Biochimica et Biophysica Acta, 688 (1982) 138–144
Elsevier Biomedical Press

BBA 71176

MODIFICATION OF ION TRANSPORT IN LIPID BILAYER MEMBRANES BY THE INSECTICIDES DDT AND DDE

DANIEL WOLFF [a] and RICARDO BULL [b]

[a] *Departamento de Biología, Facultad de Ciencias Básicas y Farmacéuticas, and* [b] *Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile, Casilla 653, Santiago (Chile)*

(Received November 10th, 1981)

Key words: DDT; DDE; Insecticide; Ion transport

In order to elucidate the mechanism of action of organochlorine insecticides on the ion transport in biological membranes, we have studied the effect of DDT and its analog DDE on the structural parameters of phosphatidylethanolamine (PE) planar bilayers. DDT and DDE increase the conductance induced by the hydrophobic ions tetraphenylarsonium ($TPhAs^+$) and tetraphenylborate ($TPhB^-$) in lipid bilayers. Neither DDT nor DDE alters the surface potential of PE monolayers. On the other hand, these organochlorine compounds increase only slightly the electric capacitance of the bilayers. These results are compatible with the hypothesis that these insecticides increase the fluidity of the membrane.

Introduction

There are several lines of evidence that organochlorine insecticides interfere with transport processes in biological membranes. They modify the normal conduction of the nerve impulse [1–4] and inhibit active transport of ions in several tissues [5–7].

Although it has been shown that the effect on active transport processes is due to an inhibition of ATPase activities of ion pumps [7], the specific mechanism by which they alter the conduction of the nerve impulse is not yet clear. DDT and other insecticides prevent the inactivation of the sodium channels in myelinated fibers and also slow down the turn-on of the potassium channels in non-myelinated fibers [2,3]. These effects might be produced either by a direct interaction of the compound with the channel structure or indirectly by changing physical properties of the lipid bilayer such as surface potential, dielectric constant and/or the fluidity.

It has been shown that DDT and other insecticides can alter properties of lipid membranes. In black lipid films, carrier-mediated ion transport is decreased [8,9]. In liposomes, nonelectrolyte permeability is increased by DDT [10]. These two results seem contradictory but could be reconciled if we accept that DDT has a dual effect on the bilayer: increases the mobility of molecules in the membrane (which explains the increase of nonelectrolyte transport) and increases the dipole potential or decreases the dielectric constant (which will decrease the positive ion concentration in the bilayer and should not affect the nonelectrolytes). Cholesterol is an example of a molecule which has a dual effect on bilayers [11].

The aim of this work was to measure the possible electrostatic effect of DDT and its analog DDE (which has no effect in nerve [4]) in lipid bilayers. From transport of hydrophobic ions we

---

Abbreviations: DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; DDE, 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene; $TPhAs^+$, tetraphenylarsonium; $TPhB^-$, tetraphenylborate; PE, phosphatidylethanolamine.

0005-2736/82/0000-0000/$02.75

can separate the combined fluidity-dielectric constant effect from the surface potential effect [12,13]. Dielectric constant was measured independently from the electric capacitance of bilayers to separate the effect on membrane fluidity.

Materials and Methods

Membrane formation

Membranes were formed at room temperature (22 ± 2°C) by apposition of two monolayers according to the technique described by Montal and Mueller [14]. The aperture in the Teflon partition separating the aqueous phases was treated with 5% squalene in hexane.

Membranes were made from bacterial phosphatidylethanolamine (Supelco Inc., Bellafonte, PA). The lipid was spread on the surface of the electrolyte solution using 10 μl of a solution containing 12.5 mg/ml of lipids in hexane.

Valinomycin (Calbiochem, La Jolla, CA), nonactin (E.R. Squibb and Sons, Princeton, NJ), tetraphenylborate (Eastman Kodak Co.) and tetraphenyl arsonium (ICN-K and K Labs, Cleveland, OH) were added to both compartments in concentrated ethanolic solutions after the membrane was formed. The final concentrations in the saline solution bathing the membrane were: valinomycin, $3.3 \cdot 10^{-7}$ M; nonactin, $1.7 \cdot 10^{-6}$ M; $TPhB^-$, $8.3 \cdot 10^{-8}$ M; and $TPhAs^+$, $1.0 \cdot 10^{-3}$ M.

