Some Properties of Sodium Channels in Neuroblastoma Cells Modified with Scorpion Toxin and Chloramine-T. Single Channel Measurements

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Abstract. Currents through sodium channels of neuroblastoma cells were measured using patch technique in outside-out configuration. Scorpion toxin (ScTX) produced 3 to 4 fold prolongation of mean open time and increased number of reopenings. The mean open times showed slow fluctuations around some average values. The distribution of channel open times for ScTX-modified channels required more than one exponential to be fitted. Chloramine-T (ChT) produced qualitatively similar, though weaker, prolongation of open times.

Key words: Neuroblastoma — Patch clamp — Sodium channel — Scorpion toxin — Chloramine-T

Introduction

Changes in sodium permeability in excitable cells in response to step depolarization proceed in two phases: fast rise called activation, and slowlier decay — inactivation (Hodgkin and Huxley 1952). The development of sodium inactivation plays the main role in the termination of action potential, and restoration of sodium permeability from inactivation determines, to a great extent, the duration of the refractory period. Much valuable information about gating mechanisms in sodium channel was obtained from measurements of macroscopic sodium currents on axons and neuronal cells treated with toxins (Koppenhöfer and Schmidt 1968; Khodorov et al. 1975; Mozhayeva et al. 1980 a,b; Meves et al. 1984; Gonoi et al. 1984; Strichartz and Wang 1986), enzymes (Armstrong et al. 1973; Rojas and Rudy 1976) and chemicals (Stämpfli 1974; Oxford et al. 1978; Wang 1984; Zaborovskaya and Khodorov 1984), which decelerate or even eliminate sodium inactivation. The patch-clamp technique (Hamill et al. 1981) has allowed to study the behavior of single normal and modified sodium channels and thereby to elucidate some features of the channel

gating mechanism which cannot be seen from records of macroscopic currents.

In the present paper we describe some properties of single sodium channels in neuroblastoma cells modified by toxin from the venom of *Buthus eupeus* scorpion and by chloramine-T.

Preliminary results of this work were published in abstract form elsewhere (Mozhayeva et al. 1988).

Materials and Methods

Experiments were performed on neuroblastoma cells (clone N 18-A) using patch clamp technique in outside-out configuration (Hamill et al. 1981). The pipettes were filled with artificial intracellular solution containing (mmol/l): 140 KF or CsF, 1 $MgCl_2$, 2.5 EGTA, 1.4 $CaCl_2$, 20 K-HEPES (pCa 7.0; pH 7.4). The extracellular solution contained (mmol/l): 160 NaCl, 2 $CaCl_2$, 1 $MgCl_2$ and 10 Tris-HCl (pH 7.5). Where appropriate, scorpion toxin (ScTX) (working designation 2001 (Mozhayeva et al. 1980 b)) was added to the external solution at 10^{-6}—10^{-5} mol/l. Chloramine-T (ChT), 0.2 mmol/l, was added to external solution of the same composition as the control one except a lower pH (6.8). The effect of ChT was irreversible; after 1—2 minutes of treatment, the ChT containing solution was replaced with control extracellular solution.

The pipettes were prepared from Pyrex glass and their resistances ranged between 4 and 10 MΩ. As soon as gigaseal has been formed and patch was excised, the tip of the pipette was transferred to a microchamber of a volume of approximately 30 μl made of glass tube.

Membrane potential (E) was taken as voltage measured inside (pipette solution) minus that measured outside. The current signal was filtered at about 1.7 kHz, digitized at 8 kHz, and stored on floppy discs. In some experiments currents were recorded with a tape recorder. Capacity transients were partially cancelled at the level of headstage (Hamill et al. 1981). Residual transients were removed by averaging records without openings and subtracting the average from each record in series.

Depolarizing pulses were applied with a pacing frequency of 1 Hz. Holding potential usually was set at -80 or -100 mV. Depolarizing test potential was preceed by 100 ms hyperpolarizing prepulse to -120 or -140 mV.

Amplitude histograms were constructed using several representative records for each potential value. Each histogram included several peaks corresponding to no, one, two, or more open channels at a time. Each peak was described by Gaussian curve and the difference between means of these curves was taken as unitary current amplitude.

Open-closed transitions were determined applying the half-amplitude threshold analysis (Colquhoun and Sigworth 1983). Mean open times (T_o) were calculated by averaging the durations of the single channel events. Open dwell time histograms were calculated using records without multiple openings. The histograms were fitted with one or more exponentials using the least squares routine. When fitting one or two bins were discarded to reduce errors from missed brief openings.

Experiments were carried out at 20—22°C.

