IONIC CHANNELS INDUCED BY SEA NETTLE TOXIN IN THE NODAL MEMBRANE

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ABSTRACT Toxin isolated from nematocysts of the sea nettle *Chrysaora quinquecirrha* (SNTX) is known to depolarize nerve and muscle membranes and to increase the miniature end-plate potentials' frequency. To investigate its mode of action at the membrane level, we have studied the toxin's effects on the frog myelinated nerve fibre. We show that SNTX creates large cation-selective channels that open and close spontaneously. The conductance of these channels, almost constant in the voltage range −100 to +50 mV, is 760 pS. The SNTX-induced channels are almost equally permeable to Na^+, Li^+, K^+, and Cs^+, but are impermeable to Ca^{++}. The open and closed times of SNTX-induced channels are voltage dependent, the open probability increasing with increased negative membrane potentials. To our knowledge, this is the first demonstration of the production of single-channel currents by a toxin, in a biological membrane.

The mode of action of the toxin extracted from the nematocysts of the jelly fish *Chrysaora quinquecirrha* was investigated on the myelinated nerve fibre of the frog *Rana esculenta* under voltage-clamp conditions. The resting potential (−70 mV) was defined as the potential at which 30% of the Na channels were inactivated (h_∞ = 0.7). Current amplitudes were calculated assuming a 10 MΩ internal resistance. Occasionally, the current through K^+ channels was abolished by external tetraethylammonium (10 mM) and replacement of internal KCl by CsCl; the current through Na^+ channels was abolished by addition of tetrodotoxin (1 μM) to the external medium. The partially purified toxin (1) was dissolved in the Ringer's solution at concentrations of 0.2–1 mg of protein/ml which were equivalent to 10–50 mouse LD_{50} (1 mouse LD_{50} = 1 mg of protein intravenously administered/kg of body weight).

External application of SNTX did not modify the Na^+ and K^+ currents. After 5–15 min of toxin application, the current trace fluctuated between two states corresponding to the opening and closing of large single channels (Fig. 1*b*). Occasionally, the fluctuations disappeared for several minutes and then reappeared. After ~30 min of toxin application, multistate fluctuations could be seen corresponding to the opening and closing of several channels. At the resting potential, transient compound inward currents corresponding to the sum of several single toxin-induced currents were observed (Fig. 1*c*). These currents were not affected by external tetrodotoxin or tetraethylammonium. This observation indicates that SNTX induces new channels.

In normal Ringer's solution, SNTX-induced currents were inward at negative voltages and outward at positive voltages (Fig. 2). The reversal potential of SNTX-induced currents was found to be +5.5 ± 2.1 mV (n = 8). Taking into consideration the uncertainty in the resting membrane potential, this value cannot be considered as noticeably different than 0 mV. The single current-voltage (I-V) relationship was almost linear with a slight tendency to rectify at large positive and negative voltages. From curves similar to Fig. 2*b*, the conductance of single SNTX-induced channels (calculated from the slope of I-V curves between −100 and +50 mV) was found to be 760 ± 40 pS (n = 8).

In low NaCl Ringer's solution (¾ of NaCl being replaced by sucrose), the SNTX-induced currents became small and brief. In three similar experiments, the reversal potential of these currents was shifted by 28.7 ± 3.5 mV towards negative voltages and the conductance was decreased to 52 ± 7% of its control value. This indicates that the SNTX-induced channels are largely cation selective (in ¼ NaCl Ringer at 16°C, the current though perfectly anion-selective channels should reverse at +31

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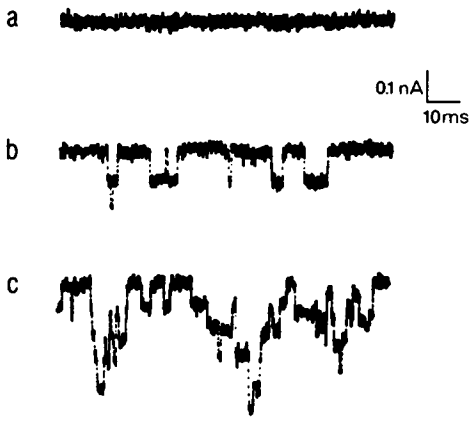

