Selective modification of the squid axon Na currents by Centruroides noxius toxin II-10

This is the author's manuscript

Original Citation:

Availability:
This version is available http://hdl.handle.net/2318/111659 since

Terms of use:
Open Access
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.
Selective modification of the squid axon Na currents by
Centruroides noxius toxin II-10*

Emilio CARBONE (1) *, Gianfranco PRESTIPINO (1), Fabio FRANCIOLINI (1), Mirna A.R. DENT (1) and Lourival D. POSSANI (2)

(1) Istituto di Cibernetica e Biofisica del CNR I-16032 Camogli ; (2) Istituto di Biologia Cellulare dell’ Università I-06100 Perugia ; (2) Instituto de investigaciones Biomedicas, U.N.A.M. México, D.F. 04510 México.

INTRODUCTION

Neurotoxins have become increasingly important tools with which to investigate the molecular components that control membrane excitability (Narahashi, 1974; Catterall, 1980). Among them, scorpion toxins have recently been shown to interact specifically with either the Na channel of various cell preparations (Romey et al., 1975; Bernard et al., 1977; Mozhaeva et al., 1980; Meves et al., 1982; Jaimovich et al., 1982; Wang and Strichartz, 1982; Barhanin et al., 1983) or with the K channel of squid giant axons (Carbon et al., 1982; Carbon et al., 1983). Toxins acting on Na channels can be divided into two classes: those affecting Na activation and those affecting Na inactivation (Hu et al., 1983; Wheeler et al., 1983). Very likely, different modes of action reflect different chemical structures of the various toxins. For instance, toxins acting on K channels appear to have rather similar molecular weight and amino acid sequence (Possani et al., 1982), which differ considerably from those of toxins acting on Na channels (Barin et al., 1975; García, 1976).

In this paper we present additional data on the purification and physiological characterization of toxin II-10 from the venom of the Mexican scorpion Centruroides noxius Hoffmann. The major effect of the toxin is that it selectively reduces the peak Na conductance of squid axon membrane leaving the K-system almost unaltered. At concentrations greater than 3 μM the peak decrement is usually accompanied by an increase in the maintained conductance, which was not clearly observed in previous studies (Carbon et al., 1982), where the concentrations used were lower than 1.4 μM.

From its mode of action and partial amino acid sequence II-10 appears to be very similar to other toxins known to act on the activation machinery of other Na channels (Jaimovich et al., 1982; Meves et al., 1982).

SUMMARY :

1° We have studied the effects of the purified toxin II-10, from the venom of the scorpion Centruroides noxius Hoffmann, on the Na and K currents of voltage clamped squid giant axons.

2° Extracellular applications of 10 μM of toxin II-10 produced a selective depression of peak Na currents, with no significant effects on the time course of K currents.

3° On pharmacologically separated Na currents, low concentrations of toxin II-10 (0.28-1 μM) caused a reversible decrease in inward and outward peak Is, with little effect on either the maintained level of the currents or their turning-off.

4° At high concentrations (>3 μM), toxin II-10 drastically reduced the peak conductance and increased both the level of the maintained conductance, and the time course of its turning-off.

5° It is suggested that, when applied extracellularly on squid axons, toxin II-10 primarily reduces the peak Na conductance by modifying the activation of fast-inactivating Na channels. At high concentrations (10 μM), the toxin also modifies the rate constants of the transition from the inactivated to the second open state of the channel (Chandler and Meves, 1970) thus producing an increased level of the maintained Na conductance. It is also very likely, however, that peak conductance and maintained conductance reflect two separate populations of Na channels on which toxin II-10 has a differentiated action. Under these conditions, toxin II-10 would be the first reported toxin which can pharmacologically separate the two types of channels.

METHODS

Axon preparation and voltage clamp. All experiments were performed on internally perfused giant axons of the squid Loligo vulgaris, available in Camogli (Italy). The perfusion technique, voltage clamp apparatus and pulse protocol for measuring Na currents were similar to those previously described (Wanke et al., 1979; Carbone et al., 1981). Axons were bathed in artificial sea water (ASW) containing (mM): 435 NaCl, 10 KCl, 10 CaCl₂, 40 MgCl₂, 20 Tris, pH 8.0. The standard intracellular medium (Na-SIS) contained (mM): 267 CsF, 307 sucrose, 50 NaF, 45 K-phosphate, pH 7.2. When both Na and K currents were recorded, the intracellular medium was 400 K-SIS containing (mM): 317 KF, 307 sucrose, 45 K-phosphate buffer, pH 7.2. The fibres were held at a holding potential of −70 mV, and preconditioned to −90 mV for 80 ms before applying the depolarizing pulses. The temperature of the bath was maintained at 5 °C throughout all the experiments. Stock solutions of the purified toxin were added directly to the external bath by means of a Hamilton microsyringe to attain the desired final concentration.