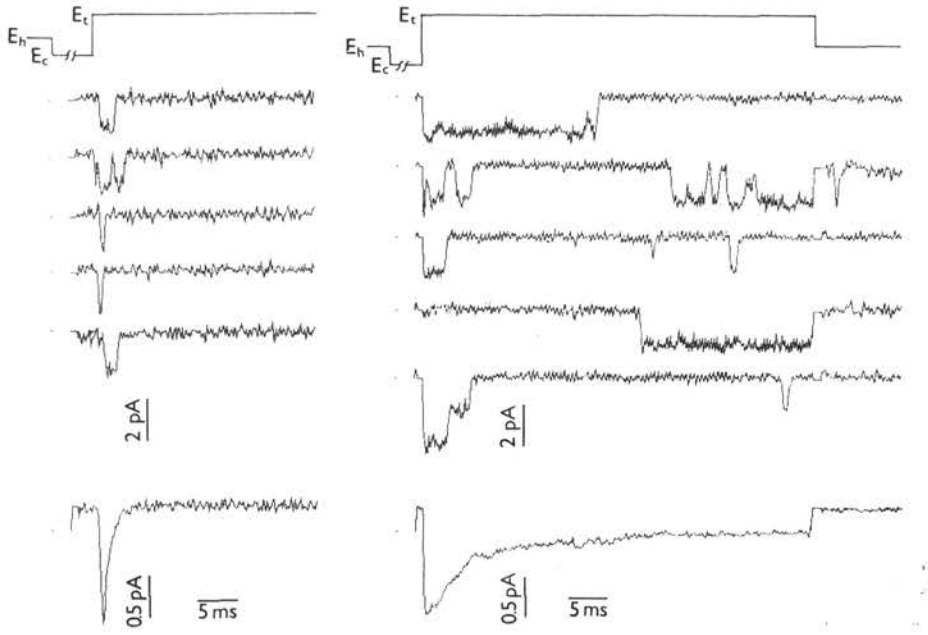

Fig. 1. Single-channel and mean currents through normal *(left column)* and scorpion toxin-modified *(right column)* channels at test potential (E_t) −30 mV. For the untreated patch holding potential (E_h) and conditioning potential (E_c) are −80 and −120 mV, respectively. The corresponding values for the toxin-modified channels are −100 and −140 mV. Mean currents are averages of 77 *(left)* and 213 *(right)* individual records.

Results

Scorpion toxin effect

Fig. 1 shows representative records for native and ScTX-treated membrane. It can be seen that channel openings in untreated membrane are short and are concentrated mainly at the very beginning of the depolarizing pulse. In the presence of ScTX openings are evidently longer and occur throughout the pulse rather than at its beginning only. Average currents show a ScTX effect pattern as seen from macroscopic measurements — fast inactivation of normal channels and a much slower and incomplete one of ScTX-modified channels (Mozhayeva et al. 1980b; Gonoi et al. 1984).

Fig. 2 presents mean open times (T_o), averaged from several experiments, of the channels in untreated and ScTX-treated membranes. Mean T_o values for normal channels were 0.63 ms and 0.77 ms at −30 to −16 mV, respectively.

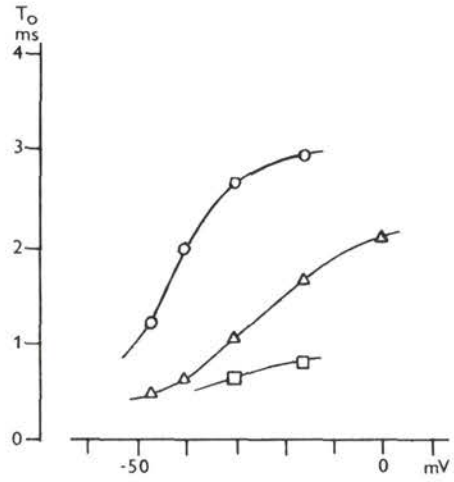

Fig. 2. Mean open times (T_o) as a function of membrane potential. Squares — control, circles — ScTX-modified channels, triangles — ChT-modified channels. Each symbol represents an average obtained from measurements on two to five patches.

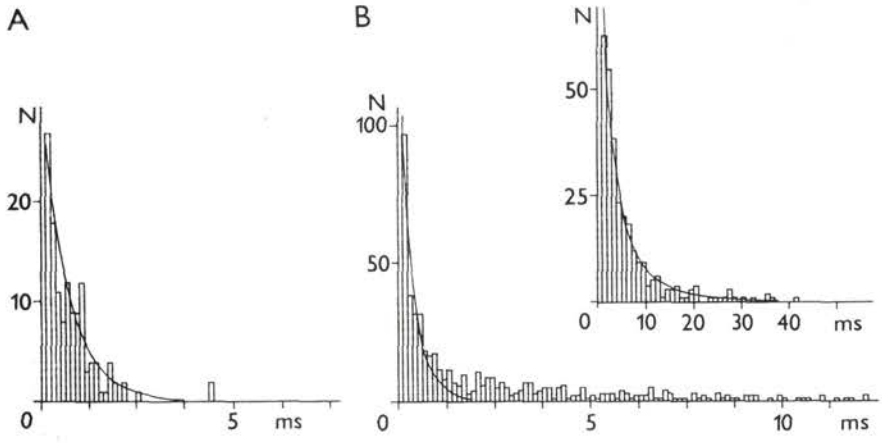

Fig. 3. Open time histograms at −30 mV obtained for untreated (*A*) and ScTX-treated (*B*) patches. *N* — the number of events per bin. The histogram for toxin-treated channels is shown with two time scales. The total numbers of events were 130 (*A*) and 293 (*B*). Continuous lines — exponential fit. The histogram for normal channel is fitted by one exponential. The histogram for ScTX-modified channel is fitted by sum of three exponentials with time constants τ_f, τ_{s1} and τ_{s2}. *A* — $\tau = 0.69$ ms; *B* — $\tau_f = 0.36$ ms, $\tau_{s1} = 2.7$ ms, $\tau_{s2} = 10.4$ ms.

