modern physiology and medicine. Stress is seen as a way to achieve the body's resistance to extreme factors of various origins. However, stress can be factor having a damaging effect on the organs and systems, leading to the development of diseases.^[1,2] An important manifestation of the stress response and the adaptive adjustment is improving of the activity of the regulatory mechanisms involved in maintaining the optimal level of intensity of metabolic processes at the level of the whole organism.^[3,4] At the same time, undoubtedly, there must be organ-specific features in the implementation of the mobilization of the various mechanisms at stress and the problem of realization of stress-reaction at the level of individual organs and tissues remains relevant. In particular, the question remains little known about the changes of metabolic processes in the development of stress reaction.

It is well known, that one of the leading damaging factors in stress, determining the development of secondary changes in organs and tissues is the intensification of free radical oxidation, which, along with this is considered as one of the universal physiological processes - oxidation of biological substrates under the action of reactive oxygen species.^[5-8] The processes of free radical oxidation in the body cells occupy one of the main places to ensure homeostasis.

Their acceleration is accompanied by the formation of large quantities of toxic super oxide radicals, which often destroy the structure of cell membranes and intracellular organelles, and slows down the process of cell division.

Some of these radicals is removed by following enzymes: superoxide dismutase, catalase, and glutathione peroxidase and glutathione reductase. These enzymes generally maintain homeostasis and reactive oxygen species. In the case when these enzymes cannot efficiently remove oxygen radicals, and they begin to accumulate in the pathologic and physiologic concentrations of these highly reactive molecules have a cytotoxic effect, causing lipid peroxidation of DNA and protein molecules. One of the products of lipid peroxidation is malondialdehyde concentration, which begins to increase during stress. Lipid peroxidation includes activation and degradation of lipid radicals, incorporation into lipids preactivated molecular oxygen double bonds in the reorganization of polyunsaturated acyl lipids and as a consequence, degradation of membrane lipids and membranes themselves. Abnormalities in the structure of biological membranes induced by lipid peroxidation, consequently, affect their function, which determines irregularities in stress. This usually results in the modification of membranes complex functions involving both the lipid bilayer and membrane enzymes.

Analysis of the current scientific literature allows to conclude that a significant number of investigations devoted to the lipid peroxidation under stress, and at that the oxidative degradation of lipids of cells and tissues of different organs in the dynamics of stress is given less attention.

It is known that in recent years a lot of attention is given to research various drugs that can reduce or prevent the damaging effects of stress on the membrane tissue and cell organelles.

2. MATERIALS AND METHODS

Among different groups of preparations, we were attracted by catacine. Catacine was isolated from a plant of *Polygonum coriarium*: molecular weight – 7500 D. The preparation is a crystal substance of incarnadine color with an aromatic smell, good soluble in water, alcohol and other organic solvents.^[9] Catacine possesses antihypoxic action in various forms of hypoxia.^[9,11] On antihypoxic activity catacine is superior such antihypoxants as gutimine, isothiobarbamine and cavergal.^[9]

Catacine inhibits oxygen consumption in the body^[10,12] and the rate of electron transfer from substrate oxidation by the respiratory chain to molecular oxygen^[11], which takes metabolism in tissues more economical oxygen consumption regime.

Due to the abovementioned facts a substantial interest is studying of catacine's influence on lipid peroxidation of different organs of animals with chronic emotional pain stress. Such studies reveal perspectives of directed correction of the structure and function of cells under stress.

Objective: 1. To carry out a comparative study of lipid peroxidation intensity in the tissues of the thymus, adrenal glands, brain and liver of rats in the dynamics of the chronic emotional and pain stress development and reveal its tissue-specific features in background activity conditions. 2. To study the influence antihypoxant – catacine on free radical oxidation of lipids in various tissues under the dynamics of chronic emotional and pain stress development.

