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ATPase INHIBITION AND ELECTROPHYSIOLOGICAL CHANGE CAUSED BY DDT AND RELATED NEUROACTIVE AGENTS IN LOBSTER NERVE

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Abstract—In an attempt to elucidate the biochemical and physiological mechanisms of action of DDT on the nerve membrane, experiments have been performed to study the effects of DDT and related chemicals on the ATPases and electrical activity of the lobster nerves. ATPases were obtained from the nerves of the walking legs of the lobster, *Homarus americanus*. The optimum conditions for the maximum ATPase activity were: Na^+ 320 mM, K^+ 20 mM, Mg^{2+} 1 mM at pH 7·6–8·0. The rigid requirements of Na^+, K^+ and Mg^{2+} suggest that the major portion of the ATPases in the lobster nerve preparations consists of Na^+-K^+-Mg^{2+} ATPases. DDT inhibited a portion of Na^+-K^+-Mg^{2+} ATPases. It was found, however, that only a portion of the DDT-sensitive ATPases could be inhibited by a high concentration of ouabain. In view of the possibility that DDT-sensitive ATPases are not the ones involved in the active transport systems, a variety of neuroactive agents which are known to induce the nerve conductance changes were examined for their actions on the ATPase preparations. The majority of chemicals that have been known to cause electrophysiological responses similar to DDT were found to be potent ATPase inhibitors. A correlation was found between the degree of DDT inhibition of ATPases and the electrophysiological symptoms of DDT poisoning.

IT HAS been long acknowledged that the poisoning mechanism of DDT on the nervous system is disruptions of Na^+ and K^+ transport processes in the nerve membrane.[1–5] Unknown is the molecular aspects of the reaction(s) through which DDT causes such disruption of the membrane function. Several reports have shown, however, that DDT probably forms a complex with certain components of the nervous system,[4,6,7] and that some protein is involved in such complex formation. The nature of the DDT-sensitive site on the nerve membrane, otherwise, remains unknown.

Recently it has been reported by two schools[8,9] that various chlorinated hydrocarbon insecticides including DDT inhibit nerve ATPases *in vitro*. The findings are important in view of the expected involvement of these nerve ATPases in the processes of ion transport across the nerve membrane.[10,11] The question whether such inhibition of nerve ATPases is causally related to the mechanism of action of DDT may be answered by the demonstration of a causal relationship between symptoms *in vivo* and the activity of ATPases at various symptomologically defined poisoning stages.

In the present report, attempts were made to correlate the degrees of ATPases inhibition with electrophysiological symptoms of poisoning as the first stage of such studies.

EXPERIMENTAL

Materials. The nerve bundles used for this study were obtained from the walking legs (pereiopods) of the American lobster, *Homarus americanus*. The nerve was isolated by simply breaking the joint between the meropodite and carpopodite of an isolated walking leg (1st, 2nd or 3rd) and pulling them apart. The nerve thus exposed (usually 5–6 cm long) was completely isolated in the artificial sea water (solution A of Table 1).

TABLE 1. COMPOSITIONS (mM) OF ARTIFICIAL SEA WATER (ASW) FOR ELECTROPHYSIOLOGICAL MEASUREMENTS

Solution	NaCl	KCl	$CaCl_2 \cdot 2H_2O$	tris HCl	pH
Standard ASW (A)	449	10	50	30	8
20 mM Ca ASW (B)	474	10	20	50	8
ASW for Ca Expts .. 100 mM Ca (C1)	374	10	100	30	8
ASW for Ca Expts .. 50 mM Ca (C2)	374	10	50	105	8
ASW for Ca Expts .. 20 mM Ca (C3)	374	10	20	150	8
ASW for Ca Expts .. 10 mM Ca (C4)	374	10	10	165	8
ASW for Ca Expts .. 0 mM Ca (C5)	374	10	0	180	8

Inhibitors used were: DDT, 1,1,1,-trichloro-2,2-*bis*(*p*-chlorophenyl) ethane of 99 per cent pure, DDE, 1,1-dichloro-2,2-*bis*(*p*-chlorphenyl) ethylene 99 per cent pure, both from Geigy Chemical Corp.; ouabain from Nutritional Biochemicals Corp.; mersalyl from sigma Chemical Co.; hemicholinium-3 from Aldrich Chemical Co.; tetraethylammonium from Eastman Organic Chemicals, *N*-acetyl imidasole from Pierce Chemical Co.; veratrine from Mann Research Laboratories, Inc., tetrodotoxin from Sankyo Co.; and grayanotoxin I from the Central Research Laboratories, Sankyo Co.

