SCORPION NEUROTOXIN - A PRESYNAPTIC TOXIN WHICH AFFECTS BOTH Na^+ AND K^+ CHANNELS IN AXONS

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SUMMARY. A neurotoxin from the venom of the scorpion,Androctonus australis Hector, affects the closing of the Na^+ channel and the opening of the K^+ channel in giant axons of crayfish and lobster nerves.It blocks both Na^+ and K^+ conductances in Sepia giant axons. Dose-response curves are markedly cooperative with all types of axons. Apparent dissociation constants for the receptor-toxin complexes are 0.25 µM, 0.7 µM and 2-4 µM for the crayfish, lobster and Sepia axons, respectively.This toxin will be probably a useful tool for biochemical investigation of Na^+ and K^+ channels.

Scorpion neurotoxins (ScTX) are basic mini-proteins (MW about 7000) consisting of a single peptide chain cross-linked by 4 disulfide bridges(1,2).They are highly toxic in the mouse(LD_{50},10-20 µg/kg) and induce spastic paralysis of skeletal muscle,tachycardia,cardiac arrythmia,excessive salivation and respiratory paralysis.The pure toxin has chronotropic and inotropic effects upon heart cells in culture at concentration as low as 10 nM(3).Detailed electrophysiological studies with total scorpion venom(4-7),which contains at least 15 different proteins including phospholipase,have shown that the neurotoxic action is presynaptic.We study in this work the effect on Na^+ and K^+ channels in axonal membranes of neurotoxin I (ScTXI) of Androctonus australis Hector venom.This toxin appears to be a useful tool to study the molecular aspects of nerve conduction.

METHODS

The giant axons were those of the crayfish Astacus leptodactylus, of the lobster Homarus gammarus (axon diameter 100 to 200 µ) and of a cephalopod,the cuttle-fish Sepia officinalis (axon diameter 200-400 µ).Giant axons from crustacea were isolated from circum-oesophagial nerve connectives,those of Sepia from stellar nerves(8). Resting and action potentials of axons were recorded by inserting a glass capillary microelectrode filled with a 3M KCl solution

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(resistance 10 megohms).Electrical stimulation was applied at one end of the nerve with a pair of silver wire electrodes.The thermostated sucrose gap apparatus for voltage clamp experiments on crayfish and Sepia axons was derived from that designed for lobster axons by Julian et al. (9).Physiological solutions were Mediterranean sea water for lobster and Sepia axons and a Van Harreveld solution(207 mM NaCl,5.4 mM KCl,13.5 mM $CaCl_2$,5.3 mM $MgCl_2$, 10 mM Tris)at pH 7.8 for crayfish axons.ScTXI was prepared pure by the method of Rochat et al.(10).

RESULTS

The resting potential of all axons was unaffected by ScTXI at 5°. A small depolarization was observed at 18°C with 5 µM toxin:3-5 mV for crustacean axons and 8-10 mV for Sepia axons.

- *Effects of ScTXI on action potentials.*

(i) Lobster and crayfish axons. ScTXI has very similar effects on both types of crustacean axons.A typical experiment is presented in Fig.1A.The action potential after ScTXI treatment presents a

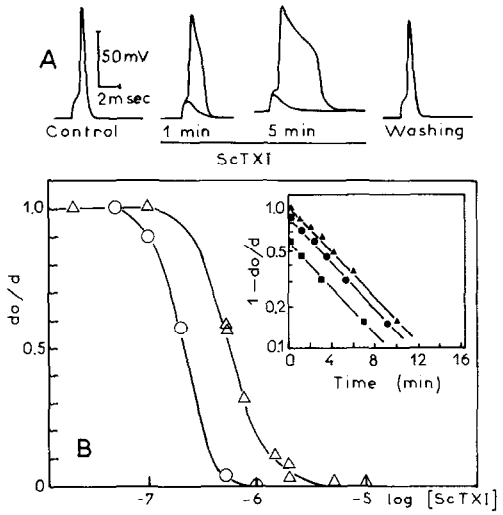

Figure 1- A- The reversible action of ScTXI(0.5 µM)on the action potential of lobster giant axons,18°C.

B- Dose-response curve of the action of ScTXI on crayfish(O) and lobster axons(Δ)(18°C).d_o represents the duration of the spike in the control measured at half-height of the action potential.d is the duration after treatment with a given concentration of ScTXI.d is measured at equilibrium i.e. when the spike shape no longer varied with time.Inset - Kinetics of the reversal of toxin action on a lobster axon poisoned with three different concentrations of ScTXI: 0.5(■),2(●),and 5 µM(▲);the axon was washed with a continuous flow of physiological solution.

marked plateau phase and resembles normal action potentials recorded with Purkinje and ventricular fibres. The neurotoxic effect is essentially reversible by washing. Dissociation of ScTXI from its membrane receptor (R) follows first-order kinetics independent of ScTXI concentration as expected for a classical interaction:

$$R + ScTX \underset{k_d}{\overset{k_a}{\rightleftharpoons}} R - ScTX$$

The value of the first-order dissociation rate constant k_d is $3.1\ 10^{-3} sec^{-1}$ (Fig. 1B inset) with the lobster axon. The fact that the kinetics of dissociation are first-order indicates a single type of receptor molecule for ScTXI in the axonal membrane. Dose-response curves for ScTXI action on lobster and crayfish axons are presented in Fig. 1B. Apparent equilibrium dissociation constants for the toxin-receptor complexes are 0.25 and 0.7 µM for crayfish and lobster axons respectively. The toxic effect is cooperative with a Hill coefficient in the dose-response curve higher than 2.

