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SOMAN AND RECEPTOR–LIGAND INTERACTION IN *ELECTROPHORUS* ELECTROPLAQUES

JAMES O. BULLOCK, DONALD A. FARQUHARSON and FRANCIS C. G. HOSKIN
Biology Department, Illinois Institute of Technology Chicago, IL 60616, U.S.A.

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Abstract—Soman, an organophosphorus cholinesterase inhibitor, interacts irreversibly with both the enzyme acetylcholinesterase and a site similar or identical to the acetylcholine receptor of the intact *Electrophorus electricus* electroplaque. The Soman-induced inhibition of esterase activity in intact electroplaque is not reversed by washing in inhibitor-free saline; indeed, the inhibition increases. This is in contrast to the slow recovery, with washing, of esterase activity in DFP- and Paraoxon-treated cells. Soman also affected the electrical and chemical excitability of the electroplaque-innervated membrane. Application of Soman to the electroplaque usually resulted in a two-phase depolarization. Coincident with the large, rapid, Soman-induced depolarization, the electrically excitable sodium ion current of the conducting membrane was completely inhibited. Dimethyl *d*-tubocurarine, an inhibitor of chemical excitation at the post-synaptic membrane, prevented but could not reverse the effects of Soman on the excitability of the innervated membrane. The rapid "aging" of the Soman–protein complex is discussed in connection with its effects on the electroplaque.

It is well known that organophosphate-inhibited cholinesterases may be reactivated by nucleophilic agents, including water and oximes, provided that the dialklyphosphorylated enzyme (or comparable structure if, for example, a phosphonate inhibitor is used) has not "aged" to the corresponding monoalkly form [1]. It has also been noted that single electroplaque cells of *Electrophorus electricus* treated with di-isopropylphosphorofluoridate (DFP) and similar organophosphates, on subsequent washing, lose their increased sensitivity to acetylcholine (ACh) in a manner paralleling the reactivation of the acetylcholinesterase activity in the same preparation [2]. These observations and conclusions fit the general concept of a role for the ACh system in synaptic transmission. On the other hand, a possible general role for ACh in conduction along excitable membranes [3] either is not supported by a sufficient body of evidence, or appears to be incompatible with some of the evidence available [4,5]. In the course of investigating these seeming contradictions, concentrations of inhibitors several orders of magnitude greater than normally required to cause inhibition of acetylcholinesterase activity in solution have been applied to nerve and muscle cells. While the observations from such experiments are seldom in dispute, the conclusions frequently are. Nevertheless, as a result of such experiments some unexpected observations have been recorded which do not appear to be directly related to the inhibition of acetylcholinesterase activity. For example, when squid axons are bathed in 10^{-3} M *O,O*-di-isopropyl *S*-(2-di-isopropylaminoethyl) phosphorothioate (Tetriso), there are rather small and readily reversible changes in action potential spike height, and a steady deterioration of the axon response over the experimental period [6]. At the remarkably high concentration of 10^{-2} M, ethyl *N,N*-dimethylphosphor-amidocyanidate (Tabun) causes a slight decrease in squid axon action potential spike height and a 10-fold increase in the threshold necessary to evoke the spike [7]. There is evidence that the organophosphates, particularly DFP, diethyl *p*-nitrophenyl phosphate (Paraoxon) and *O,O*-diethyl *S*-(2-dimethylaminoethyl) phosphorothioate (Phospholine, 217A0), interact with the ACh-receptor (AChR) in *Electrophorus* electroplaques [8]. In a somewhat different context, the phosphorylation of a certain inessential esterase ("neurotoxic esterase") is thought to be the explanation for the degeneration of axons caused by organophosphates [9]. And in a quite different system, DFP exhibits detergent-like activity on rabbit leucocytes [10]. In all of these reports, the rather slow rate of dealkylation of a protein-organophosphate, i.e. "aging," is either a factor which complicates the observations or a factor on which the observations depend.

