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IRREVERSIBLE MODIFICATION OF SODIUM CHANNEL INACTIVATION IN TOAD MYELINATED NERVE FIBRES BY THE OXIDANT CHLORAMINE-T

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SUMMARY

1. The effects of externally applied chloramine-T on the excitability of single toad myelinated nerve fibres were studied. Chloramine-T is a mild oxidant which reacts specifically with the cysteine and methionine residues of proteins.

2. Chloramine-T prolongs the action potential of a single myelinated fibre by more than 1000-fold. This effect is concentration- and time-dependent; higher concentrations and longer incubation times increase prolongation.

3. Under voltage-clamp conditions, sodium channel inactivation is markedly inhibited by chloramine-T while sodium channel activation remains normal.

4. Prolonged depolarization of the membrane leads to a maintained sodium current. The maintained sodium currents show activation kinetics, dependence on membrane potential, and reversal potentials which are similar to those of normal, inactivating sodium currents in untreated fibres. Both the maintained and the peak sodium currents are equally inhibited by tetrodotoxin.

5. After partial removal of sodium inactivation by brief exposures to chloramine-T, the voltage dependence of the steady-state sodium current inactivation (h_∞) is shifted in the depolarized direction by about 20 mV, even after correction for the non-inactivating component contributed by the maintained current.

6. The phenomena described here imply that cysteine or methionine residues are critical for the sodium channel inactivation processes. The two different modifications of inactivation, its removal shown by the maintained current, and the shift in the voltage-dependence of the remaining inactivatable channels, reveal that at least two separate residues are modified by chloramine-T.

INTRODUCTION

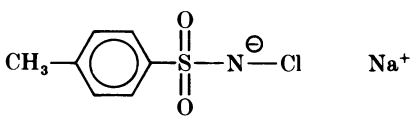
Chemical modification can yield important information on the relationship between specific structures and the functions of ion channels (for review see Brodwick & Eaton, 1982). The function of sodium channels is to allow the passage of sodium ions transiently through the membrane during a nerve impulse. Hodgkin & Huxley (1952) found that under voltage-clamp conditions sodium permeability was increased rapidly by membrane depolarization (activation) and subsequently decreased more slowly during the maintained depolarization (inactivation). In recent years many laboratories have used a variety of reagents to modify the sodium channel structure.

The results of these studies showed that both proteolytic enzymes and *N*-bromoacetamide could destroy the sodium channel inactivation in squid giant axons (Armstrong, Bezanilla & Rojas, 1973; Oxford, Wu & Narahashi, 1978). Further studies suggested that the cleavage at tyrosine and arginine carboxyl sites accessible from the axoplasmic side of the sodium channel was responsible for the removal of inactivation (Rojas & Rudy, 1976; Eaton, Brodwick, Oxford & Rudy, 1978; Oxford *et al.* 1978). Several other treatments were also found to inhibit the inactivation: for example, internal iodate on frog myelinated fibres (Stämpfli, 1974), high internal pH on squid axon (Brodwick & Eaton, 1978), and low internal pH on skeletal muscle (Nonner, Spalding & Hille, 1980). In addition, Shrager (1977) demonstrated that *N*-ethylmaleimide, a sulphydryl-specific reagent, can promote a slow inactivation process.

In the experiments reported here, I have used yet another reagent, chloramine-T, to study the functional groups for sodium channel gating. In solution at neutral and slightly alkaline pH (7·0–8·5) chloramine-T reacts preferentially with the most exposed methionine residues of soluble non-cysteine proteins and with the free sulphydryl group of cysteine residues (Shechter, Burstein & Patchornik, 1975). The effects of chloramine-T on sodium channel kinetics were studied under voltage-clamp conditions using single myelinated fibres of the toad.

METHODS

Chemicals. Chloramine-T trihydrate (mol. wt. 281·68) was purchased from Fisher Chemical Co., 461 Riverside Ave, Medford, MA 02155. Powdered chloramine-T was always dissolved in Ringer solution within 30 min before actual use. The structure of chloramine-T is shown below:



Tetrodotoxin was obtained from Calbiochem-Behring Co. All other chemicals were reagent grade from commercial sources.

Myelinated nerve voltage clamp. Single myelinated fibres from sciatic nerves of the toad (*Bufo marinus*) were isolated, mounted in a Lucite chamber and voltage clamped at 8 °C as described by Dodge & Frankenhaeuser (1958). The volume of the solution bathing the node of Ranvier was about 0·1 ml and the length of axon in this solution was about 150 μm. About 2 ml of test solution was exchanged through this pool for drug introduction and removal. Potassium current was blocked by external tetraethylammonium chloride (TEACl, 12 mM) and by internal caesium chloride (0·12 M). In some cases potassium current was measured when the solution contained no TEACl and the internodes were cut in 0·12 M-potassium chloride. The linear membrane leakage and capacitance currents were subtracted by an analogue circuit with two exponential time constants. The leak currents rarely changed after the application of chloramine-T. The holding potential was set at −95 mV to minimize slow sodium inactivation (Fox, 1976). The time course of ionic currents was recorded on a storage oscilloscope and photographed for later analysis. A graphical digitizer (Houston Instrument) in conjunction with a Horizon microcomputer (NorthStar Computers Inc., Berkeley, CA) was used to quantify the current amplitude. The peak current amplitude, calculated by assuming an internodal resistance of 20 MΩ, yielded a value of 10–25 nA at −10 mV.

