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Subconductance states of single sodium channels modified by chloramine-T and sea anemone toxin in neuroblastoma cells

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Abstract. Single channel currents of chloramine-T (Chl-T) and sea anemone toxin (ATX-II) modified sodium channels were studied in neuroblastoma cells. With both substances similar subconductance states have been observed. The conductances of the sublevels were multiples of the "unit" step which was about one-fourth of the most frequently occurring main conductance. Thus, the current levels observed were one fourth, half and five-fourths of the main current size. Both substances caused a slower decay of the averaged current compared to the current of the native channels. The main single-channel conductance was 15.2 pS ($T = 16$ °C) for the Chl-T and 10.8 pS ($T = 12$ °C) for the ATX-II modified channels. The channel open time was doubled by ATX-II, but was not increased significantly by Chl-T. The existence of the subconductance states suggests that the native channels may also have multiple open conformations.

Key words: Single sodium channels, multiple conductance states, chloramine-T, sea anemone toxin, neuroblastoma

Introduction

Multiple conductance states have been reported for acetylcholine receptor channels (Colquhoun and Sakmann 1985; Auerbach and Sachs 1983; Hamill and Sakmann 1981; Sachs 1983), inward-rectifying potassium channels in guinea-pig heart (Sakmann and Trube 1984), K^+ channels in the sarcoplasmic reticulum of the frog leg muscle (Labarca and Miller 1981), and calcium-activated potassium channels in rat muscle (Barret et al. 1982). However, no subconductance states have so far been observed in voltage sensitive sodium channels, probably because of their short open time. Substances which remove the inactivation of sodium channels offer the possibility of prolonging the open time and thus analysing the single channel current size of these channels on an expanded time scale. In the present work chloramine-T (Chl-T) and sea anemone toxin (ATX-II) were chosen to inhibit the inactivation of sodium channels in neuroblastoma cells. It is demonstrated that these channels can adopt different subconductance states.

Materials and methods

Sodium channel currents were measured in mouse neuroblastoma cells, N1E 115, by the patch-clamp method in the cell-attached configuration (Hamill et all. 1981). The cell membrane was hyperpolarized by 40 to 80 mV to remove resting inactivation. From the constant holding potential, 90 ms long depolarizing voltage pulses of different heights were applied at a frequency of 1.0–1.5/s.

Pipette and bath solutions contained (in m*M*): NaCl 150, KCl 5.0, $CaCl_2$ 0.9, $MgCl_2$ 0.4, HEPES 20, glucose 10. pH was adjusted to 7.3 and temperature (between 8° and 16 °C) was kept constant during each experiment.

In 11 experiments, cells were incubated in bath solution containing 0.5–1 m*M* chloramine-T. After 5 to 8 min the chloramine-T solution was washed out and measurements were carried out in normal bath solution.

In 8 experiments, 3–7 μ*M* ATX-II was added to the pipette solution. ATX-II from Anemonia sulcata was purchased from Ferring GmbH (Kiel, FRG).

A DEC LSI 11/23 microcomputer generated the voltage pulses, collected the data with 10 kHz sampling rate (2× the Nyquist frequency) and was used for off-line analyses. Details of the measuring system are described by Hof (1986). Analog signals were filtered at 2 kHz (− 3 dB) by a four-pole low-pass Bessel filter. Leakage and capacitive currents were compensated by an analog circuit (Nagy et al. 1983). The parameters of the recording system were estimated by the