DDT and DDE, 99% pure, (Aldrich Chemical Co., Milwaukee, WI) were added to both compartments in ethanolic solutions of various concentrations. Given values of DDT and DDE concentrations in the aqueous phase are nominal for concentrations greater than $1 \cdot 10^{-4}$ M, since the saline solution became opalescent, indicating that the organochlorine compound did not dissolve completely and the actual concentration was smaller. Control experiments showed that ethanol at the concentrations used had no effect on the membrane conductance. The aqueous solutions of electrolyte were symmetrical and consisted of 1 M KCl (in valinomycin and nonactin experiments) or 1 M NaCl (in $TPhB^-$ and $TPhAs^+$ experiments) buffered with 5 mM Tris-HCl (pH 7.0).

Electrical measurements

The system for measuring the electrical properties of the bilayers has been described in detail by Alvarez and Latorre [15] and consisted essentially of a two-electrode 'voltage clamp'. One side of the chamber was connected to a current-voltage converter through an Ag|AgCl electrode. The other side was connected to a function generator using another Ag|AgCl electrode.

The formation of the bilayer was monitored measuring the capacitance by applying a 125 Hz, 90 mV peak-to-peak triangular wave form. After membrane formation and addition of the carriers or lipophilic ions, the aqueous phase was stirred for at least 20 min to ensure equilibration. The zero-voltage conductances by $TPhAs^+$, nonactin-K^+ and valinomycin-K^+ were measured from steady-state current-voltage curves taken directly on an x–y recorder. The amplitude of the ramp was ± 15 mV and the rate of change was less than 10 mV/s to minimize hysteresis.

Since the steady-state current induced by $TPhB^-$ is limited by unstirred layers [16], current measurements were made with a voltage-pulse technique [17]. For this ion the initial current was estimated extrapolating the current transient to zero time. In order to prevent saturation of the amplifier, the transient current resulting from the charge of the membrane capacitance was subtracted using a differential ammeter [15]. One input of the ammeter was connected to the membrane and the other to a capacitor and a series resistance simulating the membrane. To improve the signal-to-noise ratio of the $TPhB^-$-induced current transients, several curves were added in digital form with the help of a signal averager and stored in diskette for later curve fitting analysis using a microcomputer. The zero-time current and the time constant, τ, were obtained fitting the best exponential to the experimental data, using the values of current after the fading on the noncompensated capacitance transient (approx. 50 μs).

The effect of the organochlorine compounds on the capacitance was tested in bare bilayers. Basal capacitance was estimated from the integral of the transient current resulting from the application of a square 10 mV step to the membrane. Because the changes in the capacitance of the bilayer induced by the insecticides are very small, the accuracy of the measurements was improved by subtracting a capacitance equivalent to the basal using the differential ammeter described before. This allowed us to increase the magnification 10-times. 320 single current-time curves were added in digital form using a signal averager. The area under the curve was calculated using a microcomputer on-line with the experimental arrangement. The precision of the measuring method was better than 0.1%.

Surface potential measurements

PE monolayers were formed on a Teflon trough divided into two compartments (19 and 1.6 cm^2) by a Teflon barrier that separated the surface of the two compartments but allowed the contact of the subphases. Measurements of the surface potentials were made by using an air-ionizing electrode of ^{210}Po which was suspended about 2 mm over the air/water interface, and an Ag|AgCl electrode was connected to the subphase. The solution of the subphase was 1 M KCl/5 mM Tris-HCl (pH 7.0). An electrometer (Keithley Instruments, Cleveland, OH, Model 602) was connected to both electrodes for the measurement of the surface potential. Before spreading the lipid in the larger compartment, the subphase was cleaned until a stable potential was obtained. 40 μl 12.5 mg/ml of PE in hexane were then added to the air/water interface and the new potential obtained was recorded. DDT and DDE were added to the subphase of the smaller compartment in appropriate amounts to obtain different concentrations. The subphase was stirred with a magnetic bar.