Solutions. Frog TEA-Ringer contained 12 mM TEACl, 110 mM-NaCl, 2·5 mM-KCl, 2·0 mM-$CaCl_2$, 5 mM-HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethane sulphonic acid), pH 7·2.

RESULTS

Effects of chloramine-T on action potentials of single myelinated fibres

Action potentials of single myelinated fibres usually had a duration of 3–8 ms at 8 °C when potassium channels were blocked by both external TEACl and internal caesium chloride (Fig. 1*A*). After treatment with chloramine-T for 10 min, at concentrations of 0·178–1·07 mM, the duration of a single action potential lasted for as long as 5 s. Chloramine-T concentration less than 45 μM had little effect on the

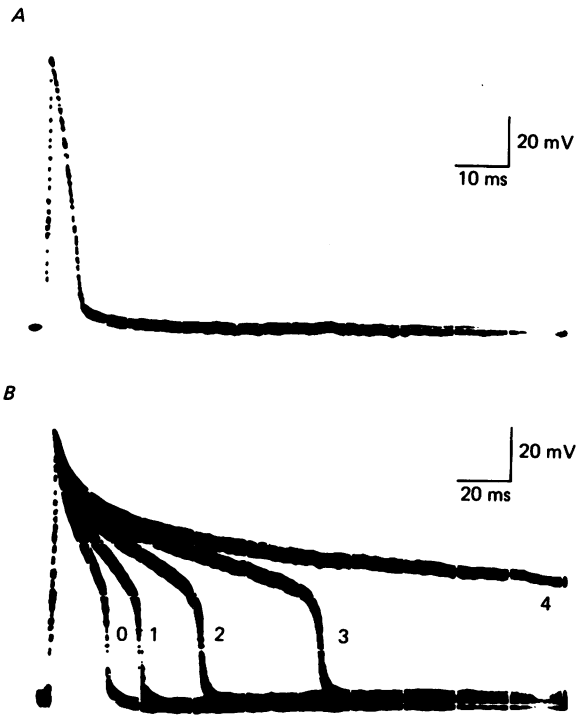


Fig. 1. Action potentials of a single myelinated fibre. *A*, control action potential in TEA-Ringer. *B*, action potentials of the same fibre after exposure for 7 min to 89 μM-chloramine-T in TEA-Ringer (trace 0), and further treatment with 178 μM-chloramine-T in TEA-Ringer (traces 1, 2, 3 and 4 correspond to 2, 4, 6 and 8 min respectively in 178 μM-chloramine-T solution). Temperature, 8 °C.

duration of action potentials; at 89 μM a 3-fold increase in duration was observed after a 7 min incubation period, as shown in Fig. 1*B*. When the chloramine-T concentration was further raised to 178 μM, the action potentials progressively lengthened. The peak amplitudes of the action potentials were not much affected at this reagent concentration for exposures of up to 8 min (Fig. 1*B*). However, longer exposures (> 20 min) not only prolonged the action potential duration to 1–5 s but also reduced the peak amplitude over time. This reduction could have been due to the accumulation of sodium ions in the axoplasm. Effects of chloramine-T were not reversed by washing, implying that covalent chemical modifications of the sodium channels had occurred.

Chloramine-T slows and removes the inactivation process of sodium channels

Under voltage-clamp conditions the most immediate effect of chloramine-T on the sodium current was the appearance of a persistent current at the end of an 8 ms test pulse, as shown in Fig. 2. Control sodium currents in the untreated node had nearly decayed to zero at the end of this depolarization. The magnitude of this maintained current increased progressively during continued chloramine-T exposure and the rate

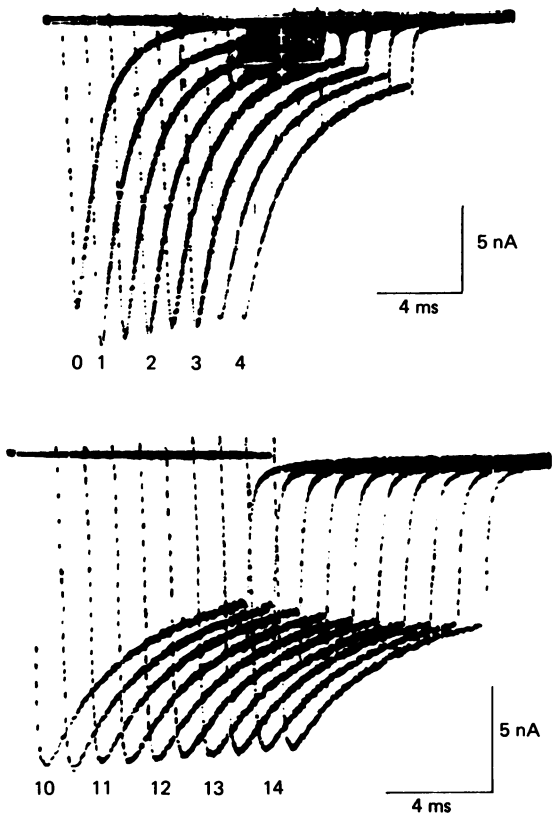


Fig. 2. Effects of chloramine-T on sodium current. Currents were measured at the membrane potential of +5 mV before and after treatment with 1·07 mM-chloramine-T. The numbers by the current traces are the drug incubation times in minutes. Consecutive currents are each displaced 1 ms apart for comparison. Holding potential, −95 mV.

of this increase was dependent on the concentration of applied chloramine-T (Fig. 3). The peak sodium current was transiently increased and then slowly reduced during exposure to chloramine-T at 1·07 mM (Fig. 2). At higher chloramine-T concentrations the decrease in the peak sodium current occurred more rapidly (Fig. 3). An increase in the sodium current peaks has also been observed on frog nerve fibres after internal application of iodate (Stämpfli, 1974), on squid axons after internal treatment with proteolytic enzymes (Rojas & Rudy, 1976) and, occasionally, after internal treatment with *N*-bromoacetamide (Oxford *et al.* 1978).

