EFFECTS OF A SCORPION TOXIN FROM Androctonus australis VENOM ON ACTION POTENTIAL OF NEUROBLASTOMA CELLS IN CULTURE

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SUMMARY. The effect of toxin II from the venom of Androctonus australis on the action potential of clone NIE-115 of mouse neuroblastoma cells was studied. The toxin increased quasi-irreversibly duration and amplitude of spike and its time derivative; half-maximum effects were obtained at 2.5, 0.3 and 0.4 nM, respectively. Toxin action was potentiated by veratridine and inhibited by tetrodotoxin. Washing suppressed the effect of veratridine and tetrodotoxin but was ineffective on scorpion toxin effect. As in nerve axons and neuromuscular preparations, the studied toxin affects the closing of the Na^+ channel and likely the opening of the K^+ channel.

Substances (neurotoxins) that specifically modify ionic permeabilities of excitable membranes are potential important tools for the characterization of the ion-transporting molecules involved in the functioning of membrane ionophores. Among these, the basic neurotoxic miniproteins (mol.wt. about 7 000) of scorpion venoms are of those which act at the lowest concentration. Of the several neurotoxins isolated from the venom of Androctonus australis Hector (1, 2), toxin II (ScTxII) is the more potent both in vivo (LD_{50}, 10 µg/kg mouse) and on chick embryo heart cells in culture in which it provokes chronotropic and inotropic effects and stimulates the passive uptake of Na^+ and Ca^{2+} at concentrations as low as 10 nM (3).

Several reports strongly suggest that scorpion toxins interact with the action potential Na^+ ionophore. Under voltage-clamp conditions, scorpion venoms from different species provoke, on axonal and neuromuscular preparations, inhibition of Na^+ current inactivation and suppression of K^+ current (4.5) and, for some venoms, a selective effect on Na^+ activation (6). In the giant axons of crayfish and lobster, ScTxI, a toxin closely related to ScTxII, was shown to affect selectively the time-course of Na^+ inactivation and to decrease the steady-state K^+ current (7). These effects are blocked by tetrodotoxin (TTX), a specific inhibitor of the action potential Na^+ current (8).

After culture in appropriate conditions, differentiated neuroblastoma cells can generate action potentials in response to electrical stimulation (9-11) and allow the measurement of Na^+ transport in relation to action potential Na^+ ionophore (12-14). Several reports suggested that an action potential

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Na^+ ionophore identical with that in nerve axons was present in these cells (15, 10). Thus, clonal lines of neuroblastoma cells offer a convenient material to study the chemical basis of the nerve Na^+ and K^+ ionophores.

In an effort to reach this goal, we have undertaken electrophysiological and biochemical studies on the action of scorpion toxins on these cells. This paper reports on our electrophysiological observations.

MATERIALS AND METHODS

Cell culture

Stock cultures of the clonal neuroblastoma cell line NIE-115 proceeded from Dr M. Nirenberg and was the kind gift of Dr Y. Netter. Cells were plated in 32 mm Falcon Petri dishes at an initial density of $5x10^4$ cells/dish. They were cultured for 3 days in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum in a humidified atmosphere of 95% air - 5% CO_2, then for 2 days in the presence of 2% dimethylsulfoxide in the same medium and return to the initial medium with 0.5% fetal calf serum only (Dr Y. Netter, personal communication). The cells were used for electrophysiological studies 1 or 2 days later.

Electrophysiological measurements

Before and during impalement, cells were superfused with Dulbecco's modified Eagle's medium buffered with 20 mM HEPES and containing 1% fetal calf serum at a turnover rate constant of 0.45 min^{-1}. Temperature was maintained at 26-28°C. On the stage of an inverted microscope a large and well differentiated cell was selected and impaled with 2 glass microelectrodes filled with 3 M KCl (resistance between 20 and 40 megohms). Membrane potential, its time derivative (dV/dt) and the stimulating current were recorded on a storage oscilloscope and photographed. In the conditions used, stable electrical measurements could be obtained during several hours.

RESULTS

Immediately after impalement spontaneous firing was sometimes observed. After a few seconds the resting potential remained constant at a value of about-40 mV (41.6 $\pm$ 7.8 mV, mean $\pm$ S.E., n = 25). Using an hyperpolarizing pulse of 3 nA, membrane resistance at resting potential was about 13 megohms (13.3 $\pm$ 3.8, n = 25). These values of the resting potential and membrane resistance agree with those measured in previous investigations (15, 10). Stimulus currents 0.1 to 2 sec long were unable to elicit an action potential at resting potential. However, after adjustment of membrane potential to -80 mV well developed (Fig. 1A) or seldom abortive (Fig. 4A) action potential were always recorded. In both cases, the maximum rates of rise (V+max) and fall (V-max) of the action potential were lower than 2 Vs^{-1}. These low values may be due to cell sampling or more probably to the temperature (26°C) at which experiments were performed.