Experiments were carried out on outbred white male rats with initial weight of 180-200 g. Animals were kept under standard vivarium conditions, on a normal laboratory diet, under conditions of free movement, and 12 - hour light regime. Approximately two weeks before the start of the experiment rats were determined by emotional test of "open field". To do this, animals suddenly were placed in a dark box in the center of the field, which is the arena of a diameter of 1.5 m, divided into squares with sides of 20 cm, illuminated mirror incandescent 500 watt, suspended in the central part at the height of 60 cm from the floor (in the center of the "open field" is created illumination 1,000 lux).^[13-16]

Rats were immobilized, placed into narrow cages and carried electrocutaneous irritation paws and tail (50 Hz current frequency, the force 30W, the pulse frequency is

7 minute, pulse length of 0.5 seconds) for 30 minutes daily. The animals were divided into following groups: 1) intact animals, 2) animals were subjected to emotional and pain stress (receiving only oil solution within 3 months intraperitoneally), 3) rats exposed to emotional pain stress with preliminary introduction of catacine (treated with oil solution of catacine for 3 months in the peritoneum at 20 mg / 100 g body weight). Depending on the group, animals subjected electrocutaneous irritation within 1,2 and 3 weeks, 1, 2 and 3 months.^[14-16] Sacrifice of animals was carried out 1 hour after the last administration of drugs in the body of animals and 10 minutes after the last electrocutaneous irritation. Control group consisted of animals, which do not expose electrical stimulation.

The speed of the lipid peroxidation process in NADPH system determined by thiobarbituric acid – a micro method developed by Y.A. Vladimirov and A.I. Archakov.^[17] The incubation mixture with volume of 1 ml contained: 0.2 μM $\text{Na}_4\text{P}_2\text{O}_7 \times 10\text{H}_2\text{O}$; 1 μM NADPH; 0.012 μM Mohr salt ($\text{FeSO}_4 (\text{NH}_4)_2 6\text{H}_2\text{O}$); 50 μl of a brain, thymus, liver and adrenal glands tissues suspension; 50 μM Tris-HCl buffer, pH 7.4. All components of medium were carried into the control sample with the exception of NADPH. They were incubated at 37°C for 20 minutes under constant shaking. The reaction was stopped by adding 1 ml of 30% trichloroacetic acid and the precipitate was removed by centrifugation for 10 min at 6000 g / min. Then 0.3 ml (0.6M) and 1.2 ml (0,12M) of thiobarbituric acid were added to 1.5 ml of centrifugate. For color development tubes placed in a water bath at 100°C and incubated for 10 minutes. The intensity of the color formation was measured with a spectrophotometer at 535 nm. The quantity of malondialdehyde was calculated using a molar extinction coefficient equal to 1.56 / 10 cm. The rate of lipid peroxidation reaction expressed in nM of malondialdehyde/ mg of protein in hour.

3. RESULTS AND DISCUSSION

Our results showed that in the initial stage of stress (Week 1, in the alarm stage) lipid peroxidation in the tissues of the thymus, adrenal glands, brain and liver increased by 72.5; 54.0; 48.8 and 29.5%, respectively, compared with the level of standards (Table. 1). When analyzing the data, obtained in the course of experiments in animal models we found tissue-specific features of lipid peroxidation. Thus, the level of the final product of lipid peroxidation in thymus tissue compared adrenal, brain and liver was higher. This means that stress affects primarily the thymus and is consistent with the data.^[18,19]

Table 1: Influence of catacine on lipid peroxidation of different organs of rats in the dynamics of stress development ($M \pm m$; $n = 8 - 10$)

Organ	Duration of the experiments	Lipid peroxidation, nM/hour mg of protein		
		Healthy animals	Experimental animals	
			Control	Catacine
Thymus	1 week	3,21 $\pm$ 0,26	5,54 $\pm$ 0,35****	3,42 $\pm$ 0,34
	2 weeks	3,27 $\pm$ 0,23	7,49 $\pm$ 0,43****	4,04 $\pm$ 0,27**

