# Isolation of Toxins from *Buthus Occitanus Sp.* Scorpion and Their Action on Excitability of Myelinated Nerve

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 10, pp. 398-400, October, 2001
Original article submitted May 18, 2001

A series of short neurotoxins (molecular weight 3500-5000 D) was isolated from Vietnamese scorpion *B. occitanus sp*. All these toxins blocked generation of action potentials (this effect depended on their molecular weight), but did not change conduction velocity and excitation threshold of the nerve.

**Key Words:** *neurotoxins; scorpion; nerve excitability; action potential; sodium channels; potassium channels*

Scorpion venom is a rich source of toxic polypeptides affecting ionic channels of excitable and nonexcitable cells. These toxins can be subdivided into four groups by specificity of their interaction with $Na^+$, $K^+$, $Cl^-$, and $Ca^{2+}$ channels [2,5-7]. Toxins of Vietnamese scorpion *Buthus occitanus sp*. are still poorly studied [4], although the preparations of this scorpion are widely used in traditional medicine. Our aim was to study the effect of toxins isolated from *B. occitanus sp*. scorpion on excitability of myelinated nerve fibers.

## MATERIALS AND METHODS

We used unpurified toxin of *B. occitanus sp*. scorpion distributed in Dong Nai province of South Vietnam. The toxin was diluted in water, protein precipitate was separated by centrifugation, while the supernatant was lyophilized and used for protein chromatography. Chromatography was performed on a Sephadex G-50 column (1×100 cm) in ammonium acetate buffer (20 mM, 20°C, pH 7.4). Toxicity of the isolated fractions was tested on C57B1/6 mice weighing 20 g. The preparations were injected intraperitoneally. The toxins were then purified by high-performance liquid chromatography (HPLC) in a Gilson gradient system on a μ-Bondapak C18 column (10 μ, 19×150 mm). The fractions were eluted with a 20-50% and 50-100% 0.1% tetrafluoroacetic acid—acetonitrile linear gradients (2×120 min) at an elution rate of 5 ml/min (0.1%). The toxin-containing fractions were chromatographed and lyophilized. Electrophoresis of scorpion toxin proteins was performed in 15% polyacrylamide gel under non-denaturating conditions (β-alanine, 0.35 M, pH 4.5) by the method of Reisfeld [9]. Molecular weight of purified toxins was determined with a Vision-20 mass-spectrometer (Thermo Bio Analysis). The effects of the test toxins were evaluated on myelinated nerve fibers isolated from *Rana temporaria* frogs. *In vitro* experiments were carried out using a standard system for recording of resting and action potentials (AP). The parameters of AP were used for evaluation of changes in spikes amplitude, threshold, and conduction velocity [1].

## RESULTS

*B. occitanus sp*. toxins were isolated by two-stage chromatography: peptide fraction obtained after gel-filtration on Sephadex G-50 was fractionated with preparative HPLC. A chromatographic elution profile of scorpion toxin on Sephadex G-50 is shown in Fig. 1. The samples were subdivided into *4* peaks. Tests on

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0007-4888/01/1324-0953$25.00 

mice showed that peaks *2* and *3* contained toxic activity. Peak *3* was processed by reverse phase HPLC (C18 column, Fig. 2), which yielded several protein fractions. Fractions *5*, *7*, *9*, *10*, *13*, *14*, and *19* exhibited high toxicity (test mice died within 0.5-3.0 h after intraperitoneal injection of these fractions). For isolation of individual toxins the fractions were repeatedly chromatographed. Homogeneity of the isolated toxins was controlled by electrophoresis. Mass-spectrometry showed that molecular weight of the toxins varied within 3500-5000 D. In the next experimental series, the effect of isolated toxins on nerve excitability was studied (Fig. 3). Toxins *5*, *7*, *9*, *10*, *13*, *14*, and *19* (the toxin number coincides with fraction number) decreased AP amplitude. Toxins *5*, *13*, and *19* produced a biphasic response: an increase in AP amplitude was followed by its decrease (Fig. 3). The changes in AP amplitude, conduction velocity, and minimum and maximum excitation thresholds varied for different toxins (Table 1).

The toxic effect ($TE_{50}$) on AP amplitude depended on the molecular weight of the toxin (Table 1). Therefore, the effect of scorpion toxins on $Na^+$ channels in myelinated fibers can be determined by their molecular weight (low-molecular-weight toxins were more effective, Table 1) [3]. Conduction velocity depends on the state of internodal myelin and the area of Ranvier nodes. We found that changes in the conduction velocity did not depend on the molecular weight of the toxin. Hence, scorpion toxins directly interact with $Na^+$ channels in the Ranvier node and this effect does not depend on the sate of myelin. No correlation was found between the molecular weight of the toxin and changes in the excitation threshold of nerve fibers (Table 1). These data suggest that scorpion toxins irrespective of their molecular weight produce an additive effect on both resting potential and AP.

