

MODE OF ACTION OF TRICHLOROETHYLENE ON SQUID AXON MEMBRANES¹

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ABSTRACT

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The mode of action of trichloroethylene on electrical properties of squid giant axons has been studied by means of voltage clamp techniques. Trichloroethylene decreased the resting membrane potential in a manner dependent upon the concentration, the depolarization by 50% saturated trichloroethylene attaining 28.4 and 32.7% of the initial value at 20 and 10°C, respectively. Leakage conductance was decreased to 34.6% of the control by 30% saturated trichloroethylene at 10-12°C. It appears that the trichloroethylene-induced depolarization is at least in part due to a decrease in resting potassium permeability. Both peak transient and steady-state conductance increases were suppressed by trichloroethylene, and the curve relating the steady-state conductance to the membrane potential was shifted in the depolarizing direction while the peak transient conductance curve was not appreciably shifted. The reversal potential for the peak transient current was greatly shifted by trichloroethylene in the direction of hyperpolarization in a manner dependent on the concentration, the maximum shift amounting to 25 mV at 10°C. This effect was less pronounced at 20°C. The shift in the reversal potential is mostly due to a decrease in selectivity of the peak transient channel and partly due to an accumulation of sodium ions inside. Analyses of dose-response relation in suppressing peak transient and steady-state conductances show that trichloroethylene interacts with receptor on a one-to-one stoichiometric basis. Steady-state sodium inactivation curve was shifted by trichloroethylene in the direction of hyperpolarization. All of these effects were partially reversed after washing the axon with anesthetic-free media. The accumulation of sodium ions inside would be much more pronounced in small nerve fibers in the brain than in giant axon and, together with the observed decrease in the selectivity of peak transient channels, would play a significant role in general anesthesia.

Voltage clamp techniques have been successfully used to separate ionic currents into sodium and potassium components during nerve excitation (Hodgkin *et al.*, 1952; Hodgkin and Huxley, 1952a) and to elucidate the mechanism of action of various neuroactive agents in terms of membrane ionic conductances. For example,

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barbiturates have been shown to decrease peak transient and steady-state conductances (Narahashi *et al.*, 1969; Blaustein, 1968). Procaine, dibucaine, lidocaine and tertiary and quaternary tropine esters also inhibit peak transient and steady-state conductances (Taylor 1959; Shanes *et al.*, 1959; Blaustein and Goldman, 1966; Hille, 1967; Narahashi *et al.*, 1969, 1972; Strichartz, 1973). Similarly alcohols decrease both conductances (Armstrong and Binstock, 1964; Moore *et al.*, 1964):

The mechanism of volatile general anesthetics on neurons remains largely to be explored. There has been no complete study dealing with the ionic mechanism of action of these anesthetics. We have performed voltage clamp experiments to study the effects of trichloroethylene on ionic conductances of squid giant axon membranes. The squid axon was chosen as a model because voltage clamp experiments could be performed with a high degree of accuracy and also because a wealth of information was available on its electrical properties.

Preliminary accounts of this study have been reported (Shrivastav and Narahashi, 1974).

Methods

Materials. Experiments were performed at the Marine Biological Laboratory, Woods Hole, Mass., using the giant axons from the squid, *Loligo pealei*. The diameter of the axons ranged from 325 to 700 μ . Isolated axons were cleaned by removing thin nerve fibers and connective tissues. For internal perfusion experiments, the axoplasm was removed by means of a small roller, and the axon was internally perfused with standard internal solution by the method originally developed by Baker *et al.* (1961) and modified by Narahashi and Anderson (1967). The desired rate of flow of the internal perfusate was achieved by adjusting the hydrostatic pressure of the standard internal solution reservoir. Depending on the diameter of the axon, the flow rate was maintained 30 to 90 μ /min. External solution was perfused continuously.

Solutions. Filtered sea water was used as external perfusate in all experiments. Anesthetic was dissolved to saturate sea water at a temperature of 21–23°C. The solubility of trichloroethylene in water at 25°C is estimated to be 8.4 mM (Adriani, 1972). Thus, when saturated in sea water, the concentration of trichloroethylene should not exceed 8.4 mM. The anesthetic-saturated sea water was then diluted with sea water to give a desired strength just before each change of anesthetic concentration. No correction of the trichloroethylene concentration in sea water was made to compensate for possible loss by evaporation during

external perfusion. The presence of anesthetic in sea water did not affect the pH, which was between 7.9 and 8. The composition of the standard internal solution was as follows: 350 mM K⁺, 50 mM Na⁺, 50 mM F⁻, 320 mM glutamate⁻, 333 mM sucrose and 15 mM phosphate buffer at pH 7.3. The ionic composition of Woods Hole sea water is 459 mM Na⁺, 9.7 mM K⁺, 535 mM Cl⁻, 10 mM Ca⁺⁺, and 52 mM Mg⁺⁺ (P. Mangelsdorf, Jr., personal communication).

