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DDT: Interaction with Nerve Membrane Conductance Changes

Abstract. *The falling phase of action potentials of lobster giant axons is prolonged by DDT; finally a plateau phase is produced like cardiac action potentials. In axons poisoned with DDT, peak transient (sodium) currents associated with step depolarizations are turned off very slowly, and steady-state (potassium) currents are markedly suppressed. These two changes would cause the prolongation of action potentials and are considered the major ionic mechanisms of DDT action.*

It has long been known that DDT prolongs the falling phase of nerve action potentials. Shanes (*1*) first observed the prolongation of negative after-potentials in external recordings from crab nerves poisoned with DDT, and this has been confirmed with cockroach nerve (*2*). Microelectrode recordings from cockroach giant axons poisoned with DDT have revealed that delayed rectification is suppressed and that the increased negative afterpotential is further augmented and prolonged, forming a plateau resembling action potentials of cardiac muscle upon removal of potassium from the bathing medium (*3*). It has been suggested that the increase in nerve membrane conductance to potassium, or the inactivation of the nerve membrane conductance to sodium, or both are inhibited, thereby increasing the negative afterpotential (*3*). To test this hypothesis, we have performed voltage-clamp experiments with the axons poisoned with DDT. Our results unequivocally point to this as the major ionic mechanism of DDT action on nerve, and it turns out that DDT may become one of the most interesting chemicals as a tool in electrophysiology.

The giant axons in the circumoesophageal connectives of the lobster *Homarus americanus* were used. Because no data were available on the effect of DDT on lobster axons, changes in action potential were first observed in the partially isolated giant axons by means of intracellular capillary microelectrodes filled with 3*M* KCl. The microelectrode experiments were done at room temperature (22°C). The sucrose-gap voltage-clamp method with the completely isolated giant axons was essentially the same as that described for squid axons (*4*), except that the chamber was modified to adapt to lobster axons. The voltage-clamp experiments were done at 7° to 10°C.

Artificial seawater containing 468 mmole of Na^+, 10 mmole of K^+, 25 mmole of Ca^{2+}, 8 mmole of Mg^{2+}, 533 mmole of Cl^-, 4 mmole of SO_4^{2-}, and 2.5 mmole of HCO_3^- per liter at *p*H 7.9 was used as the bathing medium. Purified *p,p'*-DDT was dissolved in ethanol to make up stock solution, which was in turn injected into seawater to give a suspension of 5×10^{-4} mole of DDT per liter. The concentration of ethanol was 1 percent (by volume) and in other experiments had little or no effect on the excitability of nerve.

After the lobster axon was treated with $5 \times 10^{-4}M$ DDT, its action potential was greatly augmented and prolonged, forming a plateau phase. Repetitive afterdischarge was very often superimposed on the plateau. It usually took over 20 minutes in the isolated single axons and over 1 hour in the partially isolated axons for this change of action potential to occur. The duration of the plateau depended partly on the membrane potential; when the membrane was previously hyperpolarized by an inward polarizing current passed through a second microelectrode inserted near the voltage recording microelectrode, the plateau was further prolonged, but there was

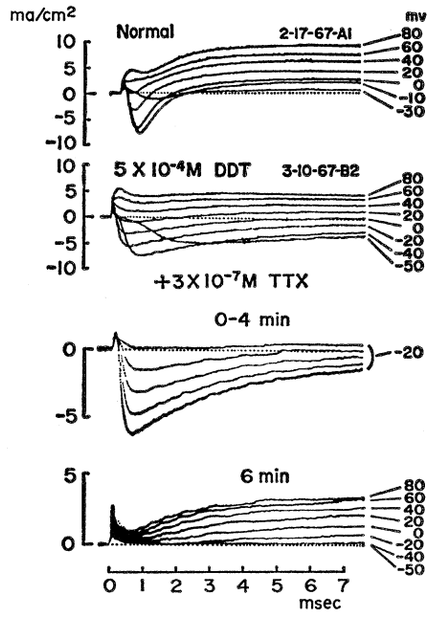


Fig. 1. Families of membrane currents associated with step depolarizations in normal axons, and those treated with DDT and with DDT and TTX. The third set of records shows changes in current during the course of TTX action. The dotted lines in each set refer to the zero base line.