In addition, sodium current inactivated more slowly following the chloramine-T treatment (see Fig. 2). After 5 min of chloramine-T treatment the half-times for inactivation of the sodium current were at least 2–5 times longer than those of the control current recorded at membrane potentials from −30 to +30 mV (data not shown). This analysis included the correction for steady-state non-inactivatable sodium current.

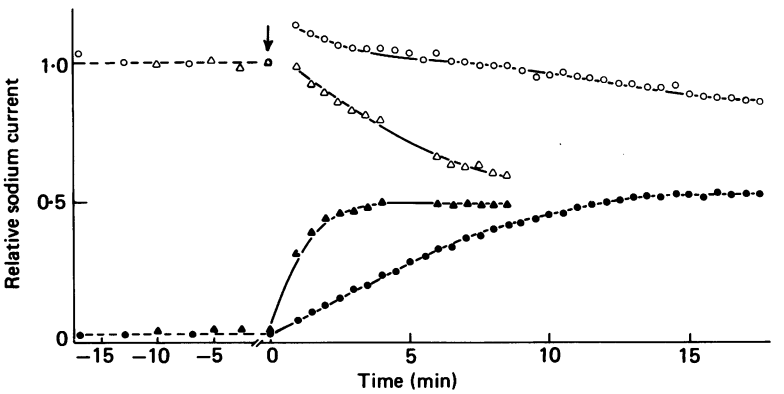


Fig. 3. Time-course of chloramine-T action on sodium currents. Chloramine-T at concentrations of 1·07 mM (○, ●) or 1·78 mM (△, ▲) was added to two different nerve fibres at time 0 (arrow). The experiments were performed exactly as described in Fig. 2. The open symbols are the relative peak sodium current compared with that measured at time 0; the filled symbols are the relative sodium current remaining at the end of an 8 ms pulse compared with the peak sodium current measured at time 0.

Voltage dependence of the chloramine-T-modified sodium channel

Sodium currents at different membrane voltages were compared on normal and chloramine-T-treated fibres (Fig. 4). The most apparent effects were the slowing of the inactivation process and a slight decrease in peak sodium current due to prolonged incubation of the nerve fibre with chloramine-T. The peak current–voltage relationships before and after chloramine-T are shown in Fig. 5*A*. The voltage dependence of peak sodium current in chloramine-T-treated fibres was very similar to that of untreated fibres; the sodium channels were activated beginning at a membrane potential near −55 mV and the reversal potentials were almost identical (see Fig. 5*A*). A slight displacement of the reversal potential by 5 mV to the left was probably due to sodium ions accumulated in the axoplasm during prolonged depolarization. The peak sodium permeability was calculated from the Goldman–Hodgkin–Katz equation and plotted against the membrane potential (Fig. 5*B*). For comparison, the voltage dependence of the maintained sodium current is also shown in Fig. 5*B*. The rising branches of these curves coincide, indicating that the activation process remained normal after chloramine-T treatment.

Other criteria for measuring the activation process in the presence of chloramine-T were also used. The turn-on of sodium currents, measured as the half-time required to reach peak sodium current, was not much changed. On average, the difference in half-time between chloramine-T-treated and control fibres was less than 20%, analysed at potentials between −30 and 20 mV. The 'tail' currents, measured upon

return to the holding potential after a 1 ms depolarization to 0 mV, had similar kinetics before and after chloramine-T treatment (ratio of decay half-times ($t_{\frac{1}{2}}$, chloramine-T)/($t_{\frac{1}{2}}$, control) = $1{\cdot}2 \pm 0{\cdot}2$, $n = 3$). These results imply that the activation process of sodium channels was insensitive to reaction with chloramine-T.

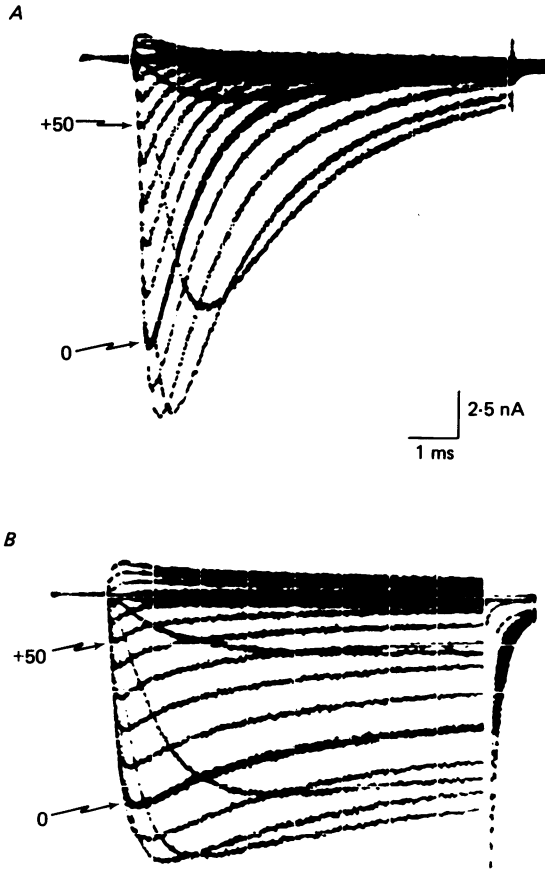


Fig. 4. Sodium currents at various membrane potentials before (*A*) and after (*B*) treatment with 1·07 mM-chloramine-T for 33 min. The holding potential was −95 mV. Numbers near the peak currents of several traces indicate the membrane potentials in millivolts for those traces. The current traces are recorded in increments of 10 mV.