Results

Effect of DDT and DDE on the conductance induced by K^+-carrier complexes

Fig. 1 shows the effect of these insecticides on the steady-state zero-voltage conductance induced by valinomycin-K$^+$ complex. In order to normalize the results obtained with different membranes which presented different basal-induced conductances, results are expressed as the ratio of the conductance in the presence of the organochlorine compound and the basal induced conductance (relative conductance). The conductance decreased by 5-fold when the concentration of DDT varied from zero to $5 \cdot 10^{-4}$ M and by 8-fold for the same change in DDE concentration. These results obtained in solvent free bilayers agree with the findings of other authors in black lipid films [8,9].

The conductance induced by the nonactin-K$^+$ complex is reduced by both DDT and DDE to a 50% at a concentration of $1 \cdot 10^{-4}$ M (Fig. 2). The effect of the pesticides is significantly larger on the valinomycin-K$^+$ complex- than on the nonactin-K$^+$ complex-induced conductance.

Effect of DDT and DDE on the conductance induced by hydrophobic ions

Fig. 3 shows the effect of DDT and DDE on the TPhAs$^+$-induced steady-state zero voltage conductance in PE membranes. DDT promotes an increase of 5-times the basal TPhAs$^+$ conductance at a concentration of $5 \cdot 10^{-4}$ M. The effect of DDE is smaller, since it produced an increase of only 2-times in the conductance at the same concentration.

Fig. 4 illustrates the time course of the TPhB$^-$-induced current in the absence and in the presence of DDT $1 \cdot 10^{-4}$ M, after a 10 mV step in potential was applied. In this example DDT promotes an increase of the initial current from 23.5 to 117 nA and reduces the time constant of the transient from 0.53 to 0.11 ms. In both cases shown in Fig. 4 the steady-state current is almost negligible (less than 1% of the instantaneous current) and the current relaxation follows a single exponential time course. These findings indicate that the current represents the transport of the hydrophobic ion across the interior of the bilayer, as indicated by Reyes and Latorre [18].

Fig. 5 shows the effect of DDT and DDE on the zero time conductance and on the time constant of the current relaxation of TPhB$^-$-doped membranes. DDT increased the conductance 7-fold at a concentration of $5 \cdot 10^{-4}$ M and decreased the time constant 10-fold. DDE increased the conductance only 4-fold and decreased the time constant 5-fold, at the same concentration. The charge translocated was reduced by 33% and 21% by DDT and DDE, respectively. The results indicate that the most important transport parameter modified by the insecticides is the rate of translocation, whereas the adsorption to the membrane is little modified.

Effect of DDT and DDE on the capacitance of PE bilayers

Table I shows the change in the capacitance of the membranes caused by DDT and DDE. It can

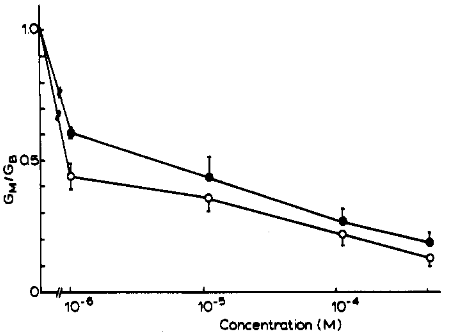

Fig. 1. Effect of DDT and DDE on the valinomycin-K^+ complex-induced conductance in PE bilayers. Results are expressed as the ratio of the conductance (G_m) in the presence of DDT (●) or DDE (○) and the basal-induced conductance (G_B). Membranes were formed in 1 M KCl/5 mM Tris-HCl (pH 7.0) at 22±2°C. Valinomycin was added to the aqueous solution to a final concentration of $3.3 \cdot 10^{-7}$ M. Each point represents the mean ±S.E. of six experiments.

