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MECHANISM OF INCREASE IN NEGATIVE AFTER-POTENTIAL BY DICOPHANUM (DDT) IN THE GIANT AXONS OF THE COCKROACH*

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Attempts have already been made to see whether dicophanum (DDT) causes changes in after-potentials in nerve. In crab nerve there was only a slight increase in negative after-potential (Shanes, 1949*b*), whereas no change was observed in squid and frog nerves (Shanes, 1949*a*, 1951). On the other hand, it was found in our previous studies that DDT causes a marked increase and prolongation of the negative after-potential in cockroach nerve (Yamasaki & Ishii, 1952; Yamasaki & Narahashi, 1957*b*, *c*, *d*). This effect of DDT, together with its unstabilizing action, is regarded as the most important feature of the toxic action of DDT (e.g. Roeder & Weiant, 1948; Welsh & Gordon, 1947; Yamasaki & Ishii, 1954). However, there is no adequate evidence to explain the increase in negative after-potential caused by DDT.

As in squid giant axons (Frankenhaeuser & Hodgkin, 1956) and in non-myelinated nerve fibres of the rabbit (Greengard & Straub, 1958), the negative after-potential of normal cockroach giant axons has been demonstrated to be explicable by an accumulation of the released potassium ions in the immediate vicinity of the nerve membrane (Narahashi & Yamasaki, 1960). The present paper gives the results of experiments whose aim was to elucidate the mechanism of the increase in negative after-potential brought about by DDT.

METHODS

The giant axons of the American cockroach, *Periplaneta americana* L., were used throughout the experiments. The methods of stimulation and recording were essentially similar to those described in our previous reports (Yamasaki & Narahashi, 1959*a*, *b*; Narahashi & Yamasaki, 1960). Stimulation was effected by a pair of silver wire electrodes, while recordings were made by a micro-electrode inserted into an axon. When polarizing currents were to be applied to the axon, a Wheatstone bridge circuit was employed, which enabled a single micro-electrode to be used both for polarization and recording. The bathing solution was changed by the method described previously (Narahashi & Yamasaki, 1960).

* Studies on the mechanism of action of insecticides XIX.

The Ringer's solution used was the same as that described in our previous papers (Yamasaki & Narahashi, 1959*a*; Narahashi & Yamasaki, 1960). DDT-Ringer's solution was prepared by introducing a small amount of ethyl alcohol containing pure DDT into Ringer's solution. The final concentration of DDT was 10^{-4} M, and that of the alcohol was 0·5 %. This concentration of alcohol was found to have no effect on the axon in any respect. All the experiments were conducted at room temperatures ranging from 16·5 to 26·5° C.

RESULTS

Changes of resting and action potentials in the course of DDT poisoning

The action potentials before and after treatment with DDT are shown in Fig. 1. After the normal bathing solution had been replaced by a Ringer's solution containing DDT at a concentration of 10^{-4} M, no noticeable change was observed in resting and action potentials for a period of several

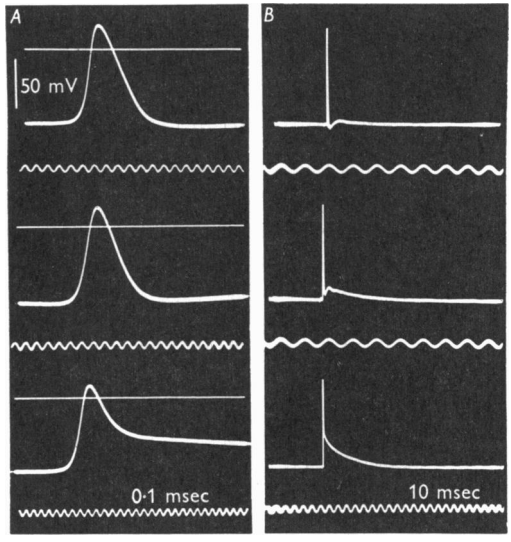

Fig. 1. Records showing changes in action potential after treatment with 10^{-4} M DDT. *A*, from top to bottom, before, 38 min after, and 90 min after treatment with DDT. The horizontal lines indicate zero potential level. *B*, as in *A*, but with slower sweep. Temperature 21·5° C.

minutes. However, after 10 min or so the negative after-potential began to grow progressively. The positive phase decreased as the negative after-potential increased. After some time, usually 15–30 min after starting treatment with DDT, the axon reached a state in which a single shock could give rise to a repetitive response (Fig. 2). With the further advance of time the negative after-potential grew larger still and the repetitive response ceased. The negative after-potential continued to grow more and more, and the positive phase could be seen as a slight dip between the spike

potential and the negative after-potential. The positive phase eventually disappeared, so that the spike potential terminated directly in the negative after-potential. The initial height of the negative after-potential finally attained a maximum value of about 30 mV or more, which stayed unchanged for some period of time. At this stage of poisoning a slight positive after-potential or undershoot sometimes followed the negative after-potential. Up to this stage the spike phase underwent little change; details will be described later. Thereafter, the rate of fall of the spike potential slowed considerably. With the further advance of time the negative after-potential began to decrease. The time of onset of this decrease varied considerably from fibre to fibre, being 3 hr or more after starting treatment with DDT. Conduction block was never observed during experiments, which were extended over 3–5 hr. The resting potential underwent little change throughout. On washing with normal Ringer's solution, these effects of DDT were found to be irreversible.