ScTX treatment prolonged T_o over the whole range of voltages tested; prolongation by the factor 4.4 and 3.8 was observed at -30 and -16 mV, respectively. Similar as T_o for normal channels, T_o for ScTX-modified channels decreased with more negative potential.

In view of the limited time resolution of our device one could propose that events which appeared as long-lasting were actually bursts of short openings separated by short unresolvable closings. The possibility that some closings were missed cannot be excluded but it seems quite improbable that this is the only, or major, reason for the occurrence of long events.

A more detailed picture of channel gating before and after ScTX treatment is provided by open time histograms. Fig. 3 shows representative open time histograms for normal and ScTX-modified channels. The distribution of open times for normal channels can be fitted by a single exponential at all potentials tested. For a single exponential distribution, time constant (τ) equals mean open time (T_o); hence values given in Fig. 2 for normal channels are simultaneously both T_o and τ. It should be noted, however, that events much longer than those expected from single exponential distribution with time constant below one ms were infrequently observed in untreated patches.

Open time histograms for ScTX-treated membrane consistently had two distinct components: a fast and a slow one. Correspondingly, they could be fitted by two (or more) exponentials with fast (τ_f) and slow (τ_s) time constants. Average time constant of the fast component was 0.32 and 0.36 ms for potentials -30 and -16 mV, respectively (five experiments).

The slow component of the distribution seems to consist of two exponential components with time constants τ_{s1} and τ_{s2}. In the experiment illustrated in Fig. 3, τ_{s1} and τ_{s2} were 2.7 and 10.4 ms, respectively. In other experiments the numbers of events were unsufficient to allow the resolution of the two slow exponential components, and the slow part of the distribution was fitted by a single exponential. Correspondingly, time constants (τ_s) varied from 3.6 to 4.6 ms at -30 mV, and from 5.6 to 7.6 ms at -16 mV.

It might be argued that the nonmonoexponential pattern of the open time distribution is due to not all channels in the patch being modified by the toxin, and that the fast and the slow components of the distribution simply present nonmodified and modified channels in the patch. However, suprasaturating ScTX concentration (10^{-6} mol/l) was used and further elevation (to 10^{-5} mol/l) neither decreased the fast components nor increased the slow components. Further, it should be noted that the time constant of the fast component of the open time distribution of the toxin-treated membrane was consistently smaller than that for normal channels. Thus, multiexponential open time distribution seems to be an intrinsic property of ScTX-modified channels.

Experiments on rat heart and frog skeletal muscles (Patlak and Ortiz 1985;

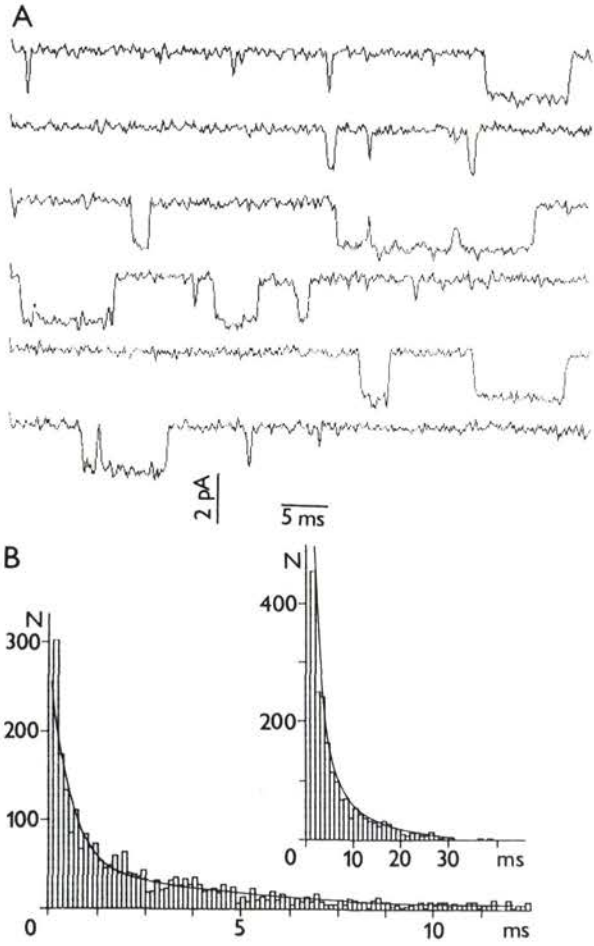

Fig. 4. Representative fragments of current records during steady-state depolarization to −30 mV (*A*) and the corresponding open time histogram (*B*). The histogram is shown with two time scales. *N* — number of events per bin. The total number of events was 2606. Continuous lines — exponential fit with sum of three exponentials. The same patch as in Fig. 3. $B - \tau_f = 0.46$ms, $\tau_{s1} = 1.67$ ms, $\tau_{s2} = 8.2$ ms.