a critical hyperpolarization beyond which the plateau started shortening. The absolute membrane potential at the plateau was kept almost constant by the hyperpolarization, as was the membrane potential at the peak of action potential. A change in membrane potential was also observed in seawater with DDT and free of K^+; upon removal of K from the bathing medium, the membrane was hyperpolarized, and the plateau was prolonged. The plateau could be changed by a polarizing current in DDT-containing seawater without K^+ in the same way as it could in seawater containing DDT. Thus it has become clear that the previous observation of the plateau formation in DDT media free of K^+ (*3*) was due to hyperpolarization by the removal of potassium.

Most of the voltage-clamp experiments were performed separately with normal axons and with axons that had been soaked in $5 \times 10^{-4}M$ DDT in seawater for a period of 40 minutes. Such separate experiments were necessary, because in most experiments with lobster axons an artificial node established by two sucrose streams did not survive beyond 20 to 30 minutes. The family of membrane currents associated with step depolarizations in a nor-

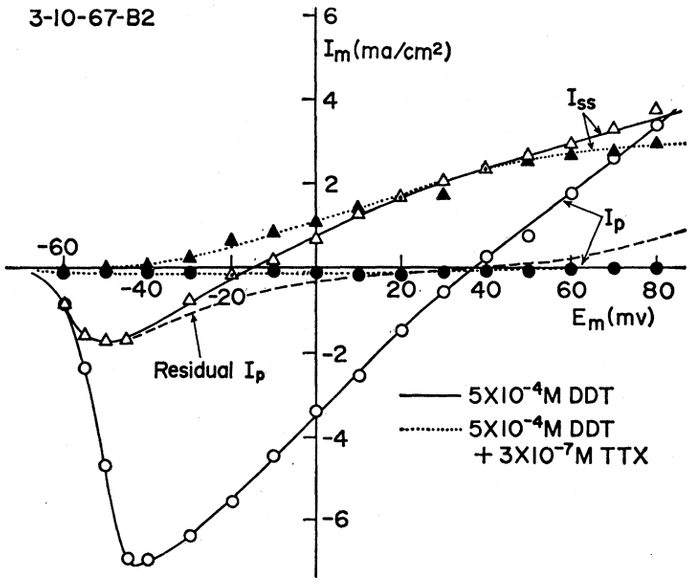
mal axon is illustrated at the top of Fig. 1. The membrane currents from the axon treated with DDT (second row of Fig. 1) are different from the normal ones in at least two respects: (i) The peak transient inward currents decline more slowly, forming into steady-state inward currents. (ii) The magnitudes of the steady-state outward currents associated with stronger depolarizatons are smaller.

Since the external potassium concentration was kept constant, the steady-state inward currents seen in the axon poisoned with DDT cannot be carried by potassium ions if the internal potassium concentration is not greatly changed. It is most likely that the steady-state inward currents are caused by a residual component of peak transient currents, which are normally carried by sodium ions. To separate the membrane current into peak transient and late steady-state currents, we applied tetrodotoxin (TTX) at a concentration of $3 \times 10^{-7} M$ on the DDT-poisoned axons. Tetrodotoxin blocks the peak transient (sodium) current without affecting the steady-state (potassium) current (*4, 5, 6*). The steady-state current, which was inward at a membrane potential of −20 mv before TTX was applied, is now converted into an outward current as the peak transient current is completely blocked by TTX. The family of membrane currents in DDT plus TTX is illustrated at the bottom of Fig. 1. All the steady-state currents are now outward in direction and are rather small in magnitude.