Biochemical characterization of toxin II-10. The venom from the Mexican scorpion Centruroides noxius Hoffmann was previously fractionated by Sephadex G-50 gel filtration followed by ion exchange chromatography on carboxymethylcellulose column in 20 mM ammonium acetate buffer pH 4.7 (Possani et al., 1981). Another chromatographic step in CM-cellulose column equilibrated with 50 mM sodium phosphate buffer, pH 6.0, was introduced (see Results). The homogeneity of the toxin was verified by polyacrylamide gel electrophoresis in the acrylamide-urea-β-alanine system of Ruisi et al., (1962) and by amino acid sequencing. The pure toxin was reduced and carboxymethylated according to the procedure described (Possani et al., 1981), and its N-terminal amino acid sequence was determined by Edman degradation in a Beckman 890 C sequencer following the method of Edman and Begg (1967). The identification of the phenithyloxydantoin amino acids (PTH) was performed by high liquid pressure chromatography and by amino acid analysis (Durum D-500) of the PTH amino acids back hydrolized, as already published (Possani et al., 1981).

RESULTS

A - Purification and partial sequence determination of toxin II-10

The toxin component II-10 from the venom of the scorpion Centruroides noxius (lethal to mice) was completely purified by a second ion exchange chromatographic step (Fig. 1). From the three toxic fractions (b, c and d) the second one was shown by amino acid analysis and by voltage clamp experiments to correspond to our previous toxin II-10, which affects the peak Na conductance of squid giant axons (Carbone et al., 1982).

The N-terminal amino acid sequence of the reduced and carboxymethylated toxin II-10 (c) (≈ 50 nmoles) was shown to be: Lys-Glu-Gly-Tyr-Leu-Val Asn-Leu-Tyr-Thr-Gly-Cys-Lys-Tyr-Glu-Cys-Phe-Lys-Leu-Gly-Asp Asn Asp Tyr-Cys-Leu... Only one amino acid was identified at each step of the sequence with an over-all yield of 95%. These results corroborate the purity of the toxin II-10 (c) (same as II-10 from Carbone et al., 1982). Toxin from this preparation was used for the present study.

B - Effects of the purified toxin II-10 on Na and K currents.

The effects of 10 μM of toxin II-10 on the time course of Na and K currents are shown in Fig. 2. Toxin II-10 produces a rapid decrease of peak I_Na at 0 mV without altering either the steady-state level of I_K or the time course of the tail currents recorded on repolarization to −70 mV. Closer inspection shows that the depression of peak currents is accompanied by a slight prolongation of the time to peak, and by a sizeable slow down of the outward current component (Fig. 2 trace T). The latter effect, observed in two other axons, can be due either to a prolongation of the time course of the outward K currents or to a slowing down of the Na inactivation process. As shown below the
second possibility can account for most of these effects, suggesting only a weak interaction, if any, of II-10 with the K channel kinetics. This point, however, was not further investigated.

C - Effects of II-10 on pharmacologically separated Na currents.

In axons internally perfused with 217 mM CsF to block K outward currents, addition of 280 nM of toxin II-10 (Fig. 3, left) reduces by 60% the size of peak I_{Na} at 0 mV with no significant effects on: (1) the time course of Na activation and inactivation, (2) the maintained level of currents at +100 mV, and (3) the time course of tail currents on repolarization. At this concentration the action is fully reversible (Fig. 3, right), and requires approximately 10 min to be complete. Larger doses of toxin (up to 1 μM) speed up the reduction of peak currents and increase the extent of the depression (see also Fig. 2d in CARBONE et al., 1982).

At large concentrations (> 3 μM) the action of toxin II-10 becomes somewhat more complex. At 10 μM (Fig. 4) the depression of peak I_{Na} is more pronounced (87% at 0 mV) while the level of the maintained currents increases by 60% at +100 mV. Comparing the records at 0 mV taken before (C) and after 8 min addition of the toxin (T), it is evident that II-10 prolongs the time to peak of I_{Na}, as well as the time constant of Na inactivation. The effects of the toxin are even more striking on the kinetics of Na channel closing. Tail currents recorded on repolarization to -70 mV become larger and markedly slower in the presence of the toxin. Usually, at these toxin concentrations leakage currents show a slight and progressive increase which, in any case, never contribute more than 15% to the level of peak outward current at +100 mV. Under these conditions, recovery was usually largely incomplete.