Electrophysiological measurements. A glass capillary of 75 μ in outer diameter and filled with 0.6 KCl solution was inserted longitudinally into the axon to measure the resting membrane potential. The capillary contained a 25- μ platinum wire to reduce high frequency impedance. The reference electrode was made of a glass capillary of about 500 μ in diameter. This capillary was filled with 2% agar in sea water. The membrane potential was continuously recorded on a strip chart recorder. For voltage clamp experiments, a 75- μ platinum wire plated with platinum black was attached "piggy-back" to the internal capillary potential electrode. This wire served as the internal current electrode. External current electrodes consisted of three platinum plates of 4-mm width coated with platinum black. The central plate was connected to an operational amplifier to measure currents, and the adjacent plates were directly grounded to serve as guard electrodes. The voltage clamp circuit developed by Moore and Cole (1963) was used in the form described by Wang *et al.* (1972). During voltage clamp, the membrane potential was held at a slightly hyperpolarized level to remove sodium inactivation. Command depolarizing pulses were applied and membrane currents associated with these pulses were recorded. Occasionally the membrane was unclamped to record the resting membrane potential and the action potential. The membrane potential of intact axons was not corrected for junction potentials. Leakage currents associated with step hyperpolarizations were also recorded. Peak transient and steady-state current were not corrected for leakage current. Membrane potential measurements were not corrected for junction potential of the internal electrode which was of the order of 10 mV (Curtis and Cole, 1942).

Temperature. The experiments were conducted at 20 or 10–12°C.

Statistical tests. Student's *t* tests were employed to evaluate the difference between the means.

Results

Resting membrane potential. Resting membrane potential decreased after external application of trichloroethylene to the intact axon. The higher the concentration of anesthetic, the greater was the membrane depolarization. Figure 1 illustrates the dose-response relationships

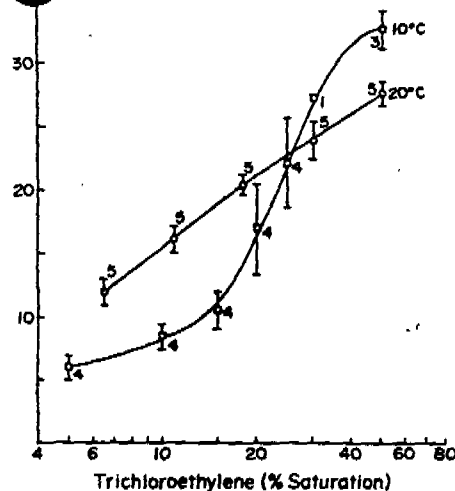


FIG. 1. Effects of external application of different concentrations of trichloroethylene on the resting membrane potential of squid giant axons at 20 and 10°C. Measurements were made at the steady state, but 15 minutes for each change of anesthetic concentration. Ordinate represents the percentage of decrease in membrane potential (E_m), and abscissa represents the percentage of saturation of trichloroethylene. Each point represents the mean $\pm$ S.E.M. with the number of axons as indicated. Curves were drawn by

the increase in resting membrane potential induced by trichloroethylene. The concentration of trichloroethylene was increased cumulatively, and after introduction of a new concentration, measurements were made when the resting membrane potential attained a steady state in about 15 minutes. The percentage of depolarizations of the membrane at 20°C ranged from $10.9 \pm 1.4\%$ (mean $\pm$ S.E.M., 5 axons) by 6.5% saturated trichloroethylene solution (from mean of -57.0 to -50.8 mV) to $28.4 \pm 1.2\%$ (5 axons) by 50% saturated solution (to -38.8 mV). At 10°C, the percent decrease in membrane potential was $5.9 \pm 1.2\%$ (4 axons) from mean value of -67.7 to -63.7 mV and $16.1 \pm 1.6\%$ (3 axons) (to -46.0 mV) by 5 and 30% saturated solutions, respectively. Thus, the trichloroethylene-induced depolarization was greater at 20 than at 10°C at concentrations of 6.5% saturation or less, but the reversed effect was seen as concentrations of 30% saturation or greater. After washing the nerves for about 1½ hours with anesthetic-free sea water, the resting membrane potential was restored to $85.9 \pm 1.2\%$ (5 axons) and $82.9 \pm 1.3\%$ (4 axons) of the control at 20 and 10°C, respectively.