The peak transient currents and the steady-state currents are plotted as a function of membrane potential in Fig. 2. Also included in Fig. 2 are the current-voltage relations from a typical normal axon. A comparison of normal axons, those treated with DDT, and those treated with DDT and TTX, reveals the following: (i) The current-voltage relation for the peak transient current undergoes little or no change with DDT. (ii) The steady-state current-voltage curve for axons treated with DDT intersects the curve for those treated with DDT and TTX at the membrane potential where the peak transient current changes its polarity, namely at the equilibrium potential for the peak transient current. The residual component of the peak transient current was obtained by subtracting the steady-state current in DDT plus TTX from that in DDT. This is illustrated by a broken line which reverses its polarity at the equilibrium potential for the peak transient current. This finding supports the idea that the steady-state inward current in DDT is the residual component of peak transient current, because no peak transient current, and hence no residual current, flows at its equilibrium potential. (iii) The steady-state current beyond the equilibrium potential for the peak transient current is smaller in DDT plus TTX than in DDT, because in this potential range the peak transient current in DDT is outward in direction, causing the apparent steady-state current to increase. (iv) In the axon treated with DDT, the maximum value for the steady-state (potassium) current, after correction for the residual component of peak transient (sodium) current by application of TTX, is much smaller than that from the normal axon.

The membrane conductance (g_p) at the peak transient current (I_p) was calculated by the equation, $g_p = I_p/(E-E_p)$, where E is the membrane potential and E_p is the equilibrium potential for I_p. The mean value for the maximum g_p from 11 preparations treated with DDT was estimated as 76 ± 8.7 mmho/cm² (standard error of mean); the mean value from 18 normal fresh preparations is 93 ± 7.3 mmho/cm². The small decline of g_p in DDT may be due to deterioration during the prolonged 40-minute soaking in seawater containing DDT.

The possible shift of the g_p-E relation along the potential axis was also studied. The mean membrane potential where g_p attained half-maximum was estimated to be -40 ± 4.1 mv (seven preparations) for axons treated with DDT; that for the normal axons was -42 ± 4.5 mv (11 preparations).



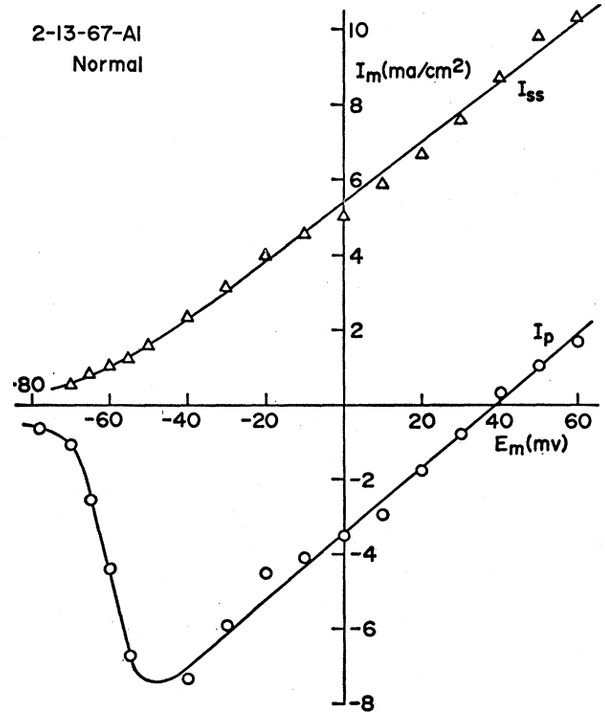


Fig. 2. Current-voltage relations for peak transient (I_p) and steady-state (I_{ss}) currents in normal axons, and in those treated with DDT or with DDT and TTX. The broken line shows the residual component of the peak transient current and was obtained by subtracting I_{ss} in DDT plus TTX from I_{ss} in DDT.

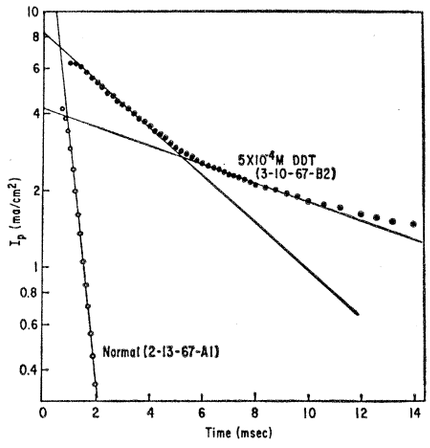


Fig. 3. Semilogarithmic plot of the decline of peak transient current at the membrane potential of −20 mv in normal axons and those treated with DDT. The true peak transient current of the normal axon was obtained by subtracting the current under the influence of $3 \times 10^{-7}M$ saxitoxin from the apparent peak transient current before the addition of saxitoxin. The true peak transient current of the axon treated with DDT was similarly obtained by use of $3 \times 10^{-7}M$ tetrodotoxin. The straight lines were drawn by eye.