Patlak et al. 1986) have shown that sodium channels occasionally can show long-lasting openings when the duration of the depolarizing pulse well exceeds the time usually required for nearly complete channel inactivation. In light of these data it may be proposed that in ScTX-treated membrane of neuroblastoma cells long-lasting events are typical for prolonged or steady-state depolarization and short ones for just early depolarizing pulses.

We did not estimate the distribution of short and long events during depolarizing pulse; however, visual inspection of the records suggests that both

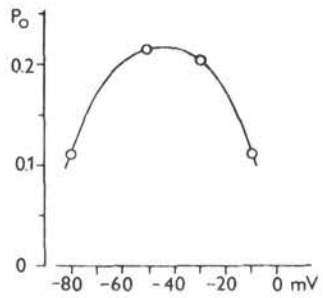

Fig. 5. Steady-state probability for the toxin-modified channel to open (P_o) as a function of membrane potential. Each potential value was set for several minutes.

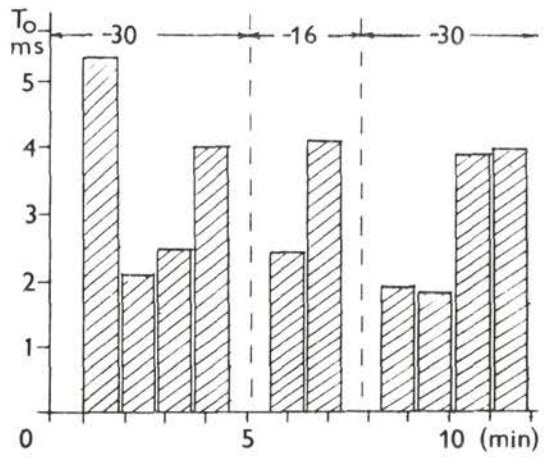

Fig. 6. Fluctuation of mean open times of ScTX-modified channels during the experiment. Each bar corresponds to a series of 100 records. The figures on the top indicate potential (mV) during depolarizing pulse. Holding potential −100 mV, conditioning potential −140 mV. See text for explanations.

long and short events occur at the beginning of the depolarizing step as well as tens of milliseconds later (see e.g. Fig. 1).

Fig. 4 shows representative fragments of current records and an open dwell time histogram obtained from steady-state measurements when the membrane was depolarized for several minutes. Both fast and slow components are present. The time constant of the fast component of the open time histogram is close to that obtained in the same experiment with pulse measurements from holding potential −100 mV and with a prepulse to −140 mV. Thus, we believe that the channel can open both for brief and for long periods throughout depolarization.

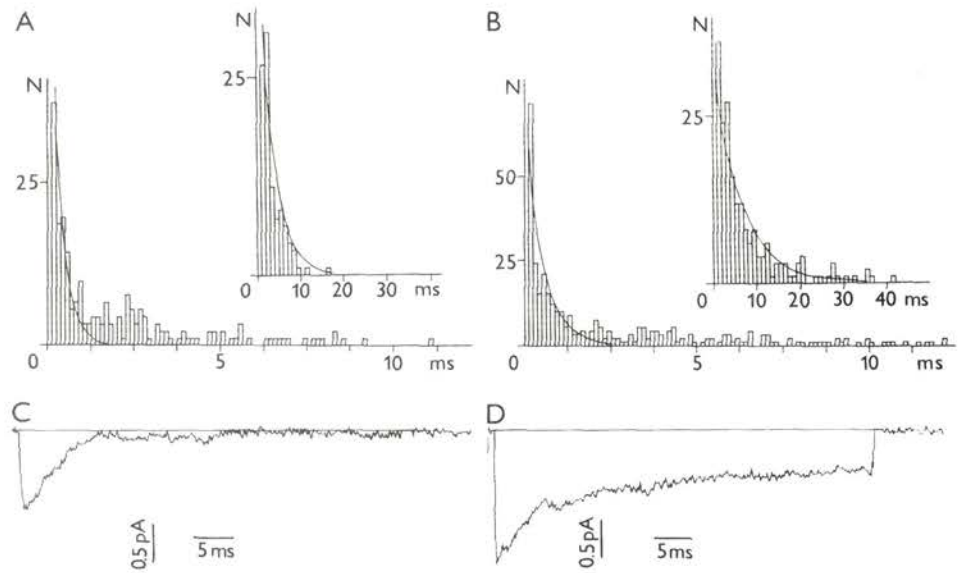

Fig. 7. Open time histograms (*top*) and mean currents (*bottom*) obtained from four series of measurements with "short" (*A*, *C*) and four series with "long" (*B*, *D*) openings at test pulse −30 mV. See Fig. 6 and the text. Open time histograms are shown with two time scales. Continuous lines — fast and slow exponentials. The same experiment as in Fig. 6.
A − $\tau_f = 0.31$ ms, $\tau_s = 3.13$ ms; *B* − $\tau_f = 0.52$ ms, $\tau_s = 6.4$ ms.

