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ISOLATION OF A Ca-CHANNEL BLOCKER FROM THE VENOM OF THE SPIDER *Agelena labirintica*

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From the venom of the spider Agelena labirintica *we have isolated the neurotoxin Agl1 (IV-6-1) with a molecular mass of 1000 Da, which effectively blocks the Ca channels of presynaptic membranes of frog nerve–muscle synapses.*

Calcium channels are characteristic for the majority of membranes of excitable cells and play an important role in the regulation of the transmembrane transfer of ions and the majority of cell processes. In spite of the great significance of calcium channels in both the physiological and the pharmacological respects, the properties and chemical nature of these channels have been studied inadequately. This is due to the fact that for a long time it was impossible to find compounds forming tools having the high specificity of interaction with calcium channels that are necesary for use as probes and ligands.

Only recently has it been possible to find neurotoxins of natural origin that interact specifically with the calcium channels of excitable membranes. Thus, conotoxin, which blocks the calcium channels of presynaptic membranes, has been isolated from mollusks [1]. Toxin AQ I, which blocks the calcium action potentials of dendrites, has been isolated from the venom of the spider *Agelenopsis aperta* [2]. The venom of the spider *Plectreurus tristes* causes blocking of presynaptic calcium channels [3], but this venom does not block the calcium channels of frog presynaptic membranes. This shows that the calcium channels of presynaptic membranes differ substantially in different animals.

We have previously [4] found that the venom of the spider *Agelena labirintica* causes a reduction in the frequency of miniature end-plate miniature potentials (MEPPs) and substantially lowers the amplitude of frog end-plate potentials (EPPs). The spider venom also effectively reduces the frequency of the MEPPs at the calcium depolarization level. An analysis of experimental results shows that the venom of the spider *Agelena labirintica* contains components selectively blocking the calcium channels of the presynaptic membranes of frog nerve–muscle synapses.

With the aim of isolating the component responsible for blocking the presynaptic calcium channels, we have subjected the venom of *A. labirintica* to fractionation. In the preliminary selection of the conditions for separating the venom we used Sephadexes G-50, G-75, and G-100. We found that the best separation was achieved on a column of Sephadex G-75 with the use of a 0.05 M ammonium bicarbonate, pH 7.9-5.2, buffer system. The whole venom was dissolved in 0.05 M ammonium bicarbonate, pH 8.2, the solution was centrifuged to precipitate insoluble impurities, and the supernatant was subjected to gel filtration. When it was passed through a column of Sephadex G-75 the venom was separated into four fractions. Fractions I and II, issuing in the first 70 ml of eluate, were inactive and contained high-molecular-mass components of the venom. Fraction III, issuing in the next 20 ml of eluate, was also inactive, while fraction IV, issuing in the next 50 ml of eluate, possessed the activity characteristic of the whole venom. The yield of fraction IV was 35-40% of the total weight of the venom. According to the theory of gel filtration, from its elution volume it may be concluded that this fraction included components with molecular masses of up to 5000 Da. It consisted of peptides with comparatively low molecular masses, free amino acids, and other low-molecular-mass components.

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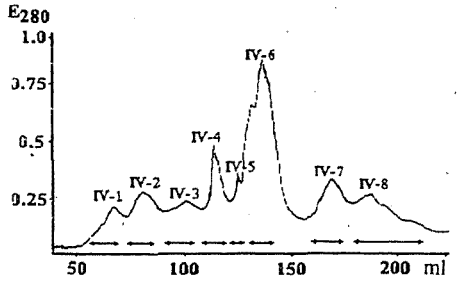

Fig. 1

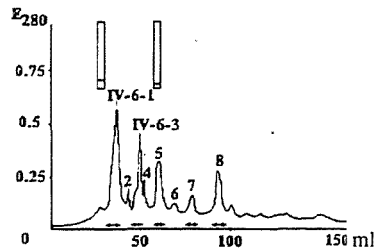

Fig. 2

Fig. 1. Gel filtration of fraction IV of the venom of the spider *Agelena labirintica* on Ultragel AcA_{202}. Column 0.9 × 120 cm, 0.05 N ammonium bicarbonate buffer, pH 8.2. Rate of elution 6 ml/h. The arrows show the limits of combination of the fractions.

Fig. 2. Gel filtration of fraction IV-6 of the venom of the spider *Agelena labirintica* on TSK-HW 40 F (column 0.9 × 120 cm, 0.15 M bicarbonate buffer, pH 8.2. Rate of elution 15 ml/h).

