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EFFECTS OF SOME ORGANOPHOSPHORUS INSECTICIDES ON CALCIUM, POTASSIUM AND CHLORIDE EFFLUX FROM ISOLATED PIG LYMPHOCYTES

Using an isotope labeling technique, we investigated the release of potassium, calcium, and chloride ions from pig lymphocytes *in vitro* under influence of some organophosphorus compounds used as insecticides. Two of used agents, viz. methyl parathion and methyl-bromphenvinphos produced significant (often exceeding 20%) changes in the quantity of the efflux of all tested ions, while the remaining two: malathion and dichlorvos changed the efflux in lesser degree. The phenomenological character of experiment did not allow to draw conclusion concerning the mechanism of observed changes. The obtained results extend on some ions, our previous finding concerning changes in transport of nucleic acids and DNA/RNA modifying enzymes through lymphocytes membrane caused by organophosphate insecticides.

1. INTRODUCTION

Organophosphorus insecticide are widely used in agricultural production. Due to their low water solubility they can interact with structures which are rich in lipids, such as biological membranes. It was confirmed by the results of experiments in which there were observed changes in activity of membrane bound enzymes [5, 9, 13, 14, 18], quantitative changes in lipid fraction [7, 10, 12], changes in fluidity and membrane charge [4, 9, 11], changes in permeability for electrolytes [2, 3] and nonelectrolytes [1, 2] under the influence of organophosphorus insecticides.

Previously we showed that the lymphocytes released an increased amount of nucleic acids and DNA/RNA modifying enzymes into the extracellular environment, following incubation with insecticide malathion. The observed changes were associated with depolimerization of nuclear DNA and variation of amount of DNA in subcellular fractions [21, 22]. The mechanism underlying these phenomena is unknown yet, but the results of research on influence of insecticides on biological membranes suggest that the changes may be associated with disturbances of membrane transport processes.

In this work we try to check whether organophosphorus insecticide can affect also transport of potassium, calcium and chloride ions through the lymphocyte membrane.

2. MATERIALS AND METHODS

Porcine blood was obtained from a local slaughtery. Peripheral blood lymphocytes were isolated according to the method of Parkes and Bradley [20]. The lymphocytes were suspended at a concentration of 10^8 cells per ml in a phosphate saline buffer (PBS) at pH 7.4. Cell viability was estimated by the ability of the cell populations to exclude Trypan Blue and was never less than 95%.

Using isotope labeling technique we investigated the efflux of potassium ($^{86}Rb^+$), calcium ($^{45}Ca^{2+}$), and chloride ($^{36}Cl^-$) from the isolated lymphocytes in the presence of various organophosphorus insecticides: methyl-bromphen-vinphos (2-bromo-1-(dichlorophenyl)vinyl dimethyl phosphate), dichlorvos (2,2-dichlorovinyl dimethyl phosphate), malathion (S-1,2-bis-(ethoxycarbonyl)ethyl O-O-dimethyl phosphorothioate, and methyl parathion (O,O-dimethyl O-p-nithrophenyl phosphorothioate). Investigations were carried out at three final concentrations of each insecticide: 10^{-5}, 10^{-4} and 10^{-3} M. The insecticides were derived from concentrated (50 mM) solutions in ethanol. The control group received only ethanol at a concentration appropriate for the maximal concentration of the particular insecticide (never more than 0.92%). One group got neither any insecticide nor ethanol.

Each isotope was added to the cells suspended in PBS at a concentration of 10^8 per ml in such amount that the final specific activity was 2 μCi/ml for ^{86}Rb and ^{45}Ca and 1 μCi/ml for ^{36}Cl. The lymphocytes were incubated for 1 h at 37°C in the presence of a particular isotope, cooled to 0°C and washed four times in ice-cooled PBS; each washing was followed by 10 s centrifugation at 10,000 g. 0.3 ml aliquot of the lymphocyte suspension was collected in small tubes, each containing 1 ml PBS and an insecticide at a desired concentration. After 1 h releasing an isotope at room temperature, the samples were centrifuged at 10,000 g for 30 s, 0.5 ml aliquot of supernatant was taken, mixed with 3 ml of scintillation cocktail and counted in a scintillator counter (LKB Wallac RackBeta 1209) to estimate the amount of radioactive isotope released by the lymphocytes. Ten thousand counts were collected for each probe to ensure a standard deviation of $\pm$ 100 counts or 1%. The scintillator counter automatically converted the counts, with a correction for dead time, to counts per minute (cpm).