1,2,2-Trimethylpropyl methylphosphonofluoridate (Soman) is an extremely potent inhibitor of acetylcholinesterase activity which "ages" by the loss of the trimethylpropoxy group from the phosphorylated esterase at a rate too rapid to measure by usual means [1], thus eliminating one variable. It is also known to have effects on biological systems not solely attributable to the inhibition of acetylcholinesterase activity, as for example the block of frog nerve conduction at 1.5×10^{-2} M, and reversal on washing [11], the interference with respiration of tissue slices and inhibition of some of the enzymes of intermediary metabolism at 5×10^{-4} to 10^{-3} M [12], and degenerative changes in motor end-plate regions after intraperitonal administration [13]. And finally it would be predicted (although the experiments have not been done and might be difficult to do) that Soman would bind to the active site of the so-called "neurotoxic esterase" and subsequently elicit nerve degeneration by virtue of its very rapid dealkylation [9].

Because of this body of inconclusive but lingering and suggestive evidence of additional effects of organophosphorus cholinesterase inhibitors, we have considered it worthwhile to attempt to examine the

underlying basis for these phenomena. To do this we have combined the use of a specific chemical probe, Soman, a sensitive physical method, the voltage clamp, and a biological preparation which permits the examination of several aspects of membrane function, the single electroplaque cell of *Electrophorus electricus* [14–16].

MATERIALS AND METHODS

Preparation of single cells (electroplaques). Electric eels (*Electrophorus electricus*) were purchased from World Wide Scientific Animals, Ardsley, N.Y., and were held at the Shedd Aquarium, Chicago,, Ill. before a final transfer to this laboratory. The relatively gross dissection of rows of electroplaques from the Organ of Sachs, and the finer dissection of single electroplaques free from adjoining cells, connective tissue, and almost all extracellular material except for adhering nerve endings are essentially the procedure described by Schoffeniels [14] and by Higman and Bartels [15].

Enzyme determination. It is assumed that the activity of a number of different esterases is being measured when the ACh-splitting activity of whole electroplaques is being followed. However, the esterase activity of cleanly dissected electroplaque should represent mostly the activity of the enzyme EC 3.1.1.6, acetylcholinesterase (AChE) [17]. AChE activity was measured by the colorimeter method of Ellman *et al.* [18]. For our purposes, intact electroplaques were incubated with AChE inhibitors for 30 min, rinsed briefly, and thereafter washed in a volume of 100 ml/electroplaque of inhibitor-free saline solution for varying periods of time. The electroplaques were then placed in 3 ml saline containing the substrate, acetylthiocholine (ATC) at a concentration of 5×10^{-4} M, and the reagents of the color reaction. The hydrolysis of this substrate by intact electroplaques was then followed for 15 min.

Mounting of electroplaques and electrical recording. A thin plastic sheet with a "window" cut in its center was pressed against the innervated face of a single electroplaque. This allowed 0.75 mm^2 of the cell surface to be exposed to a perfusion medium passing through an adjacent 0.05-ml chamber at a rate of 0.5 to 1.0 ml min^{-1} [19]. The electroplaque was impaled through its non-innervated membrane with a glass microelectrode filled with 3 M KCl, and having a resistance of 5–15 megohms. Membrane conductance was measured by means of a voltage clamp. The voltage across the innervated membrane was again measured with a KCl-filled glass microelectrode positioned inside the cell and a 3 M KCl agar bridge in contact with the perfusion chambers outside the cell. Short lengths of platinized platinum wire on either side of the electroplaque were used as the electrodes for supplying and measuring current. The series resistances between the two sets of electrodes were compensated for by separate feedback circuits [20]. The series resistance between the two voltage electrodes was estimated by observing the size of the sharp voltage step that accompanies the slower voltage response of the parallel capacitance and resistance components during a square current pulse. The series resistance between the current electrodes, but outside the potential sensing electrodes, was compensated for by feeding back that portion of the total current necessary to minimize the rise time of a critically damped response. The rise time of the clamp was approximately 35 μsec.

Reagents and solutions. ACh bromide and ATC bromide were purchased from Sigma Chemical Co. Dimethyl *d*-tubocurarine iodide was a gift from Eli Lilly & Co. DFP was purchased from Aldrich Chemical Co. Paraoxon was a gift from Dr. Robert A. Neal of Vanderbilt University. Soman was synthesized in this laboratory [7].

Dissection of the electroplaque cells and the experiments themselves were performed in a saline solution, having the following composition and mM concentrations: NaCl, 188; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; NaH_2PO_4, 0.15; Na_2HPO_4, 1.45; glucose, 5 [21]. The pH was 7.4. Substrates and inhibitors were dissolved in this medium.