Modified sodium channels are sensitive to tetrodotoxin

Modified sodium channels did not inactivate completely, even during a long depolarization of 400 ms, as illustrated in Fig. 6*A*. In fact, the rate of inactivation after 25 ms of depolarization was too slow to be measured (half-times > 2 s), producing a truly steady-state sodium current thereafter. Under normal conditions the sodium current would have been inactivated rapidly and totally by the same maintained depolarization (Chiu, 1977; and for toad see Fig. 2).

The peak sodium current and the maintained sodium current were both sensitive

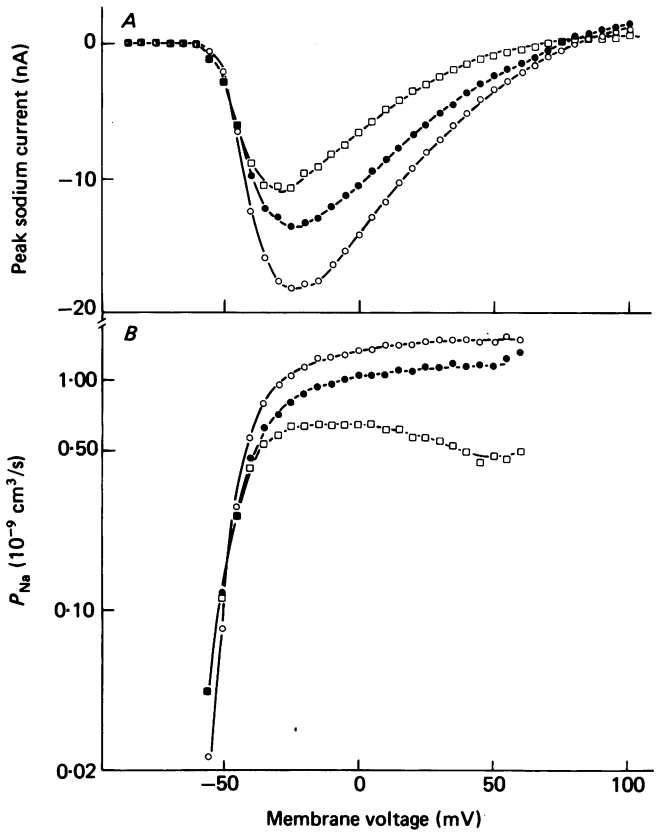


Fig. 5. *A*, relationship between peak sodium current and membrane voltage before (○) and after (●) treatment with 1·07 mM-chloramine-T. This experiment was performed as in Fig. 4 and some of the data shown here were obtained from Fig. 4. *B*, the peak sodium currents in *A* were converted to the sodium permeability (P_{Na}) according to the Goldman–Hodgkin–Katz equation:

$$P_{Na} = I_{Na}\, RT\,(e^{EF/RT} - 1)/F^2 E\, Na_o\,(e^{(E-E_{Na})F/RT} - 1).$$

Open and filled circles indicate before and after treatment with chloramine T. The relationship between maintained current (measured at 8 ms) and membrane voltage (□) is also shown in both *A* and *B*.

to tetrodotoxin (TTX) as shown in Fig. 6*B–D*. The currents were completely inhibited by 1 μM-TTX. At lower concentrations, the peak and maintained currents were equally sensitive to TTX, each being reduced by 40 % at 5 nM-TTX and by 65 % at 10 nM-TTX. From this result an equilibrium dissociation constant of 5·4–7·5 nM for TTX was calculated, a value which is slightly higher but comparable to that of unmodified frog sodium channels (Schwarz, Ulbricht & Wagner, 1973; Hahin & Strichartz, 1981), showing that the TTX binding site was not significantly modified by chloramine-T. This was further confirmed by a direct binding study using

[^{3}H]saxitoxin as described previously (Strichartz, 1982). A reduction of less than 10% of the total saxitoxin binding sites was found in a membrane preparation from rabbit brain after treatment with a similar chloramine-T concentration.

Maintained sodium currents and conditioning pulses

The results reported above clearly showed that sodium channels modified by chloramine-T could be activated normally and remain open during a long depolarization. It seemed likely, then, that these sodium channels would not be sensitive to a 'conditioning' pulse preceding the test depolarization. According to Hodgkin &

