



## REGULATION OF PHOSPHOLIPASES ACTIVITY OF MEMBRANES OF LIVER MITOCHONDRIA

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### ABSTRACT

It has been studied the influence of catacine *in vivo* conditions on activity of PLA<sub>2</sub>, lysophospholipase A1 and phospholipase D of membranes of rat liver mitochondria. It has been established that catacine inhibits the hydrolytic activity of PLA<sub>2</sub>, lysophospholipase A1 and phospholipase D of membranes of liver mitochondria. Catacine increases the formation of "bilayer" structures in the membrane. "Bilayer" is a poor substratum for phospholipases and lipid peroxidation.

### KEYWORDS:

### 1. INTRODUCTION

At present, an interest to study of phospholipases in membrane structures has increased significantly. There are data that membrane phospholipases are not only "nurses", leading to degradation of damaged membrane sites and whole organoid, but also regulators of the phospholipid composition and functional activity of membranes.<sup>[1,2]</sup> Intensive investigations of recent years on membrane lipolytic enzymes from various membranes of animal cells have led to the important conclusion that the main purpose of such enzymes is the regulation of the phospholipid composition, the integrity of the membrane structure and functions.<sup>[3,4,5]</sup> It is known, that under the influence of phospholipases, primarily hydrolysis reactions, but simultaneously reactions of synthesis, transacylation and transalkylation can take place.<sup>[2]</sup> As a result, the ratio of "bilayer" and "nonbilayer" sites in membranes varies significantly. In addition, under the influence of phospholipases, the physical characteristics of membranes change: viscosity, hydrophobicity, electrical parameters. Alteration in structural and physical-chemical properties, in turn, should affect in the functional state of membranes: the activity of membrane-bound enzymes, permeability for various substances, etc.

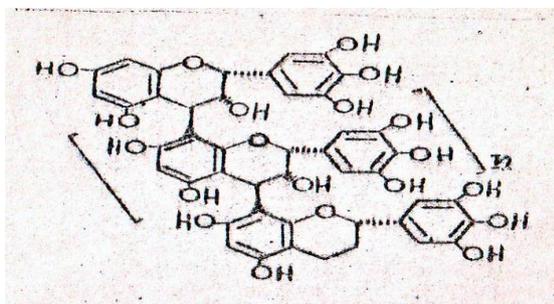
Mitochondria are the most important intracellular structures that play a significant role not only in the functioning of human and animal cells under normal conditions, but also in various pathological processes.<sup>[6]</sup> The study of the role of mitochondria in the vital activity of cells is complicated by the multiplicity of functions performed by mitochondria and the intertwining of

intracellular and external factors that determine the interaction between mitochondria and other intracellular structures.<sup>[2,3]</sup> This suggests that mitochondrial phospholipases are endogenous regulators that support homeostasis in mitochondria, regulate their functional activity under changing physiological conditions and play a role in adaptation processes to stressors.

The preservation of human life, maximal preservation of his mental and physical power - the main problem of modern physiology and medicine. Modern molecular physiology should achieve a level of development that would ensure the reliability of experimental biology. It should be borne in mind, however, that despite the successes of modern biological sciences, many processes occurring at the organismic, cellular and molecular levels have not been studied sufficiently. It is necessary to enrich, expand and generalize the modern ideas of physiology, biophysics and biochemistry about the physiological alterations in the organism. One of the main problems of molecular physiology, pharmacology and medicine is the search for new drugs that increase the organism's life resistance to various negative factors. Of course, along with the already known drugs, new drugs are being developed that are more effective. The category of such substances include catacine isolated from *Aconogonon coriarium*. However, up to the present time, questions concerning influence of catacine on phospholipase activity of mitochondrial membranes remain studied insufficiently.