Thus, the time course of DDT poisoning can be divided into at least four stages: (1) from the beginning of the increase in negative after-potential to the end of repetitive discharge; (2) from the end of repetitive discharge to the maximum negative after-potential; (3) from the beginning of marked slowing of the rate of fall of the spike potential to the beginning of marked decrease in negative after-potential; and (4) the stage of marked decrease in negative after-potential. Unless otherwise stated, the present paper describes the results of experiments on stages (1) and (2), which are thought to be indicative of the primary action of DDT. Experiments on stages (3) and (4), which seem to some extent to be secondary to stages (1) and (2), will be described elsewhere.

Nature of repetitive response induced by DDT

Figure 2 shows records of the action potential taken before, during and after the state of repetitive response under the influence of DDT. Transition from the state where there was only a single response to that where there was a repetitive response occurred abruptly when the negative after-potential had increased to some extent. There appeared to be no intermediate state. The repetitive response started at a frequency of 200–400/sec and ended abruptly after a period of 30–70 msec. The interval between the first and second impulses was relatively large, between the second and the third impulses it was shortest, and it gradually grew towards the end of the train. On the other hand, transition from the state of repetitive response back to the state of single response was not always abrupt. This transition was invariably accompanied by a further increase in the negative after-potential. On some occasions the interval between the spikes was prolonged and the number of spikes decreased until the

response became single. In other cases, however, the state of single response was established abruptly. After the repetitive period, small potential changes were frequently seen on the declining phase of the large negative after-potential, as is shown in Fig. 2. These small potentials were found from close inspection to be damped oscillations starting at the peak of the negative after-potential and ending with completion of repolarization. The oscillations later decreased in magnitude as the negative after-potential increased further with the advance of time.

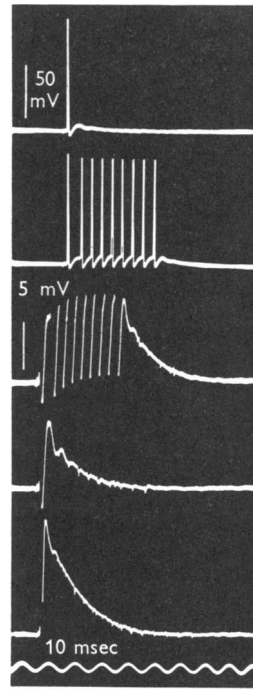

Fig. 2. Records of the action potential induced by a single stimulus before, during and after the repetitive stage in the course of DDT poisoning. From top to bottom, 20, 22, 26, 26·5 and 34 min after treatment with 10^{-4} M DDT. Temperature 21·5° C.

Repetitive response could also be induced in the DDT-poisoned axon by a long-lasting cathodal current. The normal cockroach giant axon did not give rise to a repetitive response even when a long-lasting cathodal current of suprathreshold intensity was applied. However, when the axon reached a state in which a single shock could give rise to a repetitive response, the long-lasting cathodal current was effective in producing a repetitive response (Fig. 8).

Effect of DDT on the configuration of the spike potential

The magnitude of the action potential was little affected by DDT (Fig. 1). Although there was a slight tendency for the action potential to decrease in height during the course of long-term experiments which extended to stages (3) and (4), it seems unlikely that this was due to any direct effect of DDT. The active membrane potential was probably kept almost constant throughout, because, as already mentioned, the resting potential underwent little change.

Graphical analyses of records of the action potential, such as those shown in Fig. 1*A*, revealed that the maximum rates of rise and fall were only slightly decreased by DDT, the effect on the latter being greater than that on the former. This result suggests that DDT has little effect on the mechanism by which the spike phase of the action potential is generated. However, during later stages of poisoning (stages (3) and (4)), the rate of fall of the action potential decreased so much that discrimination between the spike and the negative after-potential became difficult.

Records of trains of impulses at varying frequencies are illustrated in Fig. 3*A*. Although the DDT-poisoned axon could respond to repetitive stimulation at a frequency as high as 200–300/sec, the spike height was greatly reduced for all impulses except the first one. In the normal unpoisoned axon the spike height was also reduced during a train of impulses, but to a much less extent. This may imply that the axon is in a partially inactivated state after the first impulse.

Effect of DDT on negative after-potential

Negative after-potential following a single impulse. The time course of the decay of a negative after-potential following a single impulse was plotted on a logarithmic scale against time, as is shown in Fig. 4. Before treatment with DDT the decay could be expressed as a single exponential term. After treatment with DDT, however, the decay was not a single exponential function. The initial brief period of the decay could be described by an exponential term, which finally terminated in a faster phase. Since the height of the negative after-potential changed progressively during the course of DDT-poisoning, measurements were made when the maximum value for the negative after-potential was achieved in each series of experiments. The time constant for the initial phase thus estimated was between 14·6 and 40·4 msec in different axons, with a mean of 24·3 msec. This mean time constant is much longer than that of the normal unpoisoned axon, which has been estimated as 9·2 msec in our previous report (Narahashi & Yamasaki, 1960). The terminal fast phase also declined exponentially, with

a time constant ranging between 3·4 and 34·1 msec, and a mean of 14·7 msec.