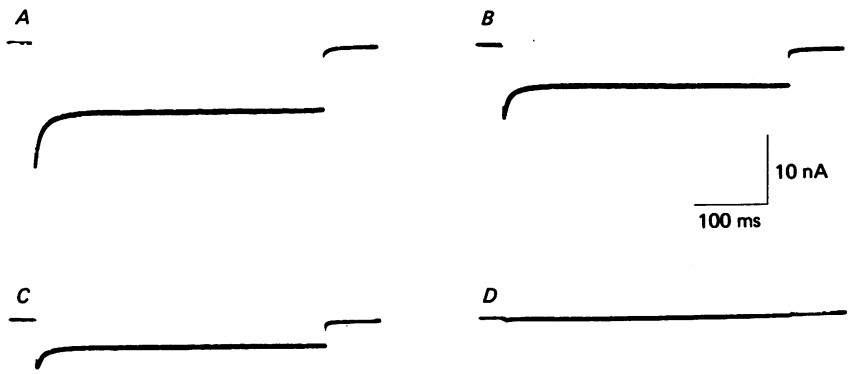


Fig. 6. Maintained sodium current during a prolonged membrane depolarization. The fibre was treated with 1·07 mM-chloramine-T in TEA-Ringer for 12 min, then washed with TEA-Ringer to remove chloramine-T. Sodium current was measured before (*A*) and after exposure to tetrodotoxin at 5 nM (*B*), 10 nM (*C*) and 1 μM (*D*) in TEA-Ringer for 5–10 min. Holding potential, −95 mV; test pulse potential, −15 mV.

Huxley (1952), a depolarizing conditioning pulse of sufficient duration will decrease the sodium conductance in the test pulse. An example of selective inactivation of the transient sodium currents by a conditioning prepulse is shown in Fig. 7. A prepulse of 100 ms duration to a voltage of either −95 mV or −45 mV made no difference to the magnitude of the maintained sodium current measured after 25 ms, although the size of the peak transient sodium current was reduced by the less negative conditioning prepulse.

Since the maintained currents were not sensitive to the conditioning pulses, it was possible to separate these currents from the total current by applying a conditioning pulse sufficient to inactivate the transient sodium currents. The turn-on kinetics of the non-inactivatable current could be measured once the transient inactivatable currents had been eliminated, and compared with the kinetics of the total currents as shown in Fig. 8.

To permit a measurement of activation kinetics uncontaminated by residual maintained currents activated during the preceding conditioning pulse, a brief repolarization of 1 ms was inserted between conditioning and test pulse. During this repolarization almost all sodium channels closed, but due to the large value of the membrane time constant, τ_h (8–11 ms at the holding potential), changes in the degree of inactivation were less than 10%.

At all membrane voltages the turn-on kinetics of the non-inactivating current were similar to those of the total current whether the conditioning pulse was to −45 mV (see Fig. 8*A* and *B*) or to +5 mV (data not shown). From these results it was concluded that the activation kinetics of the maintained currents were similar to those of the transient, inactivatable currents in chloramine-T-treated fibres. Thus, the activation kinetics of both total current and maintained current in the chloramine-T-treated fibres are indistinguishable from those of untreated channels.

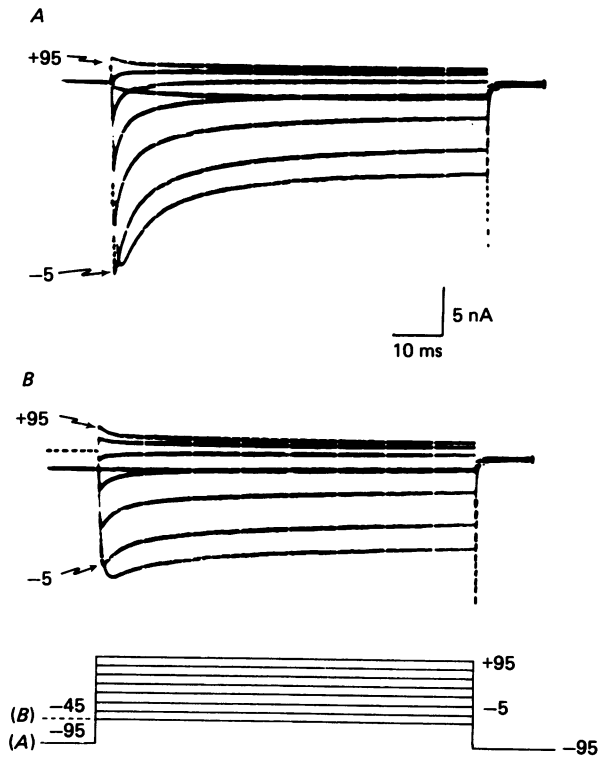


Fig. 7. Sodium currents measured following a 100 ms conditioning prepulse to −95 mV (*A*) or −45 mV (*B*). The currents were recorded after the fibre was treated with 1·07 mM-chloramine-T for 25 min. The currents measured after 25 ms of depolarization in *A* can be superimposed on those in *B*. Numbers are the membrane potentials of test pulses in millivolts. Current traces are recorded in increments of 20 mV.

Shift of voltage dependence of steady-state sodium channel inactivation

Chloramine-T not only irreversibly modified the time course of sodium channel inactivation as described above, it also modified the voltage dependence of remaining sodium channel inactivation. The steady-state sodium current inactivation (h_∞) was measured by applying long (50 ms) prepulses to various membrane potentials followed directly by a 1 ms repolarization and a standard test pulse to assay the fraction of non-inactivated channels. Fig. 9*A* shows that in normal nodes sodium channels are inactivated by increasingly more positive conditioning prepulses. This

voltage dependence, however, was modified by chloramine-T, resulting in a shift of inactivation in the depolarizing direction (Fig. 9*B* and *C*). This shift was easily measured when the relative peak sodium currents were plotted against the various conditioning pulses as shown in Fig. 10. It became apparent that chloramine-T-treated fibres differed from the control fibres in two respects. First, there was always a significant non-inactivating component present at more positive potentials, usually larger than 50% of the relative peak current, depending upon the chloramine-T