The steady-state measurements revealed that in the presence of ScTX the channel is active at potentials as negative as −60 to −80 mV. However, at these potentials openings became too short to be safely resolved, but the probability for a channel to open (P_o) could be approximately estimated as the fractional square of the amplitude histogram (Kolb et al. 1986). Fig. 5 shows the voltage dependence of steady-state P_o obtained in one experiment. P_o has a maximum at about −50 mV. It decreases at more positive potentials, evidently due to slow inactivation, and possibly because of slow ScTX dissociation (Mozhayeva et al. 1980b); the decrease at more negative potentials is evidently due to the growing rate of closing.

It should be noted that values of T_o (Fig. 2) and of time constant of dwell time distributions given in Fig. 3 and in the text above are means obtained by averaging data from, as a rule, several series of measurements. A closer inspection shows that parameters obtained in different series of measurements varied. It can be seen from Fig. 6 that mean open time of ScTX-modified channels

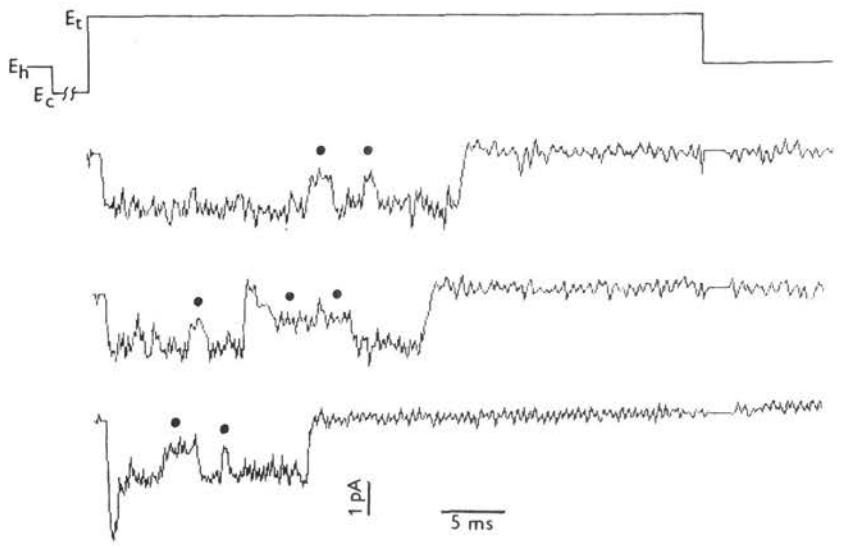

Fig. 8. Representative single channel current records with current sublevels. Sublevels are indicated by dots. Holding potential −100 mV, conditioning potential −140 mV.

fluctuated between approximately 2 and 4 ms during the period of measurements of about 12 min. Open time histograms from series with "short" (about 2 ms) and "long" (about 4 ms) mean times were summed separately (Fig. 7). The most notable difference between both histograms is that in the slow components. The first distribution, designed "short" has the time constant of the slow component of 3.1 ms, and the other, "long", has the slow time constant of 6.4 ms.

Series with "long" and "short" distributions gave different mean currents. "Long" series gave mean current which inactivated only partially during 60 ms pulse, whereas mean current from "short" series inactivated completely. These data are far from being quantitative but they demonstrate that gating properties of the channels undergo some slow reversible changes over time.

Both normal and ScTX-modified channels of neuroblastoma cells have conductances of about 15 pS at room temperature and at Na concentration of 160 mmol/l. This estimate is true for most events observed. However, current levels of lower amplitudes were sometimes observed in addition to "normal" most probable current levels. Examples are shown in Fig. 8. These sublevels were approximately half the normal ones.

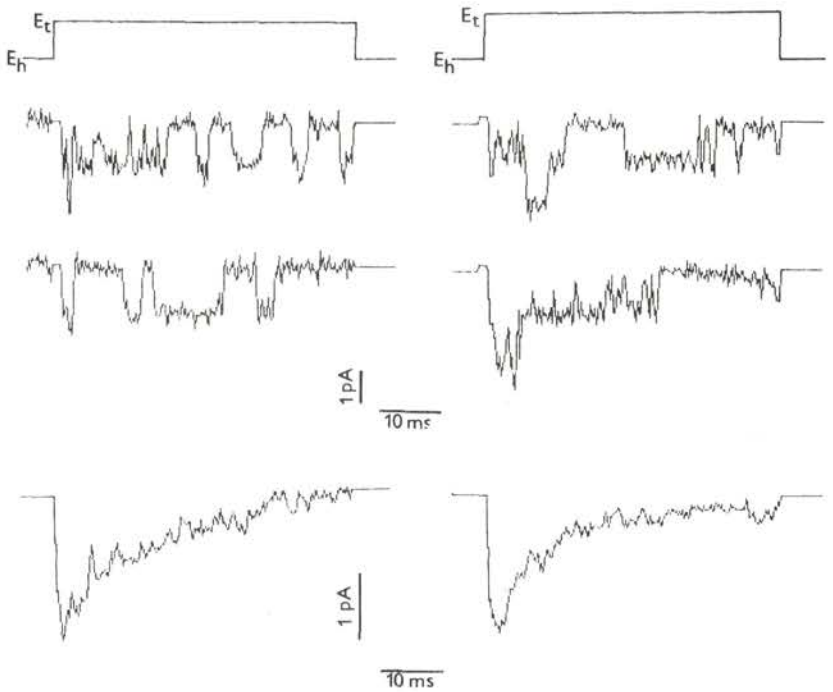

Fig. 9. Single channel and mean currents through chloramine-T modified channels at $E_t - 30$ mV (*left*) and -16 mV (*right*). Holding potential -80 mV.

