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Purification and characterization of a β-toxin from the venom of the African scorpion *Leiurus quinquestriatus*

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The venom of the African scorpion *Leiurus quinquestriatus* was subjected to high-performance ion-exchange chromatography. Among a large number (>25) of small proteins and other substances, a protein component of approx. 6500 Da was purified. The effect of this toxin was tested on single myelinated nerve fibres of the frog *Rana esculenta*. Toxin concentrations less than 10 nM produced clear effects. Activation rather than inactivation of the voltage-dependent sodium channel was strongly affected. Thus, this toxin from an African scorpion acts like the β-toxins present in the venom of North American scorpions.

β-Toxin; Na^+ channel; Voltage clamp; (*Leiurus quinquestriatus*, Nerve fiber, Frog node)

1. INTRODUCTION

The venom of the African (mideast) scorpion *Leiurus quinquestriatus* markedly inhibits inactivation of voltage-sensitive sodium channels in excitable membranes [1]. The venom of the North American species *Centruroides sculpturatus* acts quite differently [2]. Among the toxins isolated from this venom [3], only toxin V causes a drastic inhibition of inactivation [4], while toxins I, III, IV, VI and VII of *C. sculpturatus* produce a transient shift of Na activation to more negative potentials [5].

According to binding studies, the two types of scorpion toxins bind to different sites of the sodium channel [5,6]. The toxins which inhibit inactivation (preferably from 'Old World' scorpions) are called α-toxins. The toxins which affect activation (from 'New World' scorpions) are named β-toxins.

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In this report, the action of a toxic component purified from *L. quinquestriatus* is described. This toxin from an Old World scorpion acts like a β-toxin; i.e. Na activation is strongly affected while Na currents (at pulse potentials $\geqslant -40$ mV) inactivate almost normally.

2. MATERIALS AND METHODS

The venom of *L. quinquestriatus* (lot nos 59C-0430 and 123-F-4011) was obtained from Sigma, Diesenhofen, FRG. Morpholinopropanesulfonic acid (Mops) and the protein standard were from Serva, Heidelberg. All other reagents were of analytical grade or of the purest form available and were purchased from Merck, Darmstadt, FRG. Ultrafiltration membranes (YM 2) were from Amicon, Witten, FRG.

2.1. *Purification of the toxin*

10 mg of the venom were suspended in 30 ml deionized water and centrifuged at 30000 × *g* for 30 min [7]. The supernatant was concentrated to 1 ml by ultrafiltration. Fractionation of the water-solube extract of the venom was carried out by

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cation-exchange high-performance liquid chromatography using an LKB 2150/2151 apparatus with a wavelength fixed at 280 nm. The sample was loaded onto an Ultropac SP-5 PW (10 μm) column (0.75 × 7.5 cm) equipped with an Ultropac SP-5 PW (20-30 μm) guard column (0.6 × 1.0 cm) equilibrated with 10 mM NaH_2PO_4/Na_2HPO_4, pH 6.0. Elution was carried out with a linear gradient of 500 ml total volume from 0 to 500 mM NaCl, containing 10 mM NaH_2PO_4/Na_2HPO_4, pH 6.0. Flow rate was 11.4 ml/h. The peak that apeared at 375 mM NaCl was pooled and stored at −20°C until further use. The effects of other venom components eluted are currently being studied.

Gel electrophoresis was carried out according to [8], and silver staining as described in [9]. Toxin concentrations were estimated using a molar extinction coefficent of 20 $mM^{-1} \cdot cm^{-1}$ at 280 nm.

2.2. *Electrophysiological measurements*

The effect of the toxin purified was tested on single nerve fibres of the frog *Rana esculenta*. A node of Ranvier was voltage-clamped at 12°C [10]. The toxin-containing stock solution was prepared with an appropriate NaCl-deficient Ringer. Membrane currents were recorded on a computer and corrected for capacitative and leakage currents as described in [4,11,12].

3. RESULTS

The water-soluble extract of the venom from *L. quinquestriatus* was fractionated by high-performance cation-exchange chromatography. As shown in fig.1, the peak that appeared at 375 mM NaCl was homogeneous on SDS-polyacrylamide gel electrophoresis. The toxic component thus appears to be pure, unless the venom contains a further component of very similar charge and size. By comparison with the molecular mass values of standard proteins a molecular mass of 6500 Da is estimated for the purified venom component.