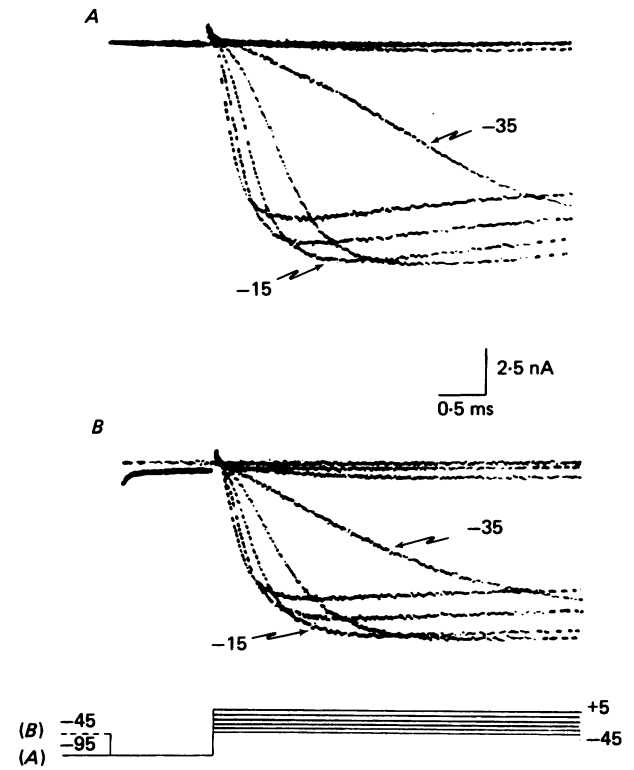


Fig. 8. Turn-on kinetics of total sodium current (*A*) and of maintained current (*B*). This experiment is similar to that shown in Fig. 7 except that only the rising phases of the sodium currents were revealed by a faster sweep speed. A 1 ms gap between conditioning pulse and test pulse was employed in *B* to eliminate most of the tail current contamination. (For explanation see text.) Chloramine-T concentration, 1·07 mM. Incubation time, 28 min. Numbers are the test pulse potentials in millivolts.

exposure time. This component was saturated around a membrane voltage of 0 mV. Secondly, there was an apparent shift in the voltage dependence of the inactivatable sodium current; after correction for the non-inactivating component this shift was measured as about 20 mV in the depolarizing direction. Table 1 shows the shift in the voltage dependence produced by chloramine-T in different fibres. It was interesting to observe that, although the magnitude of the non-inactivated component grew continuously with increasing exposure time, the voltage shift in h_∞ seemed to reach a constant value after only 3–5 min of reaction.

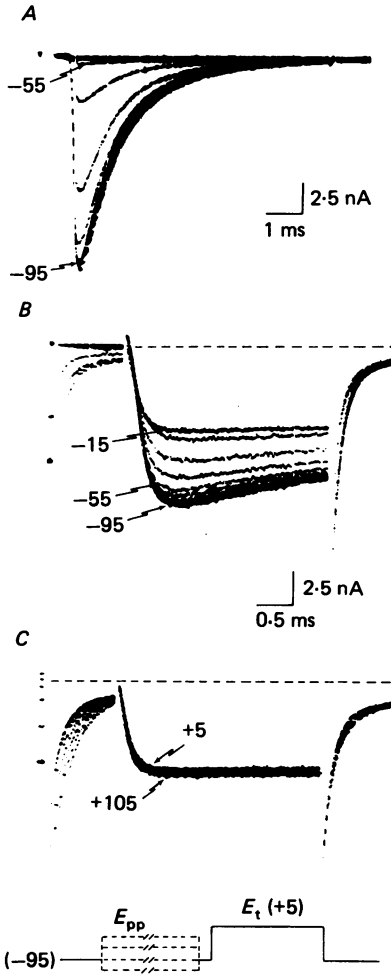


Fig. 9. Inactivation of sodium currents by prepulses. Sodium currents were measured following various conditioning pulses of 50 ms duration applied in 10 or 20 mV increments. *A*, control, steady-state sodium current inactivation. The potential of the conditioning prepulse in millivolts (from −125 mV to −5 mV) is shown by the numbers beside some of the traces. *B* and *C*, sodium currents after the nerve was treated with 1·07 mM-chloramine-T for 20 min and then washed with TEA-Ringer. A 1 ms gap between test pulse and conditioning pulse was employed in *B* and *C*, but not in *A*, to minimize the tail current contamination. Test pulse to +5 mV. The pulse programme is shown at the bottom of the Figure. Conditioning prepulses in *B* are from −125 mV to −5 mV, and in *C* from +5 mV to +105 mV. Note that sodium current traces include currents during the last 75 μs of the conditioning pulse and a 1 ms repolarization gap.

Effects of chloramine-T on potassium channels

Potassium currents were measured under the conditions described in Methods when sodium currents were blocked by 1 μM-TTX. In three experiments the potassium currents were reduced to 70–90% of their original magnitude after application of 1·07 mM-chloramine-T. There were no changes in the gating kinetics of the potassium currents after chloramine-T treatment (data not shown).