Assay method for ATPase in vitro. The nerve bundles were picked up from the artificial sea water, blotted on a filter paper and weighed. They were then transferred into chilled modified Skou's enzyme medium[12,13] containing 725 mM sucrose (isotonic to the artificial sea water), 30 mM imidazole (Nutritional Biochemical Corp.) 1 mM EDTA and 0·1 per cent of deoxycholic acid at pH 8·0. The volume of the medium was adjusted to give 20 mg of neural matter (wet weight) per milliliter of medium. The nerves were first cut into small pieces by means of a pair of fine dissecting scissors and then homogenized in a small Teflon–glass Potter–Elvehjem homogenizer. The homogenate was centrifuged for 10 min at 1000 *g* to remove fibrinous materials and used as the standard enzyme source. For the measurement of ATPase activities, a 0·2-ml aliquot of the above enzyme preparation was pipetted into a 10-ml test tube containing 1·6 ml of the assay buffer. The ionic composition of the standard assay mixture was: 320 mM NaCl, 20 mM of KCl, 3 mM of $MgCl_2$ and 50 mM of tris–HCl at pH 8·0. The inhibitors to be studied were added to the enzyme mixture with 18 μl of ethanol (final ethanol concentration 1 %) or distilled water by using a Hamilton's microsyringe and the system was maintained at 24° for 10 min. The reaction was initiated by the addition of 1 μmole of ATP (disodium salt in most cases, tris-ATP salt for the cases where the presence of Na^+ ions were undesirable) with 0·2 ml of distilled water. The ambient temperature was maintained at 37° throughout the assay

period (30 min). The reaction was stopped by the addition of 2 ml per tube of a modified Fiske–Subarrow's phosphorus assay mixture. Thus developed blue color, because of inorganic phosphorus liberated by ATPases, was measured at 620 mμ against the color blank (see below) by using spectrophotometer (Spec 20, Bausch & Lomb). This method caused some degradation of ATP which was compensated by preparing a color blank which consisted of 1 μmole of ATP in 1·8 ml of the assay buffer. The blank was first incubated similarly for 30 min at 37° and then 2 ml of phosphorus assay mixture and 0·2 ml of the enzyme preparation were added in rapid succession. The system was further incubated for 30 min at 37° for color development as before.

ATPase assay method for intact and sliced nerves. For the experiments with the intact nerves, each nerve from the 2nd walking leg was transversely cut into two pieces (2·5 cm each), and each piece was directly transferred into a 10-ml test tube containing 1·8 ml of artificial sea water in which Ca^{2+} concentration was lowered from 50 mM (standard) to 20 mM to enhance the activity of DDT. DDT with 18 μl of ethanol was added to one tube and 18 μl of plain ethanol was added to the other tube containing the other half of the matching nerve which served as the control. Both tubes were maintained for a specified time period at 24°, after which period the tubes were transferred to a water bath at 37° and 1 μmole of ATP with 0·2 ml of distilled water was added to assay the ATPase activities. After the assay, the nerves were taken out and weighed to obtain the values for the ATPase activity per milligram of neural matter.

To prepare tissue slices, the nerves from various walking legs were directly suspended in the standard ATPase assay mixture at the same weight–volume ratio as the homogenates (20 mg wet wt./ml). With a fine pair of scissors they were cut into small pieces. By using a 2-ml pipette with a broadened tip, 1·8-ml aliquots of the suspension of tissue slices were transferred into reaction tubes. These sample tubes were then handled in exactly identical manner as the homogenate samples for the assessment of ATPase activities.

For centrifugal separation of nerve components, an International Refrigerated Centrifuge (PR-1) with a rotor-269 and a Beckman Spinco (model L-2) wth a rotor-40 were used. The homogenate in a modified Skou's enzyme medium (with 725 mM sucrose) was centrifuged at 1000 *g* for 10 min to obtain the "crude precipitate" fraction. The supernatant fraction was further centrifuged at 100,000 *g* for 3 hr to obtain the "cell membrane" and "supernatant" fractions.

Electrophysiological studies. Propagated action potentials were recorded externally from the isolated nerve and were taken as a measure of nervous activity. Experiments were performed at a room temperature of 22°.

Physiological saline solutions. Several kinds of external solutions were used depending on the purpose of experiments (Table 1). Solution A was used for general purpose such as for isolating and keeping the nerve. Solution B contained less concentration of calcium and was used for many of the DDT experiments. Solutions C1 through C5 contained different concentrations of calcium with a fixed concentration of sodium and were used when the effect of calcium concentration on the DDT action was to be compared.