It is known that catacine has an antihypoxic effect at various forms of hypoxia.<sup>[7,8,9]</sup> According to antihypoxic

activity, catacine is superior to known antihypoxanthos gutimine, isothiobharmine and cavergal.<sup>[8]</sup> At chronic emotional stress catacine inhibiting the activity of phospholipases, stabilizes the membranes.<sup>[10,11]</sup> In this regard, the study of reactions involving PLA2, lysophospholipase A1 and phospholipase D of mitochondrial membranes of liver in the presence of catacine is very important in connection with the



## 2. MATERIALS AND METHODS

Wistar rats weighing 240-250 g were used in investigations. Animals were kept in vivarium at ~ 20-25 °C in standard cells without restriction of water and food. Catacine was injected into the body of healthy animals intraperitoneally 50 mg/kg of body weight and after one hour, mitochondria were isolated from the brain, heart, and liver and determined the activity of phospholipases.

Mitochondria were isolated from liver of white rats according to the generally accepted method of differential centrifugation<sup>[12]</sup> with some modifications.<sup>[13]</sup> Rats were sacrificed and the isolated liver was immersed in a beaker with isolation medium of the following composition: sucrose 300 mM, tris-HCl-10 mM, EDTA 2 mM.<sup>[13]</sup> The organs were cleaned of foreign tissue (fat, connective tissue), then their weight was determined by weighing, cut by scissors and placed in a tenfold volume compared to the organs of the pre-cooled isolation medium and homogenized for 30-40 seconds in a homogenizer with Teflon pestle. For homogenization, it was used a combined homogenizer with a Teflon pestle having a threaded knife block with a tissue supplying device. An important condition for homogenization is the temperature of the solutions and used materials, which should not exceed 0° – + 4°C, because these procedures result in partial liberation of lysosomal enzymes and a decrease pH.

The obtained homogenate was mixed and poured into a centrifugal cup, centrifuged for 7-10 minutes at 1400-1500 rpm (liver) 1500-2000 rpm (heart and brain) (at 0° – + 2°C) to remove cell debris and nuclear fraction. The obtained fraction was poured into a clean centrifuge beaker. The supernatant was centrifuged at 6000 rpm (liver for 15 min, brain and heart 20-25 min). Mitochondria obtained in the sediment were suspended by 0.1-fold volume of a cold isolation medium without EDTA, taken to the raw mass of the initial tissue

problem of adaptation of the organism to various physiological and pathological conditions.

However, there are no fundamental investigations in the literature concerning alterations in the activity of phospholipases and the mechanisms underlying them, depending on the action of catacine.

Catacine is isolated from *Aconogonon coriarium*.<sup>[8]</sup> Molecular weight -7500 D. The preparation is a crystalline substance of light red color with a fragrant odor, highly soluble in water, alcohol and other organic solvents.

weighed to a density of about 15-20 mg of the mitochondria (heart) protein and 30-40 mg of the mitochondrial protein (liver and brain) in 1 ml of the suspension. The mitochondrial protein was determined by the method of Lowry et al. [1951].

The activity of mitochondrial PLA2, phospholipase D, and lysophospholipase A1 was assessed by alterations in the phospholipid composition and metabolites of mitochondria during incubation of organelles at 37°C.<sup>[2]</sup> The reaction was started by addition of 0.1 ml of a cold suspension of mitochondria (immediately after their thawing) to 0.8 ml of incubation medium, thermostated at 37°C as incubation at 37°C with constant stirring with a magnetic stirrer. The phospholipids were separated by micro layer chromatography and quantified by phosphorus as described above.

The degree of hydrolysis of phospholipids of rat liver mitochondria under the influence of endogenous PLA2 was determined by using an incubation medium containing 0.25 M sucrose, 10 mM Tris-HCl buffer (pH 9.5) for 1 hour at 37°C as total phospholipids hydrolyzed and individual fractions - phosphatidylcholine, phosphatidylethanolamine (cephalin), cardiolipin, phosphatidylserine, phosphatidylinositol and by the formation of free fatty acids and lysoforms of phospholipids.

Activity of lysophospholipase A1 of rat liver mitochondria was determined on the hydrolysis of total phospholipids and lysophospholipids and by the formation of glycerophosphocholin, glycerophosphoethanolamine and free fatty acids in the incubation medium as described above, at pH 6.0.<sup>[15,16,2]</sup> Mitochondria were incubated for 1 hour at 37°C, then 3 ml of chloroform-methanol (1:2) was added, intensively stirred, then centrifuged and the centrifugate evaporated on a rotary evaporator, the dry residue was dissolved in 50 µl of chloroform-methanol-water (1:2:0.8) and

analyzed by thin layer chromatography.