Action potential. Figure 2 shows the action potentials recorded at 12°C before and during external application of 30% saturated trichloroethylene to an intact axon for 15 minutes, and after washing with anesthetic-free sea water. The threshold membrane potential became less negative after introduction of trichloroethylene, and the amplitude of the action potential was effectively suppressed with both the rising and falling phases slowed. In some cases, the nerve started firing repetitively in the anesthetic. Washing the axon with anesthetic-free sea water for about 1½ hours partially restored the amplitude of the action potential. However, the recovery of the action potential was complete if perfused axon was washed internally. The undershoot that immediately followed the falling phase of the action potential was almost absent in the trichloroethylene-treated axons. There was no recovery of undershoot on washing the axons either externally or internally, even when the spike amplitude was completely restored.

Leakage conductance. The leakage current was measured by application of hyperpolarizing pulses. The percentage of decrease in leakage conductance in trichloroethylene amounts to 34.6 ± 6.1 (mean $\pm$ S.E.M., 5 axons) at 10–12°C when the intact axons were exposed to 30% saturated trichloroethylene sea water. Recovery of leakage conductance after washing was $198.5 \pm 70.7\%$ of the control (5 axons).

Membrane ionic currents. Figure 3 shows families of membrane currents associated with

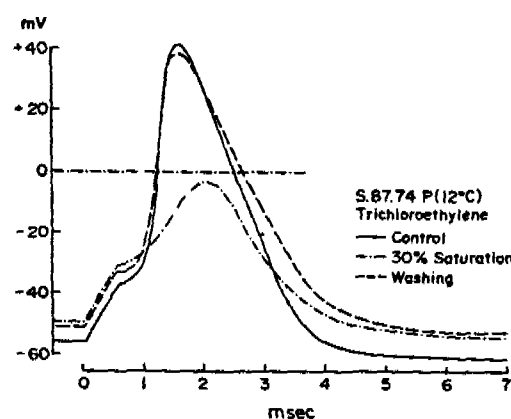


FIG. 2. Action potentials of the squid giant axon before (control) and during external application (15 minutes) of 30% saturated trichloroethylene in sea water and after washing with anesthetic-free sea water at 12°C.

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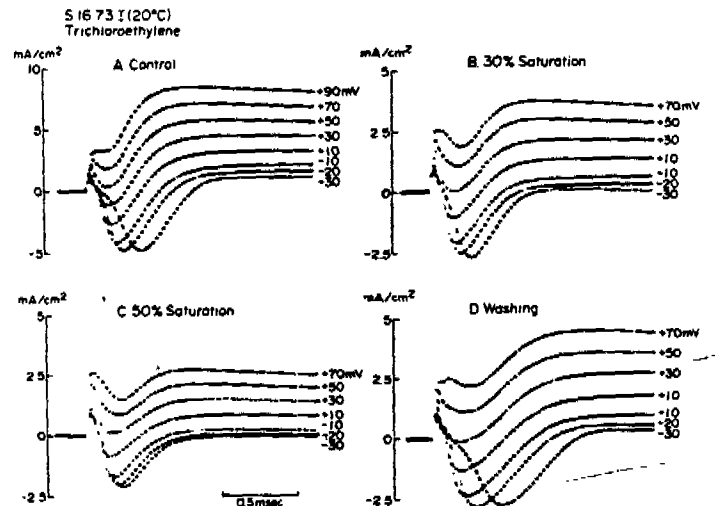


FIG. 3. Membrane currents associated with various step depolarizations from the holding membrane potential of -70 mV before (A, control) and during external application (15 minutes each) of 30% (B) and 50% (C) saturated trichloroethylene in sea water and after washing with anesthetic-free sea water (D). The membrane potential during step depolarization is given at the end of each current record (20°C).