(ii) Sepia giant axons. The effect of ScTXI on the action potential of Sepia giant axons is markedly different from that observed with crustacean axons (Fig. 2A). There is no plateau phase in the spike; instead both the amplitude and the rates of the rising and falling phases of the action potential decrease. At high concentration of

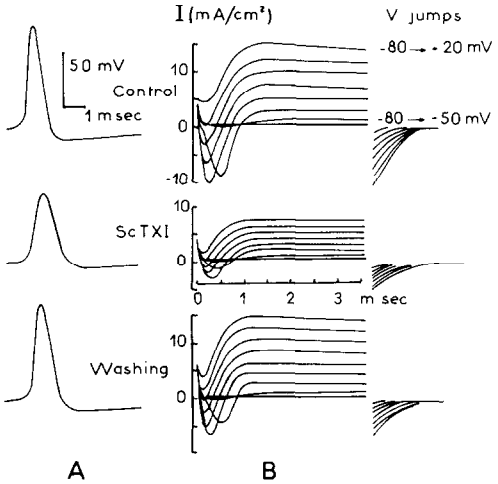

Figure 2- A- The reversible action of ScTXI (3 µM) on the action potential of Sepia giant axons, 18°C.

B- Voltage clamp analysis of toxin action on the Sepia axon. The family of kinetic profiles for ionic currents was obtained for voltage jumps from -80 mV to -50, -40, -30, -20, -10, 0 and +20 mV, 18°C. Off-current corresponds to return to -80 mV.

ScTXI(10 µM),the action potential is completely abolished.Nearly complete recovery is obtained after washing.

- *Effects of ScTXI on membrane currents:Voltage clamp analysis.*

(i) Crayfish giant axons. Giant axons were submitted to voltage jumps from a holding potential of -80 mV to -10 mV(Fig.3).The

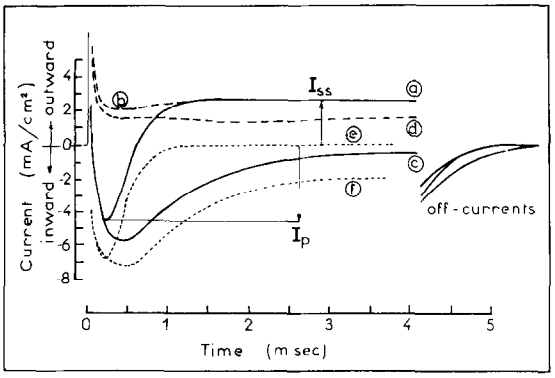

Figure 3- Voltage-clamp analysis of ScTXI action on a crayfish giant axon,18°C.The membrane potential was clamped at -10 mV after voltage jump from -80 mV.ScTXI concentration:0.2 µM(i.e.about the apparent dissociation constant measured in Fig.1).TTX concentration: 0.1 µM(the dissociation constant of the TTX-toxin receptor in this system is 3 nM(unpublished)).Off currents correspond to return to -80 mV.Traces correspond to:Control(ⓐ),Control + TTX (ⓑ), Control + ScTXI(ⓒ),Control + ScTXI + TTX(ⓓ).Dashed lines ⓔ (difference between ⓐ and ⓑ)and ⓕ (difference between ⓒ and ⓓ)represent the time course of the Na^+ current in the absence and in the presence of ScTXI.

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purpose was to examine three main events responsible for an action potential: ① the opening of the Na^+ channel, ② the closing of the Na^+ channel and ③ the opening of the K^+ channel(11).The maximum amplitude of inward current,I_p,due to opening of the Na^+ channel and Na^+ entry,is attained after 0.23 msec;then the intensity of the inward current starts to decrease.This corresponds to progressive closing of the Na^+ channel and to progressive opening of the K^+ channel.After 3 msec of voltage clamp at -10 mV a steady-state situation is attained in the control with a closed Na^+ channel and an outward current I_{ss} mainly due to K^+ efflux.Tetrodotoxin (TTX) is highly specific for blocking the Na^+ channel in most axons without affecting the K^+ channel(12).Treatment of the axon with TTX completely suppresses the peak current of sodium but leaves

the steady-state K^+ current unchanged(curve ⓑ).TTX application then permits study of the ScTXI effect upon the outward K^+ current. ScTXI treatment of the axon in the presence of TTX markedly decreases the outward K^+ current(curve ⓓ).One of the ScTXI effects is thus to alter the opening of K^+ channel(decrease of K^+permeability).Since TTX does not affect the K^+ current,the time-course of the intensity of the Na^+ current in the normal and ScTXI treated crayfish axon can be obtained by substracting the current versus time curves obtained in the absence(kinetics of the Na^+ and K^+ currents)and in the presence(K^+ current alone)of TTX.Comparison of traces ⓔ and ⓕ in Fig.3 demonstrates that,whereas ScTXI has little effect on the opening of the Na^+ channel,it slows down and partially inhibits the closing of this channel.The Na^+ channel remains partially opened even after 4 msec(inward current:2.16mA/cm^2).