---

**Toxin**

- **+100 mV**
- **0 mV**

**Washout**

- **+100 mV**
- **0 mV**
DISCUSSION

Our results show that II-10 acts on the squid axon membrane by selectively decreasing the peak Na currents. The action of the toxin is fast, develops at low concentrations (at 280 nM Na currents are nearly halved at + 100 mV) and is fully reversible (Fig. 3). Thus, the present data confirm our previous findings obtained with a similar toxin, which has been shown to affect the peak Na-conductance in a dose-dependent manner (CARBONE et al., 1982). From these observations it was concluded that toxin II-10 might interact specifically with the transiently open Na channel by fully blocking it in a one to one ratio. Such interpretation, however, is now in contrast to the present results. At large depolarizations, the block of the peak conductance is largely incomplete and the level of the maintained Na-conductance increases in the presence of 10 μM toxin (Fig. 4).

From the above arguments it is tempting to suggest that toxin II-10 acts on the Na-conductance mechanism by increasing the number of channels which go from the peak conductance state into the maintained conductance state (CHANDLER and MEVES, 1970; GILLESPIE and MEVES, 1980). But it is also possible that peak conductance and maintained conductance reflect two separate populations of Na channels (one with fast activation and inactivation, and one with slow activation and no inactivation) and that II-10 transforms one type of channel into the other (MATTESON and ARMSTRONG, 1982). In the second case, however, we can not exclude the possibility that toxin II-10 has a differentiated action on the two channels: partially blocking those fast-inactivating and decreasing the rate of closing of those slowly activating. The latter effect would account for most of the increased level of the maintained conductance as well as for the drastic slow down of tail currents during membrane repolarizations. A better discrimination between the various mechanisms of action presently requires further experimental work.

The distinct effects of toxin II-10 on the peak and maintained Na-conductance (Fig. 3, 4) are typical of other toxins known to act on Na channels. For instance, the action on the peak conductance observed at 280 nM (Fig. 3) is similar to that of II-10 from the venom Centruroides sculpturatus Ewing and Leituras quinquestratus (VUYVERBERG and LAZDUNSKI, 1983). In the case of C.s.s.II, 20 nM of toxin are sufficient to fully block the peak Na currents of frog skeletal muscles, while 100 nM of TTXγ block only 50% of the inward Na currents in neuroblastoma cells. As for II-10, the action of the two toxins is shown to be selective for the Na channel, but there is no proof of their reversibility of action. In contrast to that, the effects on the maintained conductance observed above 3 μM (Fig. 4) resemble more the actions of the venoms Centruroides sculpturatus Ewing and Leituras quinquestratus on the Na channels of squid giant axons (GILLESPIE and MEVES, 1980) and of mammal toxin I, MTI, from the venom Androctonus australis Hector on the same preparation (PICHON, 1983). In both cases, applications of 0.1 to 0.5 mg/ml of the venom C.s.E (or micromolar aliquots of MTI) produce a sizeable increase in the maintained Na-conductance and a slow down of its turnover off kinetics. Fig. 5 shows that similar effects can also be seen when 1 mg/ml of the venoms Centruroides sculpturatus sculpturatus and Centruroides noxius Hoffmann are applied on perfused axons.

**Fig. 5.** Effects on Na currents of – a: Venoms from the scorpions Centruroides noxius Hoffmann. – b: Centruroides sculpturatus sculpturatus. Lower traces were recorded with pulses to 0 mV and upper traces to + 100 mV. (C) traces were taken before the addition of 1 mg/ml of the venoms. (T) were the last records before recovery (not shown). In (a) the voltage clamp currents were recorded every 3 min; in (b) every min. Repolarizations to – 70 mV. Bars: 1 mA/cm², 2 ms. In: 50 Na-SIS, Out: ASW. Axons: A26MR82 and B03PB62. Temperature: 5 °C.
TABLE I. — Comparison of the N-terminal amino acid sequence and physiological action of toxins from scorpions of the genus Centruroides.

* Total amino acid sequence known (see reference). Abbreviations according to the scientific names of the scorpions were used: C.n.: Centruroides noxius; C.s.E.: C. sculpturatus Ewing; C.s.S.: C. saussurei saussurei; C.e.: C. elegans; C.l.I.: C. limpidus toccanus. NTX means noxious-toxin. Dash (−) was introduced in order to enhance similarities in the sequences. X means unknown amino acid.