Effect of chloramine-T

Fig. 9 shows examples of single channel recordings in membranes treated with 0.2 mmol/l chloramine-T (ChT) for 2 min at pH 6.8. The effect of ChT was irreversible, so the measurements were carried out after the ChT-containing solution had been replaced by control solution. It can be seen that, like ScTX, ChT produces long lasting events and increases the total number of events during depolarization. In accordance with macroscopic measurements (Nosyreva et al. 1987) mean currents through ChT-modified channels inactivate slowly with a time constant of about 12 ms at -30 mV. ChT increases mean open time (see Fig. 2), but to a lesser extent than does ScTX. At potentials more negative than -40 mV, T_o values for normal and ChT-modified channels are almost identical. The open time histogram for openings of ChT-modified channels at potentials -16 and 0 mV required at least two exponentials to be fitted. At -30 mV the histogram could be fitted quite reasonably by a single exponential.

Discussion

The most evident effect of ScTX and ChT on single sodium channel behavior is prolongation of open dwell times. Qualitatively similar effects have been reported for N-bromacetamide and pronase (Patlak and Horn 1982; Horn et al. 1984), cardiotropic compound DPI (Kohlhardt et al. 1986; Nilius et al. 1987), chloramine-T (McCarthy and Yeh 1987), sea anemone toxin (Nagy 1987a; Schreibmayer et al. 1987), toxin from scorpion *Leiurus quinquestriatus* (Nagy 1988). All the above agents were previously shown to decelerate or remove inactivation of macroscopic sodium channels. The prolongation of open times thus seems to indicate a decreased rate of transition of the channel from open to inactivated state. The prolongation of open times is an important but not unique determinant of the time course of macroscopic inactivation. According to our data, for example, ScTX prolonged open times more pronouncedly that did chloramine-T, but ScTX-modified channels inactivated faster than did ChT-modified channels (see Figs. 1 and 8, and the text). Further, N-bromacetamide (Horn et al. 1984) prolonged open times stronger than did ScTX (3 to 10 times and 3—4 times, respectively) but this difference is not sufficient to account for qualitative differences in inactivation between N-bromacetamide- and ScTX-modified channels. In the former inactivation is practically absent, whereas it is quite obvious in the latter. It is natural to explain differences between the three types of modified sodium channels (ScTX-, ChT- and N-bromacetamide-modified channels) by differences in the numbers of reopenings which in turn are expected to depend on the rate of transition from inactivated to noninactivated state (open or closed). Any treatment modifying inactivation seems to change both forward and backward inactivation rates, and each of them induce quantitatively different changes.

Of all the toxins capable to decelerate inactivation, the sea anemone toxin (ATX-II) bears the closest resemblance to ScTX. Like ScTX, ATX-II is a peptide; it induces deceleration of inactivation and shares a common receptor site with ScTX on the sodium channel (Bergman et al. 1976; Jacques et al. 1978; Catterall and Beress 1978; Neumcke et al. 1980; Warashina and Fujita 1983). According to Nagy (1987a) ATX-II causes an about two-fold increase of mean open time, whereas ScTX induces 3- to 4-fold increase. This quantitative difference seems to be due to weaker decelerating effect of ATX-II as compared with ScTX (Bergman et al. 1976; Neumcke et al. 1980). It cannot be excluded however that ATX-II or ScTX action is influenced by some minor difference in sodium channel properties between the two neuroblastoma clones used (N1E-115 and N18 A).

Recently, a toxin from scorpion *Leiurus quinquestriatus* was used to modify single sodium channel (Nagy 1988). The channels modified by Leiurus and

Buthus toxins displayed brief and long open times. The Leiurus toxin-modified channels have longer brief open times (0.8—1 ms) and shorter long times (2.6—3.4 ms) as compared with Buthus toxin-modified channels (see Results).