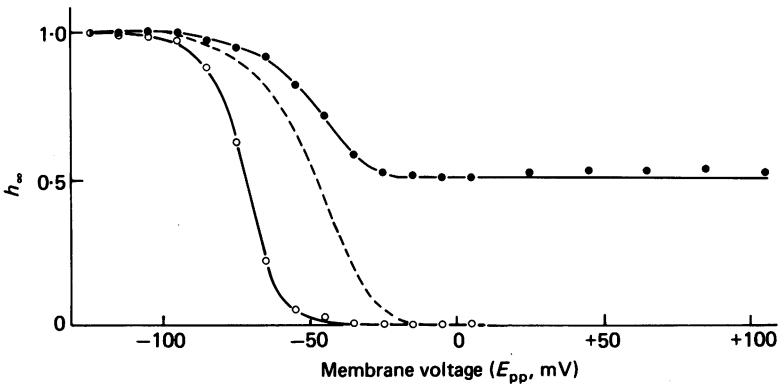


Fig. 10. Relative peak sodium currents *versus* membrane potential during conditioning pulses before (○) and after (●) treatment with 1·07 mM-chloramine-T. Some of the data points were taken from Fig. 9. The dashed line is derived from the ●—● line after subtracting the non-inactivating component.

TABLE 1. Effects of chloramine-T on steady-state sodium channel inactivation

Experiment	Time of treatment (min)	Shift in h_∞(mV)	Non-inactivated component (% of peak current)
A	1	+6	10
B	3	+20	26
C	> 10	+22	43
D	> 10	+20	54
E	> 10	+17	71
F	> 10	+18	85

Effects of chloramine-T on sodium currents when applied in the side pools

The internodes of a single myelinated fibre were cut in a solution containing 120 mM-caesium chloride and 1·07 mM-chloramine-T. Under such conditions most of the potassium currents were blocked within 10 min. No maintained sodium currents were detected within a 30 min period. A small maintained current appeared during a prolonged depolarization after half an hour; this current, however, never exceeded 10% of the total current.

DISCUSSION

In this report, evidence is presented that chloramine-T when applied externally modifies the inactivation process of sodium channels in myelinated nerve fibres and greatly prolongs the duration of action potentials. Two primary effects on sodium currents are resolved under voltage-clamp conditions. A maintained sodium current persists during a long membrane depolarization, and a shift of ~ 20 mV appears in the voltage dependence of sodium inactivation.

Maintained sodium currents, also referred to as non-inactivating or slowly inactivating sodium currents (see Meves, 1978), have been studied in detail before

by using pronase, *N*-bromoacetamide (Armstrong *et al.* 1973; Oxford *et al.* 1978), scorpion venoms (Gillespie & Meves, 1980) and deoxycholate (Wu, Sides & Narahashi, 1980). From the results presented here it is apparent that the maintained sodium currents caused by chloramine-T treatment have essentially normal activation kinetics. The maintained currents are therefore unlikely to arise from a class of slowly opening sodium channels or by reopening of the inactivated sodium channels, as suggested for the maintained currents observed under treatment with deoxycholate (Wu *et al.* 1980) or scorpion venom (Gillespie & Meves, 1980). It is more likely that the maintained currents are due to removal of the inactivation process, i.e. similar to the maintained sodium currents found after pronase or *N*-bromoacetamide treatment.

The magnitude of the apparent shift of the voltage dependence of steady-state current inactivation is ~ 20 mV (Table 1). This shift is unique to chloramine-T. Only a small shift (about +2·5 mV) is seen after brief treatment of squid axons with *N*-bromoacetamide (see fig. 4 of Oxford *et al.* 1978). It is known that by increasing the external positive surface charge of the membrane, the voltage dependence of steady-state sodium current inactivation will be shifted in the depolarizing direction (Frankenhaeuser & Hodgkin, 1957; Hille, 1968). The fact that chloramine-T does not introduce any charge differences of proteins under normal condition (Shechter *et al.* 1975) argues that this voltage shift is not due to 'non-specific' charge differences on the membrane surface. Instead, it suggests that certain specific residues of sodium channels are involved in this process. If true, chloramine-T modifies at least two separate sites on the sodium channel: one responsible for the maintained currents and the other for the shift in voltage dependence. This notion is further supported by the different rates of onset of the two effects. The voltage shift seems to be nearly complete within 5 min of chloramine-T treatment, while it takes 20–30 min to remove most of the inactivation process.

The specificity of chloramine-T has been well studied. Unlike *N*-bromoacetamide and pronase, chloramine-T does not break peptide bonds; it specifically modifies the most exposed methionine residues and sulphydryl groups of proteins in solution (Shechter *et al.* 1975). The significance of sulphydryl groups for sodium channel inactivation has been studied by using *N*-ethylmaleimide on the crayfish giant axon (Shrager, 1977). *N*-ethylmaleimide has little effect on the inactivation process when applied externally, but results in a large shift in the voltage dependence of the 'slow' inactivation, which occurs on a time scale about 50-fold slower than the 'fast' inactivation process described in this report. These results, combined with the specificity of chloramine-T, suggest that methionine residues are critical for the function of sodium channel inactivation. However, cyanogen bromide, which reacts with methionine residues, has no effect on sodium channel inactivation in the perfused squid axon (Oxford *et al.* 1978). Nevertheless, this negative result could be due to the reduced reactivity of cyanogen bromide in the pH 6·3 solution used by these investigators.