RESULTS

Inhibition of AChE. In the absence of AChE inhibitors, cleanly dissected intact electroplaques hydrolyzed ATC at the rate of 285 ± 96 nmoles hr^{-1} $cell^{-1}$ (mean of 28 cells ± standard deviation). This is lower than the previously reported values of 680 ± 390 nmoles hr^{-1} $cell^{-1}$ (mean ± standard deviation) using ACh as the substrate [22]. The smaller standard deviation for the lower value suggests a more thorough removal of connective tissue and adjoining cell debris, but factors such as seasonal variation, substrate and differences in the color reactions used may also be involved. Figure 1 shows the recovery of AChE activity at the end of 30-, 60- and 90-min washing periods after a 30-min incubation of electroplaques with 3×10^{-4} M DFP, Paraoxon or Soman. As previously reported [2], the most rapid rate of recovery was found in Paraoxon-treated cells; the rate for DFP-treated cells was much less. In marked contrast, the Soman-treated cells continued to lose activity during the washing period. Although all three inhibitors were present at concentrations at least four

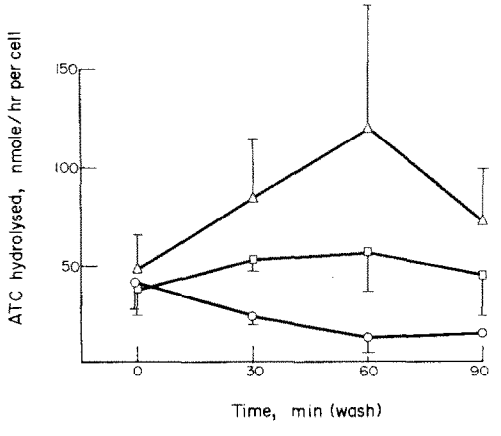

Fig. 1. Time course of inhibited electroplaque AChE reactivation. Each point is the mean of five determinations and the vertical bars represent the standard deviation where they are larger than the symbol. Paraoxon-inhibited AChE activity is represented by triangles, DFP-inhibited activity by squares and Soman-induced activity by circles.

orders of magnitude greater than would normally cause complete inhibition of purified AChE in solution (>99.9 per cent), only about 85 per cent was inhibited in the intact cells in the first 30 min of exposure to inhibitor. Similar residual activity was observed in a previous study [2], in which organophosphates were used to inhibit whole electroplaque AChE activity. It seems likely that this remaining activity results from a sparing effect due to permeability barriers which shield some fraction of the AChE activity from contact with the inhibitors (for instance see Refs. 17 and 23). This remaining activity certainly represents real hydrolysis by the enzyme since, as can be seen in Fig. 1, Soman continues to act during the wash and inhibits part of the residual activity. Also, the residual activity occurs regardless of which inhibitor is used so that it is not the result of any reactivation before zero time.

The data were subjected to linear regression analysis with respect to the changes of activity during the wash periods, and to analysis with respect to the sources of variance between inhibitors and with respect to the portion of the Organ of Sachs from which electroplaques were dissected. In summary, there is a significant difference in enzyme activity during the wash period between the treatment groups, and this difference was not influenced by any variation imposed by the anatomical source of the electroplaque cells. In addition, the analysis indicated that through the first 60 min of washing the recovery of the DFP- and Paraoxon-treated cells had significant linear components. In contrast, the Soman-treated cells showed a significant linear rate of increasing inhibition during the same period. The reason that a significant linear regression of enzyme activity on the wash time does not occur when the analysis is extended to 90 min for the DFP- and treatment groups is apparently due to a process unrelated to enzyme inhibition or reactivation. This process may be a loss of enzyme activity due to the washing process itself. Cells which were not treated with inhibitor also lost some small amount of activity after 60 min of washing. However, we feel that the conclusions drawn from Fig. 1 remain valid. These conclusions are: (1) that for DFP- and Paraoxon-treatment groups there is a significant (5 per cent level) linear regression of enzyme activity on wash time (for 0–60 min) and that this regression line has a positive slope (re-activation); (2) that the Soman treatment group also has a significant linear regression (5 per cent level) of enzyme activity on wash time (for 0–90 min) and this regression line has a negative slope (inactivation).