FIGURE 1 Traces of current at −70 mV before addition of SNTX (*a*), and 14 min (*b*), and 35 min (*c*) after addition of SNTX (10 mouse LD_{50}/ml) to the Ringer's solution of the following composition (mM): NaCl, 111.5; KCl, 2.5; $CaCl_2$, 1.8; Co_3HNa, 2.4. $CaCl_2$ Ringer's solutions were buffered with TrisCl (10 mM) or HEPES (2 mM). The temperature was 15.5°C. The fibre number was 18-2-82*a*.

mV, and the current through perfectly cation-selective channels should reverse at −31 mV). Moreover, this conclusion is confirmed by the observation that the SNTX-induced currents are not affected by replacing all but 6 mM of Cl^- by isethionate in the Ringer's solution.

Substitution of external NaCl by KCl or LiCl or internal KCl by CsCl did not modify the open channel I-V curve. When replacing external NaCl by $CaCl_2$, the SNTX-induced currents became very small and brief even at large negative membrane potentials. In these conditions, it was impossible to correctly measure the amplitude of the currents that were inward near −100 mV. These currents could be carried by Ca^{++}. However, one cannot exclude the possibility that these small and brief currents are carried by Cl^- (as suggested by the results in low NaCl Ringer's solution). After superfusion of the SNTX-treated

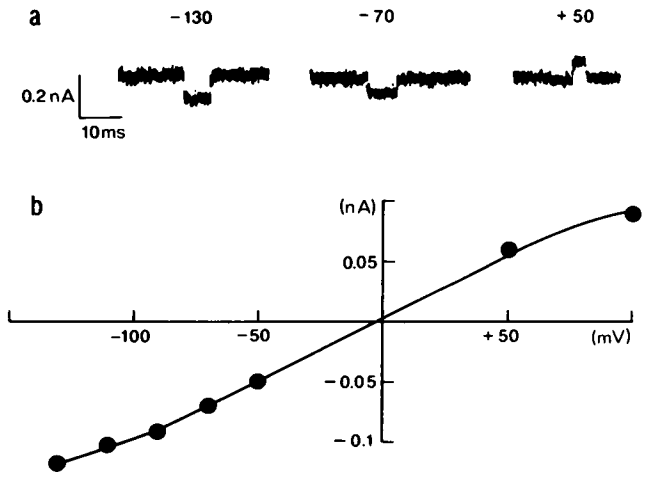

FIGURE 2 *a*, single SNTX-induced current at −130, −70, and +50 mV. *b*, single SNTX-induced current against membrane potential. The temperature was 16.5°C. The fibre number was 24-2-82*c*.

nodes by low NaCl or $CaCl_2$ Ringer's solutions, large SNTX-induced currents could be restored and observed during several minutes by washing with normal SNTX-free Ringer's solution. These observations indicate that the channels are permeable to Na^+, Li^+, K^+, Cs^+, but not (or slightly) to Ca^{++}.

Records of SNTX-induced currents at different membrane potentials revealed that the open time and frequency of opening of the channels increased at large negative voltages. This point is illustrated in Fig. 3, *a* and *b*, which shows typical SNTX-induced currents at −70, −120, and again −70 mV after 15 min (*a*) and 30 min (*b*) of toxin application. This observation indicates that the opening of the channels is voltage-dependent and increases at large negative membrane potentials. Fig. 3*c*, which presents typical histograms showing open and closed time distribution at −70 and −120 mV confirms this conclusion. In three similar experiments, the ratio of mean open time at −70 and −120 mV was 0.25, 0.22, 0.20, and the ratio of mean closed time at −70 and −120 mV was 1.54, 3.00, 3.25. The open time and frequency of opening