RESULTS

Characteristic of the ATPases of the lobster nerves. To examine the localization and composition of the ATPases in the lobster nerve preparations, an attempt was made to

first fractionate the nerve homogenates by a centrifugal method and inhibit each nerve fraction with specific inhibitors. The results shown in Table 2 indicate that the lobster

TABLE 2. SUBCELLULAR LOCALIZATION OF ATPASES AND EFFECTS OF INHIBITORS IN LOBSTER LEG NERVES

	Relative ATPase activity	10^{-5} M Ouabain % inhibition	10^{-5} M Mersalyl % inhibition
Supernatant (Sp)	57·7	48·7	100
Cell membrane (CM)	42·2	49·8	85·7
Crude supernatant (Sp + CM)	100*	50·7	93·1
Crude precipitate (Cp)	36·3	9·4	58·8

* ATP, 1·7 nmole, hydrolyzed per milligrams of tissue wet weight equivalent per minute.

nerve homogenates contained ATPases unusually sensitive to mersalyl, and that the "crude supernatant" fraction contained most of the mersalyl-sensitive ATPases. It also appears that approximately 50 per cent of the ATPases in the "crude supernatant" fraction was ouabain sensitive. Examination of the susceptibilities of ATPases in the "crude supernatant" fraction (Fig. 1) revealed that the I_{50} value for mersalyl was of the order of 6×10^{-6} M. It was also shown that the I_{50} value for the ouabain-sensitive ATPase for this inhibitor was about 2×10^{-6} M.

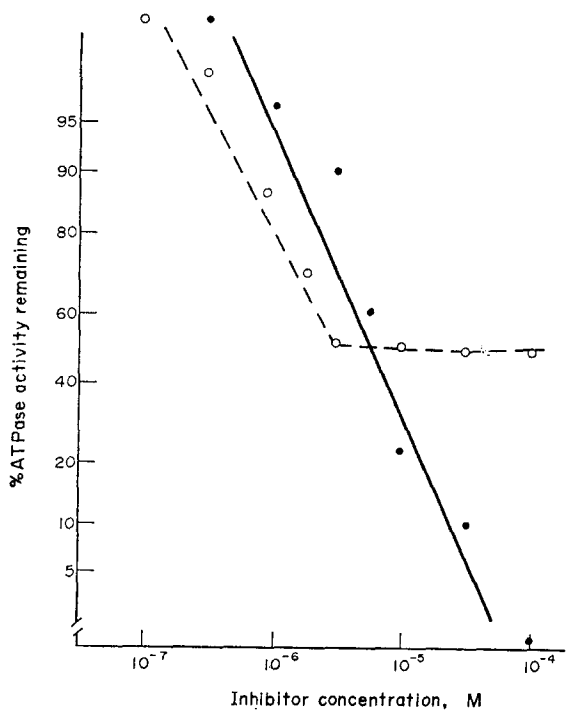

FIG. 1. Inhibition of ATPases of the crude supernatant of the lobster nerve homogenate by ouabain (open circles) and by mersalyl (closed circles). Note that approximately 50 per cent of the total ATPase activity is particularly sensitive to ouabain.

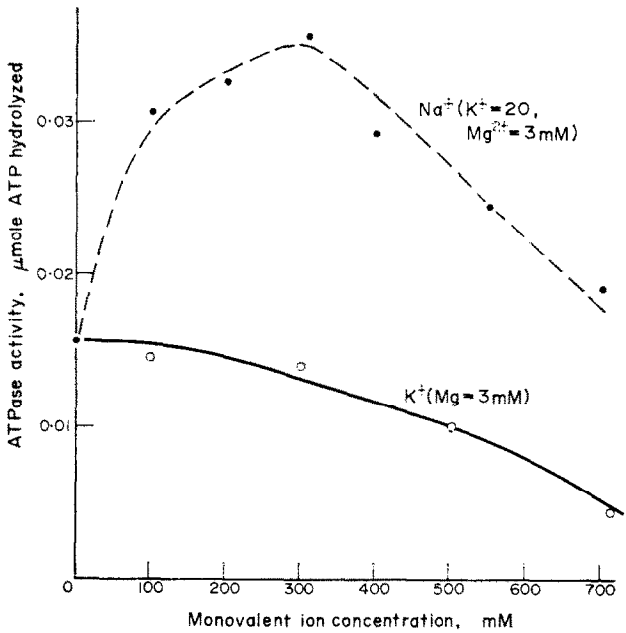

FIG. 2. The effect of the concentration of Na^+ and K^+ ions upon the ATPase activity of the crude supernatant fraction of the lobster peripheral nerves. The closed circles represent the effect of Na^+ with K^+ and Mg^{2+} fixed at a constant concentration of 20 and 3 mM, respectively, and the open circles represent the effect of K^+ with Mg^{2+} fixed at a constant concentration of 3 mM. pH was 8·0 for both cases.