Effects on membrane potential. Although normally 5×10^{-6} M ACh has no effect on the electroplaque resting potential, in the presence of the reversible AChE inhibitor physostigmine, this same concentration of ACh produces a reversible depolarization [24]. If the electroplaque is first pretreated with the irreversible inhibitor DFP, and the DFP is then removed, the same concentration of ACh again produces a response similar to that seen with physostigmine. Again each depolarization is reversed on removal of ACh. In addition, however, the magnitude of each succeeding depolarization produced decreases with time as the cell is washed with inhibitor-free saline [2]. We have repeated these DFP experiments with similar results, as shown in Fig. 2. Again, although preincubation in 3×10^{-4} M DFP did not affect the resting potential, subsequent application of 5×10^{-6} M ACh after removal of the DFP caused a depolarization of approximately 40 mV. Upon return to ACh- and DFP-free saline, the electroplaque repolarized to its original resting potential [8]. Each succeeding application of ACh evoked a smaller response until, after about 35 min the magnitude of the depolarizations was about half of the initial value. Reapplication of DFP resulted in the restoration of the ACh-obtained depolarization to its initial magnitude.

Figure 2 also shows spontaneous depolarization and repolarization of an electroplaque caused apparently (although not directly) by the prolonged treatment with AChE inhibitor and ACh. This phenomenon has previously been reported for electroplaques bathed in organophosphates, in ambenonium, and in physostigmine [8,24]. In the present study, as in the past, these effects were not always seen.

Thus far, the results with DFP are a confirmation of previous findings, and an indication that our experimental methods are comparable to those used previously. Now in contrast to this, Fig. 3 shows typical effects of preincubation with Soman on eel electroplaques. Again, the application of 5×10^{-6} M ACh produced a depolarization, but in contrast to the DFP-treated electroplaques the reversal of the depolarization on removal of ACh was incomplete, whereas succeeding application of ACh now produced depolarizations to the same voltage as the first one. After the third ACh application, the electroplaque depolarized irreversibly; in other experiments it was never possible to apply ACh more than three times without a similar slow irreversible depolarization occurring. This effect was sometimes observed after one or two ACh applications, and occasionally after as little as a 10-min exposure to Soman before any ACh had been applied. Finally, with the Soman-treated electroplaques the recovery from ACh-induced depolarization was slower than with DFP, and the brief spontaneous depolarizations and repolarizations seen with DFP did not occur.

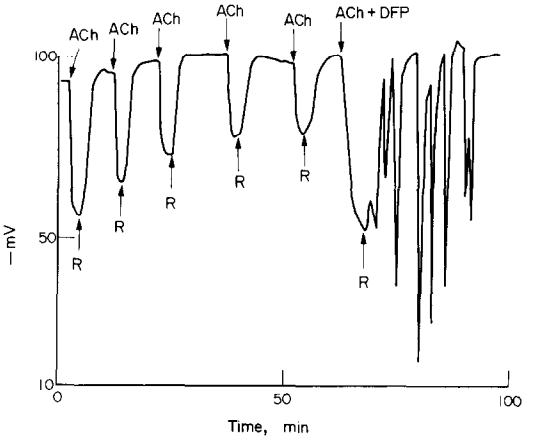

Fig. 2. ACh-induced depolarizations in the electroplaque preincubated in 3×10^{-4} M DFP for 30 min. The ACh concentration was 5×10^{-6} M. The depolarizations and recoveries seen after 70 min occurred spontaneously. R indicates eel saline.

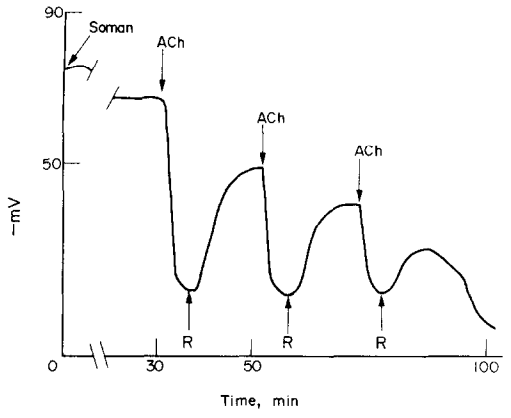

Fig. 3. ACh-induced depolarizations in an electroplaque preincubated in 3×10^{-4} M Soman for 30 min. The ACh concentration was 5×10^{-6} M. Note that Soman alone caused some slight depolarization before ACh was applied and that the cell depolarized irreversibly after partial recovery from the final ACh-induced depolarization. R indicates eel saline.

