

Influence of Catacine on Phospholipase A₂ Activity of Rat Liver in the Dynamics of Chronic Emotional-Painful Stress Development

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Abstract At a chronic emotional-painful stress phospholipase A₂ hydrolytic activity noticeably increases in mitochondria of rats' liver. Phospholipase A₂ activity increases with increase of experiment's duration. In our opinion non-bilayer sites may appear in a membrane at a stress and it depends on heaviness of stress. Thus phosphatidylethanolamine is hydrolyzed by phospholipase A₂ quicker in comparison with phosphatidylcholine. Catacine considerably delays influence of negative effect of stress and consequently phospholipase A₂ activity decreases in respect of phosphatidylethanolamine and phosphatidylcholine and comes nearer to normal rates. It means that in stress conditions catacine increases non-bilayer sites in a cellular membrane of a living cell.

Keywords Rat, Chronic emotional-painful stress, Catacine, Liver, Mitochondria, Phosphatidylcholine, phosphatidylethanolamine, Free fatty acids, Phospholipase A₂

1. Introduction

At present a problem of stress and its influence on various functional systems of an organism is still actual problem for modern physiology and medicine [1-6].

Influence of emotiogenic factors breaks the hormonal and vegetative nervous systems homeostasis. In this regard an interest of scientists in stress problem constantly grows and it isn't casual as mechanisms of development of a stress and adaptation of an organism to it are still insufficiently studied, there aren't enough data on the metabolic changes happening in tissues and bodies in stress conditions. A stress is considered as a way of achievement of resistance of an organism to action of extreme factors of various geneses. At the same time, a stress can become the factor having the damaging effect on organs and systems, conducting to development of diseases [7]. The maximum preservation of intellectual and physical powers of human in the conditions of stress is a main problem of modern physiology and medicine. All above-stated facts are also defined the purposes and problems of the present research. Stress phenomenon and adaptation to it are one of the problems facing modern physiology. Despite a large number of the investigations and publications, devoted to various aspects of

studying of this problem there are no the exhaustive data containing complete idea of stress as physiological process, mechanisms of its appearance, formation, stress stability in a modern literature; in addition approaches for prophylaxis of negative consequences of stress are not developed. It is known that recently much attention is paid to investigations of various medicines, capable to reduce or prevent the damaging action of stress on tissue membranes and cellular organelles.

Under influence of phospholipases, first of all, hydrolysis reactions are progressed, but at the same time synthesis, transacylation and transalkylation reactions can go. As a result ratio of bilayer and non-bilayer sites in membranes changes considerably. Besides, under influence of phospholipases such physical characteristics of membranes as viscosity, hydrophobicity, and electric parameters can change. Change of structural and physicochemical properties, in turn, has to affect a functional condition of membranes: membrane-bound enzymes activities, permeability for various substances.

So, for example, it is shown that functioning of potential-dependent channels essentially depends on a charge of phospholipid molecules, making a direct environment of the channel [8]. Considering above-stated facts, it is reasonable to assume that it is connected with change of the phospholipid structure of mitochondrial membranes, proceeding with participation of endogenous phospholipases. Therefore, research of a problem of mitochondrial phospholipase participation in the enzymatic

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transformations of phospholipids in mitochondrial membranes of animals, subjected to stress at change of a functional condition of organelles would be very actual.

Among different groups of preparations we were attracted by catacine. Catacine was isolated from a plant of *Polygonumcoriariu*: 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 inhibits of oxygen consumption in an organism [10, 11] and suppresses the speed of electrons transfer from oxidation substrates via respiratory chain of mitochondria to molecular oxygen [12] that transfers a metabolism in tissues to more economical mode of oxygen consumption.

Due to above- stated facts, it is essential to give consideration for influence of catacine on phospholipase A₂ activity of mitochondrial liver at a chronic emotional and painful stress before and after an injection of catacine to an organism of animals.

