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ISOLATION AND CHARACTERIZATION OF NEUROTOXINS FROM THE VENOM OF THE SPIDER *Anemesia sp.*

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Three neurotoxin fractions have been isolated from the venom of the spider Anemesia sp. *On interacting with a presynaptic membrane, two of them (An4 and An5) caused an enhancement of the secretion of the mediator and exhaustion of its reserves, while the third (An7) caused suppression of the amplitude of the synaptic potentials.*

Glutamatergic synapses play an important role in the central nervous sytem of vertebrates and the peripheral nervous system of insects [1]. Great advances in the investigation of the organization and functioning of glutamatergic synapses have been achieved thanks to the discovery of low-molecular-mass neurotoxins in the venom of a spider of the Araneidae family [2-5].

We have found that the venom of the spider *Anemesia sp.* acts selectively on the glutamatergic synapses of the locust [6]. In relatively low concentrations (1-5 μg/ml) it causes an increase in the frequency of the miniature excitatory postsynaptic potentials (MEPSPs) of the locust while at high concentrations (50 μg/ml) it completely suppresses the amplitude of the synaptic potentials. These facts show that the spider venom contains at least two neurotoxic components with presynaptic and postsynaptic effects.

In order to isolate these components, the *Anemesia sp.* venom was fractionated. First, a solution of the whole venom in 0.05 M ammonium bicarbonate, pH 7.9, was centrifuged to precipitate insoluble impurities, and the supernatant was separated on the anion-exchange resin TSK-DEAE 650 M. The venom was separated into two fractions, An^a and An^b, of which fraction An^b possessed neurotoxicity. In the following stage, fraction An^b was separated on Sephadex G-75 (Fig. 1). This gave 8 fractions (An1 – An8). The fractions issuing in the first 90 ml of eluate possessed no neurotoxicity, while fractions An4, An5, and An7 issuing between the 90th and the 210th ml of eluate possessed neurotoxicities with different action mechanisms and to different degrees. According to the theory of gel filtration, the elution volume indicated that these fractions included components with molecular masses of from 5 to 50 kDa.

By electrophoresis in 15% PAAG with SDS in the presence of standard markers, we established the approximate molecular masses of the components responsible for neurotoxicity: fraction An4, 15 kDa; An5, 5 kDa; and An7, 1 kDa (Fig. 2).

When tested on locust nerve – muscle synapses, fraction An4 caused a marked increase in the frequency of MEPSPs and a block of synaptic transmission. Fraction An5 caused the discharge of MEPCPs in the form of packets (Fig. 3). Fraction An7 caused a fall in the amplitude both of MEPSPs and of the exciting postsynaptic potentials (EPSPs) and also suppressed the response to the ionophoretic application of glutamate (Fig. 4).

The effects of the neurotoxic fractions An4 and An5 had an irreversible, and those of An7 a reversible, nature. At the same time, none of the three neurotoxic fractions exhibited any influence whatever on the parameters of vertebrate cholinergic synapses.

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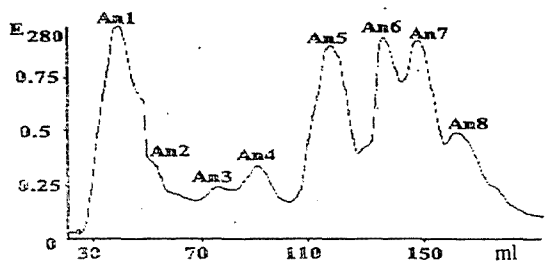

Fig. 1. Gel filtration of fraction An^b on Sephadex G-75 (column 1.25 × 90 cm) in 0.05 M ammonium bicarbonate buffer. Rate of elution 10 ml/h.

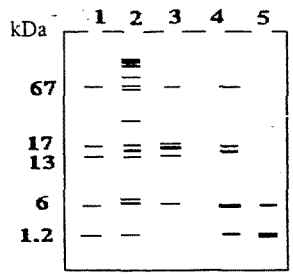

Fig. 2. Electrophoresis of the whole venom of the spider *Anemesia sp.* and its fractions in 15% PAAG in the presence of 0.1% SDS: 1) marker proteins; 2) whole venom of the spider *Anemesia sp.*; 3) fraction An4; 4) fraction An5; 5) fraction An7.

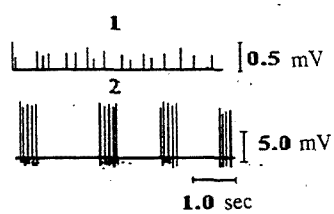

Fig. 3. Action of the neurotoxic fraction An5 of the venom of the spider *Anemesia sp.* on the spontaneous activity of a locust nerve–mucle synapse: 1) in the norm; 2) in the presence of the neurotoxic fraction An5 (10^{-6} M).

Thus, fractions An4 and An5 act at the level of the presynaptic membrane, the former enhancing the secretion of the mediator and depleting its reserves, and the latter interfering with the mechanism of the secretion of the mediator, while An7 acts at the level of the postsynaptic membrane — i.e., it interacts with the glutamate receptor. There is no doubt that these neurotoxins are new tools in the investigation of the mediator-secreting process and of the mechanisms of chemoreception in glutamatergic synapses.

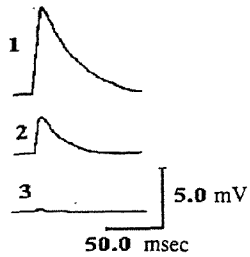

Fig. 4. Action of the neurotoxic fraction An7 of the venom of the spider *Anemesia sp.* on the glutamate reponses of a locust nerve–mucle synapse: 1) in the norm; 2) after the action of the neurotoxic fraction An7 (5×10^{-7} M) for 5 min; 3) after 10 min.

EXPERIMENTAL

The neurotoxic fractions were isolated with the aid of ion-exchange chromatography (TSK-DEAE 650 M) and gel filtration on Sephadex G-75 in accordance with the manufacturers' recommendations. The emergence of the protein fractions was monitored with the aid of an ultraviolet detector of the Uvicord S type (LKB, Sweden) at 280 nm.

Electrophoresis was conducted in 15% polyacrylamide gel over 180 min. The gel was stained with Amido Black 10 B, followed by decolorization in 7% acetic acid for 24 h. The amount of sample deposited on the plate was 0.1-0.2 mg. In the electrophoretic investigation we used Reanal reagents.

To determine the molecular masses of the fractions we used column chromatography and electrophoresis in 15% PAAG with SDS. As marker proteins we employed bovine serum albumin (67 kDa), myoglobin (17 kDa), cytochrome C (13kDa), cytotoxin (6 kDa), and bradykinin (1.2 kDa) from Serva (GFR).

The electrophysiological experiments were carried out on tergocoxal muscle of an M-120 locust. The membrane potential of the muscle fibers and the synaptic potentials were recorded by glass microelectrodes. The microelectrodes were filled with a 3 M solution of KCl and had a resistance of 5-20 Ω. As the potential amplifier we used a direct-current amplifier connected by Maksimov and Mumladze's scheme [7]. The signal from the output of the amplifier was fed to a C1-69 oscillator, photo recording from the screen of which was performed with the aid of a FOR-2 photorecorder.

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