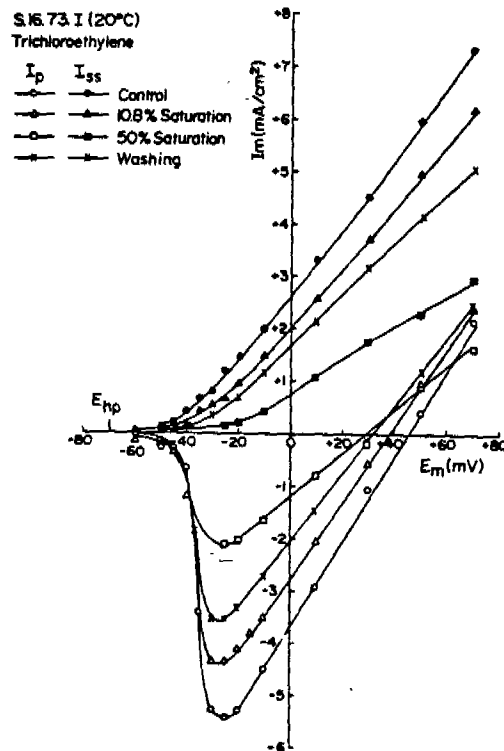


FIG. 4. Current-voltage relations ($I_m - E_m$) for peak transient current (I_p) and steady-state current (I_{ss}) before (control) and during external applications (15 minutes each) of 10.8% and 50% saturated trichloroethylene and after washing with anesthetic-free sea water. E_{hp} , holding membrane potential (20°C).

various magnitudes of step depolarizations at 20°C before and during application of trichloroethylene to an intact axon for about 15 minutes for each concentration and after washing with anesthetic-free medium. Trichloroethylene reduced inward peak transient (I_p) and outward steady-state (I_{ss}) currents (fig. 3, B and C). Another distinct change was a slight increase in outward peak transient currents. In all of the nine axon preparations used, the recovery of ionic currents after washing with anesthetic-free sea water was incomplete (fig. 3D). The time-to-peak transient current (t_p) was not affected by low concentrations of trichloroethylene but underwent a small but consistent increase by higher concentrations. Measured at $+20$ mV with 30% saturated trichloroethylene, the increase in t_p amounted to $8.9 \pm 3.5\%$ and was not statistically significant ($P > .2$, 5 axons). The onset of the steady-state potassium current was delayed after application of trichloroethylene.

Current-voltage relations. The amplitudes of peak transient and steady-state currents of the same intact axon as used for figure 3 are plotted against the membrane potentials in figure 4. The following features become immediately apparent from the current-voltage curves:

1. Trichloroethylene suppressed peak transient currents at the membrane potentials more positive than -40 mV.

It suppressed steady-state currents at all the potentials examined.

The reversal potential for peak transient current was shifted in the direction of hyperpolarization.

After external washing with anesthetic-free water, only partial recovery of both ionic currents was observed.

The recovery of the reversal potential after external washing was poor.

The reversal potentials for peak transient current of intact axons at 10 and 20°C are plotted against the concentration of trichloroethylene in figure 5. The shift in the reversal potential by trichloroethylene was much greater at 10°C than at 20°C.

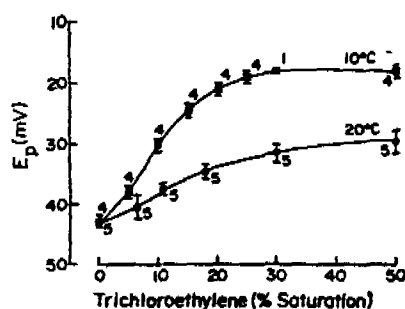


FIG. 5. The reversal potential (E_p) for peak transient current at different percentages of saturation of trichloroethylene at 10 and 20°C. Each point represents the mean $\pm$ S.E.M. with the number of axons indicated.

