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RESEARCH OF BENZONAL'S PROTECTIVE EFFECT ON THE BACKGROUND OF COBRA VENOM (NAJA NAJA OXIANA ECHWALD) EFFECT ON THE MITOCHONDRIAL PHOSPHOLIPID CONTENT

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ABSTRACT
In this article it has been studied introduction of *Naja naja Oxiana* Echwald poison into rat body. It has been defined raising of quantity of phosphatidylcholine and phosphatidyl serine, falling of quantity of phosphatidyl ethanolamine, cardiolipin, phosphatidyl inositol and phosphatide acids in heart mitochondria. After introduction of poison into rat body they were given benzonal. The quantity of phosphatidylcholine, phosphatidyl ethanolamine and cardiolipin came up to healthy animals' level. Benzonal increased phosphatidyl serine and phosphatidyl inositol quantity more than healthy animals' level.

1. INTRODUCTION
Previously we have showed that administration of benzonal into animals on the background of cobra *Naja naja Oxiana* Echwald poison increases the lifespan of animals.[1,2] Increasing of animals' resistance to cobra venom depends on the period of benzonal administration: the sooner after the inflow a drug is administered, the greater the duration of the life of animals. After introduction of benzonal on the background of cobra venom influence rate of oxygen consumption is reduced compared with control animals. This means that benzonal regulates the organism's respiratory function, creates favorable conditions for work at air hunger. In consideration of obtained results, it is interesting to study the influence of benzonal on the background of effect of cobra venom on membrane phospholipid metabolism of the heart mitochondria of rats.

2. MATERIALS AND METHODS
Experiments were carried out on white male rats of 120-130 g body weight, kept on a standard diet of the vivarium. The animals were divided into three groups of 16 animals each. The first (control group) and the second groups were injected cobra venom intramuscularly at a dose of 160 mg/kg body weight. After 2 minutes, the animals of the second group were administered additionally benzonal - 50 mg/kg of body weight. The third group received physiological (salt) solution (intact group). After 15 min, the samples of 1996 venom collection were used; they were dried in desiccators over calcium chloride. Isolation of mitochondria from cardiac tissue was performed as previously described method.[3] Phospholipids of mitochondria were extracted by method of Cates.[4] The phospholipid composition of mitochondria analyzed by two-dimensional thin layer chromatography. Protein was determined by Lowry.[5]

3. RESULTS AND DISCUSSION
After the administration of benzonal on the background of cobra venom effect the same type of character of changes occurs in the content of phosphatidylcholine, phosphatidylethanolamine and cardiolipin in heart mitochondria as the treated and untreated rats by benzonal. Percentage deviation of their content from the norm at the administration of benzonal is minimal (Table.1). So, if at the poisoning of animals by cobra venom in heart mitochondria phosphatidylcholine content increases to 1.51 times from the level of standards, phosphatidylethanolamine and cardiolipin decrease to 1.42 and 1.82 times, respectively, in the presence of benzonal phosphatidylcholine content increases only 1.41 times, cardiolipin and phosphatidylethanolamine is reduced by 1.55 and 1.22 times. Thus, benzonal contributes of the maintenance in cardiac mitochondria of poisoned animals a constant level of the main phospholipids. According to some authors[6-10]. of particular importance in maintaining of the structure of mitochondrial enzymes is cardiolipin, which plays an important role in the processes of oxidative phosphorylation.[6, 11] Evidently cardiolipin is essential for the catalytic function of enzymes and plays a crucial role in the coupling of electron transport processes.

Table 1
Effect of cobra venom on phospholipid content of rat heart mitochondria and protective effect of benzonal (% of total phospholipids M ± m, n = 16)

Phospholipids	Intact animals	Animals poisoned by cobra's venom	
		Benzonal	Control
Phosphatidylcholine	36,00±0,41	41,00±0,71	54,30±0,37
phosphatidylethanolamine	20,30±0,32	17,20±1,72	11,70±0,48
Cardiolipin	8,72±0,27	6,82±1,23	1,56±0,60
phosphatidylserine	2,71±0,27	5,38±0.28	3,46±0,91
phosphatidylinositol	3,81±0,36	7,41±0,11	2,75±0,57
Phosphatidic acid	7,00±0,28	2,09±0,20	5,00±0,32
Non identified phospholipids (NIPL)	21,46±1,97	20,10±2,33	21,22±2,03
Phosphatidylcholine/ phosphatidylethanolamine	1,78	2,36	4,64
Neutral phospholipids	36,00±0,41	41,00±0,71	54,30±0,37
Acidic phospholipids (APL)	33,82±1,23	32,08±2,40	22,91±2,28
Ratio of NIPL/APL	1,06	1,27	2,37