Negative after-potential following a train of impulses. Figure 3*B* shows records of the negative after-potential at various frequencies of stimulation, The after-potential following a single impulse was already large, and there was only a small additional build-up during a train of impulses. It seemed

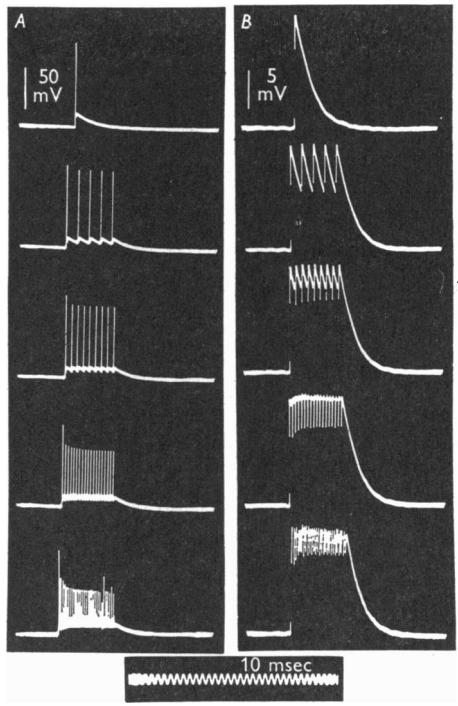

Fig. 3. Records of the action potentials induced by a single shock and by repetitive stimulation at various frequencies. These records were taken 50–62 min after starting treatment with 10^{-4}M DDT. *A*, from top to bottom, single stimulus, 50/sec, 100/sec, 200/sec and 300/sec. *B*, as in *A*, but with higher amplification. Temperature 18° C.

at first sight that the time course of the falling phase of the negative after-potential was independent of the stimulus frequency. To investigate this point further the following analyses were made.

Figure 5 shows the time course of the decay of the negative after-potential after a train of impulses at frequencies of 50 and 200/sec. The initial phase decayed approximately exponentially with a time constant close to that for the decay after a single impulse. However, there was a tendency for the declining phase to terminate in a very slow component. The initial phase continued longer than in an unpoisoned axon, but transition from the initial phase to the terminal phase occurred at a

comparable potential level in both the poisoned and unpoisoned axons. On the other hand, there was little sign of the terminal fast phase which was always observed in the negative after-potential after a single impulse.

Summarizing the various declining phases of the negative after-potential, the following patterns can be discriminated:

a, unpoisoned axon, single impulse, single fast phase;

b, unpoisoned axon, train of impulses, initial fast phase and terminal very slow phase;

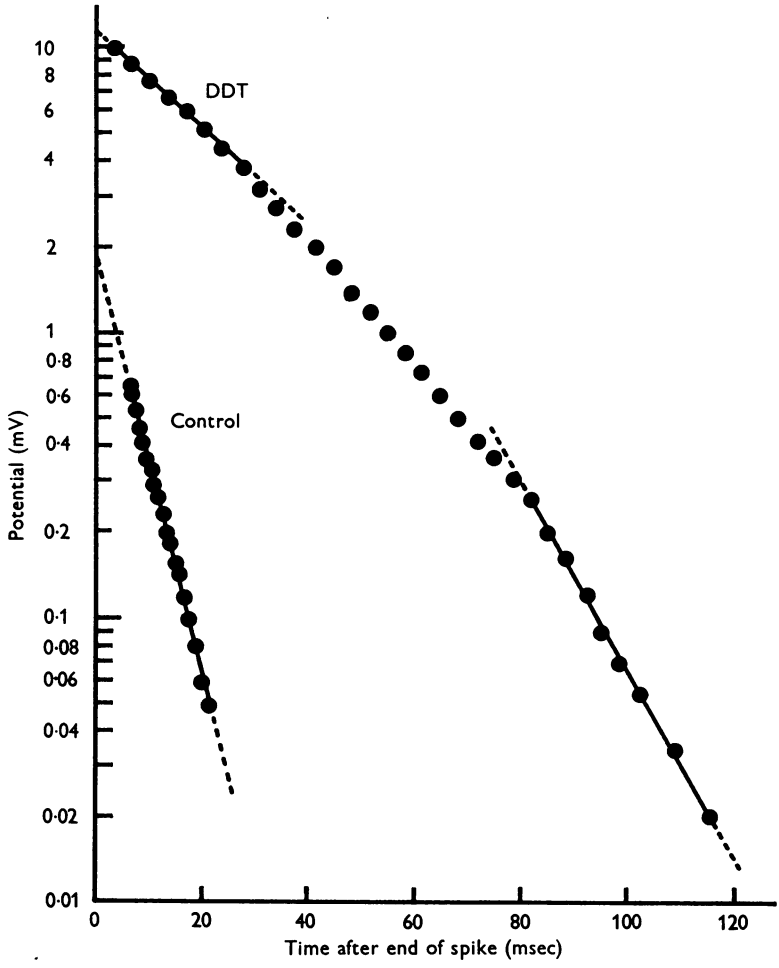

Fig. 4. Time course of the decay of the negative after-potential following a single impulse before and 53 min after starting treatment with 10^{-4} M DDT. The straight lines were drawn through the points by eye. Their time constants were: control, 5·8 msec; DDT, initial phase, 24·5 msec; DDT, terminal phase, 12·7 msec. Temperature 19° C. Semi-log scale.

c, DDT-poisoned axon, single impulse, initial slow phase and terminal fast phase;

d, DDT-poisoned axon, train of impulses, initial slow phase and terminal very slow phase.