(ii) Sepia giant axons. Fig.2B shows a family of voltage clamp profiles associated with a series of step depolarizations(Voltage jumps)from the same holding potential -80 mV.ScTXI(3 µM)decreases both the peak of the inward current I_p and the steady-state outward current I_{ss}.Current-voltage relations for I_p and I_{ss} before and after application of ScTXI are presented in Fig.4A.Both values

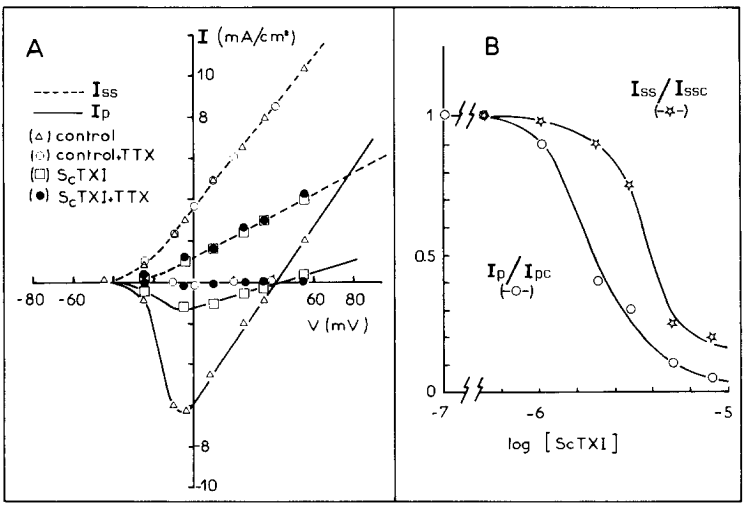

Figure 4- A- Relationships between the intensities of membrane currents I_p and I_{ss} and values of the clamped potential V without ScTXI,in the presence of ScTXI(3 µM)and in the presence of ScTXI (3 µM)plus TTX(0.1 µM)18°C.

B- Dose-response curves giving the ScTXI concentration dependence of membrane currents I_p and I_{ss}.The terms I_{pc} and I_{ssc} relate to values of I_p and I_{ss} determined for the axon in the control,i.e.in the absence of toxin.

of I_p and I_{ss} are drastically decreased at all membrane potentials. ScTXI appears to block both ionic channels through which Na^+ and K^+ normally flow during the action potential. Analysis of the toxin effect upon I_p (Na^+ current) and upon I_{ss} (K^+ current) give apparent dissociation constants of 2 µM and 4 µM respectively for the receptor -ScTXI complex (Fig. 4B). The dose-response curves here again show an important cooperativity ($n_H > 2$) with an all-or-none effect over an increase in ScTXI concentration of less than 10 fold. Fig. 4A shows that TTX still acts in the presence of ScTXI. It completely blocks the Na^+ channel already partially blocked by ScTXI (effect upon I_p). Tetrodotoxin does not affect the inhibitory effect of ScTXI on the K^+ channel (no effect upon I_{ss}).

DISCUSSION

ScTXI appears to interact with a single type of receptor site in the axonal membrane of the crayfish and of the lobster. Dissociation constants of the toxin-receptor complexes are 0.25 µM and 0.7 µM respectively. On these two membranes ScTXI selectively affects the time course of Na^+ inactivation (closing of the Na^+ channel) and drastically decreases the steady-state potassium current (opening of the K^+ channel). There are several other neuroactive agents that provoke under voltage clamp the prolongation of the sodium current and the partial suppression of the potassium current (13). However, as far as we know, ScTXI is the one which acts at the lowest concentrations. The effects previously observed by other workers with the venom itself on the excitable membrane of nodes of Ranvier of *Xenopus laevis* and of squid axon (5,7) were similar to those observed here with the pure toxin with crayfish and lobster giant axons. ScTXI acts differently on the axonal membrane of *Sepia*. It does not affect the kinetics of the ionic currents but it drastically and reversibly blocks both the Na^+ and K^+ membrane permeabilities. The effect of ScTXI on the Na^+ permeability is similar to a TTX effect. Because of its effect on both the Na^+ and the K^+ channels and because of its fairly high affinity for axonal membranes, ScTXI appears to be a potentially useful tool for the chemical characterization of molecules involved in the functioning and in the coupling of the Na^+ and K^+ ionophores in the axonal membrane. It is of interest that the binding site for ScTXI in the axonal membrane is distinct from that of TTX, which has already been used with success in the tritiated form to count the Na^+ channels in nerves (14-17).

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