Thus, it can be said that there was little or no shift.

The time for the peak transient current to reach its maximum at the membrane potential of −20 mv was estimated to be 0.77 ± 0.051 msec (18 preparations) for the normal axons. The time in the axons treated with DDT was 0.98 ± 0.085 msec (12 preparations), a 1.27-fold prolongation.

The peak transient (sodium) current normally declines exponentially while the membrane is kept depolarized (*7*). To measure the time course of the sodium inactivation, we recorded membrane currents in normal seawater and in seawater containing $3 \times 10^{-7}M$ saxitoxin (STX), and the latter current was subtracted from the former to obtain the true peak transient current. Saxitoxin, like TTX, blocks the peak transient current selectively with no effect on the steady-state current (*8*). Similar subtraction was done for the membrane currents recorded in DDT and in DDT plus $3 \times 10^{-7}M$ TTX. The falling phase of the corrected peak transient currents are plotted on a semilogarithmic scale in Fig. 3. In the normal axon, the measurements fall on a straight line except near the summit of the current, whereas in axons treated with DDT the measurements fall on two or possibly three straight lines. Furthermore, the falling phase of the normal axon is much faster than the initial falling phase of the DDT-treated axon. The measurements of the time constant (τ_h) give the mean value of 0.74 ± 0.074 msec for the normal axons (eight measurements); the mean value is for the initial falling phase of the DDT-treated axons (six measurements) 3.26 ± 0.56 msec.

Because of difficulty in estimating the steady-state equilibrium potential, we calculated the slope membrane conductance (g_{ss}) for the steady-state current (I_{ss}) by the equation $g_{ss} = dI_{ss}/dE$. The mean value for the maximum g_{ss} in the axons treated with DDT plus TTX is estimated as 29 ± 2.7 mmho/cm² (five measurements), while the normal mean value is 89 ± 5.1 mmho/cm² (18 measurements). The maximum conductance is reduced to about one-third the normal value by the treatment with DDT.

The time for the steady-state current to reach its half-maximum was measured with the steady-state current corrected for the residual peak transient current by the use of TTX or STX. The time was prolonged 1.6-fold by DDT, from the normal value of 2.19 ± 0.75 msec (seven measurements) to 3.51 ± 0.42 msec (three measurements).

Our experimental results demonstrate the validity of the hypothesis that DDT delays the turning off of peak transient (sodium) conductance or the sodium inactivation and inhibits the increase in steady-state (potassium) conductance. These two changes are naturally expected to cause a prolongation of the falling phase of the action potential according to Hodgkin-Huxley equations (*7*). Similar observations on DDT were recently made by Hille (*9*) with the nodes of Ranvier of frog. However, in squid giant axons DDT augmented the negative afterpotential only to a very small extent; an action potential having a plateau was never produced even when DDT was perfused internally.

It is interesting that under the influence of DDT the onset of the steady-state conductance increase is delayed only 1.6-fold in the face of the markedly sustained increase in the peak transient conductance. This finding suggests that the channel for peak transient current and that for late steady-state current may work independently, and is compatible with the hypothesis that there are two operationally separate channels, which was implicit in the Hodgkin-Huxley formulation and was originally evolved from experiments with TTX (*4*, *5*). However, it should be emphasized that the term "channel" does not refer to an anatomical structure but only refers to a conceptual pathway.

The mechanism whereby DDT exerts its effect on the membrane conductance change remains to be studied. It is unlikely that DDT directly plugs the channels for the peak transient current because it does not block but rather keeps the channels open. This mechanism is contrasted with that of TTX which is considered to plug the peak transient channels to block the current (*10*). Recently, it was found that DDT forms charge-transfer complexes with nerve components (*11*). Whether the formation of complexes has direct bearing on the conductance change observed in our study is not known. However, this finding suggests membrane protein components may be the binding or target site of DDT.

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