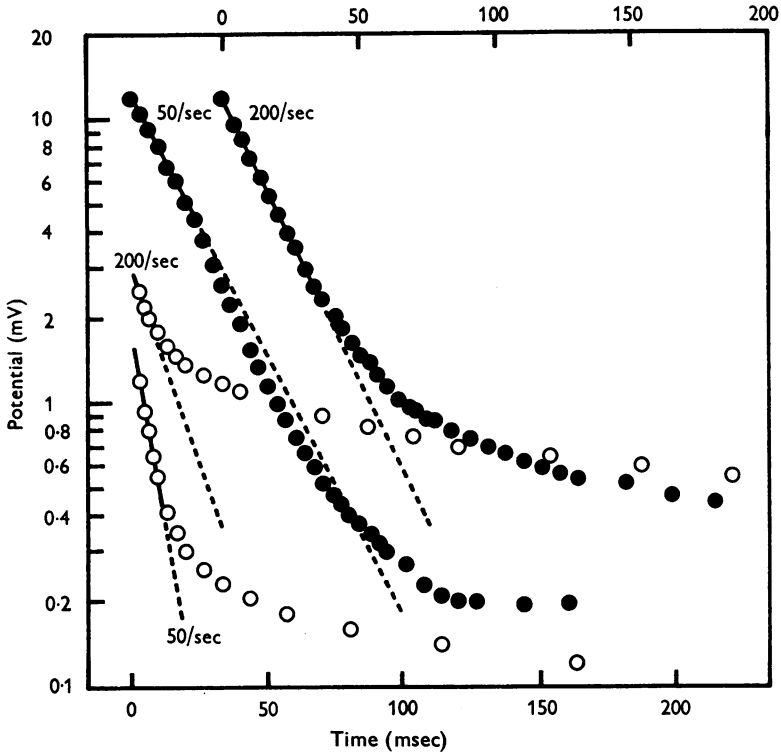

Fig. 5. Time course of the decay of the negative after-potential following a train of impulses at 50 and 200/sec before and 55 min after starting treatment with 10^{-4} M DDT. The open circles represent the potential before treatment, and the filled-in circles after treatment. The ordinate indicates the potential in mV on a logarithmic scale. The abscissae are the time in msec after the end of the last impulse in the train, the upper scale applying to 200/sec (after treatment) and the lower scale to the others. The straight lines were drawn through the points by eye. Their time constants were: before treatment, 50/sec, 8·6 msec; 200/sec. 16·3 msec; after treatment, 50/sec, 24·1 msec; 200/sec, 22·1 msec. The same axon as in Fig. 4. Temperature 19° C.

If the mechanism for the production of the terminal very slow phase of *b* is unimpaired by DDT, and if the mechanism for the production of the fast phase of *c* is also operative when the axon is excited repetitively, these two mechanisms should partly cancel each other when the DDT-poisoned axon is stimulated repetitively. This may result in the appearance of the prolonged initial phase in the repetitively stimulated DDT-poisoned axon.

Addition of negative after-potential during a train of impulses. There are two alternative explanations for the increase in negative after-potential caused by DDT. Two important features of DDT poisoning, which are the increase in the negative after-potential and the appearance of the terminal fast phase in the decay of the negative after-potential, may imply that the mechanisms by which the falling phase of the action potential is normally accelerated are delayed or partly suppressed by treatment with DDT.

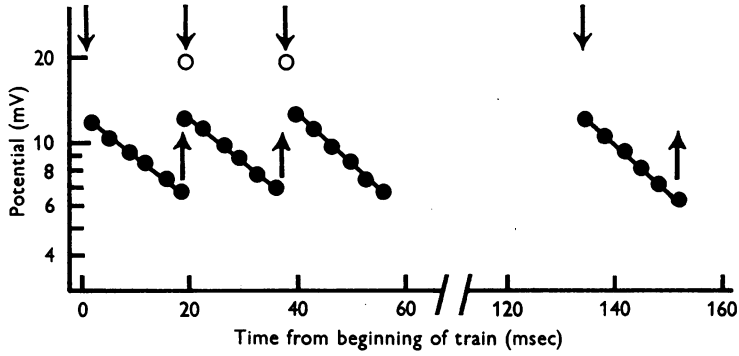

Fig. 6. Time course of the decay of the negative after-potential during a train of impulses at 54/sec, 53 min after starting treatment with 10^{-4}M DDT. The first, second, third and eighth impulses are illustrated. The filled circles are the measured points. The straight lines were drawn through the points by eye. Their time constants were: first, 29·9 msec; second, 29·6 msec; third and eighth, 26·4 msec. The open circles represent the potential at the end of the second and third spikes, calculated by adding the initial height of the first negative after-potential to the after-potential remaining at the instant when the second and third spikes arise. The upward arrows indicate the start of the spikes, and the downward ones the end of the spikes. The same axon as in Figs. 4 and 5. Temperature 19° C. Semi-log scale.