Effects of ScTxII on action potential

No modification of the resting potential was observed with low concentrations of toxin (< 10 nM). With concentrations higher than 0.1 µM, a small depolarizing effect (up to 10 mV) occured. In contrast, after hyperpolarization to

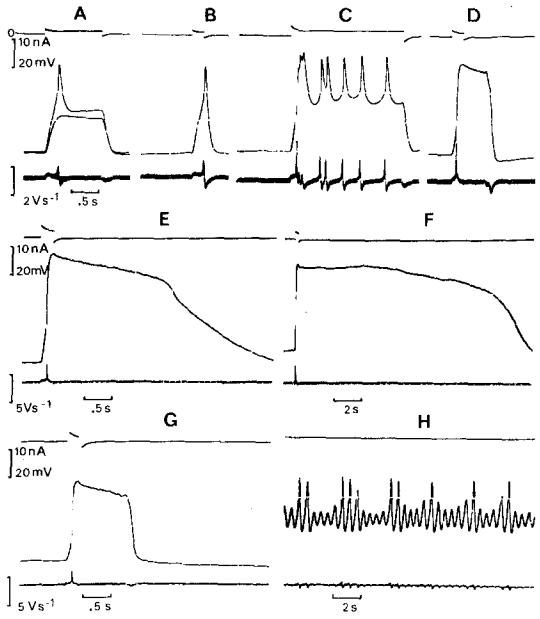

Fig. 1. Action of ScTxII and veratridine on spike electrogenesis in neuroblastoma cells of clone NIE-115. A-B, control records; C-D, effects of 0.1 nM ScTxII recorded 8 and 16 min after the beginning of perfusion with toxin containing medium; E-F, effects of 0.1 nM ScTxII + 1 μM veratridine 2 and 5 min after the beginning of perfusion; G, same as in F after 15 min washing with toxin -free medium; H, same as in F after washing for 26 min and suppression of hyperpolarization. Upper trace, stimulating current (zero membrane potential is at the beginning on left of the trace); middle trace, membrane potential; lowest trace, electronically differentiated version of the action potential (dV/dt).

-80 mV, toxin concentrations as low as 0.1 nM remarkably modified the action potential. With long stimulating pulses the cell fired repetitively (Fig. 1C). With shorter pulses, duration of the spike at half-height of the action potential (D_H), potential at spike peak (Vmax) and V+max increased. With toxin concentrations lower than 10 nM, D_H increased with time of superfusion and reached equilibrium values at 20 min. With higher concentrations, maximum effect was obtained immediately. Vmax and V+max were strongly affected even with the lowest concentrations of toxin (Fig. 1C and D, Fig. 2B to F) without modification of V-max. With 3 nM toxin, the active membrane potential became higher than +20 mV and Vmax exceeded 100 mV. Half-maximum effect on spike prolongation occured at 2.5 nM ScTxII and at 0.3 - 0.4 nM for spike amplitude and rate of rise (Fig. 3). Suppression of the hyperpolarizing current resulted in spontaneous firing lasting for several minutes (Fig. 2F), whatever the toxin concentration used was, and persisted after washing (Fig. 1H).

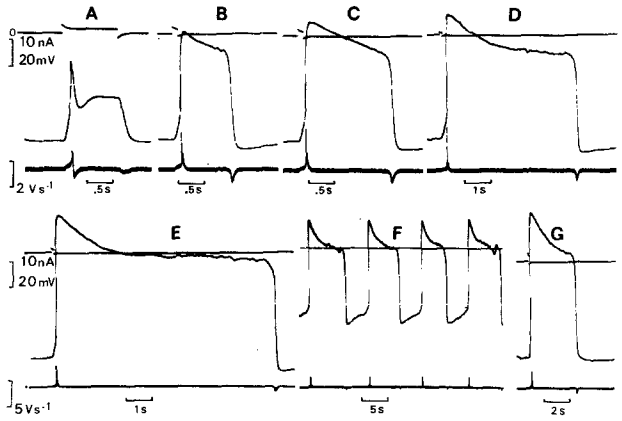

Fig. 2. Effects of increasing concentrations of ScTxII. A, control record; B-E, effects of 0.3 nM (B), 1 nM (C), 3 nM (D) and 10 nM (E) of ScTxII recorded 30, 7, 2 and 2 min after the beginning of perfusion in the presence of toxin, respectively; F, same as in E after suppression of hyperpolarization (spontaneous firing); G, same as in E after washing with toxin-free medium for 45 min.

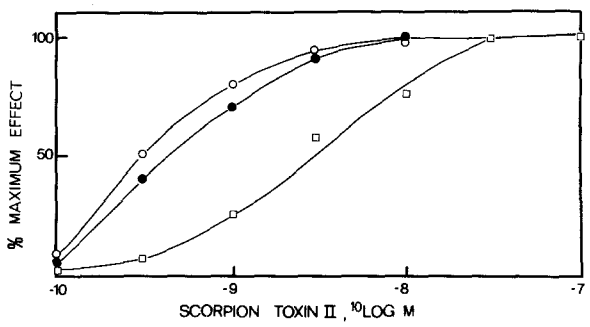

Fig. 3. Dose-response curves of the action of ScTxII on three parameters of the action potential (□, duration at half-height; ●, potential at spike peak, o, time derivative). Results are expressed as % of maximum effect. All measurements were done at equilibrium i.e. when the spike shape no longer varied with time.