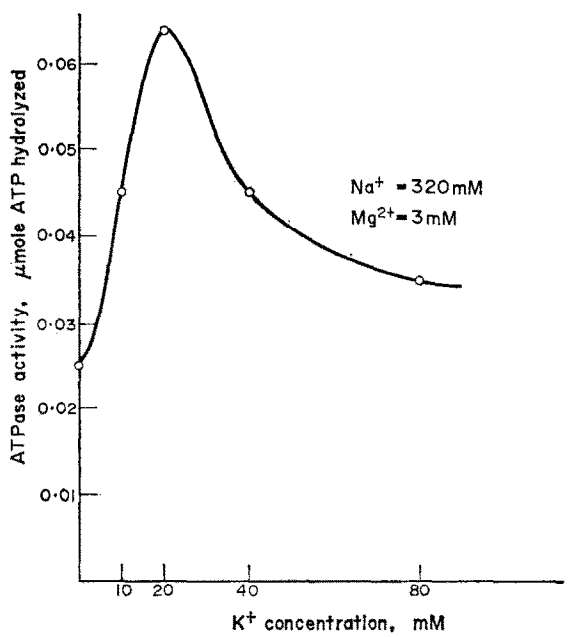

FIG. 3. The effect of the concentration of K^+ ion upon the ATPase activity of the crude supernatant fraction. In this experiment the concentration of Na^+ and Mg^{2+} are fixed at 320 mM and 3 mM, respectively, at pH 8·0.

Skou's recent work[12,13] has demonstrated the presence of a relatively pure Na^+-K^+-Mg^{2+} ATPase in the nerve preparation from a European crab species. The data obtained here suggest that the "crude supernatant" fraction from the lobster nerves contains at least a group of ATPases, i.e. Na^+-K^+-Mg^{2+} ATPase (approximately 50 per cent of the total ATPase activity) which is sensitive to ouabain and mersalyl at a concentration of 10^{-4} M. The crude supernatant fraction was, therefore, chosen for all subsequent experiments.

Attempts were then made to characterize the ion and pH specificity of the "soluble" fraction from the lobster nerve homogenate preparations. Figure 2 shows the influence of monovalent cation upon the overall activity of the ATPases in the homogenate preparation. Both Na^+ and K^+ have optimum concentrations beyond which the ATPase activities decrease. In addition, the concentration of K^+, at a fixed Na^+ concentration, also appeared to influence the total ATPase activity (Fig. 3). At a fixed Na^+-K^+ level, the effects of divalent cations (both Ca^{2+} and Mg^{2+}) were more drastic than that of monovalent cations (Table 3). Examination of pH specificity revealed that there is a broad pH range (7·6–8·2) where ATPase activities are at a maximum (Fig. 4). Since the major purpose of this work is to compare the electrophysiological responses of the nerves with the ATPase activity, the conditions closest to the composition of the artificial sea water within the range of reasonable ATPase activity were selected as the standard ATPase assay buffer, i.e. 320 mM Na^+, 20 mM K^+ and 3 mM Mg^{2+} at pH 8.

Effect of DDT and DDE on the ATPase activity in the nerve homogenate. As shown in Table 3, the degree of DDT inhibition is greatly influenced by the external ionic

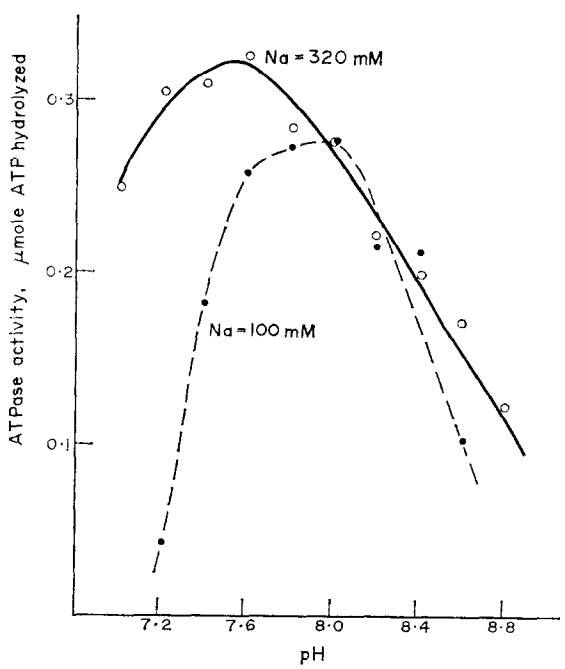

FIG. 4. The effect of pH upon the ATPase activity of the crude supernatant fraction at two different Na^+ concentrations. K^+ and Mg^{2+} concentrations are 20 and 3 mM respectively.