When the effect of Soman by itself was examined more closely it was found, from nine electroplaques, that application of 3×10^{-4} M Soman resulted in a slow depolarization of 5–10 mV over a period of 17 ± 2 (mean $\pm$ standard error) min followed by a sharp irreversible depolarization of 47 ± 5 (mean $\pm$ standard error) mV. A typical experiment is shown in Fig. 5a. The sharp depolarization coincided with a change in the excitability of the conducting membrane, as well as an irreversible change in the conductance of the membrane.

Exploration of effects by voltage clamp. To examine these effects in still more detail, electroplaques were periodically stepped to various voltages and held there briefly while the current response was recorded. This is the well-known technique of the voltage clamp, described briefly in Materials and Methods. The electronic equipment was so arranged that the membrane remained in current clamp condition (at the cell's zero current potential) except for 18-msec periods during which the electroplaque was voltage clamped. A series of such voltage steps, executed at various times during the Soman experiments, allowed the current–voltage relationship of the innervated membrane to be found. Thus, the conductance of the membrane was determined before Soman application and shortly after the rapid irreversible depolarization caused by the Soman application. This is seen in Fig. 4a. During the occurrence of the depolarization itself, we observed the current flow when the membrane was clamped repeatedly to a potential of 0 mV. This is seen in Fig. 4b. It is evident in Fig. 4b that the decline in the sodium current transient has a different time course from either the zero current potential decline or the changes in the steady state current. The interpretation of the current–voltage plot is based mainly on the work of Grundfest *et al.* [16,25], a brief summary of which is included here. In the untreated electroplaque, the resting potential is taken to be the potassium ion equilibrium potential. An extrapolation of the steady state constant conductance seen over the range of depolarizing potassium inactivation back to the potassium ion equilibrium potential allows the leak conductance to be obtained. The conductance at the peak inward current, over a range of sufficiently positive voltages, is assumed to be made up of the sodium conductance plus the