The results of comparative electrophoretic investigations of the venom of the spider *Agelena labirintica* and its fractions obtained after separation on Sephadex G-75 revealed their heterogeneity. Then 140 mg of fraction IV was dissolved in 1.5 ml of 0.005 M ammonium bicarbonate buffer with pH 8.2 and deposited on a column of Ultragel AcA 202 (Fig. 1). Eight fractions were obtained, of which fraction 6 blocked synaptic transmission most actively. On electrophoresis in 12% polyacrylamide gel (PAAG) 4-5 protein components were detected in this fraction. The further purification of fraction 6 was carried out on a column of TOYOPEARL TSK–HW40F (Fig. 2), giving 8 fractions of which only fraction IV-6-1 (Agl1) possessed the activity sought. According to the results of electrophoresis and electrofocusing, Agl1 contained a single component with a molecular mass of about 1000 Da.

In the investigation of the influence of this component on the presynaptic calcium channels we used the recording of the currents of the nerve terminal with the aid of an extracellular lead. Under these conditions we recorded the integral current passing through the presynaptic and postsynaptic membranes, this being the sum of the Na and K currents participating in the generation of the presynaptic action poential, the Ca current of the presynaptic membrane, and the end-plate current (EPC). These experiments showed that Agl1 exerted no influence on the Na and K components of the signal.

In order to study the influence of Agl1 directly on the Ca currents of the nerve terminal, the K current and the EPC were blocked by the addition of tetraethylammonium (10 mM) and tubocurarine (10^{-3} M) to the solution. The addition of Agl1 to the solution then led to a considerable lowering of the Ca current. The degree of suppression of the Ca current depended on the concentration of Agl1 (Fig. 3). When the preparation was washed with normal Ringer solution not containing Agl1, partial restoration of the amplitude of the Ca current was observed.

Thus, from the venom of the spider *Agelena labirintica* we have isolated a neurotoxin with a molecular mass of about 1000 Da that effectively blocks the Ca channel of the presynaptic membranes of frog nerve–muscle synapses. There is no doubt that this neurotoxin will find wide use as a tool in the study of features of the functioning of the Ca channel of the presynaptic membranes of vertebrates.

EXPERIMENTAL

The neurotoxin component from the venom of the spider *Agelena labirintica* was isolated with the aid of gel fitration on Sephadex G-75, Ultragel AcA 202, and TSK-40F in accordance with the manufacturers' recommendations. The yields of the protein fractions were monitored with the aid of an ultraviolet detector of the Uvicord S type (LKB, Sweden) at 200 nm.

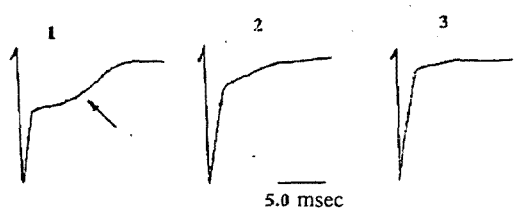

Fig. 3. Action of a neurotoxin (Agl1) from the venom of the spider *Agelena labirintica* on the presynaptic membrane of a frog nerve–muscle synapsis: *1*) in the norm; *2*) action of Agl1 (10^{-6} M) on the calcium current; *3*) action of Agl1 (10^{-5} M) on the calcium current. The arrow shows the calcium current of the nerve termination.

Electrofocusing was conducted on standard polyacrylamide plates with an Ampholine gradient in the pH interval of 3.5-9.5 (LKB).

Electrophoresis was performed in 12% polyacrylamide gel at 10 V/cm by Laemmli's method [5]. Amido Black was used for staining, followed by decoloration with 7% acetic acid for 24 h. The amount of sample deposited on a plate was 0.1-0.2 mg. In the electrophoretic investigations we used Reanal reagents.

To determine the molecular masses of the fractions, we employed chromatography and electrophoresis in 12% PAAG with SDS. As marker proteins we took bovine serum albumin (67 kDa), myoglobin (17 kDa), cytochrome C (13 kDa), melittin (2.5 kDa) and bradykinin (1.2 kDa) from Serva (GFR).

The evoked signals were extracted from the nerve termination extracellularly with the aid of microelectrodes having fused tips. The resistance of the microelectrodes was 1-2 mΩ. Depending on the aim of the experiment, the microelectrodes were filled with normal Ringer solution or 10 mM $CaCl_2$. After amplification, the evoked signals of the primary termination were photographed from the screen of an oscillograpoh (CI-69) with the aid of a FOR-2 photo recorder. The experiments were carried out at room temperature.

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