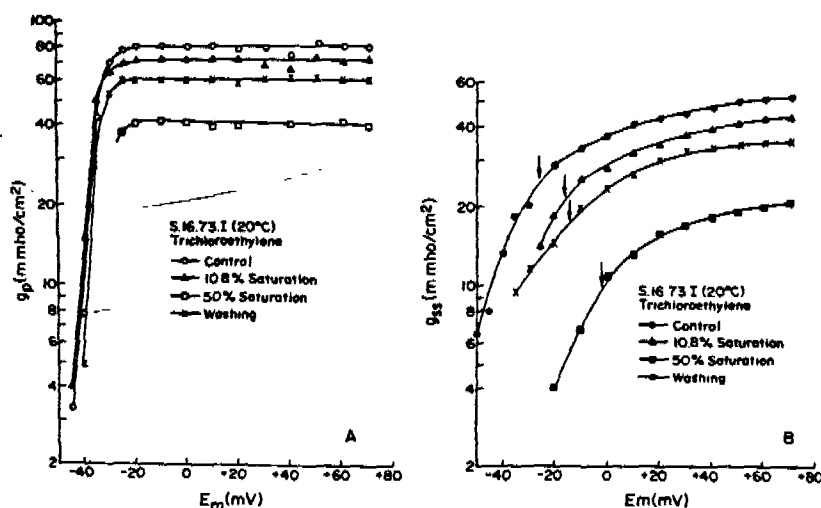


FIG. 6. Peak transient conductance (g_p) (A) and steady-state conductance (g_{ss}) (B) plotted in a logarithmic scale as a function of the membrane potential (E_m) before control and during external application (15 minutes) of 10.8 and 50% saturated trichloroethylene and after washing with anesthetic-free sea water (20°C). Arrows in B indicate the membrane potentials where g_{ss} attains a half-maximal value.

In the internally perfused axons the reversal potential for peak transient current was estimated to be 46.0 ± 2.0 mV (mean $\pm$ S.E.M., 4 axons, 10–12°C). When the axons were externally exposed to 30% saturated trichloroethylene while suspending the internal flow, the reversal potential was changed to 26.5 ± 4.9 mV. Upon resuming the internal flow without removing anesthetic from the external perfusate, the reversal potential quickly recovered to a value of 44.2 ± 1.4 mV. Then the anesthetic was removed from the external perfusate solution, and the reversal potential remained almost unchanged at 43.7 ± 1.1 mV. These results suggest the possibility that the drug acts from inside of the axon to shift the reversal potential.

Membrane ionic conductances. The membrane conductances for peak transient and steady-state currents were calculated by equations:

$$E_p = \frac{I_p}{E_m - E_p} \quad (1)$$

$$E_{ss} = \frac{I_{ss}}{E_m - E_{ss}} \quad (2)$$

where g , I and E with subscript refer to the conductance, current and reversal potential for peak (p) and steady-state (ss) components, respectively, and E_m refers to the membrane potential. The steady-state slope conductance,

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g_{ss} (slope), was also calculated from the equation

$$g_{ss} \text{ (slope)} = \frac{dI_{ss}}{dE_m} \quad (3)$$

The membrane conductances of the same intact axon as used for figures 3 and 4 are plotted on a logarithmic scale against the membrane potential in figure 6. After application of trichloroethylene for 15 minutes, the peak transient conductance curve was shifted downward along the conductance axis, but was not appreciably shifted along the potential axis (fig. 6A). The membrane potentials at which the peak transient conductance attained a half-maximal value were estimated to be -37.5 ± 0.9 mV in the control, and -36.2 ± 1.7 and -35.5 ± 2.7 mV after application of 18 and 50% saturated trichloroethylene sea water, respectively (4 axons). Neither of the latter two values is different significantly from the control value ($P > .2$). The steady-state conductance curve was also suppressed downward and was shifted in the direction of depolarization (fig. 6B). The membrane potentials at which the conductance attained a half-maximal value (arrows, fig. 6B) were estimated as follows (5 axons): control -28.0 ± 3.3 mV, 6.5% saturated trichloroethylene -20.6 ± 2.9 mV ($P > .02$), 18% saturated -12.0 ± 2.7 mV ($P > .001$), 50% saturated -4.0 ± 2.3 mV ($P > .001$), after washing -17.0 ± 4.9 mV ($P > .02$).

Dose-response relationship. The dose-response curves for the suppression of peak conductance of intact axons by trichloroethylene at 20 and 10°C are illustrated in figure 7A. The effect on the peak conductance was not greatly affected by temperature change, and the small difference at the highest concentration used (50% saturation) was not statistically significant ($P > .1$). The data on the steady-state conductance are plotted in figure 7B. Again there was no marked difference between 10 and 20°C in the percentage of suppression at the highest concentration ($P > .1$). It can be seen from figure 7, A and B, that trichloroethylene suppressed the peak transient and steady-state conductances to approximately the same extent at either 10 or 20°C.

Figure 8 illustrates Hill's plots for the data at 20°C according to the equation:

$$\log \frac{V}{V_{max} - V} = n \log [S] - \log K \quad (4)$$

where V is the response or percentage of inhibition of conductance, V_{max} is the maximum response (100% inhibition), $[S]$ is the percent saturation of trichloroethylene, n is the interaction coefficient and K is the apparent dissociation constant.

