PROTECTIVE ACTION OF CHOLESTEROL AGAINST CHANGES IN MEMBRANE FLUIDITY INDUCED BY MALATHION

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Organophosphorus insecticides are widely used in agriculture and food production in the control of insect pests. They are less persistent than organochlorine insecticides and many of them can be used by laypersons. However, both organochlorine and organophosphorus insecticides are toxic for mammals [1]. Acute poisonings caused by organophosphorus insecticides are manifested by disturbances in the function of the central nervous system resulting from the inhibition of acetylcholinesterase [2]. However, some markers of chronic toxicity in mammals cannot be related to acetylcholinesterase inhibition [3].

Most of the organophosphorus insecticides are poorly soluble in water and show high affinity towards lipids. Thus, biomembranes can be the target of action of these agents [4]. Body fluids should be taken into account as the primary site of interaction. Therefore blood seems to be the appropriate material for studies on the toxic action of organophosphorus insecticides.

Insecticides can modulate fluidity of the membrane [5], which may be quantitatively assessed by changes in the microviscosity and lateral diffusion coefficient. The latter parameter can be evaluated spectrofluorimetrically with pyrene as a probe, by measuring the ratio of the intensity of excimer (I₀) to the intensity of monomer state (Iₘ), because [6 - 9]:
\[ \frac{I_d}{I_m} \text{ const } \times \text{DAQ} \]

where DAQ is the lateral diffusion coefficient.

Pig erythrocyte membranes were obtained according to the method of Dodge et al. [10] and unitamellar liposomes were formed either of phosphatidylcholine or from equimolar amounts of phosphatidylcholine and cholesterol. Protein was determined according to Bradford [11]. A suspension of liposomes or erythrocyte membranes was incubated for 1 h at 37°C in the presence of the organophosphorus insecticide, malathion (\(O,O\)-dimethyl \(S\)-(1,2-dicarboxyethyl)phosphorodithionate). Fluorescence spectra were measured in a Perkin-Elmer spectrofluorometer, Model LS5-B. The excitation was set at 334 nm and the emission spectra were taken in the range of 350 - 500 nm. The spectra were corrected for light scattering, using controls with membranes but without added probe. One-way analysis of variance was used in the statistical analysis. The differences between means were compared by the Scheffe’s multiple comparison test [12].

The dependence of the excimer to monomer ratio on malathion concentration for erythrocyte membrane, liposomes of pure phosphatidylcholine and liposomes enriched with cholesterol is displayed in Fig. 1.

Malathion caused a statistically significant lowering of the \(I_d/I_m\) ratio for erythrocyte membranes (25 and 48% at malathion concentration of 50 and 100 \(\mu\)M, respectively) and for phosphatidylcholine liposomes (18 and 23%, respectively) whereas the insecticide did not affect this ratio for liposomes enriched with cholesterol.

These results indicate that the presence of cholesterol may be of importance in the interaction of organophosphorus insecticides with biological membranes. Cholesterol action may be a result of either competition for similar or the same interaction sites or changes in the structural organization of phospholipids.

Antunes-Madeira & Madeira [13] showed that partition coefficient of malathion in egg phosphatidylcholine bilayers decreased linearly with temperature in the range at which the lipid was in the liquid-crystalline state; addition of 50 mol% cholesterol strongly decreased incorporation of malathion and abolished the temperature dependence. Partition values in native membranes decreased sequentially as follows: rabbit sarcoplasmic reticulum, rat liver mitochondria, pig brain microsomes, myelin and erythrocytes; this dependence reflects the relative content of cholesterol.
Fig. 1. The ratio of intensity of fluorescence of excimer ($I_d$) to monomer ($I_m$) state of pyrene for erythrocyte membranes (V), liposomes prepared from phosphatidylcholine (O), and liposomes made from phosphatidylcholine and cholesterol mixed in equimolar amounts (●) as a function of malathion concentrations. Error bars denote ± S.D., each experimental point is the mean for five replications.

Since cholesterol diminishes the ability of insecticides to bind to the membrane, the obtained results concerning influence of insecticides on the lateral diffusion of lipids may be due to the decreased degree of binding of insecticides in the membranes in the presence of this sterol either by competitive occupation of the interaction sites or by changes in the structural organization of the phospholipids. The results obtained in this work point to this second possibility as the source of the changes observed in the effect of organophosphorus insecticides in the presence of cholesterol; nevertheless, changes in membrane structure could also be the cause of the recorded disturbances.

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REFERENCES