TABLE 3. EFFECTS OF VARIOUS IONIC COMPOSITIONS AND INHIBITORS UPON THE ATPASES OF THE LOBSTER LEG NERVES AS GIVEN BY THE PERCENTAGE OF THE ATPASE ACTIVITY RELATIVE TO THAT IN THE STANDARD ASSAY BUFFER

Ionic compositions	Control	Plus DDT (10^{-5} M)	(Per cent inhibition)	Plus DDE (10^{-5} M)	(Per cent inhibition)
Na (320) K (20) Mg (3)	100	38·8	(61·2)	54·9	(45·1)
Na (320) K (0) Mg (3)	39·1	28·0	(28·4)	32·8	(16·0)
Na (320) K (20) Mg (0)	15·2	2·5	(83·6)	0	(100)
Na (320) K (20) Mg (1)	116·0	29·6	(74·5)	47·5	(59·0)
Na (320) K (20) Ca (1)	25·0	18·1	(27·6)	25·8	(0)
Na (0) K (100) Mg (3)	41·5	19·5	(53·0)	24·1	(40·9)
Na (0) K (300) Mg (3)	39·0	17·4	(55·4)	18·5	(52·5)
Na (0) K (0) Mg (3)	43·4	30·8	(29·0)	35·6	(17·8)
	Ouabain $2·5 \times 10^{-4}$ M				
Na (320) K (20) Mg (3)	57·5	24·0	(58·3)	31·8	(44·7)
	Mersalyl 2×10^{-5} M				
Na (320) K (20) Mg (3)	13·9	2·9	(79·2)	0	(100)

compositions. However, it appears from the table that the per cent of DDT inhibition is highest at the optimum condition for Na^+-K^+-Mg^{2+} ATPases. To determine the I_{50} values for DDT and DDE inhibition, the enzyme in the standard assay buffer was incubated with various concentrations of DDT and DDE. Figure 5 illustrates the

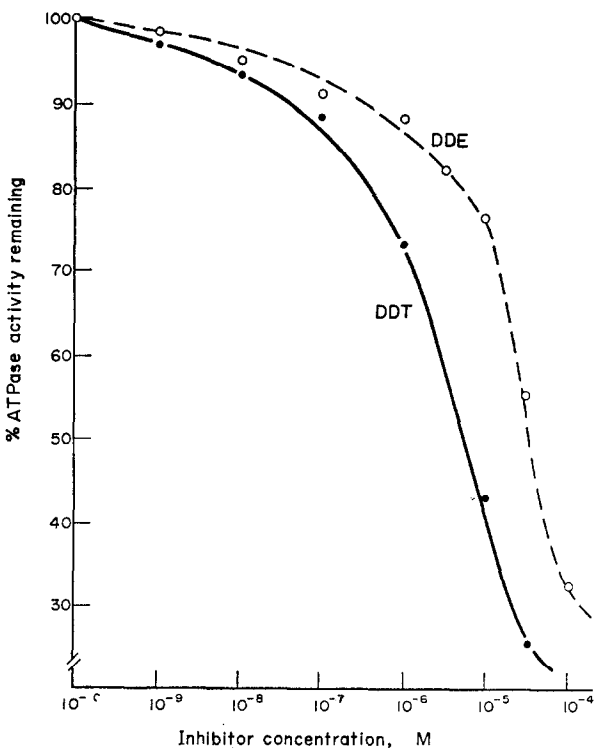

FIG. 5. The relationship between the concentration of DDT or DDE in the external assay medium and the ATPase activity in the crude supernatant fraction at 37°.

concentration–inhibition relationships for DDT and DDE at 37°. The I_{50} values for DDT (3×10^{-6} M) are approximately in the same order of magnitude as those necessary to cause electrophysiological effects on the nerve.

Effects of DDT and DDE on the electrical activity. Propagated action potentials recorded from the isolated leg nerve are composed of at least two components; the initial spikes of short duration are followed by a larger and slower depolarization. An example of the records is shown in Fig. 6A. The initial spikes are the responses of large nerve fibers with faster conduction velocity, whereas the slow component is the response of smaller nerve fibers with slower conduction velocity.

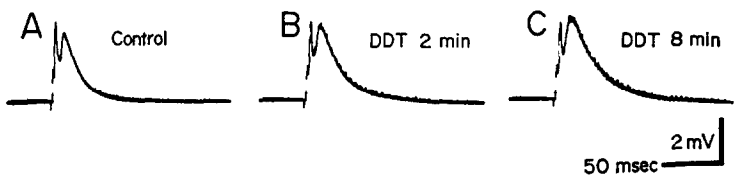

FIG. 6. The effect of 10^{-5} M DDT on the action potential produced by a single stimulus in a lobster leg nerve. The standard artificial sea water containing 50 mM Ca^{2+} is used as the bathing medium. A: control; B: 2 min after application of DDT at 22°; C: 8 min after application.