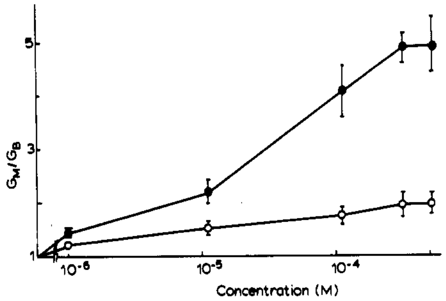

Fig. 3. Effect of DDT and DDE on $TPhAs^+$-induced conductance in PE bilayers. Results are expressed as the ratio of the conductance (G_m) in the presence of DDT (●) or DDE (○) and the basal-induced conductance (G_B). Membranes were formed in 1 M NaCl/5 mM Tris-HCl (pH 7.0) at 22±2°C. $TPhAs^+$ was added to the aqueous solution to a final concentration of 1 mM. Each point represents the mean ±S.E. of six experiments.

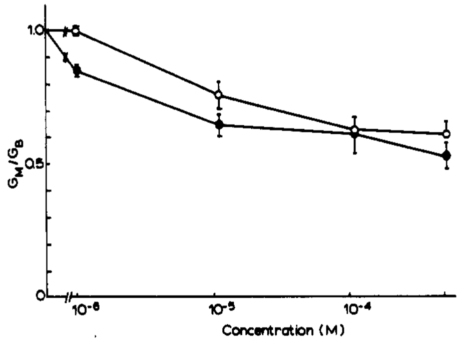

Fig. 2. Effect of DDT and DDE on the nonactin-K^+ complex-induced conductance in PE bilayers. Results are expressed as the ratio of the conductance (G_m) in the presence of DDT (●) or DDE (○) and the basal-induced conductance (G_B). Membranes were formed in 1 M KCl/5 mM Tris-HCl (pH 7.0) at 22±2°C. Nonactin was added to the aqueous solution to a final concentration of $1.7 \cdot 10^{-6}$ M. Each point represents the mean ±S.E. of six experiments.

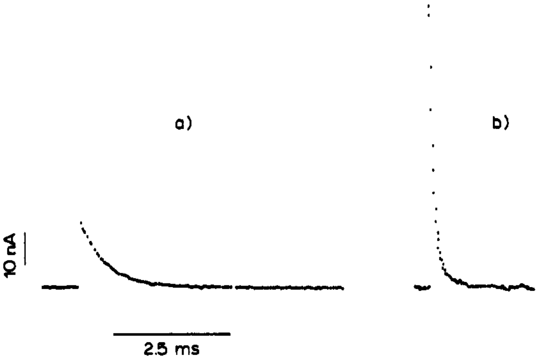

Fig. 4. (a) $TPhB^-$-induced current of PE bilayer after applying a 10 mV step in potential. The membrane was formed in 1 M NaCl/5 mM Tris-HCl (pH 7.0). $TPhB^-$ was added to the aqueous phase to a final concentration of $8.3 \cdot 10^{-8}$ M. (b) $TPhB^-$-induced current after adding DDT to a final concentration of 0.1 mM DDT. Other experimental conditions were the same as in (a).

TABLE I

EFFECT OF DDT AND DDE ON THE CAPACITANCE OF PE BILAYERS

The values (expressed as percent of change with respect to the basal capacitance), represent the means ±S.E. of at least five experiments. The membranes were formed in 1 M KCl/5 mM Tris-HCl (pH 7.0) at room temperature.

	Concentration (M) ($\times 10^4$)				
	0.01	0.11	1.11	3.11	5.11
DDT	0.26±0.11	0.75±0.11	1.72±0.22	2.16±0.20	2.06±0.30
DDE	0.09±0.08	0.27±0.09	0.59±0.06	0.81±0.14	0.99±0.17

