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AN INVESTIGATION OF THE PHOSPHOLIPASE ACTIVITY OF THE VENOM OF THE SPIDER *Eresus niger*

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It has been shown previously that the venom of the spider *Eresus niger* effectively blocks the nerve-muscle synapses of the frog, interacting at the level of the presynaptic membrane [1]. An analysis of the action of the spider venom on synaptic transfer has revealed much in common with the action of the presynaptic neurotoxins of snakes, which possess phospholipase activity [2]. In view of this, we have studied the phospholipase activity of the venom of the spider *Eresus niger*.

Lyophilized *Eresus niger* venom was obtained from the Central Asian Zoological Combine. Phospholipase activity was determined by the potentiometric titration method [3]. The reaction medium contained: phosphatidylcholine or phosphatidylinositol — 1.5 mM; NaCl — 150 mM; $CaCl_2$ — 10 mM; Triton X-100 — 8 mM.

On the use of phosphatidylcholine and phosphatidylinositol as substrates the venom hydrolyzed the phosphatidylcholine more effectively, with a specific activity of 2.6 μmole/min per 1 mg of protein at the rate of 0.24 μmole/min. The hydrolysis of phosphatidylcholine took place optimally at pH 8.0. With an increase in the concentration of the spider venom, the rate of hydrolysis of phosphatidylcholine increased proportionally (Fig. 1a). The curve of the dependence of the effiency of hydrolysis on the concentraion of the substrate had the form of a hyperbola (Fig. 1b) and was described by a Michaelis–Menten equation. The Michaelis constant K_M and the maximum rate of the reaction V, calculated on the basis of a Lineweaver–Burk graph, were 2.55 mM and 7.7 μmole/min per 1 mg of protein, respectively.

The efficiency of the hydrolysis of phosphatidylcholine depended on the presence of calcium ions in the medium, and at 10 mM the rate of hydrolysis doubled as compared with a control. Sr^{2+}, Mg^{2+}, Ba^{2+}, and Co^{2+} ions had practically no influence on the efficiency of hydrolysis.

The enzymatic reaction was conducted at pH 8.0, the total amount of protein was 1 mg, and the amount of substrate 20 mg, dissolved in 10 ml of ether, 1.5 ml of 0.1 M Tris buffer, and 0.2 ml of 0.1 M $CaCl_2$. The reaction was continued until

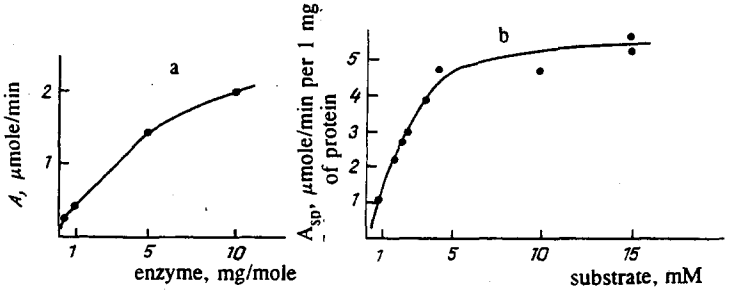

Fig. 1. Kinetics of the hydrolysis of phosphatidylcholine by the phospholipase from the venom of the spider *Eresus niger*: a) dependence of the activity of the enzyme, A, on its concentration; b) dependence of the initial rate of the reaction on the concentration of the substrate (fixed pH value of 8.0; incubation temperature 37°C).

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the substrate had been hydrolyzed completely. TLC was conducted on silica gel in the chloroform–methanol–20% NH_4OH (70:30:5) system. The hydrolysis products were separated as described in [4].

Analysis of the products of the hydrolysis of phosphatidylcholine with the aid of GLC showed that the venom split out predominantly the fatty acid residues in the sn-1 position:

Source of phospholipase	16:0	18:1	18:2	ES	EU
Bee venom					
obs.	20,4	25,0	54,6	20,4	79,6
sn-1	38,8	23,2	38,0	38,8	61,2
sn-2	2,0	26,8	71,2	2,0	98,0
Spider venom					
sn-1	36,9	24,1	39,0	36,9	63,1

Thus, the results presented show that the venom of the spider *Eresus niger* possess phospholipase activity, which possibly plays an important role in the blocking of synaptic transfer.

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