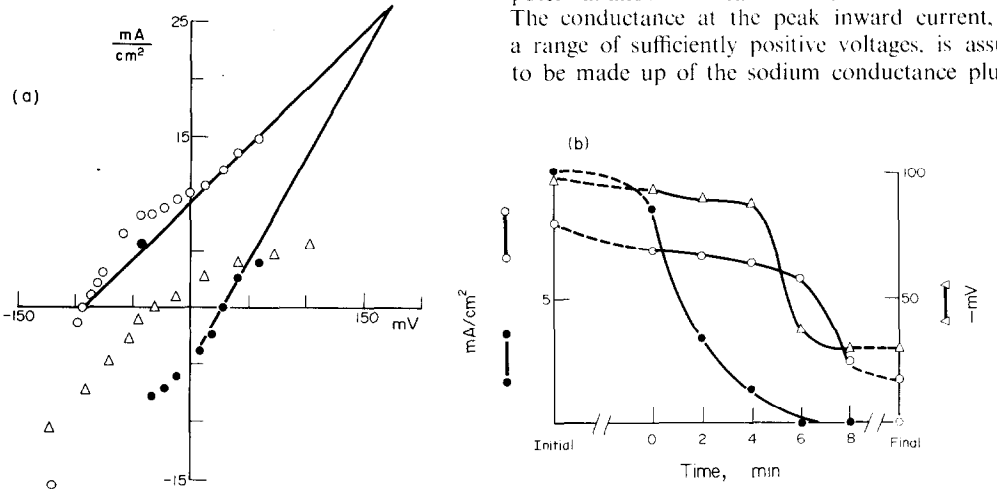

Fig. 4. Soman-induced changes in the ionic currents and the conductance of the innervated membrane. (a) Current measurements obtained using the voltage clamp, before Soman application (the filled and unfilled circles) and after the Soman-induced rapid depolarization (unfilled triangles). The unfilled circles and triangles represent the total ionic current at the end of an 18-msec pulse (steady state current). The filled circles represent the total ionic current at the peak of the sodium ion transient (at approximately 1 msec after the start of an above-threshold pulse). (b) The steady state (unfilled circles) and peak sodium ion (filled circles) currents at zero membrane potential and the corresponding innervated membrane resting potential (triangles) before, during and after the Soman-induced rapid depolarization. "Initial" refers to the measurements taken shortly after Soman was first applied (approximately 15 min before zero time). "Final" refers to values taken at the same time as the final depolarization phase in Fig. 4a. The values in between represent a sampling of the three parameters at 2-min intervals taken during the actual Soman-induced rapid depolarization. The measurements in 4a and 4b are from the same cell.

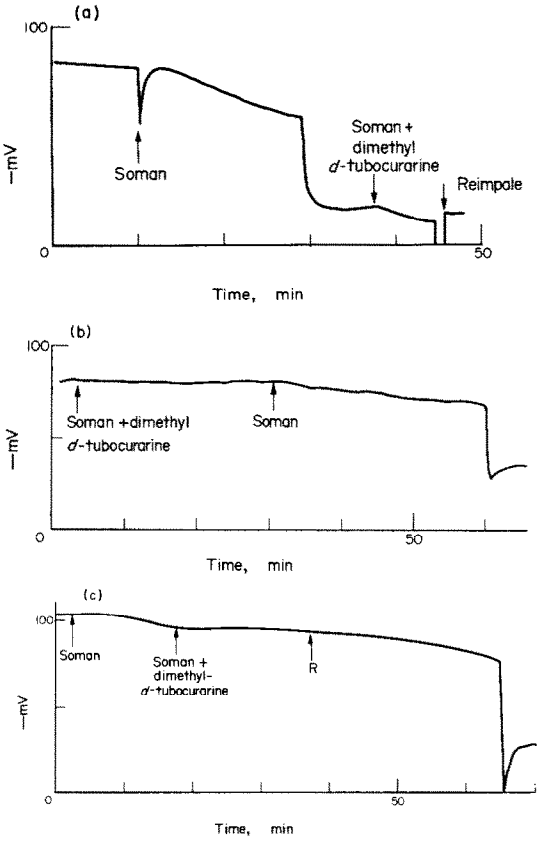

Fig. 5. Interaction of Soman and dimethyl *d*-tubocurarine. (a) Soman-induced slow and rapid depolarization of the innervated membrane. The potential change coincident with Soman application is an artifact of the perfusion system. After the depolarization, the microelectrode was deliberately removed and reinserted in order to check the impalement. (b) Dimethyl *d*-tubocurarine prevents the depolarizing effects of Soman. (c) Dimethyl *d*-tubocurarine applied after Soman but before a Soman-induced rapid depolarization. R indicates eel saline. In 5a, 5b, and 5c the Soman concentration was 3×10^{-4} M and the dimethyl *d*-tubocurarine concentration was 5×10^{-5} M.

leak conductance (the normal potassium ion conductance having been drastically lowered at these voltages by depolarizing potassium inactivation). An extrapolation of this second constant conductance line to its intersection of the steady state leak conductance gives an estimation of the sodium ion equilibrium potential.

The most striking effect of the Soman treatment, as revealed by the voltage clamp studies, was an apparent increase in membrane resistance accompanying the rapid depolarization event. We noted a similarity of this membrane resistance increase to the resistance increase which has been observed after activation of the electroplaque synaptic membrane by carbamylcholine.* Therefore, it was of interest to determine whether the effects of Soman could be modified by a ligand which binds to the AChR. Such a compound is dimethyl *d*-tubocurarine, an inhibitor of AChR activation, a muscle relaxant, and a compound well characterized chemically, especially in view of recent advances in our understanding of the structure of *d*-tubocurarine [26].