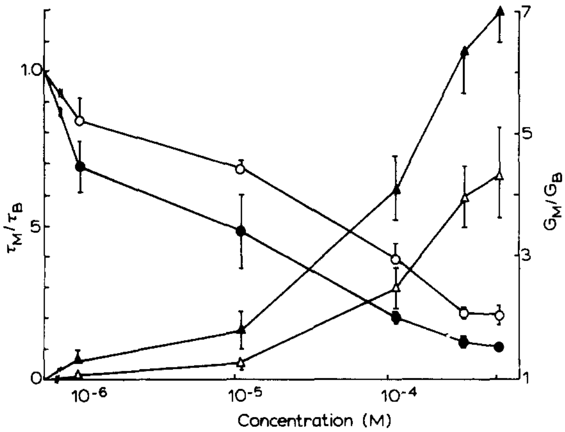

Fig. 5. Effect of DDT and DDE on TPhB$^-$ initial conductance and on the time constant of TPhB$^-$ current decay in PE bilayers. Conductance is expressed as the ratio of the conductance (G_m) in the presence of DDT (▲) or DDE (△) and the basal-induced conductance (G_B). The time constant decay is expressed as the ratio of the time constant (τ_M) in the presence of DDT (●) or DDE (○) and the time constant in the absence of the insecticides (τ_B). Membranes were formed in 1 M NaCl/5 mM Tris-HCl (pH 7.0) at 22°C. TPhB$^-$ was added to the aqueous phase to a final concentration of $8.3 \cdot 10^{-8}$ M. Each point represents the mean and the S.E.

be appreciated that the capacitance increased 2% with DDT $5 \cdot 10^{-4}$ M and 1% with the same concentration of DDE. Experiments were done in bilayers which presented very stable capacitance values. Controls showed that the same volume of ethanol as that added with the organochlorine compounds did not produce any detectable change in the capacitance.

TABLE II

EFFECT OF DDT AND DDE ON THE SURFACE POTENTIAL OF PE MONOLAYERS

The values are expressed as the change in the surface potential (mV) of the monolayer and are represented as the mean ± S.E. of four experiments. The subphase solution was 1 M KCl/5 mM Tris-HCl (pH 7.0). Experiments were performed at room temperature. The average of the basal surface potential of the PE monolayers was 464 ± 4.9 (S.E.) (mV), ($n = 8$).

	Concentration (M) ($\times 10^4$)	
	2.5	5.0
DDT	-0.25 ± 2.50	-0.25 ± 3.30
DDE	-0.63 ± 0.69	-2.30 ± 1.63

Effect of DDT and DDE on the surface potential of PE monolayers

The surface potential of saturated PE monolayers was found to be 464 ± 4.9 mV, air-phase-positive, which is in accordance with the findings of other authors [18,19]. Neither DDT nor DDE, even at concentrations up to $5 \cdot 10^{-4}$ M in the subphase, significantly altered the surface potential of PE monolayers (Table II).

Discussion

A compound may alter the membrane conductance, modifying the surface potential or the intrinsic conductance [11]. It is possible, in principle, to identify which of them is affected by using opposite-charged ionic probes [12,13]. TPhAs$^+$ and TPhB$^-$ are the most widely used molecules since they have the same size and hydrophobic coat. These ions have been used by other authors to test the effect of transport inhibitors [20], herbicides [21,22] and anesthetics [18] on artificial membranes.

The modification of these two factors can be evaluated quantitatively using the following expressions developed by Szabo [11]:

$$(\bar{G}_+/\bar{G}_-)^{-1/2} = \exp(F\Delta\psi/RT) \qquad (1)$$

$$(\bar{G}_+ \cdot \bar{G}_-)^{1/2} = \frac{\bar{u}}{\bar{d}} \exp(-\Delta\Delta\mu^\circ/RT) \qquad (2)$$

Eqn. 1 represents the change in the electrostatic potential term and Eqn. 2 the change in the intrinsic conductance term when the membrane is modified. $\bar{G}_+$ and $\bar{G}_-$ are the relative conductances for the hydrophobic cation and anion, respectively. $\Delta\psi$ is the change in surface potential of the membrane, $\Delta\Delta\mu^\circ$ is the change in the difference of the chemical potential of the hydrophobic ion between the aqueous and the membrane phases, $\bar{u}$ is the relative mobility of the ion in the membrane interior and $\bar{d}$ is the relative membrane thickness. F, R and T have their usual meanings.