The location of the modified residues which are critical for sodium channel inactivation cannot be specified easily. More than one residue may be involved. One could be located at or near the extracellular surface of sodium channels since chloramine-T bears a negative charge in solution and this species should be

impermeant (Montelaro & Rueckert, 1977). Alternatively, the chloramine-T anion could be protonated and this neutral molecule ($RH/R^- = 4{\cdot}2 \times 10^{-5}$, at pH 7; Bishop & Jennings, 1958) could permeate the membrane and react with residues within the membrane and on the cytoplasmic surface. The fact that chloramine-T has little effect on sodium current inactivation when applied internally does not prove that it is not active from the internal surface since all the chloramine-T molecules could react with substrates in the internode before they can reach the nodal membrane. It has been reported that pronase does not remove sodium channel inactivation when injected rather than internally perfused into squid giant axons, probably due to a large amount of non-specific substrate in the axoplasm (Armstrong *et al.* 1973).

Finally, like other chemical reagents used to modify sodium channel inactivation, chloramine-T does not significantly alter the activation process of sodium channels. This is consistent with previous reports that the activation 'machinery' is not sensitive to chemical modification, either from the external or internal membrane surface (Armstrong *et al.* 1973; Oxford *et al.* 1978), or to changes in internal pH (Brodwick & Eaton, 1978; Nonner *et al.* 1980). Thus, the activation processes can be chemically separated from the inactivation processes even without cleaving the peptide bonds.

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REFERENCES

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.

Bishop, E. & Jennings, V. J. (1958). Titrimetric analysis with chloramine-T. I. The status of chloramine-T as a titrimetric reagent. *Talanta* **1**, 197–212.

Brodwick, M. S. & Eaton, D. C. (1978). Sodium channel inactivation in squid axon is removed by high internal pH or tyrosine specific reagents. *Science, N.Y.* **200**, 1494–1496.

Brodwick, M. S. & Eaton, D. C. (1982). Chemical modification of excitable membranes. In *Progress in Clinical and Biological Research*, vol. 79, ed. Haber, B., Perez-Polo, J. R. & Coulter, J. D., pp. 51–72. New York: Alan R. Liss.

Chiu, S. Y. (1977). Inactivation of sodium channels: second order kinetics in myelinated nerve. *J. Physiol.* **273**, 573–596.

Dodge, F. A. & Frankenhaeuser, B. (1958). Membrane currents in isolated frog nerve fibre under voltage clamp conditions. *J. Physiol.* **143**, 76–90.

Eaton, D. C., Brodwick, M. S., Oxford, G. S. & Rudy, B. (1978). Arginine specific reagents remove sodium channel inactivation. *Nature, Lond.* **271**, 473–476.

Fox, J. M. (1976). Ultra-slow inactivation of the ionic currents through the membrane of myelinated nerve. *Biochim. biophys. Acta* **426**, 232–244.

Frankenhaeuser, B. & Hodgkin, A. L. (1957). The action of calcium on the electrical properties of squid axons. *J. Physiol.* **137**, 218–244.

Gillespie, J. I. & Meves, H. (1980). The effect of scorpion venoms on the sodium currents of the squid giant axon. *J. Physiol.* **308**, 479–499.

Hahin, R. & Strichartz, G. (1981). Effects of deuterium oxide on the rate and dissociation constants for saxitoxin and tetrodotoxin action. *J. gen. Physiol.* **78**, 113–139.

HILLE, B. (1968). Charges and potentials at the nerve surface. Divalent ions and pH. *J. gen. Physiol.* **51**, 221–236.

HODGKIN, A. L. & HUXLEY, A. F. (1952). The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. *J. Physiol.* **116**, 497–506.

MEVES, H. (1978). Inactivation of the sodium permeability in squid giant nerve fibres. *Prog. Biophys. molec. Biol.* **33**, 207–230.

MONTELARO, R. C. & RUECKERT, R. R. (1977). A mechanism and an evaluation of surface specific iodination by the chloramine-T procedure. *Arch. Biochem. Biophys.* **178**, 555–564.

NONNER, W., SPALDING, B. C. & HILLE, B. (1980). Low intracellular pH and chemical agents slow inactivation gating in sodium channel of muscle. *Nature, Lond.* **284**, 360–363.

OXFORD, G. S., WU, C. H. & NARAHASHI, T. (1978). Removal of sodium channel inactivation in squid giant axons by *N*-bromoacetamide. *J. gen. Physiol.* **71**, 227–247.

ROJAS, E. & RUDY, B. (1976). Destruction of the sodium conductance inactivation by a specific protease in perfused nerve fibres from *Loligo*. *J. Physiol.* **262**, 501–531.

SCHWARZ, J. R., ULBRICHT, W. & WAGNER, H. H. (1973). The rate of action of tetrodotoxin on myelinated nerve fibres of *Xenopus laevis* and *Rana esculenta*. *J. Physiol.* **233**, 167–194.

SHECHTER, Y., BURSTEIN, Y. & PATCHORNIK, A. (1975). Selective oxidation of methionine residues in proteins. *Biochemistry* **14**, 4497–4503.

SHRAGER, P. (1977). Slow sodium inactivation in nerve after exposure to sulfhydryl blocking reagents. *J. gen. Physiol.* **69**, 183–202.

STÄMPFLI, R. (1974). Intra-axonal iodate inhibits sodium inactivation. *Experientia* **30**, 505–508.

STRICHARTZ, G. S. (1982). Structure of the saxitoxin binding site at sodium channels in nerve membranes. *Molec. Pharmacol.* **21**, 343–350.

WU, C. H., SIDES, P. J. & NARAHASHI, T. (1980). Interaction of deoxycholate with the sodium channel of squid axon membranes. *J. gen. Physiol.* **76**, 355–379.