Alternatively, such an increase in negative after-potential could be caused by an increased accumulation of potassium near the membrane during activity.

If the second explanation were correct, the negative after-potentials in poisoned axons would add in a linear manner, as in unpoisoned axons. To make this argument clear, the successive negative after-potentials during a train of impulses at a frequency of 54/sec were plotted on a logarithmic scale against time, as illustrated in Fig. 6. The initial height of the second negative after-potential, estimated by adding what remained of the first after-potential at the moment when the second spike arose to the initial height of the first negative after-potential obtained by extrapolation, was $6{\cdot}6 + 12{\cdot}4 = 19{\cdot}0$ mV, as against the observed value of 12·5 mV. The calculated initial height for the third negative after-potential was $6{\cdot}3 + 12{\cdot}4 = 18{\cdot}7$ mV, as against the observed value of 12·7 mV. Further,

the amount by which the eighth impulse in a train declined in the same period as the stimulus interval was not equal to the contribution of a single impulse, the former value being 12·4 − 6·0 = 6·4 mV as against the latter of 12·4 mV. Hence it is concluded that the increase in negative after-potential caused by DDT does not arise from a greater amount of potassium release during activity. The small build-up of the negative after-potentials during a train of impulses, however, is attributable to the accumulation of released potassium.

Effect of DDT on the rectifying property of the membrane

The delayed rectification of the cockroach nerve membrane has already been demonstrated in one of our previous reports (Yamasaki & Narahashi, 1959*b*). Since the action potential of the cockroach giant axons has been shown previously (Yamasaki & Narahashi, 1959*a*) to be accounted for by the ionic theory (Hodgkin, 1957), the rectification can be interpreted on the assumption that the potassium conductance, a rise of which is responsible for the falling phase of the action potential, is kept increased during a flow of cathodal current. It was suggested in the preceding section that the increase in negative after-potential caused by DDT might arise from a delay or partial suppression of the mechanism by which the falling phase of the action potential is accelerated. The falling phase of the action potential is caused by an inactivation of the sodium-carrying system, which leads to a decline of sodium conductance, and by a rise in potassium conductance. Hence, if the rise in potassium conductance during activity was delayed or partly suppressed by DDT, it would follow that the delayed rectification would be affected.

Figure 7 shows records of the electrotonic potential from a normal axon induced by equal and opposite long-lasting currents. In accordance with our previous observation (Yamasaki & Narahashi, 1959*b*), the delayed rectification became apparent when the intensity of current exceeded a certain level. There also appeared a graded response with subthreshold cathodal currents. It should be noted that the decay of the catelectrotonic potential was faster than that of the corresponding anelectrotonic one, and terminated in a positive phase. The faster decay and the positive phase can be interpreted as indicating that there is an appreciable rise in potassium conductance during a flow of cathodal current.

Records of the electrotonic potential under the influence of DDT are illustrated in Fig. 8. The axon was in a state of repetitive response when these records were taken. The bottom record was taken with a single shock applied to the end of the nerve preparation by external electrodes. The top record is an electrotonic potential induced by a just subthreshold cathodal current, and the pair of records in the middle are for equal and opposite

threshold currents. It will be seen that the graded potential, which rose and fell as quickly as normal, was followed by a gradual rise in potential which then declined to a lower steady state after attaining a summit. On cessation of cathodal current the electrotonic potential decayed slowly without any sign of positive overshoot. A similar time course for the catelectrotonic potential was also seen when a threshold current was applied. Thus it became clear that in the DDT-poisoned axons the rectification occurred on application of cathodal current with a delay much

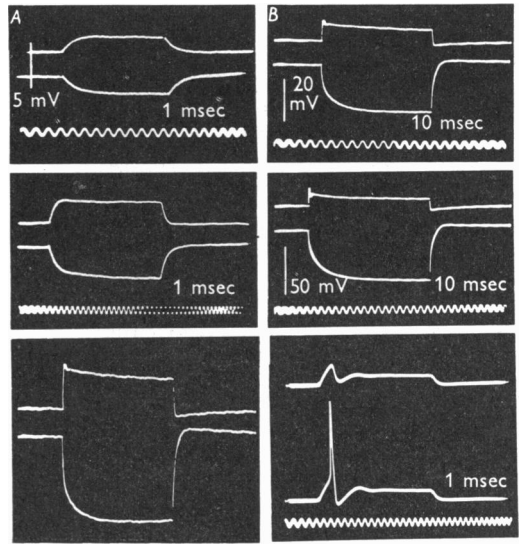

Fig. 7. Records of the electrotonic potential induced by equal and opposite currents with various intensities in a normal unpoisoned axon. The intensity of current was increased in steps from the top of column *A* to the bottom of column *B*. Upper tracing in each record shows catelectrotonic potential, and lower tracing anelectrotonic potential, except for the bottom record of *B* where just subthreshold and threshold catelectrotonic responses are shown. The time marker of the top of *B* also applies to the bottom of *A*. Temperature 20° C.

greater than normal. The degree of rectification was also depressed by DDT. This is undoubtedly responsible for the slower decay and the absence of the positive phase following break of cathodal current. The slower decay of the catelectrotonic potential was also observed in our previous study which was made with external electrodes (Yamasaki & Narahashi, 1957*d*).