One of the most interesting features of ScTX-modified channels is the occurrence of both brief and long-living openings. The presence of more than one exponential component in open time distribution can be explained by the interconversion of channels between two or more modes of activity with different open times (Hess et al. 1984). Then, the multiexponential distribution of open times simply results from simultaneous activity of several channels which interconvert between "modes" independently of each other. The evidence for the occurrence of two or more modes of activity were presented both for normal (Patlak and Ortiz 1985; Patlak et al. 1986) and for drug modified (Nilius et al. 1987) sodium channels. The slow fluctuations of mean open times observed in the present work seem to reflect interconversion of ScTX-modified channels between at least two "modes" of activity with different gating parameters. It should be noted however, that the different "modes" of activity of sodium channels described earlier (Patlak and Ortiz 1985, 1986; Patlak et al. 1986; Nilius et al. 1987) correspond to different time intervals of depolarization: brief openings were observed within first ten milliseconds of depolarizing step, and long-living ones were seen during prolonged depolarizations and were termed "late" currents. In our experiments brief openings were seen both within the first milliseconds of the depolarizing step, and many seconds later. The same was true for long-lasting events. Thus, if coexistence of brief and long openings is due to the ability of ScTX-modified channel to display more than one "mode" of behavior, these "modes" seem to be distinct from those described earlier for sodium channels. They have more resemblance to "mode" 1 (brief openings) and "mode" 2 (long-lasting openings) of Ca channels in heart (Hess et al. 1984).

Another way to explain the nonmonoexponential distribution of the open times is to assume that there are more than one open state (conformation) the channel can assume. The data in favour of this idea were obtained both from macroscopic measurements (Chandler and Meves 1970; Armstrong and Bezanilla 1977; Mozhayeva et al. 1980a; Sigworth 1981) and from single channel measurements (Nagy 1987b). The final conclusion concerning the explanation of the multiexponential distribution of open times will require sufficient data obtained from measurements on patches containing only a single channel.

Sublevels of unitary currents seems to be the general property of ion channels (Geletyuk and Kazachenko 1985; Fox 1987). As to sodium channels, the heterogeneity of current amplitudes was reported by Nagy and coauthors (1983). The lower level accounted for about 0.78 of the most probable one. In our experiments the sublevel was approximately a half of the main level. Similar results were reported in a study with toxin from scorpion *Leiurus quinquestriatus*

(Nagy 1988). Scorpion toxins themselves do not seem to produce current sublevels; rather, they make them easier to be observed due to the increased total probability for channels to open.

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References

Armstrong C. M., Bezanilla F. (1977): Inactivation of the sodium channel. II. Gating current experiments. J. Gen. Physiol. **70,** 567—590

Armstrong C. M., Bezanilla F., Rojas E. (1973): Destruction of sodium conductance inactivation in squid axons perfused with pronase. J. Gen. Physiol. **62,** 375—391

Bergman G., Dubois J. M., Rojas E., Rathmayer W. (1976): Decreased rate of sodium inactivation in the node of Ranvier induced by a polypeptide toxin from sea anemone. Biochim. Biophys. Acta **455,** 173—184

Catterall W. A., Beress L. (1978): Sea anemone toxin and scorpion toxin share a common receptor site associated with the action potential sodium ionophore. J. Biol. Chem. **253,** 7393—7396

Chandler W. K., Meves H. (1970): Evidence for two types of sodium conductance in axons perfused with sodium fluoride solution. J. Physiol. (London) **211,** 653—678

Colquhoun D., Sigworth F. J. (1983): Fitting and statistical analysis of single channel records. In: Single-Channel Recording (Ed. B. Sakmann and E. Neher), pp. 191—263, Plenum Press, New York, London

Fox J. A. (1987): Ion channels subconductance states. J. Membrane Biol. **97,** 1—8

Geletyuk V. I., Kazachenko V. N. (1985): Single Cl^- channels in molluscan neurones: multiplicity of the conductance states. J. Membrane Biol. **86,** 9—15

Gonoi T., Hille B., Catterall W. A. (1984): Voltage clamp analysis of sodium channels in normal and scorpion toxin-resistant neuroblastoma cells. J. Neurosci. **4,** 2836—2842

Hamill O. P., Marty A., Neher E., Sakmann B., Sigworth F. J. (1981): Improved patch-clamp technique for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch. **391,** 85—100

Hess P., Lansmann, J. B., Tsien R. W. (1984): Different modes of Ca channels gating behaviour favored by dihydropyridine Ca agonists and antagonists. Nature **311,** 538—544

Hodgkin A. L., Huxley A. F. (1952): The dual effect of membrane potential on sodium conductance in the giant axon of Loligo. J. Physiol. (London) **116,** 497—506

Horn R., Vanderberg C. A., Lange K. (1984): Statistical analysis of single sodium channels. Effect of N-bromacetamide. Biophys. J. **45,** 323—335

Jacques Y., Fosset M., Lazdunsky M. (1978): Molecular properties of action potential Na ionophore in neuroblastoma cells. Interaction with neurotoxins. J. Biol. Chem. **253,** 7383—7392

Khodorov B. I., Peganov E. M., Revenko S. V., Shishkova L. D. (1975): Sodium currents in voltage-clamped nerve fibre of frog under combined action of batrachotoxin and procaine. Brain Res. **84,** 541—546

Kohlhardt M., Frobe H., Herzig J. W. (1986): Modification of single cardiac Na channels by DPI 201-106. J. Membrane Biol. **89,** 163—172

Kolb H. A., Brown C. D. A., Murer H. (1986): Characterization of a Ca-dependent maxi K channels in the apical membrane of a cultured renal epithelium (ITC-12.P3). J. Membrane Biol. **92,** 207—215