The slopes of the curves provide the value of n , which are estimated to be 0.9 and 1.0 for the maximum peak transient conductance and the steady-state conductance at 60 mV, respec-

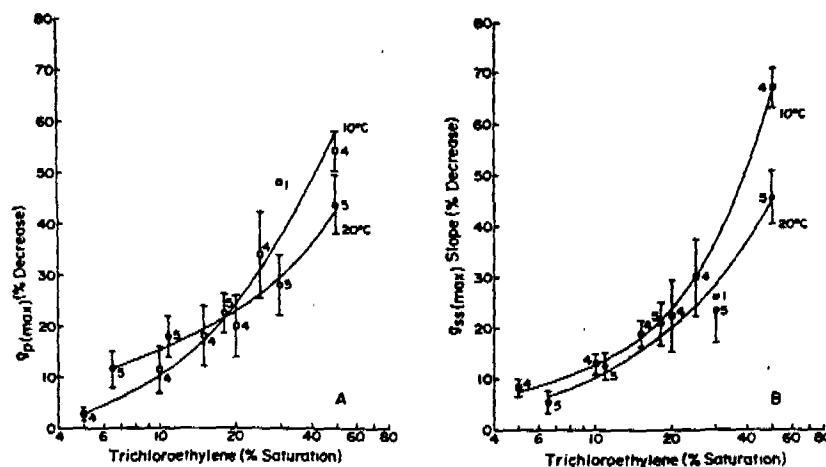


FIG. 7. Dose-response relations for the action of trichloroethylene in suppressing the maximum peak transient conductance ($g_p(max)$) (A) and the maximum steady-state slope conductance ($g_{ss(max)} \text{ slope}$) (B) at 10 and 20°C. Measurements were made at the steady state (15 minutes) after application of trichloroethylene. Each point represents the mean $\pm$ S.E.M. with the number of axons as indicated. Curves were drawn by eye.

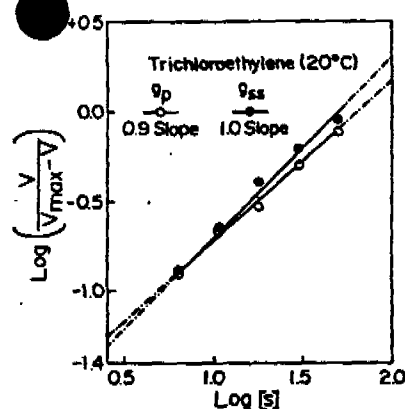


FIG. 8. Hill's plots for the action of trichloroethylene in suppressing the maximum peak transient conductance (g_p) and the steady-state conductance (g_{ss}) at +60 mV. Each point represents the mean value of 5 measurements. V , percentage of decrease of conductance; V_{max} , maximum percentage of decrease of conductance (100%); $[S]$, percentage of duration of trichloroethylene. The slopes of the straight lines are 0.9 and 1.0 for g_p and g_{ss} , respectively (20°C).

ly. This indicates that trichloroethylene binds to its receptor on a one-to-one stoichiometric basis thereby blocking the conductances. The apparent dissociation constants for the peak transient and steady-state conductances were estimated to be 41 and 50% saturation of trichloroethylene, respectively.

Steady-state sodium inactivation. The effect of trichloroethylene on the steady-state sodium inactivation was also examined at 10°C using intact axons. Prepulses of various amplitudes and 45-msec duration were followed by a depolarizing pulse of +50 or +60 mV with a 1-msec duration. The amplitude of peak transient current associated with the test pulse was measured and plotted as a ratio to its maximum value against the membrane potential of the pulse. Figure 9 depicts such steady-state sodium inactivation curves before and during application of 10% and 20% saturated solutions of trichloroethylene for 15 minutes in each case, and after washing with anesthetic-free solution. The measurements were fitted by the equation

$$h_{\infty} = \frac{1}{1 - \exp\left(\frac{E_m - E_n}{k}\right)} \quad (5)$$

where h_{∞} represents the ratio of the peak sodium current associated with the test pulse to maximum value, E_n represents the mem-

brane potential for $h_{\infty} = 0.5$ and k represents the slope factor (Hodgkin and Huxley, 1952b). Trichloroethylene shifted the curve in the direction of hyperpolarization. The shift of the curve, measured at half-maximal value, was 5.6 ± 2.0 mV for 10% and 5.9 ± 2.0 mV for 20% saturated toward the control level, the difference between the recovery and control curves being estimated to be 1.1 ± 1.6 mV (mean $\pm$ S.E.M., 5 axons). Trichloroethylene also increased the slope factor k by 1.4 ± 0.6 and 3.1 ± 0.5 in 10 and 20% saturated sea water (mean $\pm$ S.E.M., 5 axons). After washing, the increase in k remained by 1.0 ± 1.1 (mean $\pm$ S.E.M., 5 axons).