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Sequence</th>
<th>Reference</th>
<th>Action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.n. II-10</td>
<td>KEGYL VNL YT GCKY ECFKL GDNDY - CL...</td>
<td>POSANI et al., 1981</td>
<td>Na activation</td>
<td>CARBONE et al. (see text)</td>
</tr>
<tr>
<td>C.n. II-9.2.2</td>
<td>KEGYL VDKNT GCKY ECFKL GDNDY - CL...</td>
<td>POSANI et al., 1981</td>
<td>No effects</td>
<td>CARBONE et al.</td>
</tr>
<tr>
<td>C.n. II-13</td>
<td>KEGYL VDYHDG GCKY ECFKL GDNDY - CL...</td>
<td>POSANI et al., 1981</td>
<td>No effects</td>
<td>CARBONE et al.</td>
</tr>
<tr>
<td>C.n. II-14</td>
<td>KDOYL VDAK - GCKKNCY ECFKL GDNDY - CL...</td>
<td>POSANI et al., 1981</td>
<td>Na activation</td>
<td>CARBONE et al.</td>
</tr>
<tr>
<td>C.s.E. I*</td>
<td>KDGYLVKT - GCKKNCY ECFKL GDNDY - CL...</td>
<td>BABBIN et al., 1975</td>
<td>Na activation</td>
<td>MEYES et al., 1982</td>
</tr>
<tr>
<td>C.s.E. VI*</td>
<td>KEGYLVKKS DQCKY DCFWL GKENHNTCE...</td>
<td>BABBIN et al., 1974</td>
<td>Na activation</td>
<td>MEYES et al., 1982</td>
</tr>
<tr>
<td>C.s.E. V*</td>
<td>KEGYLVNKS TGCY VCLKL GENE GNCCE...</td>
<td>BABBIN et al., 1974</td>
<td>Na inactivation</td>
<td>MEYES et al., 1982</td>
</tr>
<tr>
<td>C.s.E. V*</td>
<td>KEGYLVKKS DQCKY VCLKL GENE GNCCE...</td>
<td>FONTECILLA-CAMPAS et al., 1980</td>
<td>Na inactivation</td>
<td>MEYES et al., 1982</td>
</tr>
<tr>
<td>C.s.S. I</td>
<td>KEGYLVS KS TGCY ECFKL GDNDY - CL...</td>
<td>GARCIA, 1976</td>
<td>Na inactivation</td>
<td>JAIMOVICH et al., 1982</td>
</tr>
<tr>
<td>C.s.S. II*</td>
<td>KEGYLVS KS TGCY ECFKL GDNDY - CL...</td>
<td>GARCIA, 1976</td>
<td>Na activation</td>
<td>JAIMOVICH et al., 1982</td>
</tr>
<tr>
<td>C.e. II-6.3</td>
<td>KZGYLVBHS TGCKY CFCFL GBBBY - CL...</td>
<td>RAMIREZ et al., 1981</td>
<td>Na activation</td>
<td></td>
</tr>
<tr>
<td>C.I.I. II-9.3</td>
<td>KGZXLVBH SKECP...</td>
<td>POSANI et al., 1980</td>
<td>Na activation</td>
<td></td>
</tr>
<tr>
<td>C.n. NTX*</td>
<td>TAI NVKCTS PKQCS KPCCKLYS GAGAKH...</td>
<td>POSANI et al., 1982</td>
<td>K activation</td>
<td>CARBONE et al., 1982</td>
</tr>
<tr>
<td>C.n. II-10.2</td>
<td>TFI DVKCGS SKKECP...</td>
<td>POSANI et al., 1982</td>
<td>K activation</td>
<td>CARBONE et al., 1982</td>
</tr>
</tbody>
</table>

axons, indicating that the increase in the maintained conduction and related phenomena are a general property of most scorpion venoms when applied to the giant axon of the squid. It is noteworthy that the venoms C.s.E and L.q. applied to myelinated fibres produce rather different effects (CAHALAN, 1975; MEYES et al., 1982). The reason for this has still to be clarified.

Finally we would like to comment on the above physiological data with respect to the information available on the primary structure of the toxins from the venom of the scorpion Centruroides. In Table I we have reported the partial N-terminal amino acid sequence of 14 toxins from the scorpions of the genus Centruroides. As shown, toxins acting on Na channels appear to have very similar structure, which differs remarkably from that of K-toxins: NTX and C.n.II-10.2 (POSANI et al., 1982). Among Na-toxins the similarity between II-10, C.s.S.II and C.n.II-14 is remarkable. The latter, at high concentrations was observed to have a slight effect on the peak Na-conductance, while toxins C.n.II-9.2.2 and C.n.II-13 were found to be insensitive to both Na and K currents of the squid giant axon (CARBONE, PRESTIPINO, WANKIE, POSANI and MAELICKE, unpublished observations).

A more detailed discussion of the effects of toxin II-10 compared to other Na-toxins requires of course more work and the full knowledge of its amino acid sequence. Since it is very likely that minor changes or deletions condition the fine mechanism of action of each toxin, it is conceivable that their identification will allow for a better understanding of the mode of action of the toxins and of the structure of their receptors at the channel site. In this respect, toxin II-10 seems to have all the prerequisites for such an approach: selectivity of action and high affinity for the channel.

REFERENCES


Vol. 79, nos 4, 1984 SCORPION TOXINS AND Na CHANNELS IN SQUID 183