Koppenhöfer E., Schmidt H. (1968): Die Wirkung von Scorpiongift auf die Ionenströme des Ranvierschen Schnürrings. II. Unvollständige Natrium Inaktivierung. Pflügers Arch. **303,** 150—161

McCarthy W. A., Yeh J. Z. (1987): Chloramine-T removes closed and open Na-channel inactivation in NIE-115 neuroblastoma cells: a single channel/multi-channel patch study. Biophys. J. **51,** 436 a

Meves H., Rubly N., Watt D. D. (1984): Voltage-dependent effect of a scorpion toxin on sodium current inactivation. Pflügers Arch. **402,** 24—33

Mozhayeva G. N., Naumov A. P., Nosyreva E. D. (1980a): Kinetics of sodium currents decay on repolarization of axonal membrane under normal conditions and in the presence of scorpion toxin. Neirofisiologiya (Kiev) **12,** 549—557 (in Russian)

Mozhayeva G. N., Naumov A. P., Nosyreva E. D., Grishin E. V. (1980b): Potential-dependent interaction of toxin from venom of the scorpion *Buthus eupeus* with sodium channels in myelinated fibre. Voltage clamp experiments. Biochim. Biophys. Acta **597,** 587—602

Mozhayeva G. N., Naumov A. P., Nosyreva E. D., Kuryshev Yu. A. (1988): The study of kinetics of single sodium channels with decelerated inactivation. Abstr. 5th Soviet-Swiss Symp. Biol. Membr. Structure and Function. Riga, p. 74

Nagy K. (1987a): Kinetic behaviour of chloramine-T and sea anemone toxin modified single sodium channels in neuroblastoma cells. Pflügers Arch. **408,** Suppl., R 37

Nagy K. (1987b): Evidence for multiple open states of sodium channels in neuroblastoma cells. J. Membrane Biol. **96,** 251—262

Nagy K. (1988): Mechanism of inactivation of single sodium channels after modification by chloramine-T, sea anemone toxin and scorpion toxin. J. Membrane Biol. **106,** 29—40

Nagy K., Kiss T., Hof D. (1983): Single Na channels in mouse neuroblastoma cells membrane. Indication for two open states. Pflügers Arch. **399,** 302—308

Neumcke B., Schwarz W., Stämpfli R. (1980): Modification of sodium inactivation in myelinated nerve by Anemonia toxin II and iodate. Analysis of current fluctuations and current relaxations. Biochim. Biophys. Acta **600,** 456—466

Nilius B., Benndorf K., Markwardt F., Franke T. (1987): Modification of single cardiac sodium channels by DPI 201-106. Gen. Physiol. Biophys. **6,** 409—424

Nosyreva E. D., Grishchenko I. I., Negulyaev Yu., A. (1987): Effect of chloramine-T on activation and inactivation of sodium channels of neuroblastoma cells. Neirofisiologiya (Kiev) **19,** 789—794 (in Russian)

Oxford G. S., Wu C. H., Narahashi T. (1978): Removal of sodium channel inactivation in squid axons by N-bromacetamide. J. Gen. Physiol. **71,** 227—247

Patlak J., Horn R. (1982): Effect of N-bromacetamide on single sodium channel currents in excised membrane patches. J. Gen. Physiol. **79,** 333—351

Patlak J. B., Ortiz M. (1985): Slow currents through single sodium channels of the adult rat heart. J. Gen. Physiol. **86,** 89—104

Patlak J. B., Ortiz M. (1986): Two modes of gating during late Na^+ channel currents in frog sartorius muscle. J. Gen. Physiol. **87,** 305—326

Patlak J. B., Ortiz M., Horn R. (1986): Open time heterogeneity during bursting of sodium channels in frog skeletal muscle. Biophys. J. **49,** 773—778

Rojas E., Rudy B. (1976): Destruction of the sodium conductance inactivation by specific protease in perfused nerve fibres from Loligo. J. Physiol. (London) **262,** 501—531

Schreibmayer W., Kazerani H., Tritthart H. A. (1987): A mechanistic interpretation of the action of toxin II from *Anemonia sulcata* on the cardiac sodium channel. Biochim. Biophys. Acta **901,** 273—282

Sigworth F. J. (1981): Covariance of nonstationary sodium current fluctuations at the node of Ranvier. Biophys. J. **34,** 111—132

Stämpfli R. (1974): Intraaxonal iodate inhibits sodium inactivation. Experientia **30,** 505—508
Strichartz G. R., Wang G. K. (1986): Rapid voltage-dependent dissociation α-toxins coupled to Na channel inactivation in amphibian myelinated nerves. J. Gen. Physiol. **88,** 413—435
Wang G. K. (1984): Modification of sodium channel inactivation in single myelinated nerve fibres by methionine-reactive chemicals. Biophys. J. **46,** 121—124
Warashina A., Fujita S. (1983): Effect of sea anemone toxins on the sodium inactivation process in crayfish axons. J. Gen. Physiol. **81,** 305—323
Zaborovskaya L. D., Khodorov B. I. (1984): The role of inactivation in cumulative blockage of voltage-dependent sodium channels by local anaesthetics and antiarrythmics. Gen. Physiol. Biophys. **3,** 517—520.

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