Discussion

When applied externally to intact axons at 10°C, 30% saturated trichloroethylene depolarized the squid axon membrane from the mean value of -67.7 to -48.0 mV. The observed decrease in leakage conductance by 34.6% is in keeping with the depolarization, for the leakage conductance is largely composed of potassium conductance. Since the depolarization attained a steady-state level relatively rapidly (about 10 minutes), it can be assumed that the change in

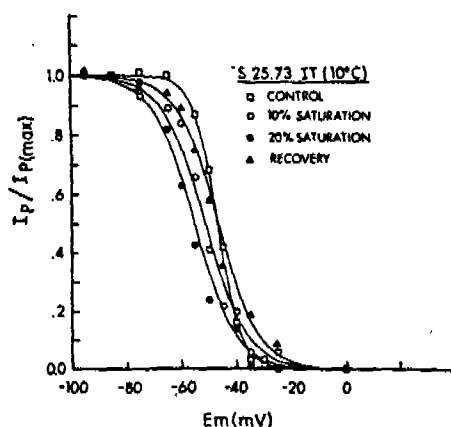


FIG. 9. Steady-state inactivation curve before ($\square$) and during external application (15 minutes) of trichloroethylene at 10% ($\circ$) and 20% ($\bullet$) saturation levels, and after washing with anesthetic-free sea water ($\blacktriangle$). I_p , peak current associated with test step depolarization; $I_{p(max)}$, the maximum value of I_p . The abscissa represents the membrane potentials of conditioning pulses. The measurements were fitted by equation 5 with the aid of Hewlett-Packard 9821A calculator. The E_n and k values in equation 5 for control, 10 and 20% of trichloroethylene saturation and after washing were -46.8, -51.3, -55.1 and -46.5 mV, and 4.3, 7.5, 7.4, 7.2, respectively. Temperature, 10°C.

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internal ionic concentrations is negligible. Thus, if one assumes that the decrease in leakage conductance is entirely due to a decrease in potassium permeability, the change in resting sodium permeability may be calculated from the constant field equation (Hodgkin and Katz, 1949):

$$E_m = \frac{RT}{F} \ln \frac{P_K [K]_o + P_{Na} [Na]_o + P_{Cl} [Cl]_i}{P_K [K]_i + P_{Na} [Na]_i + P_{Cl} [Cl]_o} \quad (6)$$

where P is the permeability to ions in subscript, $[]$ is the concentration of ions outside (o) or inside (i) of the axon, and R , T and F have their usual meaning. Taking $[K]_i = 335$ mM, $[Na]_i = 52$ mM and $[Cl]_i = 135$ mM (Rosenberg, 1973) and the values for the ionic concentrations in natural sea water as described in "Methods," the sodium permeability after exposure to 30% saturated trichloroethylene at 10°C is calculated to be 2.55-fold of the initial control value.

When applied externally, trichloroethylene caused a shift of the reversal potential for peak transient current in the direction of hyperpolarization (figs. 4 and 5). The possible causes of the shift are as follows:

Increase in internal sodium concentration.

The influx of radioactive sodium in normal intact axons was estimated to be 29.0×10^{-12} mol/cm²sec (unpublished data). For an axon with a diameter of 500 μ and exposed to trichloroethylene for 10 minutes, the increase in internal sodium concentration due to a 2.55-fold increase in resting sodium permeability is calculated to be 3.5 mM. This will shift the reversal potential in the direction of hyperpolarization only by 1.6 mV.

Change in ionic selectivity of the sodium channel. The ratio P_K/P_{Na} during peak transient current can be calculated by the constant field equation. Since the contribution of chloride permeability to peak transient current is negligible, equation 6 can be reduced to describe the reversal potential (E_p):

$$E_p = \frac{RT}{F} \ln \frac{(P_K/P_{Na}) [K]_o + [Na]_o}{(P_K/P_{Na}) [K]_i + [Na]_i} \quad (7)$$