Veratridine, an alkaloid which causes repetitive action potentials (16) and in some cases, persistent depolarization of nerves, provoked at 0.1 mM an increase in duration of neuroblastoma action potential with prolongation of its falling phase (not illustrated). No effect was observed at 1 μM. In the presence of 1 μM veratridine and 0.1 nM scorpion toxin, a marked modification of the spike was observed (Fig. 1E and F). Spike duration increased enormously while Vmax and V+max remained unchanged. Membrane potential failed

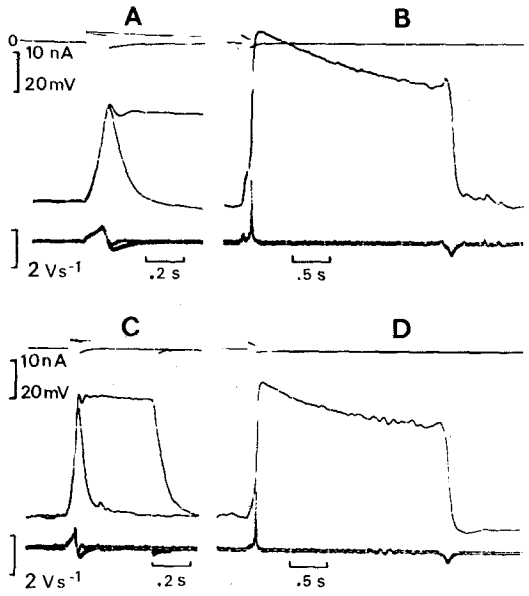

Fig. 4. Effects of ScTxII and tetrodotoxin. A, control record; B, effect of 1 nM ScTxII recorded 15 min after the onset of perfusion with toxin; C, 1 nM ScTxII + 0.2 µM TTX (5 min after onset of perfusion); D, same as in C after 7 min washing with toxin -free medium.

to decrease quickly as it does with toxin alone. After washing, the electrical activity returned to the pattern observed with toxin alone and especially, spontaneous firing could be recorded again after suppression of the hyperpolarizing current (Fig. 1G and H).

TTX provoked a dose-dependent decrease of ScTxII - induced D_H, Vmax and V+max enhancement. Half-maximum effect was obtained at 50 nM. With concentrations higher than 0.2 µM, the action potential was almost completely abolished (Fig. 4C). TTX effects quickly disappeared by washing and action potential characteristics of ScTxII-treated cells reappeared (Fig. 4D).

Tetraethylammonium (TEA), which blocks action potential K^+ current, modified evoked action potential in the same way as ScTxII. However at a concentration as high as 10 mM, duration of the action potential was increased only 3-times. This concentration had only a slight effect on the duration of the spike elicited by 1 nM ScTxII or more.

DISCUSSION

ScTxII modifies quasi-irreversibly the electrical activity of neuroblastoma cells by increasing the amplitude and duration of the action potential,

half-maximum effect being obtained at concentrations of 0.4 and 2.5 nM, respectively. These concentrations are close to those observed for the dissociation constant of binding of ^{125}I-labeled scorpion toxin from Leiurus quinquestriatus venom to the same cells and of enhancement of activation of the action potential Na^+ ionophore by veratridine by this toxin (14).

The interaction of ScTxII with cell receptors is potentiated by veratridine and antagonized by TTX. These neuroactive agents bind to sites different from ScTxII sites as shown by the suppression of their effect by washing whereas, in the same conditions, persistent effects of ScTxII are observed.

Since ionic mechanisms of neuroblastoma cells are still unknown, no complete interpretation of our results are possible. However, by reference to axon membranes, it is clear that ScTxII provokes a huge decrease or a blockade of Na^+ inactivation. Such a mechanism has been shown for scorpion venom or scorpion toxins in several nerve or neuromuscular preparations under voltage clamp conditions (4, 5, 7, 17, 18). The maintainance of a low value of the Na^+ inactivation parameter even at high values of the membrane potential could explain the increase of Vmax and V+max.

A drastic change in sodium activation of frog myelinated nerve fibres by the venom of Centruroides sculpturatus (6) has been observed. With Androctonus (7) and Leiurus (5) toxins sodium activation is only slightly affected. Indeed, after repolarization at the end of the spike, the membrane potential falls as promptly as in control cells which is not the case with drugs such as veratridine affecting both Na^+ activation and inactivation.

Under voltage-clamp conditions, K^+ current is reduced by scorpion venom in squid axon (4) and by ScTxI in crayfish and lobster giant axons (7). TEA also depresses K^+ current; it prolongs action potential and triggers repetitive firing. However, enlargement of action potential by ScTxII is always greater than in the presence of excess TEA, suggesting that an other mechanism must be involved to explain the prolongation of the falling phase of the action potential. A quantitative interpretation of ionic mechanisms and scorpion toxin effects in neuroblastoma cells must await voltage-clamp data.

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