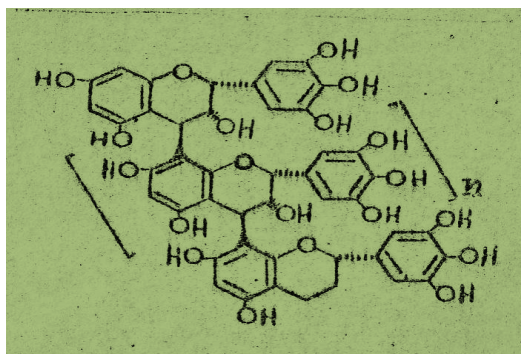


Figure 1. Catacine [9]

2. Material and Methods

Experiments were carried out on outbred white female rats, with an initial body mass of 180-200 g. Animals were contained in standard conditions of a vivarium, on usual laboratory diet, in the conditions of free movement and 12 hrs. light conditions. Approximately in two weeks prior to experiment at rats emotionality was determined by the test of an "open field".

High-emotional rats were used for research. Rats were immobilized, placing them in cramped cages and carried out electrodermal irritation of paws and a tail of the immobilized animals (frequency of current of 50 Hz, force 30 V, frequency of impulses 7 per minute, length of an impulse of 0,5 sec.) within 30 min. daily. Depending on group of animals, they were subjected electrodermal irritation within 1, 2 and 3 weeks and 1, 2 and 3 months [13-15]. In other series of experiments against the background of proceeding electrostimulation of a rat daily, it was received intraperitoneal preparation of catacine in a dose of 20 mg/kg, dissolved in vegetable oil, within 3 months. After administration of the studied preparation in an hour animals were subjected to electrodermal irritation. Animals were sacrificed in 10 min. after electrodermal irritation. Control

group of animals were received 1 ml of vegetable oil in the same terms.

The control group was made up by the animals that weren't subjected to electrostimulation.

Mitochondria were isolated by method of the differential centrifugation. The mitochondria of rat liver cells, when released by homogenization into water or isotonic NaCl solution, are altered to such an extent that they are not morphologically identifiable. When released into strong solutions of sucrose (0.8 to 1.0 M), however, they show the morphological and staining properties characteristic of normal intracellular mitochondria. Cytological studies of rat liver homogenates prepared in solutions of sucrose and other compounds at several concentrations indicate that the proportion of morphologically intact mitochondria, both extracellular and intracellular, increases with increasing concentration of solute and is related to the osmotic pressure of the medium. Sedimentation of mitochondria from homogenates prepared in 0.88 M sucrose and washing with the same medium does not result in any change in their morphological or staining properties. A procedure for the centrifugal fractionation of rat liver homogenates prepared in 0.88 M sucrose is described. By this procedure it is possible to obtain mitochondria in suspensions that are apparently free from other cellular components. In the experiments reported, 65 to 82 per cent of the succinoxidase activity of the original homogenate was recovered in the mitochondria, the remainder of the enzyme activity being present in a fraction containing nuclei and unbroken liver cells. On the basis of cytological observations and additional chemical data, it is concluded that mitochondria contain all of the succinoxidase present in the cytoplasm of the liver cell and probably all of the succinoxidase present in the entire cell. It is further estimated that 34 per cent of the total nitrogen and 19 per cent of the pentosenucleic acid (PNA) of whole liver can be accounted for in mitochondria. The succinoxidase Q02 (microliters of 02 per hour per mg. of nitrogen) and the ratio, micrograms of PNA phosphorus to mg. of nitrogen, are approximately 2100 and 13, respectively, for mitochondria. These values remain constant on repeated sedimentation and resuspension of mitochondria in 0.88 M sucrose. Submicroscopic particulate material of a diameter of approximately 100 mμ or greater was also isolated from 0.88 M sucrose homogenates of rat liver by means of prolonged centrifugation at high speed. Suspensions of this material are opalescent, show birefringence of flow, and contain no succinoxidase activity. The PNA content of the submicroscopic particles is characteristically high, the ratio, micrograms of PNA phosphorus to mg. of nitrogen, being approximately 58 for this fraction. It is estimated that the submicroscopic particles account for approximately 20 per cent of the total nitrogen and 50 per cent of the PNA of whole liver [16]. Liver, taken from several white rats, using as the environment of isolation of 0,25M of sucrose, 10mm of ethylene diaminetetraacetate (EDTA), 10mm of tris-HCl, pH-7,4 [17]. Ablution of mitochondria was carried out by centrifugation at 6000g within 20 min., using the isolation

environment without EDTA. All procedures on isolation of mitochondria were carried out at 0–2°C.