We have calculated the effect of the insecticides on the intrinsic conductance and on the electrostatic potential using Eqns. 1 and 2. The result of these calculations is shown in Fig. 6. It can be appreciated that there is a negligible effect on the

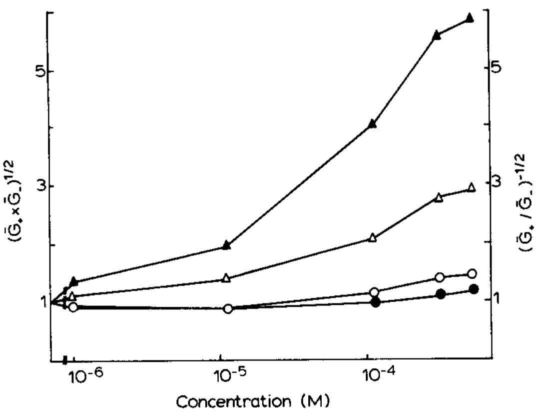

Fig. 6. Effect of DDT and DDE on the calculated intrinsic conductance term $(\bar G_+ \cdot \bar G_-)^{1/2}$ and the electrostatic potential term $(\bar G_+ / \bar G_-)^{-1/2}$ of conductance of PE bilayers. Intrinsic conductance: ▲, DDT; △, DDE. Electrostatic potential: ●, DDT; ○, DDE.

electrostatic term; however, the intrinsic conductance is increased by a factor of 5.6 and 2.8 in the presence of $3 \cdot 10^{-4}$ M DDT and DDE, respectively. Direct measurements of surface potentials in PE monolayers corroborate that the organochlorine compounds do not alter the electrostatic potential term.

The intrinsic conductance term, according to Eqn. 2, includes the mobility of the ion in the membrane, the membrane thickness and the chemical free energy of partition of the ionic probe. In the case of TPhAs$^+$ and TPhB$^-$, the chemical free energy of partition depends on the hydrophobic energy barrier and the image force energy barrier in the membrane. This last term depends on the dielectric constant and the thickness of the membrane [23]. So, the increase in the intrinsic conductance induced by the organochlorine compounds may reflect an increase in the fluidity, the dielectric constant, and/or a decrease in the thickness of the membrane. Changes in these last two parameters can be estimated by measuring the geometric capacitance (C_g), which is given by the equation

$$C_g = \frac{\epsilon_0 \epsilon_m A}{d} \tag{3}$$

where ϵ_0 is the permittivity of the free space, ϵ_m is the dielectric constant, A is the area and d the thickness of the membrane.

We found an increase of membrane capacitance of 2% for DDT and 1% for DDE at $5 \cdot 10^{-4}$ M (Table I). According to Eqn. 3 this increase in the capacitance could be accounted for by an increase in membrane area, dielectric constant and/or a decrease in the thickness of the membrane.

A change in membrane area would produce a proportional change in the conductance. So, if we attribute all the change in capacitance to an increase in membrane area we cannot explain the large increase in the conductance found.

To analyse the effect of changes in membrane thickness and dielectric constant on the conductance we have to calculate the change in the image force energy barrier. According to Parsegian [23] the difference in the peak potential energy barrier $W(\epsilon, d)$ between two membranes of thickness d_1 and d_2 and dielectric constant ϵ_{m1} and ϵ_{m2} is given by

$$\Delta W(\epsilon, d) = \frac{(z_i e_0)^2}{8\pi\epsilon_0 r}\left(\frac{1}{\epsilon_{m2}} - \frac{1}{\epsilon_{m1}}\right) - \frac{(z_i e_0)^2}{4\pi\epsilon_0}\ln 2 \times \left(\frac{1}{d_2 \epsilon_{m2}} - \frac{1}{d_1 \epsilon_{m1}}\right) \tag{4}$$

where e_0 is the electronic charge and r and z are the radius and the valence of the ion, respectively. The change in conductance can be estimated from the following expression [11]

$$\frac{G_M}{G_B} \propto \frac{1}{\bar d} \exp(-\Delta W/kT) \tag{5}$$

where G_M and G_B are the conductances of the membrane modified by the insecticide and of the unmodified membrane, respectively. If all the change in capacitance is attributed to an increase in the dielectric constant, it would explain only 13% of the modification observed in the intrinsic conductance. On the other hand, if all the change in capacitance is attributed to a decrease in membrane thickness we would explain only about 3% of the observed effect. Since changes in area, dielectric constant or thickness of the membrane can explain only 13% of the conductance modification, at least 87% of the change should be attributed to an increase in membrane fluidity. This conclusion is in accordance with the studies of Antunes-

Madeira and Madeira [10], who found that DDT increases nonelectrolyte permeability in liposomes.