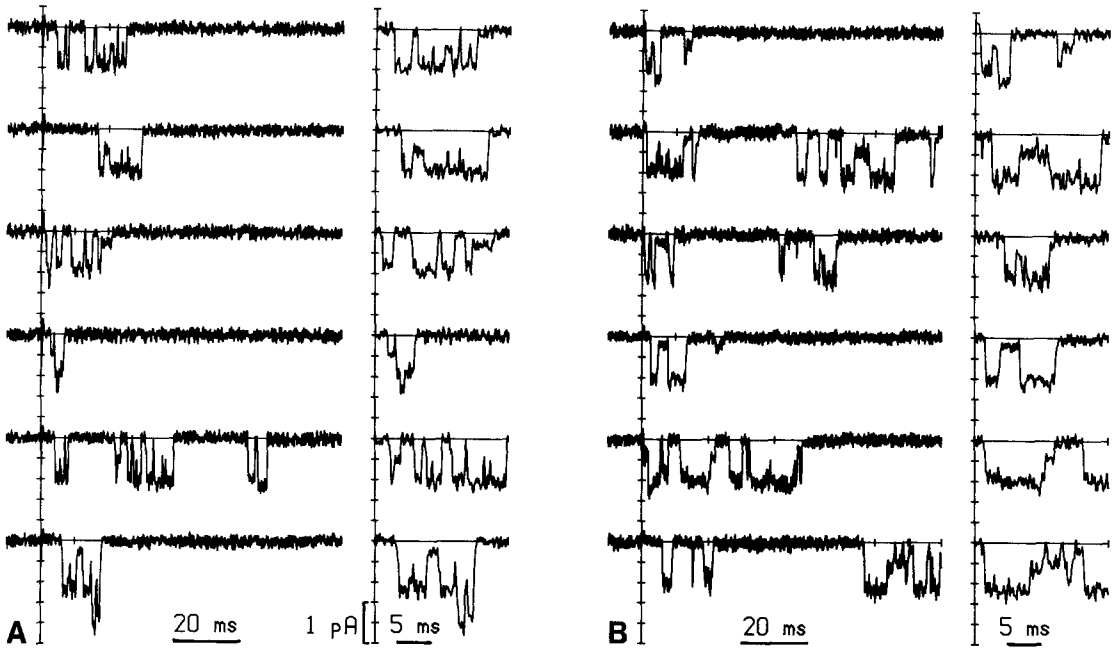

Fig. 1 A and B. Selected single channel current records from sodium channels modified by chloramine-T (**A**) or sea anemone toxin (**B**). The pulse length was 90 ms (left columns in A and B). 20 ms segments of records having sublevels are displayed on an expanded time scale in the right columns. For **A**: $V_m = RP + 10\,\text{mV}$, $V_H = RP - 70\,\text{mV}$, the main single channel current size was 0.94 pA. For **B**: $V_m = RP + 20\,\text{mV}$, $V_H = RP - 60\,\text{mV}$, the main single channel current size was 0.98 pA. Temperature 12 °C

equations of Colquhoun and Sigworth (1983), yielding 0.166 ms for the rise time and 0.090 ms for the dead time. The peak of a unit pulse was reached if the pulse duration was longer than 0.562 ms. The signal-to-noise ratio was $\geqq 8.33$.

Current records having sublevels longer than 0.56 ms were selected. On these records current points of segments of different amplitudes were averaged separately. Mean values of segments were compared to the mean value of the most frequently occurring current size (see Fig. 2 B).

To compare the averaged current records of different patches (Fig. 2 A) records at the same pulse potential would be required. Because the absolute value of the resting potential was not known I made use of the strong potential dependence of the fast time constant of the first latency density and selected records in which this time constant was identical.

Results

Chloramine-T has an irreversible effect on the sodium channels in neuroblastoma cells similar to that reported for myelinated nerve fibres (Wang 1984) and squid axons (Wang et al. 1985). It slows the decay of the averaged single channel current (Fig. 2 A). However, unlike the effect of pronase and *N*-bromoacetamide (Patlak and Horn 1982) the channel open time is only slightly increased. The mean open time (measured at the threshold of the half-value of the main current size regardless of the current levels) was 1.05 ± 0.23 ms ($n = 5$) at $V_m = RP = 20\,\text{mV}$ ($T = 15\,°\text{C}$), which is close to the value of 0.86 ± 0.10 ms ($n = 7$) obtained for the native channels at the same temperature and relative potential. Channels open and close several times during a depolarization (see Fig. 1 A), resulting in a slower decay of the averaged current as shown in Fig. 2 A. The integral of the open channel probability was increased by a factor of 2.8 compared to the native channels. The single channel conductance of the chloramine-T modified channels (estimated from the most frequent current size) was 15.2 ± 2.4 pS ($n = 5$) at 16°C, which is similar to the conductance of the native channels (14.6 pS at 16 °C calculated from Nagy et al. (1983) with the $Q_{10} = 1.28$).