Chemical modification of Soman's effects. When dimethyl *d*-tubocurarine and Soman were applied together to an electroplaque, depolarization did not occur. This is seen in Fig. 5b. Furthermore, Fig. 5c shows that dimethyl *d*-tubocurarine applied after the Soman treatment, but before the occurrence of the rapid depolarization, prevented further depolarization. Removal of the dimethyl *d*-tubocurarine resulted, after a delay, in the occurrence of the rapid depolarization. We consider it significant that this removal of the dimethyl *d*-tubocurarine did not result in an immediate sharp depolarization, but instead this occurred after a passage of time almost identical to the time required for the rapid depolarization to occur with Soman alone. This time delay occurred even if the sequence of applications was first Soman, then dimethyl *d*-tubocurarine and Soman, followed by Soman alone (i.e. if Soman was present at all treatment times). If dimethyl *d*-tubocurarine is always present (i.e. if dimethyl *d*-tubocurarine is present before, during a 50-min exposure to Soman, and after Soman is removed), no depolarization is observed, even when the membrane potential is monitored for 55 min after the Soman was removed. Note that although while present dimethyl *d*-tubocurarine can completely inhibit Soman's depolarizing ability, dimethyl *d*-tubocurarine cannot repolarize the membrane once the Soman-induced depolarization has taken place. This is shown in Fig. 5a.

Finally, it should be noted that, after the application of dimethyl *d*-tubocurarine and Soman, the presence of Soman was not necessary for the sharp depolarization to occur (Fig. 5c). That is, it appeared that although dimethyl *d*-tubocurarine was able to prevent the effects on the membrane of a prior application of Soman, the effects of the original Soman application were realized once the dimethyl *d*-tubocurarine was removed.

DISCUSSION

The reactivation of organophosphate-inhibited AChE as a major (if not the sole) explanation for the decline in the sensitivity of the organophosphate-treated electroplaque to ACh has been confirmed by the use of Soman. Whether new AChE is synthesized or not [2] now appears more than ever to be a moot point. The role of the Soman as an irreversible AChE inhibitor in this particular explication is specifically related to the aging process [1]. In this, 1,2,2-trimethylpropyl methylphosphonyl enzyme degrades to methylphosphonyl enzyme (a form which for all practical purposes represents irreversibly inhibited AChE) at an immeasurably greater rate than the corresponding reaction whereby DFP would yield monoisopropylphosphoryl enzyme.

Treatment of the electroplaque cells with organophosphate AChE inhibitors at concentrations which are orders of magnitude greater than would cause essentially complete inhibition of AChE in solution leaves a substantial proportion of the measurable

* D. A. Farquharson, manuscript in preparation.

AChE activity uninhibited. It is probable that a whole complex of factors is involved in this, among them the time-dependent nature of the inhibition reaction [27], the variety of ACh-hydrolyzing enzymes involved [17], the differential solubilities of inhibitors and substrate in different parts of the cellular substructure [7,28] and, for Paraoxon and DFP at least, the simultaneous reactions of inhibition and reactivation. It may be seen that Soman treatment of intact electroplaques resulted in a slightly greater inhibition of measurable cholinesterase activity and, even more significantly, this inhibition continued to increase after the removal of the Soman-containing bathing medium. This is probably due to a retention of unreacted Soman in the cellular substructure, especially in the lipid portions of the plasma membrane.

The undiminished ability of ACh to depolarize the Soman-treated electroplaque is the consequence of this irreversible inhibition of the AChE. The steady decrease in the resting potential of the Soman-treated cell is an effect which, while different from many of those listed in the beginning of this paper, is similar to them in that it does not appear to be attributable exclusively to the ability of Soman to inhibit AChE. Nevertheless, the opinion has been advanced that prolonged exposure to AChE inhibitors may, finally, have secondary irreversible effects on function [29]. Now, to some degree, the ambiguity resulting from the parallel between AChE activity and membrane function has been reduced. The use of the voltage clamp shows that Soman causes an apparent increase in membrane resistance which follows the large (40–50 mV) depolarization, and we note that certain aspects of the current–voltage relationships during Soman treatment resemble those seen during carbamylcholine treatment.* Finally, the competition between Soman and the receptor ligand dimethyl *d*-tubocurarine shows that, in addition to Soman's inhibition of AChE, it also interacts with the AChR in an irreversible manner. Whether the irreversibility is due to an "aging" reaction is an open question. Evidence for the interaction of DFP with the AChR has been reported previously [8] and while the possibility of di-isopropylphosphoryl-AChR aging was not raised at that time, judging from the interaction of DFP with AChE, it might be speculated that such a reaction would be slow. The receptor-blocking action of DFP appears to be reversible and probably competitive in nature [8]. Organophosphate compounds, known inhibitors of AChE (DFP, Tetram and two others), have been found to block the binding of labeled nicotine and decamethonium to macromolecules thought to be AChR proteins [30]. This interaction of purified AChR and organophosphate compounds also was reversible. These observations indicate that Soman and DFP differ in their actions on the AChR in at least two aspects: the reversibility of the effect and the quality of the effect itself.