Activity of mitochondrial phospholipase A₂ was estimated on change of phospholipids structure during an incubation of organelles at 37°C [18, 19]. Reaction was begun with addition of 0,1 ml of cold suspension of mitochondria (immediately after their unfreezing) to 0,8 ml of the environment of incubation, thermostated at 37°C.

Degree of hydrolysis of mitochondrial phospholipids of liver of control and experimental animals under the influence of endogenous phospholipase A₂ was defined using the incubation environment, containing 0,25M of sucrose, 10mm of Tris-HCl buffers, pH 9,5 within an hour at 37°C of free fatty acids, lysophosphatidylcholine, lysophosphatidylethanolamine formation, and quantitatively determined by phosphorus. Specific activity of phospholipase A₂ was expressed by microgram/hour of mg of protein. Mitochondrial proteins were determined by Lowry *et al.* [20]. Statistical data manipulation of results was carried out by Fischer and Student's method.

3. Results and Discussion

Mitochondria have been used as a substrate in the research. Change of phospholipids in mitochondrial membrane is a

dignity of this approach.

Influence of stress on activity of phospholipase A₂ of rat liver mitochondria is given in tab. 1. At a stress, activity of phospholipase A₂ of liver mitochondria was increased. So, if after 1, 2 and 3 weeklong stress phospholipase A₂ activity of liver mitochondria by formation of free fatty acids was increased 1,44; 1,97 and 1,98 times from norm, in 1, 2 and 3 months of stress – 1,98; 1,91 and 1,96 times respectively.

In norm, phospholipase A₂ of liver mitochondria most efficiently hydrolyzed phosphatidylethanolamine, than phosphatidylcholine. So, if activity of an enzyme by lysophosphatidylcholine formation makes up 13,27±1,46 microgram/hour of mg of protein, by lysophosphatidylcholine formation – 19,67±2,00. These results conform to data of authors of other works [19, 20].

For substrate specificity definition at stress condition we defined phospholipase A₂ activity of liver mitochondria by lysophosphatidylcholine and lysophosphatidylethanolamine formation. So, if after 1, 2 and 3 weeklong stress activity of phospholipase A₂ of liver mitochondria of cerebrum in relation to phosphatidylcholine increased to 1,28; 1,80 and 1,78 times from norm, in relation to phosphatidylethanolamine – 1,67; 2,31 and 2,40 times respectively.

Table 1. Influence of catacine on phospholipase A₂ activity of rat liver mitochondria in dynamic of chronic stress development (M ± m; n = 8 – 10)

Period of experiment	Variants	Activity, microgram/h of mg of protein		
		Products of hydrolysis		
		Free fatty acids	lysophosphatidylcholine	lysophosphatidylethanolamine
1 st week	Control	3,44±0,32	13,27±1,46	19,67±2,00
	Experiment	4,96±0,64***	17,05±1,69****	32,85±2,33****
	Catacine	3,67±0,39	13,75±1,12	22,17±2,16
2 nd week	Control	3,58±0,55	13,30±1,80	19,45±1,97
	Experiment	7,04±2,87****	23,99±3,76****	44,97±6,45****
	Catacine	4,17±0,39	15,61±2,45	24,12±2,67
3 rd week	Control	3,64±0,59	13,38±1,88	19,50±2,88
	Experiment	7,21±1,98****	23,83±3,39****	46,76±9,76****
	Catacine	4,15±0,35	15,16±2,12	23,30±2,95*
1 st month	Control	3,50±0,84	13,22±1,97	20,05±3,90
	Experiment	6,92±1,90****	22,56±3,86****	45,79±8,73****
	Catacine	4,06±0,38	14,92±2,45	23,60±3,33
2 nd month	Control	3,64±0,97	13,49±1,94	19,98±3,97
	Experiment	6,96±1,76****	23,67±3,99****	48,35±9,80****
	Catacine	4,14±0,47	14,86±1,24	23,93±1,87
3 rd month	Control	3,59±0,88	13,49±2,20	19,95±3,65
	Experiment	7,23±1,98****	24,05±4,88****	47,56±7,95****
	Catacine	3,95±0,42	14,55±1,36	22,44±2,56

All analyses are the mean of triplicate measurements ± standard deviation. Means not sharing a common letter were significantly different at P ≤ 0.05.