	3 weeks	3,25±0,25	6,69±0,36****	3,83±0,31*
	1 month	3,19±0,28	6,20±0,38****	3,67±0,27
	2 mos.	3,24±0,25	6,10±0,41****	3,65±0,30
	3 mos.	3,20±0,23	5,77±0,33****	3,63±0,28
Adrenal	1 week	3,13±0,22	4,82±0,30****	3,43±0,24
	2 weeks	3,10±0,20	6,20±0,32****	3,61±0,23*
	3 weeks	3,14±0,25	5,93±0,32****	3,60±0,26
	1 month	3,12±0,23	5,60±0,33****	3,55±0,21*
	2 mos.	3,13±0,21	5,59±0,31****	3,48±0,29
	3 mos.	3,12±0,20	5,64±0,30****	3,43±0,22
Brain	1 week	3,43±0,22	5,10±0,28****	3,59±0,20
	2 weeks	3,37±0,24	6,14±0,34****	3,93±0,28
	3 weeks	3,34±0,21	5,66±0,29****	3,86±0,23*
	1 month	3,38±0,26	5,63±0,28****	3,81±0,21
	2 mos.	3,42±0,23	5,62±0,27****	3,78±0,24
	3 mos.	3,39±0,21	5,36±0,33****	3,77±0,23
Liver	1 week	3,46±0,29	4,48±0,34****	3,60±0,30
	2 weeks	3,33±0,28	5,52±0,56****	3,73±0,43
	3 weeks	3,40±0,27	5,38±0,49****	3,81±0,33
	1 month	3,39±0,29	5,42±0,38****	3,73±0,31
	2 mos.	3,36±0,23	5,15±0,40****	3,64±0,36
	3 mos.	3,42±0,35	5,12±0,37****	3,73±0,35

Note: here and other tables confidence factor is shown as: *P<0,05; **P< 0,02; ***P<0,01; ****P<0,001.

As further studies shown, with increase of stress action's duration increase of lipid peroxidation increases. For example, if after two weeks' process thymus, adrenal, brain and liver tissues increased up to 129.5; 100.0; 82.2 and 65.5%, 3 weeks of stress - to 105.5, 88.8; 69.5 and 58.4% respectively from the normal level. Thus, the maximum stress at the rate of formation of malondialdehyde in the tissue of different organs occurs in the alarm stage.

Further investigations showed that the stage of resistance (1 month stress), lipid peroxidation in tissues of different organs of rats compared to 3-week experiment, slightly slowed down, however, compared to the norm, was high. So, if after 1 month stress the process of lipid peroxidation in the tissues of the thymus, adrenal glands, brain and liver compared to control increased respectively 94.4; 79.5; 66.7 and 60.0%. This means that in the process of resistance increasing of lipid peroxidation in tissue organs compared to the alarm stage slowed.

Studies have shown that in exhaustion stage increasing of lipid peroxidation process slowed compared to the resistance stage. So, if, after 2 months of experiment lipid peroxidation process in the tissues of the thymus, adrenal glands, brain and liver compared to the control increases respectively - 88.2; 78.8; 64.5 and 53.2%, in the 3-month experiment 80.5; 70.9; 58.2 and 49.8%.

Thus, in conditions of chronic pain emotional stress lipid peroxidation increased in thymic, adrenal, brain and liver tissues. Action of stress on peroxidation is more pronounced in the thymus tissue. On formation of

malondialdehyde abovementioned organs located in the following sequence: thymus> adrenals> brain> liver.

The next stage of our work was to study the effect of antihypoxant - catacine to identify its possible protector or regulatory influences on oxidative processes in tissues under stress conditions. Studies on the effect of antihypoxant - catacine on lipid peroxidation of different organs in rats showed that catacine is very strong anti-stressor. Injection of catacine to organism of experimental rats, exposed to stress, significantly reduced the intensity of the process of malondialdehyde formation in the tissue of various organs and closer to that of normal animals. Thus, catacine is exogenous antioxidants having the ability to prevent the emergence of free radicals under stress and neutralize reactive oxygen molecules.

4. CONCLUSION

Analyzing the results, it can be concluded that the emotional - painful stress increases lipid peroxidation in tissues of different organs of rats, the most significant increase in lipid peroxidation observed in the alarm stage. It should be noted that under these conditions of stress increasing of lipid peroxidation particularly noticeably occurs in the thymus tissue, then the adrenal, brain and liver. After injection of catacine into the organism of experimental rats exposed to stress effects, significantly reduces the intensity of the process of malondialdehyde formation in the tissue, and is close to that of normal animals.

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