These results strongly support the view that the rise in potassium conductance during activity was delayed and partly suppressed by DDT. Despite this change in delayed rectification the initial falling phase of the spike potential undergoes little change. It seems therefore reasonable to assume that DDT does not inhibit the inactivation process occurring

during the falling phase of the action potential, at least in the initial stages of poisoning.

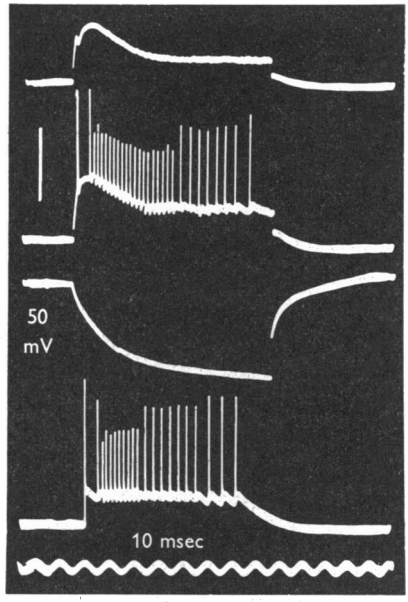

Fig. 8. Responses of a DDT-poisoned axon to long-lasting currents and a single shock. 41–44 min after starting treatment with 10^{-4} M DDT. From top to bottom, catelectrotonic potential induced by a just-subthreshold current; catelectrotonic potential with superimposed repetitive spikes induced by a threshold current; anelectrotonic potential with same threshold current; train of impulses induced by a single shock. Temperature 26·5° C.

Effects of potassium and calcium on the DDT-induced negative after-potential

Effect of changing the external potassium concentration. Figure 9 shows records of the action potential taken in 0, 3·1 and 10·0 mM-K solutions each containing DDT at a concentration of 10^{-4} M. The DDT-induced negative after-potential was very effectively depressed by high potassium solution. In a potassium-free solution it was greatly augmented and a plateau appeared. A small positive after-potential followed the plateau. There occurred a fall of resting potential when the potassium concentration was raised and a rise when it was lowered, as in unpoisoned axons. These effects of potassium were completely reversible upon washing with normal Ringer's solution. It should be noted that the action potential from the axon bathed in a potassium-free DDT solution resembles the cardiac action potentials from mammals in that there appears to be a marked plateau phase.

The delay and suppression of the rectification by DDT were also observed in potassium-free solutions.

Effect of changing the external calcium concentration. As is shown in Fig. 10, when an axon had been treated with 10^{-4}M DDT and was in a state giving rise to an increased negative after-potential, a rise in external calcium concentration from 1·8 to 18 mM caused a further increase in

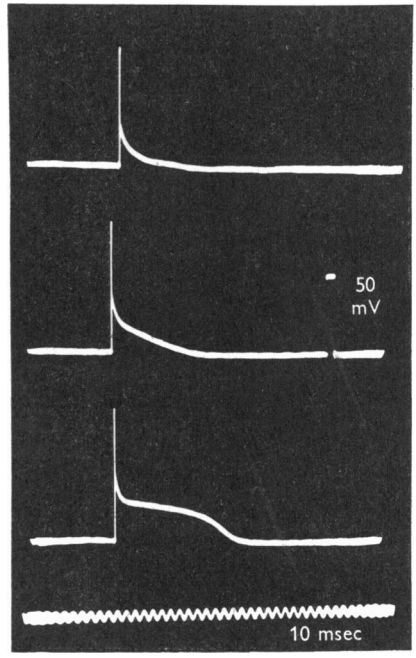

Fig. 9. Effect of changing the external potassium concentration on the action potential of the DDT-poisoned axon. These records were taken 85–112 min after starting treatment with 10^{-4}M DDT. Top record, 10 mM-K; middle record, 3·1 mM-K (normal); bottom record, 0 mM-K. Voltage calibration is given by a brief square pulse in the middle record. Temperature 26·5° C.

negative after-potential. The spike potential in the DDT-poisoned axon was affected in the same way as in an unpoisoned axon (Narahashi & Yamasaki, 1960), the maximum rate of rise being slowed down. These effects of calcium were completely reversible on washing with normal Ringer's solution.

Figure 11 shows records of repetitive activity as induced by a single stimulus in DDT-Ringer's solutions containing 1·8 mM (normal) and 18 mM-Ca. Two points are worth noting: (*a*) in spite of a tenfold increase in [Ca], the DDT-poisoned axon was still able to fire repetitively in response to a single stimulus. (*b*) The spike height was reduced progressively during a train of impulses in 1·8 mM-Ca, whereas it was little affected in 18 mM-Ca.