Thus, the toxins isolated from *B. occitanus sp.* scorpion block AP, and this effect depends on the molecular weight of the toxin. By contrast, the effect of scorpion toxins on nerve excitation threshold does not depend on their molecular weight. Probably, the effect of these toxins is determined by direct interaction with $Na^+$ and $K^+$ channels in the Ranvier nodes of myelinated nerve fibers.

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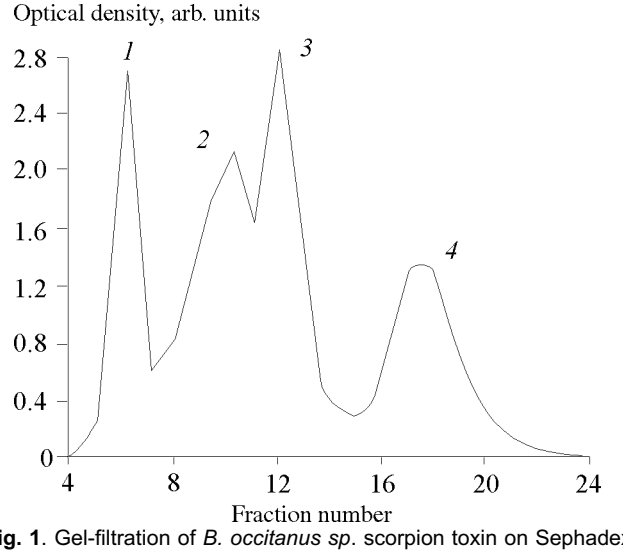


**Fig. 1**. Gel-filtration of *B. occitanus sp.* scorpion toxin on Sephadex G-50 column.

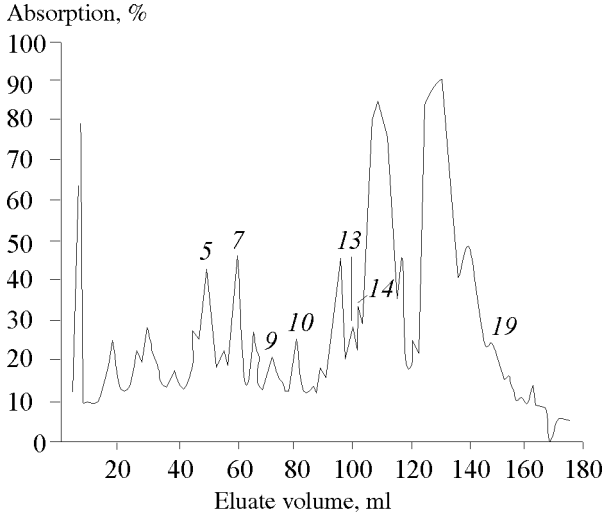


**Fig. 2**. HPLC elution profile on C18 column. Here and in Fig. 3: numbers indicate individual fractions.

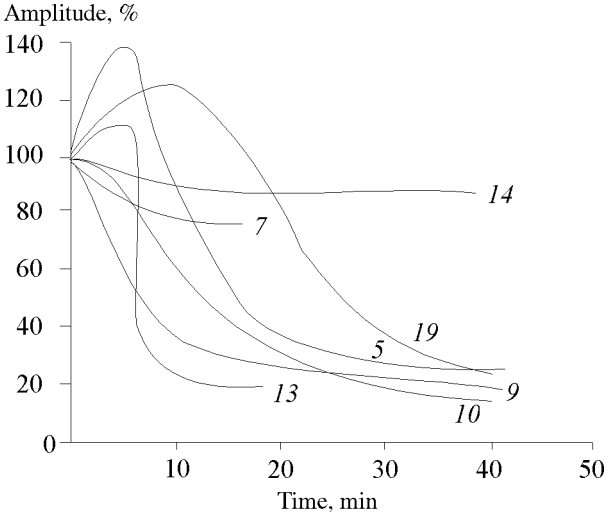


**Fig. 3**. Effect of scorpion toxins on the dynamics of AP amplitude.

**TABLE 1**. Effect of *B. Occitanus Sp*. Scorpion Toxins on Excitability of Myelinated Nerve Fibers

| $TE_{50}$ | | Toxin number (molecular weight, D) | | | | |
|---|---|---|---|---|---|---|
| | | 3-9 (4947.6) | 3-5 (4315.7) | 3-7 (4053.9) | 3-14 (3842.8) | 3-10 (3812.2) |
| AP, min | | 4 | 6 | 7 | 8 | 10 |
| AP velocity, min | | 16 | 19 | 10 | 50 | 10 |
| Excitation threshold, min | min | 50 | 68 | 1 | 35 | 30 |
| | max | 49 | 70 | 20 | 51 | 10 |

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