Fig.2 compares Na inward currents before (A) and during the application of toxin (B,C). Without a conditioning pulse (fig.1B), the toxin merely reduces the size of the currents, while the strong inhibition of inactivation known to be caused by venom [11], is absent. However, with a depolarizing conditioning pulse (fig.2C), Na currents are activated at much smaller test pulses. At depolarizations to −80 and −70 mV, the Na current does not inactivate during a 14 ms pulse. At somewhat larger depolarizations the toxin-induced currents inactivate slowly. Those currents which were already present before toxin application (> −50 mV) decay quite rapidly. Plotting peak I_{Na} vs pulse potential (fig.3) clearly reveals the effect of the toxin. With a depolarizing conditioning pulse a >30 mV shift of the descending branch of the I_{Na}-E curve to more negative potentials is observed. Furthermore, the conditioning pulse restores the original size of the maximum of the Na current and the reversal potential (E_{Na}) is shifted in the hyperpolarizing direction.

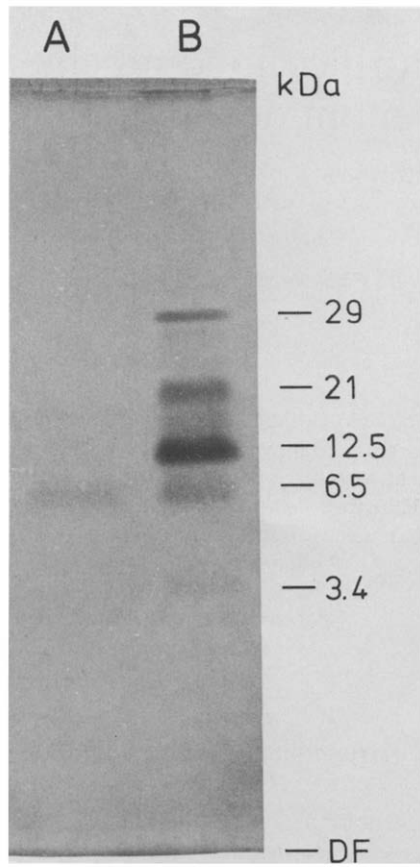

Fig.1. Analysis of the toxin (≃0.6 μg) purified by ion-exchange HPLC on an SDS gel: silver-stained polyacrylamide gradient gel (15–30%). Toxin in lane A. Marker proteins (in B): carbonic anhydrase, trypsin inhibitor from soybean, cytochrome *c*, aprotinin and insulin B chain. DF, dye front.