Two-dimensional chromatography was used for the separation of phospholipids in chloroform-methanol-28% ammonia (65:25:5) solvent systems in the first direction, and chloroform-acetone-methanol-acetic acid-water (6:8:2:2:1) in second, lysophosphatidylcholine and glycerophosphocholine, glycerophosphoethanolamine and lysophosphatidylethanolamine were separated by one-dimensional chromatography in the system: chloroform-methanol-3M trichloroacetic acid-water (4:6:2:1). The values of *R<sub>F</sub>* in this system were 0.8 for lysophosphatidylcholine and 0.4 for glycerophosphocholine. Identification of glycerophosphocholine was carried out on the basis of a positive reaction with Dragendorff reagent and also with the help of a "witness" obtained as a result of hydrolysis of lysophosphatidylcholine, phospholipase A1 from venom of large hornet. Quantitatively, phospholipids and glycerophosphocholine (or glycerophosphoethanolamine) were determined by phosphorus as described above. For control, the initial content of phospholipids, free fatty acids, glycerophospholipids was determined before incubation.

Hydrolytic activity of mitochondrial phospholipase D was determined by the formation of phosphatidic acid,

choline or ethanolamine in 0.25 M sucrose and 2 M Tris-HCl buffer (pH 7.4) after incubation at 30°C for 60 min. The reaction was stopped by the addition of 1.5 ml of chloroform. After centrifugation, the aqueous layer was separated and the choline content was determined with an iodine reagent.<sup>[15,16,2]</sup> Before determining the ethanolamine content in the aqueous layer, it was preheated in a boiling water bath for 15 minutes (this procedure is necessary for the denaturation of proteins that interfere with the determination of ethanolamine). After centrifugation, the ethanolamine concentration was determined by the ninhydrin reagent. For control the initial content of choline and ethanolamine in the incubation mixture was determined before incubation. Specific activity of phospholipases expressed in µg/h ml of protein.<sup>[15,16,2]</sup>

### 3. RESULTS AND DISCUSSIONS

Table 1 shows the results of measuring the hydrolytic activity of phospholipase A2, lysophospholipase A1 and phospholipase D of the mitochondria of the liver after the administration of catacine into the body of rat.

**Table 1: Influence of catacine on the activity of phospholipases of rat liver mitochondria (M±m; n = 8-12)**

Readings	Hydrolysis products	Activity, µg/h mg protein		
		Control	Catacine	%
PLA2	Lyso phospholipid	46,12 ± 3,55	37,77±4,02*	75,4
	Free fatty acids	4,54 ± 0,43	3,28±0,22***	72,2
	Lysophosphatidylcholine	11,97 ± 1,86	9,21±0,65**	77,0
	Lysophosphatidylethanolamine	19,56 ± 1,28	12,79±0,87****	65,4
	%	163,4	138,9	
	Lysocardioliipin	17,49 ± 2,56	10,53±0,66****	60,2
	%	146,1	114,3	
Lysophospholipase A <sub>1</sub>	Glycerophospholipid	9,54 ± 0,33	6,37 ± 0,38****	66,8
	Free fatty acids	1,26 ± 0,11	0,86 ± 0,12****	68,2
	Glycerophosphocholine	4,77 ± 0,15	3,08 ± 0,08****	64,7
	Glycerophosphoethanolamine	3,77 ± 0,21	2,28 ± 0,16****	60,4
Phospholipase D	Common phospholipid	12,55±0,64	10,67±0,30**	85,0
	Phosphatidic acid	9,14±0,40	7,99±0,20**	85,0
	Choline	0,61±0,08	0,55±0,06	91,2
	Ethanolamine	1,12±0,09	0,98±0,08*	81,3