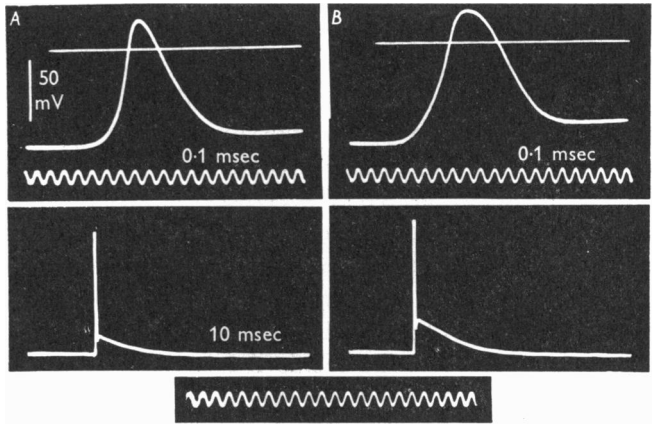

Fig. 10. Effect of changing the external calcium concentration on the action potential of the DDT-poisoned axon. These records were taken 69–78 min after starting treatment with 10^{-4} M DDT. The horizontal lines indicate zero potential level. *A*, 1·8 mM-Ca (normal); *B*, 18 mM-Ca. Temperature 19° C.

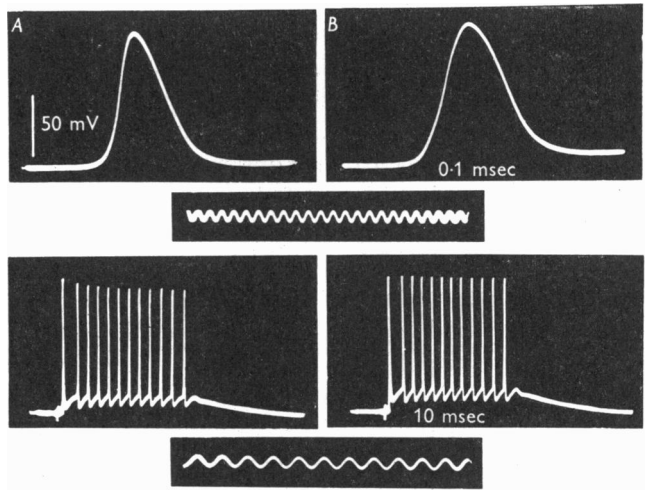

Fig. 11. Effect of changing the external calcium concentration on the train of impulses induced by a single stimulus in a DDT-poisoned axon. *A*, 1·8 mM-Ca (normal); *B*, 18 mM-Ca. Temperature 21·5° C.

DISCUSSION

It seems probable that the moderately increased negative after-potential is partly responsible for the repetitive discharge, because the state of repetition is first established when the negative after-potential has grown to some extent. As suggested for the production of repetition in myelinated nerve fibres by veratrine (Tasaki & Mizuguchi, 1948), and in

muscle fibres by tetraethylammonium (TEA) (Hagiwara & Watanabe, 1955), re-stimulation by an increased negative after-potential can be regarded as one of the mechanisms involved. However, the existence of other important mechanisms is suggested by the fact that normal unpoisoned axons of the cockroach do not give a repetitive response on application of a long-lasting cathodal current (Yamasaki & Narahashi, 1959*b*). Hence, it can be stated that DDT establishes a condition in which a repetitive response arises from any long-lasting depolarization, such as that provided by an increased negative after-potential.

It is interesting to note in this connexion that damped oscillations are observed on the decay of the negative after-potential immediately after the repetitive period is over. It seems reasonable to assume that the above-mentioned condition, which favours repetition when the negative after-potential increases, is related to an augmentation of oscillatory potentials. Thus, if the tendency for the axon membrane to set up oscillatory potentials in response to cathodal currents is increased by DDT, it follows that the increased and prolonged negative after-potential following a spike generates large oscillatory potentials, which are able to give rise to action potentials if they are of sufficient amplitude. The second impulse likewise sets up the third impulse and so on. With the advance of time the state of repetition is suppressed as described before. At this time the oscillatory potentials are so decreased in amplitude that they fail to give rise to action potentials. They can be observed as damped oscillations superimposed on the declining phase of the negative after-potential. In view of these considerations it may be stated that the induction of repetition by DDT which is observed in the initial stage of poisoning can be interpreted as being due to the two factors, i.e. moderately increased negative after-potential, and augmented oscillatory potentials.

Oscillations of membrane potential have received considerable attention (Arvanitaki, 1939, 1943; Cole & Curtis, 1941; Cole, 1941; Hodgkin & Rushton, 1946; Shanes, 1949*a*; Sjodin & Mullins, 1958). Cole & Curtis (1941) and Hodgkin & Rushton (1946) have shown that oscillatory property of the membrane depends on the apparent inductance, resistance and capacity of the membrane. Thus the damped oscillations observed during a flow of cathodal current in *Carcinus* axons were attributed to a decrease in membrane resistance upon depolarization (Hodgkin & Rushton, 1946). This explanation seems to be applicable to the present case; a decrease in membrane resistance caused by an augmented negative after-potential would cause oscillations, if the three parameters of the membrane property were altered by DDT in such a manner as to favour oscillations. However, since no data are available for alteration of the membrane constants by DDT, it is unwise to speculate further. It should be added that

Shanes (1949*a*) observed the after-potentials of the normal squid giant axon to be oscillatory.

Various hypotheses have been put forward for the mechanism of action of DDT on nerve. Welsh & Gordon (1947) and Gordon & Welsh (1948) carried out a series of experiments on the repetitive response of crayfish axons under the influence of DDT and various concentrations of calcium. They found that in the DDT-treated axon a single stimulus gives rise to a train of impulses. This effect was accelerated by low [Ca], and was similar to that produced by low [Ca]. They concluded that DDT is adsorbed at or near the axon surface and acts as a barrier layer which hinders the normal reaction of calcium ions with the surface.

