AN INVESTIGATION OF THE VENOM OF RENARD'S VIPER Vipera ursinii renardi CH. V. PHOSPHOLIPASE A_2 WITH ANTICOAGULANT PROPERTIES

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The anticoagulant action of snake venoms is usually connected with their influence on the level of fibrinogen and, accordingly, with the activity of the proteolytic enzymes contained in the venom [1]. At the same time, it is assumed that the anticoagulant effect of the venoms can be explained by the breakdown of the phospholipids, which play the role of procoagulants in the process of blood clotting. On this basis, anticoagulant properties of phospholipases A_2 have long been predicted [2] but only recently have pure enzyme preparations of phospholipase A_2 actually capable of prolonging the time of recalcification of citrate plasma been obtained from venoms [3].

The list of homogeneous phospholipases A_2 possessing anticoagulant properties is growing continuously [4]. In this connection, it appeared of interest to evaluate the anticoagulant action of two phospholipases A_2 which we have obtained in the pure form from the venom of Renard's viper Vipera ursinii renardi Ch. [5]. To measure the recalcification time we used citrate plasma from human donor blood to which was added the material under test diluted with physiological solution: In control experiments, physiological solution was added in place of the enzyme solution.

The diagram (Fig. 1) demonstrates the dose dependence of the anticoagulant action of phospholipase A_2 III-3. The other phospholipase A_2 (III-2) from this venom, which differs in a number of properties, structure, dimensions, and pI values [6], possessed no anticoagulant effect.

It must be mentioned that all known anticoagulant phospholipases A_2 from snake venoms are characterized by a pI value of 9.5; nevertheless, basicity is not considered as a necessary condition for the manifestation of the anticoagulant action of an enzyme, since other basic phospholipases A_2 of snake venoms have been described in the literature which are not capable of acting as anticoagulants [9]. This situation is completely

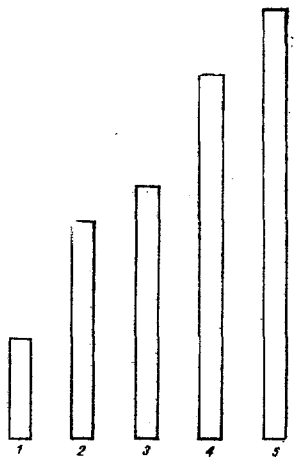

Fig. 1. Anticoagulant activity of phospholipase A_2 III-3. Reaction mixture (0.3 ml): 0.1 ml of citrate plasma from human donor blood, 0.1 ml of 0.277% $CaCl_2$, and 0.1 ml of the material under test. 1) Control (0.1 ml of plasma + 0.1 ml of $CaCl_2$ + 0.1 ml of physiological solution). Additions of phospholipase A_2 III-3: 2) 10 μg; 3) 25 μg; 4) 50 μg; 5) 100 μg.

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confirmed by our results revealing anticoagulant activity in phospholipase A_2 III-3 with pI 6.73 and not detecting it in phospholipase A_2 III-2 with pI 7.57.

It may be assumed that other features of their structure are important for the manifestation of anticoagulant properties in phospholipases A_2.

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A LOW-MOLECULAR-WEIGHT CHANNEL-FORMING COMPONENT OF THE NEUROTOXINS OF THE VENOMS OF SPIDERS OF THE FAMILY THERIDIIDAE

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The presynaptic effect of the venoms of spiders of the genus Latrodectus (family Theridiidae) is well known [1]. It is considered [2] that it is due to high-molecular-weight toxins which, on interacting with a membranous nerve termination cause the massive liberation of a mediator. It has been shown [3, 4] that the whole venom of the spider Latrodectus mactans and a purified toxin isolated from it with a molecular weight of 130,000 form conductivity channels for K^+, Na^+, and Ca^{2+} ions in a model membrane, and this has been made the basis of their presynaptic action.

We have investigated the venom of the spider Lithyphantes paykulliana from another genus of the family Theridiidae. In preliminary experiments it was shown that like the venom of spiders of the genus Latrodectus, this venom acts toxically on mammals and insects, with LD_{50} for white mice and cockroaches of 5.5 and 5.2 mg/kg, respectively. Using nerve-muscle preparations of the frog and locust it has been established that the venom of the spider Lithyphantes paykulliana also causes a massive liberation of mediator, and in a model membrane it forms a homogeneous population of conductivity channels for cations with an amplitude of 20-350 pS, depending on the concentration and type of cation in the medium.

In order to ascertain the components responsible for the presynaptic and channel-forming effect of the venom, it was separated on Sephadex G-100. On using 0.01 M phosphate buffer with pH 8.0 containing 0.01 M sodium chloride, it was possible to separate the whole spider venom into four fractions containing proteins of the following molecular sizes: 100,000 dalton and above – fraction I; 60,000-70,000 dalton – fraction (II); 30,000-40,000 dalton – fraction (III); and 5000 ± 500 dalton – fraction (IV).

Test on preparations of the synapses from mammals and insects showed that fraction (I) increased the frequency of MEPPs and acted presynaptically only on vertebrates, while fraction (III) acted similarly only on the synapses of insects. All four fractions, including the one that contained components with a molecular weight of about 5000 dalton and below increased the integral conductivity of bilayer membranes and formed channels characterized by the same ionic selectivity and the same amplitude.

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