The cause of the resistance increase which accompanies the Soman-induced rapid depolarization is not known. For carbamylcholine-induced depolarizations, the resistance increase follows an initial resistance decrease. The resistance increase induced by carbamylcholine is probably due in part to an inactivation of the AChR. This inactivation is the desensitization phenomenon. For the Soman-induced and carbamylcholine-induced depolarizations, there are factors affecting the resistance in addition to this desensitization process which can result in a final membrane resistance that is greater than it was before exposure to these drugs. For instance, in Fig. 4a the cord conductance of the resting cell decreased from 180 mmho/cm^2 to a slope conductance of 114 mmho/cm^2 over the same voltage range. In the present study, no resistance decrease has been observed accompanying the Soman-induced depolarization. This would have been difficult because the exact time at which the rapid depolarization did occur after exposure to Soman was difficult to gauge. However, it is assumed that a brief resistance decrease does occur and that the immediate result of this resistance decrease is the Soman-induced rapid depolarization. Recent work in this laboratory has confirmed that Soman produces a discrete increase and decrease in membrane resistance relative to the normal membrane resistance.*

The initial slow depolarization caused by Soman and the effects of dimethyl *d*-tubocurarine on this are less clear, but they also seem to fit into the framework of Soman effects not directly related to AChE inhibition. On the one hand, we have not seen clear-cut changes in the ionic conductances accompanying this initial slow depolarization phase, as with the later sharp phase. For example, no consistent or significant changes occurred in the ionic conductance components or their respective reversal potentials. On the other hand, dimethyl *d*-tubocurarine again blocked this slow depolarization even in the presence of Soman. The recommencement of the slow depolarization phase after removal of the dimethyl *d*-tubocurarine, now even in the absence of re-added Soman, suggests either, as already mentioned, that unreacted Soman has been retained in the lipid regions of the excitable membrane or that irreversible reactions had already occurred, the final effects of which were forestalled by the dimethyl *d*-tubocurarine, but were not reversed. Whether this initial slow depolarization is due to a reaction of Soman with AChR is not clear, nor is it clarified by the competition with dimethyl *d*-tubocurarine since that diquaternary compound binds with many components of electric tissue [31].

In conclusion, previous studies have expanded the spectrum of organophosphate's specific interactions at the neuromuscular junction to include a direct inhibitory action on the AChR [8]. Here we report the first instance of an organophosphate being able to clearly activate an excitable membrane. This activation is apparently mediated through or at least is in conjunction with the AChR, since dimethyl *d*-tubocurarine can effectively block the activation. Since structurally similar organophosphorus cholinesterase inhibitors, such as DFP and Paraoxon, have no such activating ability on the innervated membrane of the electroplaque, but do bind to the AChR [30], it seems reasonable to conclude that the rapid aging ability exhibited by Soman may be a prerequisite for organophosphate-induced AChR activation. The ability of Soman to rapidly age in the environment of a similar active site (AChE), on the intact electroplaque membrane, was demonstrated in this report. The apparent

* D. A. Farquharson, manuscript in preparation.

irreversibility of applying Soman to the innervated membrane also favors the hypothesis that the resulting Soman-induced depolarization is dependent on a rapid aging of a Soman–receptor complex. Other organophosphates, structurally very similar to Soman, such as DFP, act on the purified or *in vivo* AChR reversibly (by blocking binding sites or activation) [8,30]. With these facts in mind, it becomes interesting to speculate that it is the methyl-phosphonylation of the AChR by Soman which results in its activation. Regardless of such speculation one of the important questions remaining concerns how the temporal sequence of Soman-induced activation (the slow and rapid depolarizations) can be understood in terms of the accepted model for post-synaptic chemical excitation.

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