Action of Some Insecticides on Membranes of Mouse Liver Mitochondria

by

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There is a growing body of evidence that insecticides act through interference with nerve transmission and that the interference occurs as a result of alterations in ion transport across the excitable membrane (MATSUMURA and O'BRIEN, 1966). More precisely, it appears that insecticides, including DDT and allethrin, interfere with the normal conductance of univalent cations involved in nerve transmission (NARAHASHI and HAAS, 1967), and it is not surprising that the same compounds interfere with activity of the plasma membrane Na^{+}-K^{+}-ATPase (DAVIS et al., 1972) which is responsible for energy-linked counter movement of these ions in plasma membranes generally. We have undertaken to examine the influence of insecticides on membranes by employing purified subcellular organelles. For example, mitochondria represent attractive material for such a study, since a great deal of information is available about ionic flux across the mitochondrial membrane (see, for example, PRESSMAN, 1970). We have been encouraged in this direction by finding that low concentrations of several insecticides markedly stimulate mitochondrial ATPase (PAYNE, HERZBERG and HOWLAND, 1973). The present communication extends these observations by presenting evidence for stimulation of coupled respiration and dramatic light-scattering changes produced by the addition of insecticides to suspensions of mitochondria.

METHODS

Liver mitochondria were isolated in 250 mM sucrose from mice (strain 129 J, Jackson Laboratory, Bar Harbor, Maine (MYERS and SLATER, 1957). Oxidation and ATP synthesis was measured polariographically as described previously (HOWLAND, 1967) and the reaction medium contained 250 mM sucrose, 6 mM sodium succinate, 12 mM potassium phosphate and 6 mg of mitochondrial protein. The reaction was carried out at 20° at pH 7.4 in 1.5 ml using a Clark electrode and a Gilson Medical Electronic Oxygraph. Light scattering was recorded at a wavelength

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of 540 nm employing a Gilford spectrophotometer equipped with a recorder essentially as described earlier (SETTLEMIRE et al., 1968) and a reaction medium containing one milligram of mitochondrial protein in 3 ml of 0.25 M sucrose. Pesticides were obtained from the K & K Labs, Inc., Plainview, New York, except for DDT which was from Aldrich Chemical Co., Milwaukee, Wisconsin. The pesticides were dissolved in DMSO (dimethylsulfoxide), except allethrin which was in ethanol.

RESULTS AND DISCUSSION

A number of compounds that stimulate mitochondrial ATPase also stimulate respiration when ADP or phosphate is omitted from the reaction medium. These uncoupling compounds also prevent ATP synthesis and their action is interpreted as resulting from the discharge of the high energy state of mitochondria (see SLATER, 1971). It is clear from Table I that the action of the insecticide heptachlor differs importantly from that of uncouplers (similar results having also been obtained with aldrin, dieldrin and DDT). In the first place, insecticides fail to stimulate respiration in the absence of ADP but do so in their presence. In the second place, their addition does not significantly alter the efficiency of ATP synthesis as reflected in the ATP/O ratio.

TABLE I

The effect of heptachlor on mitochondrial oxidation of succinate

conditions	respiration rate µatoms O_2/min	ATP/O
control	0.089	
+ heptachlor 54 nmoles	0.081	
+ ADP 0.52 µmoles	0.288	1.40
+ ADP + heptachlor	0.367	1.44

Efforts were made to determine the basis for the stimulation of respiration in the presence of ADP, a number of explanations being considered and rejected. For example, it was considered that the higher rates of oxidation might result from an increased influx of ADP into the mitochondria but direct measurement of uptake of ^{14}C-labeled ADP failed to confirm this. A clue as to the possible basis was found in the form of the induction of light-scattering changes in suspensions of mitochondria upon addition of insecticides.

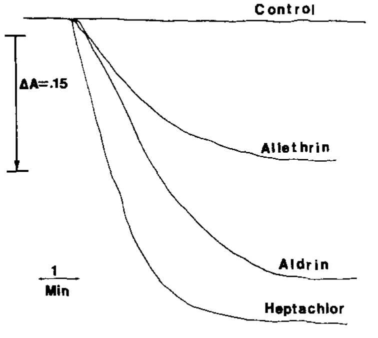

Figure 1. Light scattering changes induced by pesticides. Deflection in the direction of the arrow indicates a decrease in absorbence.

As indicated by the data in Figure 1, two chlorinated hydrocarbons (heptachlor and aldrin) and a pyrethroid, allethrin, induce similar light scattering responses to mitochondria suspended in isotonic sucrose. The concentration of the insecticide in each of the experiments was 2.2, 1.8, and 1.7 X 10^{-5} M for allethrin, aldrin and heptachlor, respectively. DDT and dieldrin have been shown in other experiments to induce similar light scattering changes. Addition of as much as 40 µl of the solvent (DMSO or ethanol) did not induce any light scattering changes. The response is dependent upon the amount of insecticide present, as shown for one of the insecticides, heptachlor in Figure 2.

Other reports by CHAPPELL and CROFTS (1966) and BRIERLEY and SETTLEMIRE (1968) have indicated that light scattering changes reflect mitochondrial volume changes, an increase in volume being associated with a decrease in light scattering. The swelling observed by others has, in turn, been shown to be an osmotic response to movement of salts. The origin of the decrease in light scattering in these experiments is unclear since the suspending medium contained only sucrose, a molecule not taken up by the mitochondrion.

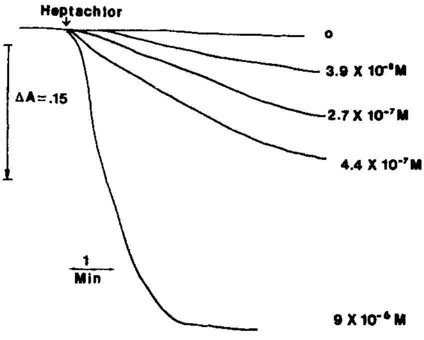

Figure 2. Effect of the concentration of heptachlor on light scattering.

A possible explanation is that the light scattering changes do not indicate volume changes but changes in the surface characteristics of the membrane, an interpretation of this nature having been used by COHEN and LANDOWNE (1971) in relating light scattering to structural changes in nerve axons. It is also possible that the changes are related to the movement of ions that have leaked out of the mitochondria upon suspension in sucrose. In either case, it seems clear that the insecticides, when added at low concentrations, produce dramatic changes in the membrane of mammalian mitochondria. It appears likely that these changes also form the basis for the increase in respiration noted in the table, either by increasing the permeability of the membrane to succinate or by producing conformational changes of such a nature as to increase the intrinsic activity of the respiratory chain, which resides within the coupling membrane.

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