An increase in the intrinsic conductance should be reflected in an increase in the carrier-mediated transport. We found that the insecticides decrease carrier-induced conductances. This result cannot be accounted for by the changes in the membrane physical parameters we postulate. However, it is possible that the organochlorine compounds have a direct effect on the carriers that mask their effect on membrane properties. As it has been proposed by Jackson and Gardner [9], a hydrophobic interaction between the insecticides and the carriers in the aqueous solution, would decrease the effective bulk concentration of the carriers and therefore their concentration in the membrane. A decrease in the concentration of the carrier-K^+ complex in the membrane should produce a decrease in the induced conductance.

Some authors have indicated that the lipid milieu surrounding the ionic channels might influence their gating properties in nerve [3,24]. Although it is attractive to correlate changes in fluidity with the neurotoxic effect of the insecticides, the different extent to which DDT and DDE modify the fluidity of the bilayer seems to be insufficient to explain the different effects of DDT and DDE on sodium channels.

Acknowledgements

This work was supported by research grant No. B-1224-8113 of the Servicio de Desarrollo Científico, Artístico y de Cooperación Internacional, Universidad de Chile. The authors are grateful to Dr. Osvaldo Alvarez and Mrs. Ximena Cecchi for helpful criticism of the manuscript.

References

1 Shanes, A.M. (1949) J. Gen. Physiol. 33, 75–102

2 Narahashi, T. and Haas, H.G. (1968) J. Gen. Physiol. 51, 177–198

3 Hille, B. (1968) J. Gen. Physiol. 51, 199–219

4 Arhem, P. and Frankenhaeuser, B. (1974) Acta Physiol. Scand. 91, 502–511

5 Janicki, R.H. and Kinter, W.B. (1971) Science 173, 1146–1148

6 Doherty, J.D. and Matsamura, F. (1975) Pestic. Biochem. Physiol. 5, 242–252

7 Iturri, S. and Wolff, D. (1982) Comp. Biochem. Physiol. 71C, 131–134

8 Hilton, B.D. and O'Brien, R.D. (1970) Science 168, 841–843

9 Jackson, D.A. and Gardner, D.R. (1978) Pestic. Biochem. Physiol. 8, 129–136

10 Antunes-Madeira, M.C. and Madeira, V.M.C. (1979) Biochim. Biophys. Acta 550, 384–392

11 Szabo, G. (1974) Nature 257, 47–49

12 McLaughlin, S.G.A., Szabo, G., Eisenman, G. and Ciani, S.M. (1970) Proc. Natl. Acad. Sci. U.S.A. 67, 1268–1275

13 Szabo, G., Eisenman, G., McLaughlin, S.G.A. and Krasne, S. (1972) Ann. N.Y. Acad. Sci. 195, 273–290

14 Montal, M. and Mueller, P. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 3561–3566

15 Alvarez, O. and Latorre, R. (1978) Biophys. J. 21, 1–17

16 LeBlanc, O.H., Jr. (1969) Biochim. Biophys. Acta 193, 350–360

17 Ketterer, B., Neumcke, B. and Lauger, P. (1971) J. Membrane Biol. 5, 225–245

18 Reyes, J. and Latorre, R. (1979) Biophys. J. 28, 259–280

19 Hladky, S.B. (1974) Biochem. Biophys. Acta 352, 71–85

20 Melnik, E., Latorre, R., Hall, J.E. and Tosteson, D.C. (1977) J. Gen. Physiol. 69, 243–257

21 Smejtek, P. and Paulis-Illangasekare, M. (1979) Biophys. J. 26, 441–466

22 Smejtek, P. and Paulis-Illangasekare, M. (1979) Biophys. J. 26, 467–488

23 Parsegian, V.A. (1969) Nature 221, 844–846

24 Bean, B.P., Schrager, P. and Goldstein, D.A. (1981) J. Gen. Physiol. 77, 233–253