As it can be seen, catacine reduces mitochondrial PLA2 activity of liver compared with the control. So, the total fractions of lysophospholipids with catacine decrease 1.25 times, free fatty acids - 1.28 times. In control, phosphatidylethanolamine and cardioliipin are the most preferred substrate for PLA2 of liver mitochondrial membranes. The activity of PLA2 in comparison with phosphatidylcholine with respect to phosphatidylethanolamine and cardioliipin increases only 1.63 and 1.46 times and agrees with the data of the authors of the works.<sup>[16,17]</sup> After the introduction of catacine in the body, the hydrolytic activity of PLA2 decreases, but the nature of substrate specificity persists. In this case, the activity of PLA2 with catacine by

hydrolysis of phosphatidylcholine, phosphatidylethanolamine and cardioliipin decreases, respectively, in 1.23, 1.34 and 1.39 times. In this case, the activity of PLA2 in comparison with phosphatidylcholine with respect to phosphatidylethanolamine and cardioliipin decreases 1.37 and 1.20 times.

After the introduction of catacine into the animals' organism in liver mitochondria, the activity of lysophospholipase A1 by the formation of glycerophospholipids, glycerophosphoethanolamine, glycerophosphocholine decreases, respectively, by 1.33, 1.40 and 1.31 times the control level and the

accumulation of free fatty acids by 1.31 times. This means that catacine inhibits the activity of lysophospholipase A1 of mitochondrial membranes. It is known that usually lysophosphatidylcholine and free fatty acids in membranes are present in minor concentrations.<sup>[2,19]</sup> An increase in the concentration of this lysophospholipid and fatty acids contributes to the disruption of the structure of biological membranes.<sup>[2,3]</sup> However, at the present time such properties of low concentrations of lysophosphatidylcholine have been discovered, such as the ability to enhance or even induce cellular proliferation, stimulate adhesion and differentiation of lymphoid cells, have a mitogenic effect on macrophages, activate T-lymphocytes, initiate monocyte chemotaxis, reduce myocardial sensitivity to cholinergic effects, inhibition of the contractility of the smooth muscle tissue of the arteries, etc.<sup>[19]</sup>

The introduction of catacine into the animal body leads to a slight decrease in the catalytic activity of the phospholipase D mitochondria by hydrolysis of total phospholipids 1.15 times the control level, phosphatidic acid production 1.15 fold; phosphatidylcholine 1.09 times, phosphatidylethanolamine 1.19 times. Catacine does not affect the substrate specificity of phospholipase D of liver mitochondria. Under these conditions both in control and phospholipase D, a more preferred substrate for liver phospholipase D is phosphatidylethanolamine than phosphatidylcholine, which agrees with the data of the works.<sup>[18,2]</sup>

Thus, catacine inhibits the hydrolytic activity of PLA2, lysophospholipase A1 and phospholipase D of liver mitochondrial membranes. Catacine increases the formation of "bilayer" structures in the membrane. It is known that the activity of lipolytic enzymes is largely determined by the structural state of the membrane, the presence of defects and non-bilayer structures in it. That is why suspensions of phospholipids in water-organic systems are used as substrates for the study of phospholipase of various origins. "Monolayers" are the preferred sites for the action of the phospholipase, and an increase in the order of the "bilayer" leads to a decrease in the rate of hydrolysis of the phospholipase.<sup>[20,21]</sup>

At the level of mitochondrial membranes, observed regularities – hydrolase-transferase reactions of phospholipases are the main links of a single mechanism of compensatory-adaptive reactions of the organism to various effects and physiological states of the organism. The components of the lipolytic system of mitochondrial membranes are interrelated with each other; they carry out enzymatic transformations of phospholipids, which lead to a change in the structural and functional parameters of the membranes. On the other hand, the activity of the phospholipases, as well as the type of catalyzed reaction (hydrolysis, transacylation, transalkylation) is largely determined by the structural state of the membrane.<sup>[2]</sup> Analyzing the obtained results, it can be concluded that catacine can be one of the

perspective approaches for the development of pathways of increase of the resistance of the organism to unfavorable environmental conditions, in particular, hypoxia, ischemia and the action of various toxic substances and poisons.

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