Application of DDT at a concentration of 10^{-5} M prolonged the falling phase of the compound action potential (or increased and prolonged the negative after-potential) and initiated after-discharges. When the standard artificial sea water (solution A in Table 1) containing 50 mM Ca was used, these symptoms of poisoning started appearing within 2–3 min (Fig. 6B) and increased in intensity with time (Fig. 6C) and reached a maximum within 20–30 min. After about 60 min, these effects were still observable, but in some preparations the magnitude of the action potential decreased. This is probably because of deterioration which also occurs in normal unpoisoned nerves.

Repetitive discharges caused by application of DDT have been found to be related to calcium concentration, lowering the calcium intensifying the repetitive discharges.[14,15]

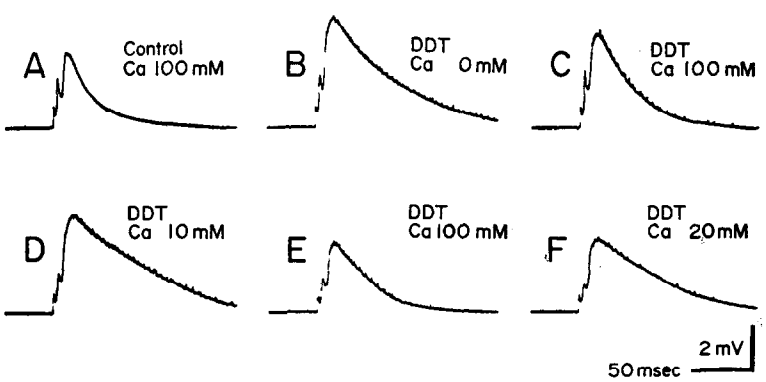

FIG. 7. The effect of changing Ca^{2+} concentration on the DDT-induced (10^{-5} M) prolonged action potential in a lobster leg nerve. The concentration of Ca^{2+} is changed while maintaining the Na^{+} concentration constant (artificial sea water C1, C3, C4 and C5 in Table 1). Records B, C, D, E and F were taken 3, 7, 9, 13 and 15 min after application of DDT respectively. Note that the effect of DDT is enhanced by lowering the Ca^{2+} concentration.

In the present study, both the increase in negative after-potential and the repetitive after-discharges were augmented by lowering the calcium concentration. An example of such experiments is illustrated in Fig. 7. The effect of calcium was reversible unless the nerve preparation was left exposed to Ca-free medium for a long period of time, so that the experiments such as shown in Fig. 7 could be repeated many times with the same preparation. The DDT-poisoned nerve very often fired spontaneously in relatively low calcium concentrations (0–20 mM).

Because of intense DDT action at low calcium concentrations, an artificial sea water containing 20 mM Ca was chosen for the study on the relationship between the nervous symptoms of poisoning and the ATPase activity. Calcium lower than 20 mM was more effective to intensify the DDT action, but the nerve deteriorated more quickly.

Application of DDE at a concentration of 10^{-5} M had little or no effect on the action potential. In some preparations, the falling phase of the action potential was slightly prolonged, but after-discharges were never initiated.

ATPase activity in the DDT-poisoned nerves. The nerves at various stages of DDT-poisoning were homogenized in 2 ml of the standard assay buffer and the ATPase activities were measured. It was found that in all experiments (0 to up to 60 min of