In Fig. 1 A selected single channel currents from a single patch are shown. Besides the most frequent amplitude of 0.94 pA different sublevels can be observed. One level is around 0.5 pA, which can be reached either from the closed (record 4) or from the open state (record 1, 2 and 5). In selected records of different patches this current level was observed in 4% to 12% of openings. Ocassionally ($< 4\%$ of all openings) a "partially closed" state (~ 0.25 pA) appeared (record 3 and 6 in Fig. 1 A). Often definitely larger currents than the most frequent were measured, as records 4 to 6 demonstrate.

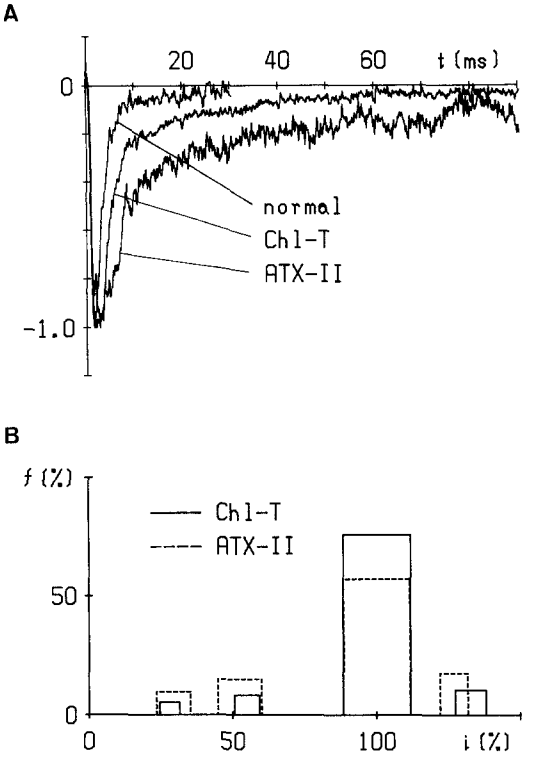

Fig. 2. **A** Averaged single channel currents. The mean currents are normalized to the peak value. Temperature 12 °C. **B** Comparison of the size of the sublevels (*i*) with the main current size. On the ordinate the relative occurrence (*f*) of the sublevels longer than 0.56 ms is plotted calculated on selected current records (see Materials and methods). For Chl-T the mean values of the currents (± SD) were (in %) 28.0 ± 3.4, 55.0 ± 4.3, 100.0 ± 11,7 and 132.7 ± 5.4 with the occurrence of (in %) 5.3, 8.4, 75.8 and 10.5, respectively. The total number of events was 178 from four patches. For ATX-II the currents (± SD) were (in %) 29.4 ± 5.9, 52.4 ± 7.6, 100.0 ± 11.5 and 126.8 ± 4.8 with the relative occurrence of (in %) 9.8, 15.0, 57.1 and 17.5, respectively. The sum of events was 114 from three patches

Current amplitude histograms constructed from all digitized points showed an asymmetrical bell-shaped curve as described previously for the native channels (Nagy et al. 1983). However, in these histograms the contribution of sublevels cannot be recognized due to their rare occurrence and short life time. Therefore, single events were calculated as described in Materials and methods.