At poisoning of animals by cobra venom the content of phosphatidylinositol reduced to 1.28 times from the level of norm in heart mitochondria and the level of phosphatidylserine, on the contrary, increased to 1.28 times. After the benzonal administration on the background of cobra venom effect phosphatidylinositol level exceeds the norm by 1.95 times, and phosphatidylserine content much more increased (1.98 times). Hereby, benzonal accelerates the synthesis of phosphatidylinositol and phosphatidylserine in cardiac mitochondria. It is known that phosphatidylinositol - is the most common representative of phosphoinositides. It is a big part of thephosphoinositides sum, extracted from different organs and tissues. They play an important role in the organization and operation of cellular structures.[12,13]

Phosphoinositides involved in the intracellular signaling, and are a source of universal low-molecular regulators: inositol-1,4,5-triphosphate and 1,2-diacylglycerol[14]. The first mobilizes by quantum manner Ca^{2+} ions release from intracellular stores of non-mitochondrial nature, mainly of the endoplasmic reticulum. The second regulates activity of enzymes of protein kinase C. At various forms of cancer it was observed decrease of phosphoinositides corresponding stage of the disease in the blood of patients.[15] Inositol bearing six phosphorus atoms (IP_6) reduces the growth of transplanted mouse fibrosarcoma that increases the survival of mice with tumors, reduces the number of metastases in the lung tissue[18], prevents and inhibits breast carcinoma.[19-22] prostate.[16] and intestine.[22] IP_6 reduces the population of erythro-leukemic K-562 cells to 19-36% and increases the synthesis of hemoglobin.[23]

It is an allosteric effector of hemoglobin and is able to modify the oxyhemoglobin dissociation curve.[24] Erythrocytes artificially loaded in IP_6 assimilate and transport more oxygen, which favorably affects to the function of myocardium[25]. In the presence of human IP_6, neutrophils enhance of superoxide anion outflow.[26]. IP_6 is an important antagonist of Ca^{2+}, that in excess use can be intensively accumulated in the aortic wall. In this regard, other Ca^{2+} antagonists are less effective compared with IP6.[27] It stimulates the uptake of $^{45}Ca^{2+}$by purified mitochondria isolated from rat liver.[28] IP6 is one of the most effective natural antagonists.[29, 30]

At poisoning of animals by cobra venom phosphatidic acid content decreases 1.29 times from the normal level in the cardiac mitochondria. Reduction of given phospholipid level apparently is due to the action of phospholipase A_2 of cobra venom or weakening of the synthesis. Administration of benzonal on the background of cobra venom effect causes a significant reduction in the content of phosphatidic acid (2.89 times). It is known that one of the central metabolites of phospholipid metabolism - phosphatidic acid is synthesized in the outer mitochondrial membrane. Reduction of phosphatidic acid content under the influence of benzonal associated with inhibition of the hydrolytic activity of phospholipase D or suppression of its synthesis.

After the administration of benzonal on the background of cobra venom effect changes were found in cardiac mitochondria in a ratio of phosphatidylcholine/phosphatidylethanolamine. Thus, in the absence benzonalthis coefficient increases by 2.6 times in the presence - only 1.32 times.

At poisoning of animals by cobra venom in cardiac mitochondria neutral phospholipid content increases to 1.51 times from the level of standards, and the level of acidic phospholipids, on the contrary, decreases to 1.32 times. After the administration of benzonal the background of poison action acidic phospholipid content is not differs from the norm, meanwhile the level of neutral phospholipids approaches to its level.

4. CONCLUSION

Analyzing the obtained results, we can conclude that benzonal removes action of cobra venom against the major phospholipids: phosphatidylcholine, phosphatidylethanolamine and cardiolipin of cardiac mitochondria of rats. Under the influence of benzonal

phosphatidylinositol and phosphatidylserine content significantly increases, and phosphatidic acid level decreases.

Thus, on the background of cobra venom effect benzonal forwards to maintain in cardiac mitochondria a constant level of neutral and acidic phospholipids as two structurally and functionally distinct categories of phospholipids, necessary to maintain the physicochemical properties of the functioning membrane. Prevention of possible oscillations in the qualitative and quantitative content of phospholipids and increase of the level of phosphatidylinositol in biological systems is one of the main conditions to ensure their normal physiological status.

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