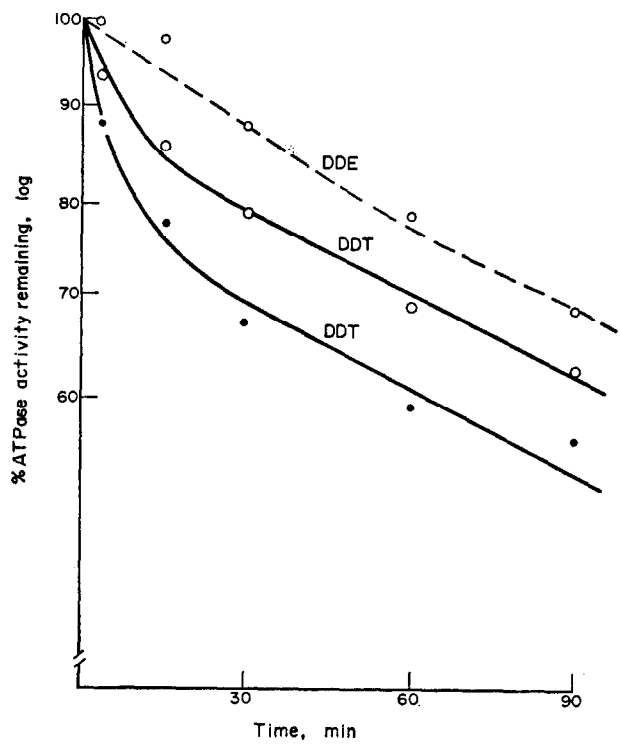

FIG. 8. The effect of externally applied DDT or DDE (both 10^{-5} M, final concentration) upon the ATPase activity of the intact and sliced nerves in the artificial sea water containing 20 mM Ca^{2+} (solution B of Table 1). The middle curve (semi-closed circles) represents the effect of DDT on the intact nerves in the artificial sea water (B); also, identical results were obtained by using the standard assay mixture for ATPases. The bottom curve (closed circles) represents the effect of DDT on the sliced nerve preparations in the standard assay buffer. Temperature 24°.

poisoning) the ATPase activity of the homogenate from the treated nerves was no lower than that of the untreated controls. To study the possibility that only a small portion of the ATPase which is located at certain vital sites on the membrane is affected by DDT *in situ*, the intact nerves at various poisoning stages were directly assayed (without homogenization) for their ATPase levels by using the artificial sea water containing 20 mM Ca (solution B of Table 1). The results shown in Fig. 8 show that ATPase activity in the intact nerves was moderately affected by DDT (semi-closed circles). An almost identical level of inhibition was also observed when the nerves were assayed for the ATPase activity in the standard assay buffer. The effect of DDT was highest when the nerves were sliced to small pieces and tested for the ATPase activity in the standard assay buffer (closed circles of Fig. 8). On the other hand, the degree of DDE inhibition appeared to be less marked (open circles of Fig. 8).

ATPase and electrical activity in the nerve poisoned with other pharmacological agents. To further study the relationship between the nervous symptoms of poisoning and the inhibition of ATPases, several chemicals of pharmacological interest were tested. The results of present experiments on the ATPase and electrical activities, together with some data collected from the literature, are summarized in Table 4.

TABLE 4. EFFECTS OF VARIOUS PHARMACOLOGICAL AGENTS ON THE ELECTRICAL AND ATPASE ACTIVITIES OF NERVE FIBERS

Chemicals	ATPase activity	Electrical activity			
		Minimum effective concn*			
	I_{50} *in vitro*	Externally	Internally†	Action potential‡	Ionic conductances inhibited§
DDT	3×10^{-6} M	3×10^{-6} M		NAP, RD	$g_{Na}\downarrow$, $g_K\uparrow$[2,3]
DDE	3×10^{-5} M	1×10^{-5} M		No effect	
Hemicholinium-3	5×10^{-5} M	1×10^{-2} M‖	1×10^{-3} M‖	Block[16]	$g_{Na}\uparrow$, $g_K\uparrow$[16]
Tetraethylammonium	1×10^{-3} M	3×10^{-1} M‖	6×10^{-4} M‖	NAP[17,18]	$g_k\uparrow$[17,18]
N-acetyl imidazole	5×10^{-5} M	5×10^{-4} M¶		NAP, RD**	
Veratrine	1×10^{-4} g/ml	$2{\cdot}4 \times 10^{-5}$ g/ml‖		NAP, RD[19–23]	
Tetrodotoxin	$>2 \times 10^{-4}$ M	1×10^{-8} M‖	$>1 \times 10^{-5}$ M‖	Block[24,25]	$g_{Na}\uparrow$[24,25]
Grayanotoxin I	$>1{\cdot}2 \times 10^{-3}$ M	$2{\cdot}4 \times 10^{-4}$ M¶		NAP, RD[26]	None[26]

* The lowest concentration range that causes the definite pharmacological effects on the electrical activity of nerve.

† Data with internally perfused squid giant axons.

‡ NAP, negative after-potential is increased and prolonged; RD, repetitive discharges are produced.

§ $g_{Na}\uparrow$, the mechanism by which the membrane sodium conductance is increased upon depolarization;
$g_{Na}\downarrow$, the mechanism by which the membrane sodium conductance is decreased during depolarization.
$g_K\uparrow$, the mechanism by which the membrane potassium conductance is increased upon depolarization.

‖ Data with squid giant axons.

¶ Data with crayfish giant axons.

** W. R. Kem, personal communication.

DDT has been demonstrated to affect the ionic conductance of the lobster nerve membrane.[2,3] The major effects are the slowing of the sodium inactivation and the suppression of the potassium conductance increase. These changes in conductances

can account for the prolongation of the action potential and the latter initiates repetitive after-discharges.