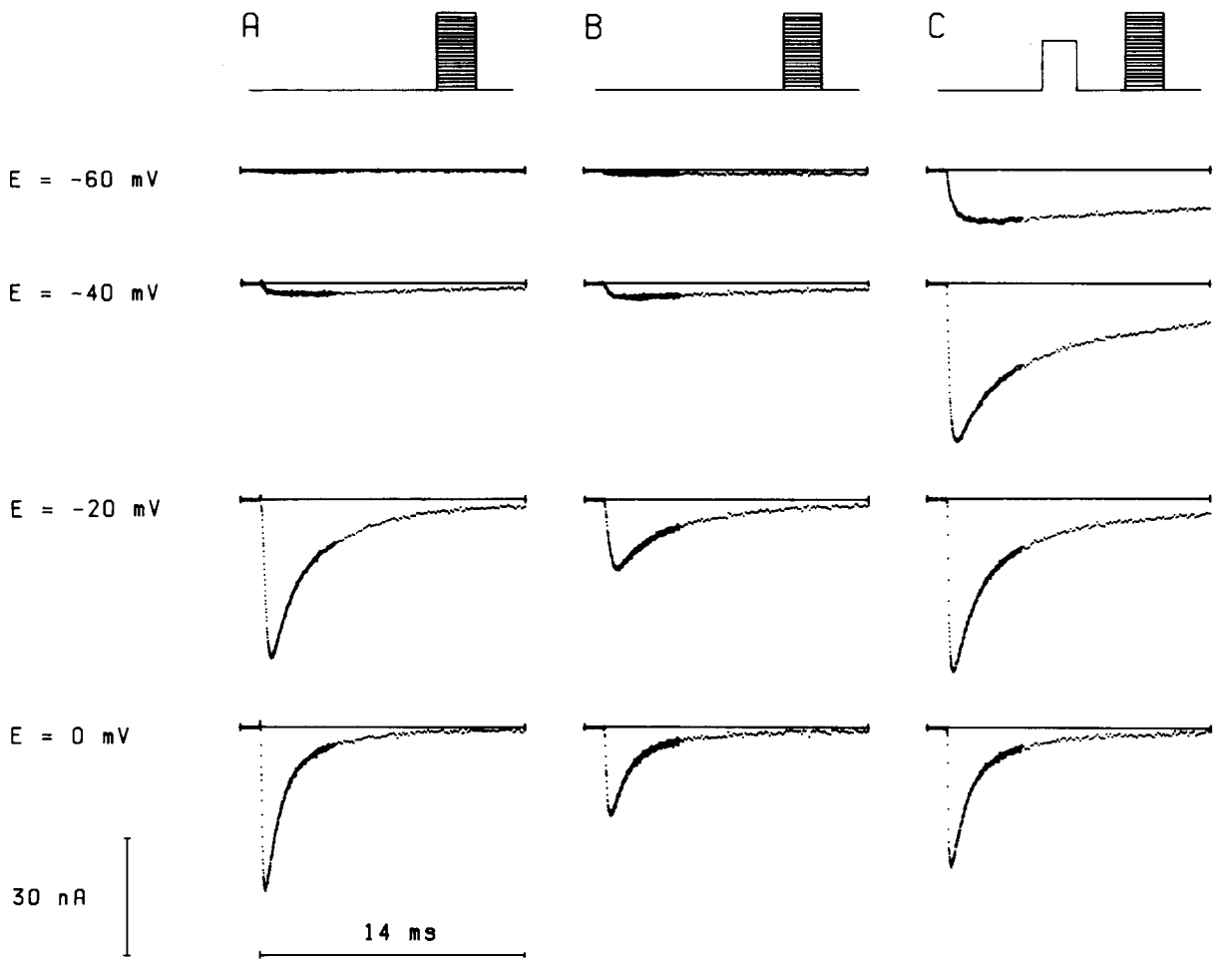

Fig.2. Na currents associated with 14 ms test pulses from the holding potential (−110 mV) to potentials between −60 and 0 mV (20 mV steps). A, B: no conditioning pulse. C: 15 ms conditioning pulse to 20 mV, pause between conditioning and test pulse 20 ms (see pulse programme in inset). A, control; B and C, during the application of toxin (25 nM). K currents blocked by internal Cs and external tetraethylammonium.

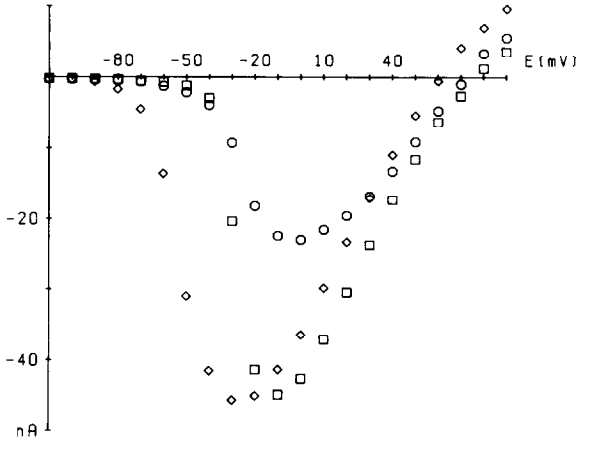

Fig.3. Peak Na current-voltage relation, I_{Na}-E, before (□) and during the application of toxin (○,◇). (○) No conditioning pulse; (◇) 15 ms conditioning pulse to 20 mV, 20 ms pause between conditioning pulse and test pulse. Data from the same experiment as in fig.2.

4. DISCUSSION

The experiments show that the venom of the scorpion *L. quinquestriatus* contains a component that acts preferentially on the activation of Na currents of nerve. Thus, this toxin acts like a β-toxin. So far, toxins of β-class have been found exclusively in North American scorpions [5,6]. Analysis by cation-exchange HPLC and SDS gel electrophoresis showed that the toxin is a basic protein of molecular mass equal to that of other scorpion toxins (~7000 Da). It remains to be elucidated whether other Old World scorpion venoms contain β-toxins and how far β-toxins from the Old and the New World are similar in structure.

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