With the values $[K]_i = 335$ mM, $[Na]_i = 52$ mM, $[K]_o = 9.7$ mM and $[Na]_o = 459$ mM, the ratio P_K/P_{Na} for control axons at 10°C is esti-

mated to be 1:12.9 for E_p equal to 43.2 mV (not corrected for junction potential) and 1:5.1 for E_p equal to 33.2 mV (corrected for a 10-mV junction potential). Similarly, taking $[Na]_i = 55.5$ mM and $[K]_i$, $[K]_o$ and $[Na]_o$ unchanged as above for axons treated with 30% saturated trichloroethylene at 10°C for 10 minutes, selectivity, ratio P_K/P_{Na} is calculated to be 1:2.0 for E_p equal to 18.5 mV (not corrected for junction potential) and 1:1.2 for E_p equal to 8.5 mV (corrected for a 10-mV junction potential). In both cases, therefore, the selectivity ratio P_K/P_{Na} of the peak transient channels is increased by a factor of 4 to 6 as a result of exposure of 30% saturated trichloroethylene at 10°C.

Leakage conductance. The leakage conductance in the control axons is small and is decreased by trichloroethylene. However, there is evidence that the leakage current shows a rectification in depolarized membranes (Adelman and Taylor, 1961). Thus, if trichloroethylene greatly augmented the rectification, then the reversal potential for peak transient current as estimated without correction for the leakage current would be shifted in the hyperpolarizing direction.

Steady-state potassium current. If the steady-state potassium current started flowing earlier than normal, it would contribute significantly to the total peak transient current, thereby causing a shift in E_p . However, this can be ruled out because trichloroethylene retards the onset of the potassium currents rather than accelerates it (fig. 3).

Halothane applied externally to squid giant axons in low concentrations also depolarized the membrane and decreased the reversal potential for peak transient current (Shrivastav and Narahashi, 1974).

Trichloroethylene possesses some unique features of nerve blocking action which are lacking in pentobarbital and procaine (table 1). They include depolarization of the membrane, decrease in leakage conductance, absence of the shift of the curve-relating peak transient conductance to membrane potential, absence of the change in the time-to-peak current and shift of the steady-state sodium inactivation curve in the hyperpolarizing direction. It should be noted that trichloroethylene has some effects on the resting membrane characteristics as exemplified by the resting potential and the leakage

TABLE 1

Effects of trichloroethylene, pentobarbital and procaine on various properties of squid axon membranes

O, no change; ↓, decrease; ↑, increase; →, shift in the depolarizing direction; ←, shift in the hyperpolarizing direction.

	Trichloroethylene	Pentobarbital*	Procaine*
Resting potential	↓	O	O
Action potential	↓	↓	↓
Peak conductance	↓	↓	↓
Steady-state conductance	↓	↓	↓
Leakage conductance	↓	O	↓
Peak conductance-membrane potential curve	O	→	→
Time-to-peak current	O	↓	↓
Reversal potential for peak current	→	O	→
Steady-state Na inactivation curve	→		O

* From Narahashi et al. (1969).

* From Taylor (1959) and Shanes et al. (1959).

conductance, as well on the membrane characteristics during activity. On the other hand, entobarbital and procaine affect primarily the membrane characteristics during activity.

In human and dog, the blood levels of trichloroethylene during surgical anesthesia have been estimated to about 0.8 and 2.0 mM, respectively (Adriani, 1972), which correspond to 9.5 and 3.8% saturated trichloroethylene in water at 5°C, respectively. Therefore, the trichloroethylene concentrations of 10 to 50% saturation in sea water at 10 and 20°C used in the present study are of the same order of magnitude as those in the blood during surgical anesthesia.

A nerve fiber with a diameter much smaller than that of the squid giant axon has a very large surface area/volume ratio. Therefore, these concentrations of trichloroethylene will more quickly increase the internal sodium concentration as a result of an increase in resting sodium permeability. If one assumes that the same degree of the sodium permeability increase occurs in a small fiber as in a squid giant axon, the internal sodium concentration increases from 52 to 81.6 mM in a fiber with a diameter of 1 μ in 10 seconds as against 55.5 mM in a fiber with a diameter of 500 μ in 10 minutes. This factor by itself will cause a shift of the reversal potential for peak transient

current by as much as 10.9 mV in the small fiber exposed for only 10 seconds as against the shift of only 1.6 mV in the giant axon exposed for 10 minutes. Such a large shift will drastically curtail the peak of the action potential. In anesthetized cats exposed to high pressures of inert gases, a cation imbalance has also been found in their spinal fluids (Bennett and Hayward, 1967). In addition, trichloroethylene decreases the selectivity of the sodium channel during activity, suppresses peak transient conductance and depolarizes the nerve membrane. All these factors together will have a profound depressing effect on the electrical activity of small nerve fibers and nerve endings which are found in abundance in the brain.

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