Thus at stress, free fatty acids and lysoform phospholipids content raises. At stress the most preferable substratum for phospholipase A₂ of liver mitochondria, as well as in norm is lysophosphatidylethanolamine, than lysophosphatidylcholine. It is known that lysoform phospholipids like monoacyl compounds, possess more expressed hydrophily in comparison with diacyl forms and their accumulation in membranes promotes weakening of hydrophobic interactions.

In work [21] it was shown that, increase of lysophosphatidylcholine contents in membranes already twice of itself led to formation of new hexagonal type of packing of phospholipids molecules. It was resulted, first, by increase in membranes permeability, and secondly, strength of membrane proteins communication with hydrophobic area of membrane was broken that forwards their migration to polar surfaces of membrane.

It was noted also that with increase of lysophosphatidylcholine contents in membranes possibility of formation of intermolecular inserts in membrane proteins was increased. Free fatty acids are blocked transport of ATP and ADP through an internal membrane of mitochondria, inhibiting adenine nucleotide translocase [22-24].

The investigations conducted by us detected inhibiting action of catacine on hydrolytic activity of phospholipase A₂ of liver mitochondria of rats. In the alarm stage (in 2 weeks of stress) activity of phospholipase A₂ of liver mitochondria in relation to formation of free fatty acids increases to 1,97 times, in the presence of catacine enzyme activity was almost completely normalized, i.e. increased only to 1,16 times from norm respectively. In these experimental conditions catacine approximately identically inhibits activity of phospholipase A₂ of liver mitochondria in relation to lysophosphatidylcholine and lysophosphatidylethanolamine. So, if in absence of catacine enzyme activity increases on hydrolysis of lysophosphatidylcholine and lysophosphatidylethanolamine to 1,80 and 2,31 times, in the presence of catacine – in 1,17 and 1,24 times respectively.

In the exhaustion stage (in 2 months of stress) activity of phospholipase A₂ of liver mitochondria increases by free fatty acids formation to 1,93 times, in the presence of catacine – to 1,14 times. Under these experimental conditions activity of phospholipase A₂ of liver mitochondria in absentia of preparations by hydrolysis of lysophosphatidylethanolamine and lysophosphatidylcholine increased to 2,42 and 1,75 times from norm, in the presence of catacine – to 1,20 and 1,10 times respectively. Thus, catacine almost completely removed a negative effect of chronic emotional and painful stress in relation to liver mitochondria in an organism of experimental animals. So, it is known that changes of a functional condition of internal membrane of mitochondria are accompanied both reorganization (phospholipid structure disturbance, the relations of lipids/protein, a lipid/lipid, phospholipid/protein, phospholipid / lysophospholipid), and change of physical and chemical characteristics (membrane potential, electric resistance of a membrane, permeability for ions) [25].

4. Conclusions

At a chronic emotional and painful stress hydrolytic activity of phospholipase A₂ raises in mitochondria of rats' liver. With increase of experiment duration activity of phospholipase A₂ increase was enhanced. In our opinion at stress in a membrane it was appeared non bilayer sites and it depends on heaviness of stress. Any defects of the bilayer organization are preferable sites for hydrolytic action of phospholipase. Thus, phospholipase in comparison of phosphatidylcholine rapidly hydrolyzed lysophosphatidylethanolamine. In general, phosphatidylcholine is the least preferable substratum than lysophosphatidylethanolamine for many intracellular phospholipases [26, 27]. Catacine essentially slowed down an action of negative effect of stress; therefore activity of phospholipase A₂ in relation to lysophosphatidylethanolamine and phosphatidylcholine decreased and came nearer to normal indicators.

At stress, in the presence of catacine oxidative phosphorylation and ADP/O normalized. That means stress has not influence on organism functions.

It means that catacine in the conditions of stress raises bilayer sites in mitochondrial membrane. Thus, defects of the bilayer organization are preferable sites for action of phospholipases. On the contrary, the increase in bilayer orderliness conducts to reduction in the rate of its hydrolysis by phospholipases.

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