The ATPase activity was effectively inhibited by hemicholinium-3 (HC-3) with an I_{50} value of 5×10^{-5} M. When applied to the inside of the squid giant axon, HC-3 is able to block the action potential by the inhibition of the mechanisms, whereby the membrane sodium and potassium conductances are increased upon depolarization.[16]

Tetraethylammonium (TEA) also inhibited ATPases. It affects the action potential of the squid giant axon only from inside the membrane.[17,18] The falling phase of the action potential is greatly prolonged forming a plateau like cardiac action potentials, and the mechanism by which the membrane potassium conductance is increased upon depolarization is inhibited.

N-acetyl imidazole was able to inhibit ATPases fairly effectively. In crayfish giant axons, it prolongs the falling phase of the action potential and initiates repetitive after-discharges by a single stimulus.* Although no data on ionic conductances are available, it seems quite possible that the sodium inactivation, the potassium conductance increase, or both are affected by this chemical.

The ATPase activity was also blocked by veratrine. Veratrine has long been known to prolong the falling phase of the action potential and produces repetitive discharges. However, the ionic mechanism of veratrine remains to be studied.

Tetrodotoxin (TTX) was without effect on the ATPase activity. This toxin is one of the most effective nerve blocking agents and inhibits the mechanism of sodium conductance increase without affecting other conductance mechanisms.[24] However, it is totally ineffective when applied inside of the membrane.[25]

Grayanotoxin I (GTX) had no effect on the ATPase activity. Although it produces large negative after-potentials and repetitive after-discharges, the conductance mechanisms including Na increase, Na inactivation and K increase are not affected.[26] However, with repetitive depolarizing pulses a change in sodium conductance can be detected.

DISCUSSION

The ionic and pH requirements of Na^+-K^+-Mg^{2+} activated ATPases in the lobster peripheral nerves indicate that the system(s) requires the intermediate conditions between the external and internal phases. The standard artificial sea water contains 450 mM Na^+, 10 mM K^+ and 50 mM Ca^{2+} at pH 8·0, and the normal internal phase (axoplasm) contains 50 mM Na^+, 400 mM K^+, a trace of Ca^{2+} and 10 mM Mg^{2+} at pH 7·35. The optimum conditions for the nerve ATPases are 300 mM Na^+, 20 mM K^+, and 1 mM Mg^{2+} at pH 7·6–7·8. Under these conditions, if the Na-K-Mg ATPases are located near the internal surface of the nerve membrane which is exposed to the axoplasmic ionic environment, they can be activated upon excitation because Na^+ ions flow inward across the membrane causing a transient increase in local Na concentration near the internal membrane surface and K^+ ions flow outward causing a transient decrease in local K^+ concentration there.

The most crucial question here is whether the DDT-induced membrane conductance changes are causally related to the phenomenon of ATPase inhibition *in vitro*

* W. R. Kem, personal communication.

by DDT. This point is worthy of consideration because, if the action of DDT is solely upon the ATPase for active transport, the end result of DDT action *in vivo* should merely be a slow blocking of general nerve activity, such as the one caused by ouabain. Careful examination of the data shown in Table 3 clearly indicates, however, that the DDT-sensitive ATPase(s) cannot be identical to the ouabain-sensitive Na^+-K^+-Mg^{2+} ATPase, i.e. even in the presence of $2{\cdot}5 \times 10^{-4}$ M ouabain, the per cent inhibition of the ATPases by DDT and DDE (58 and 45 per cent respectively) did not significantly vary from the values obtained in the absence of ouabain (61 and 45 per cent, respectively). At least a certain portion of the DDT-sensitive ATPases present in this system, therefore, should be serving for some other function than the active transport of ions. In this connection, however, it is of interest to see in Table 4 that many of the compounds capable of inhibiting the ATPases affect the mechanism, whereby membrane sodium conductance is inactivated ($g_{Na}\downarrow$), or the mechanism whereby membrane potassium conductance is, increased ($g_K\uparrow$), or both (DDT, HC-3 and TEA). The other two ATPase inhibitors (*N*-acetyl imidazole and veratrine) produce large negative after-potential and repetitive after-discharges which are most likely because of the inhibition of Na inactivation, K activation, or both. Those which do not affect either of these two mechanisms do not inhibit the ATPases (TTX, GTX). Although the data presented in Table 4 do not demonstrate the causal relationship (e.g. HC-3 is a much more potent ATPase inhibitor than is expected by its neurotoxicity), they leave the possibility that the ATPases are somehow related to the two conductance mechanisms.

Another possible explanation is that the systems involved in such changes of conductance in the axonic membrane do possess very similar properties as ATPases studied here. In this case, the ATPase inhibition by DDT should be regarded as merely representing an analogous situation to what actually occurs during the process of DDT inhibition in the nerve membrane.

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