In our previous studies the effects of DDT, potassium and certain metabolic inhibitors on the resting and action potentials of the cockroach nerve were examined with external electrodes (Yamasaki & Narahashi, 1957*a*, *d*). Unlike potassium and metabolic inhibitors, DDT had little or no effect on the resting potential and impulse conduction, though a marked increase in negative after-potential was caused. It was suggested that DDT affects the nerve function by changing the ionic permeability of the nerve membrane.

The experimental evidence described in the present paper provides convincing evidence that a marked delay and partial suppression of the rise in potassium conductance during activity are responsible for the increase in negative after-potential caused by DDT. The sodium inactivation process which occurs during the latter half of the spike is unlikely to be inhibited by DDT. This view strongly supports our previous hypothesis. It is also consistent with Welsh & Gordon's (1947) hypothesis in the sense that DDT exerts its effect at the surface membrane of the axon.

However, the present results, together with our observations on calcium action (Narahashi & Yamasaki, 1960), do not seem to fit with Welsh and Gordon's hypothesis, in that the action of DDT cannot be linked with calcium in the manner suggested by the hypothesis. The reasons for this supposition are as follows: (*a*) The resting potential, the magnitude and the maximum rates of rise and fall of the action potential, and the height of the negative after-potential, are all reduced by lowering the external calcium, whereas these changes are not caused by DDT. (*b*) The giant axon of the cockroach, even when treated with low-calcium solutions, neither gives rise to repetitive activity in response to a single stimulus nor discharges impulses spontaneously; this is in striking contrast with the results for lobster and crayfish axons, with which repetitive or spontaneous activity is observed when the external calcium is lowered (Adelman, 1956; Gordon & Welsh, 1948). (*c*) The repetitive response of the DDT-poisoned cockroach axon is not suppressed by raising the external [Ca]; this is also contrary to the results for crayfish axons, with which the repetitive response produced

by DDT is effectively suppressed by raising [Ca] (Gordon & Welsh, 1948). All these three arguments are incompatible with the idea that DDT competes with calcium for sites at the surface membrane.

The spike height during a repetitive response induced by DDT is increased by raising calcium. Since the proportion of the sodium-carrying system which is in an inactive state is decreased by raising calcium (Frankenhaeuser & Hodgkin, 1957), the improvement can be accounted for by a removal of inactivation.

Prolongation of the nerve action potential is known to be produced by treatment with certain drugs or ions such as veratrine alkaloids (Shanes, 1958), TEA (Burke, Katz & Machne, 1953; Tasaki & Hagiwara, 1957), high concentrations of glycerin (Mueller, 1958), and cobalt and nickel ions (Matsumoto, 1959; Sasaki, 1959). Among these, TEA was demonstrated to depress delayed rectification in the nerve cells of *Onchidium* (Hagiwara & Saito, 1959). It is apparent that TEA resembles DDT in the sense that the rectifying property of the membrane is suppressed. On the other hand, the veratrine-induced negative after-potential is different from that induced by DDT in that the former declines with a single exponential term (Shanes, Grundfest & Freygang, 1953), and may involve an increase in sodium conductance (Shanes, 1958).

It is interesting to note that the DDT-poisoned axon, when treated with potassium-free solution, gives rise to action potentials which resemble those of mammalian cardiac muscle fibres. The behaviour of the membrane potential under these conditions will be further described elsewhere.

SUMMARY

1. After treating a cockroach giant axon with DDT, the positive phase following the spike decreased and finally disappeared, and the negative after-potential increased until it attained a maximum, although the spike phase underwent little change. There was a brief period during which a repetitive response could be induced by a single shock. Thereafter, however, the rate of fall of the spike potential slowed considerably, and the negative after-potential finally began to decrease. Conduction block was never observed, and the resting potential underwent little change throughout.

2. Immediately after the repetitive period, damped oscillations were often seen on the declining phase of the increased negative after-potential.

3. In normal axons the time course of the decay of the negative after-potential following a single impulse could be expressed as a single exponential term. After treatment with DDT, however, the decay was initially slower than normal, and terminated in a faster phase.

4. After a train of impulses in a DDT-poisoned axon, the declining phase terminated in a very slow component which was less marked than in a normal axon, and there was little or no sign of the terminal fast phase seen after a single impulse.

5. Unlike normal axons, the negative after-potentials in the DDT-poisoned axon did not add linearly during a train of impulses.

6. Delayed rectification, which was always observed in the normal axons when cathodal current was applied, was found to be further delayed and partly suppressed in the DDT-poisoned axons. On cessation of cathodal current the electrotonic potential decayed more slowly in the DDT-poisoned axons than in the normal ones.

7. A rise in external potassium concentration reduced the DDT-induced negative after-potential. In potassium-free solutions it was greatly augmented and a plateau appeared.

8. A rise in external calcium concentration caused a further increase in the DDT-induced negative after-potential.

9. It is concluded that the increase in negative after-potential caused by DDT is not caused by a rise in potassium accumulation near the nerve membrane, but by a delay and suppression of the rise in membrane potassium conductance which accelerates the falling phase of the action potential.

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