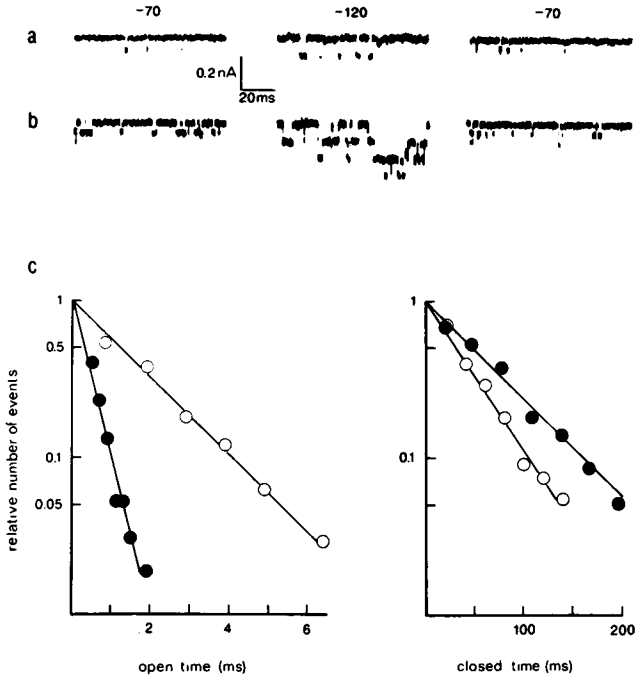

FIGURE 3 *a* and *b*, traces of current at −70, −120, and again −70 mV after 15 min (*a*) and 30 min (*b*) of SNTX application (10 mouse LD_{50}/ml). *c*, semilogarithmic representation of open and closed time distribution of SNTX-induced channels at −70 mV (●) and −120 mV (○). In these records, 150 current steps were recorded at −70 mV and 170 current steps were recorded at −120 mV. The current traces were stored on a tape recorder and digitized at 7,200 Hz on a computer, LSI11/23 (Minc, Digital Equipment). The mean open and closed times were measured in traces where there was no more than one channel open at a given time. In the measurements, durations briefer than 300 μs were neglected. The straight lines indicate the best fit to the distributions. The mean open time, τ_o, was 0.44 ms at −70 mV and 1.73 ms at −120 mV. The mean closed time, τ_c, was 71 ms at −70 mV and 46 ms at −120 mV. The temperature was 16.5°C. The fibre number was 1-3-82.

of the channels seemed to be modified by the change in membrane potential without any delay or induced inactivation during pulses.

From the present results, it appears that SNTX molecules can be incorporated into biological membranes and form large voltage-dependent channels. These channels are very similar to those induced by alamethicin incorporation into the nodal membrane (2). Although alamethicin channels do not discriminate between anions and cations, they are activated at negative voltages and have a conductance of 600 pS (2).

The depolarizing effect of SNTX observed on nerve and muscle (3) is likely to be due to a Na^+ inward current through SNTX-induced channels. However, in the node of Ranvier, concentrations of toxin of 0.2–1 mg of protein/ml were unable to induce a steady-state inward current and a maintained depolarization. The reason was that a maximum of five to six channels could be incorporated into the nodal membrane. On the same preparation, 100–1,000 alamethicin channels could be incorporated (2). An explanation for this discrepency is that the molecule of SNTX is a much larger molecule (MW = 100,000) than alamethicin (MW = 2,000). Because there is a high density of Na^+ and K^+ channels in the frog nodal membrane, only a few molecules of SNTX are likely to be incorporated whereas many SNTX molecules could be incorporated into nonmyelinated nerve fibre membranes and muscle membranes. There are at least two possibilities to explain the relation of the SNTX-induced channels to the effects of the toxin on presynaptic terminals: (*a*) the toxin might act by depolarizing the membrane, thus opening the voltage-dependent Ca^{++} channels and allowing entry of Ca^{++} with a subsequent release of transmitter (4); (*b*) Na^+ influx or K^+ efflux through SNTX channels might lead to exocytosis of transmitter vesicles by causing release of Ca^{++} from intracellular stores (5,6). Further investigations are required to specify the mechanism by which SNTX induces transmitter release.

Like other toxins from coelenterates (7–10), SNTX increases the membrane permeability to monovalent cations. However, SNTX offers the advantage of creating (at least in the nodal membrane) a few large ionic channels whose properties can be studied with classical voltage-clamp techniques. From this point of view, SNTX can be used as a tool to study the transmitter release and to change the internal ionic composition of cells under membrane voltage control.

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