In Fig. 2 B the realtive size and occurrence of sublevels (on selected records) are compared to the main current size. The occurrence of the sublevels was ~ 4% of the total number of openings in patches inspected. The plot in Fig. 2 B shows that the smallest sublevel is about one-fourth of the main size. Twice as large is the next sublevel size. The largest level is 32.7% larger than the main current size, and the difference is also close to the one-fourth "unit" step. It is not clear whether the large standard deviation of the main size is due to the undistinguishable three-fourth "unit" step.

Similar results were obtained with ATX-II modified sodium channels. The decay of the averaged single channel currents was slower with ATX-II than with chloramine-T (see Fig. 2 A), suggesting that it is a more effective blocker of inactivation in neuroblastoma cells. Channel openings can occur several times, some of them very late, during a depolarization (Fig. 1 B). The integral of the open channel probability was 5.5 times larger than that of the native channels. The mean channel open time (measured at the half-value of the main current size) was 2.3 ± 0.18 ms ($n = 4$) at $V_m = RP + 20$ mV ($T = 12$ °C), about twice as long than that of the native channels. The single channel conductance (calculated from the main current size) was 10.8 ± 1.8 pS ($n = 4$) at 12 °C, which is slightly lower than that of the chloramine-T modified and native channels (Nagy et al. 1983).

In single channel current records (Fig. 1 B) sublevels similar to those of the Chl-T modified channels can be observed. Because the channel open time is prolonged by ATX-II the occurrence of sublevels longer than 0.56 ms increased (see Fig. 2 B). In the three patches inspected about 7% of all openings displayed sublevels. Figure 2 B shows that the mean values of the sublevels are similar to those of the Chl-T modified channels. However, in the amplitude histograms constructed from all digitized points the presence of the sublevels could not be recognized.

Discussion

The present work demonstrates that chloramine-T and sea anemone toxin modified sodium channels can have subconductance states. These open states can be reached from both the closed and the main open state. The conductance of the sublevels are multiples of about one-fourth of the most frequent conductance. The main conductance of both modified channels is similar to that of the native channels. A recent paper by Chinn and Narahashi (1986) reported that deltamethrin strongly prolongs the open time of sodium channels in neuroblastoma cells and thus made the observation of a subconductance state possible.

The fact that similar sublevels are observed with different substances suggests that the sublevels probably exist in the unmodified channels too, but due to their short life times and rare occurrence are overlooked. The relative occurrence of sublevels for the native channels (supposing the same relative probability for occurrence) can be estimated from the presented results. The integral of the open channel probability was increased by a factor of 2.8 and 5.5 for the Chl-T and ATX-II modified channels, respectively. The rela-

tive occurrence of sublevels (longer than 0.56 ms) for the two channel types were ~ 4% and ~ 7%. From these values the estimated occurrence of sublevels for the native channels is about 1% of all openings.

The open time of the ATX-II modified channels, but not the open time of the Chl-T modified channels was increased. This suggests that the two substances may alter the kinetic properties of the sodium channels differently. Frequent reopening of channels was observed for both substances suggesting that the absorbing property of the inactivated state is changed. This observation suggests a different mechanism than expected from the macroscopic current measurements. The inhibition of the macroscopic inactivation is usually interpreted by the inhibition of the open-inactivated transition resulting in a prolonged open time. The presented results suggest that the rate constant for the inactivated-open transition is increased by both substances (making the revisiting of the open state possible), but the open-inactivated transition rate is definitely reduced only by ATX-II.

The effect of ATX-II on neuroblastoma cells is similar to that of pronase on chick dorsal root ganglion neurons (Carbone and Lux 1986), in which preparation both longer open times and reopenings of channels were observed. Similarly, ATX-II causes a prolongation of the channel open time and reopenings on rat ventricular cells (Schreibmeyer et al. 1986). By contrast, Patlak and Horn (1982) found a strong prolongation of the open time, but no reopening on rat myotubes treated with pronase or *N*-bromoacetamide. These differences indicate different kinetics of the sodium channels in different preparations.

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