Each experiment was performed in 10 replications. The average radioactivity in the group which received neither any insecticide nor ethanol was used to normalize the radioactivity in the remaining groups. A quantity obtained in this way was further called „efflux". One-way analysis of variance was used to test for effects of insecticides.

3. RESULTS AND DISCUSSION

Table 1 gives the results of efflux of $^{86}Rb^{+}$ ions from the lymphocytes. At all tested concentrations of methyl-bromphenvinphos a statistically significant enhanced efflux of $^{86}Rb^{+}$ was observed. The enhancement ranged from 20% (10^{-5} M) to nearly 30% (10^{-3} M). An enhancement of efflux caused by dichlorvos was independent of its concentration and averaged 6%. Malathion had no statistically significant effect on the release of $^{86}Rb^{+}$. Methyl parathion produced a decrease in the releasing of $^{86}Rb^{+}$ at all tested concentrations. The decrease ranged from 21% (10^{-5} M) to 16% (10^{-3} M).

Table 1

Efflux of $^{86}Rb^{+}$ from lymphocytes under influence of organophosphorus insecticides (mean ± SD, n = 10, all exps.). For the explanation of the term „efflux" see Material and Methods

Insecticide	Concentration [M]	Efflux	p*
Methyl-bromphenvinphos	0	1.0007 ± 0.0529	
	10^{-5}	1.2041 ± 0.2023	<0.01
	10^{-4}	1.2738 ± 0.1516	<0.001
	10^{-3}	1.2997 ± 0.1296	<0.001
Dichlorvos	0	0.9666 ± 0.0209	
	10^{-5}	1.0261 ± 0.0326	<0.001
	10^{-4}	1.0193 ± 0.0441	<0.005
	10^{-3}	1.0318 ± 0.0405	<0.001
Malathion	0	0.9629 ± 0.0361	
	10^{-5}	0.9812 ± 0.0160	>0.1
	10^{-4}	0.9540 ± 0.0202	>0.5
	10^{-3}	0.9768 ± 0.0209	>0.2
Methyl parathion	0	1.0213 ± 0.0665	
	10^{-5}	0.8055 ± 0.0397	<0.001
	10^{-4}	0.8272 ± 0.0323	<0.001
	10^{-3}	0.8594 ± 0.0704	<0.001

* – Scheffe's multiple comparison method.

The exposure of the lymphocytes to methyl-bromphenvinphos at concentrations 10^{-4} and 10^{-3} M produced a significant reduction of calcium efflux (9 and 12% respectively) – see Table 2. Dichlorvos did not cause any statistically significant changes but malathion did – a 10% enhancement at 10^{-3} M. Methyl parathion produced an enhancement in the releasing of calcium at concentrations 10^{-4} M (30%) and 10^{-3} M (28%).

Table 2

Efflux of $^{45}Ca^{2+}$ from lymphocytes under influence of organophosphorus insecticides (mean ± SD, n = 10, all exps.). For the explanation of the term „efflux" see Material and Methods

Insecticide	Concentration [M]	Efflux	p*
Methyl-bromphenvinphos	0	0.9822 ± 0.0462	
	10^{-5}	0.9520 ± 0.0633	>0.2
	10^{-4}	0.8977 ± 0.0305	<0.001
	10^{-3}	0.8617 ± 0.0451	<0.001
Dichlorvos	0	1.0070 ± 0.0615	
	10^{-5}	0.9646 ± 0.1003	>0.2
	10^{-4}	1.0456 ± 0.0719	>0.2
	10^{-3}	0.9859 ± 0.0713	>0.5
Malathion	0	0.9804 ± 0.0689	
	10^{-5}	0.9775 ± 0.0926	>0.5
	10^{-4}	1.0175 ± 0.1489	>0.2
	10^{-3}	1.1641 ± 0.1434	<0.002
Methyl parathion	0	0.9901 ± 0.1108	
	10^{-5}	1.0454 ± 0.1691	>0.2
	10^{-4}	1.2910 ± 0.1777	<0.001
	10^{-3}	1.2688 ± 0.1833	<0.001

* – Scheffe's multiple comparison method.

The exposure of the lymphocytes to methyl-bromphenvinphos resulted in diminishing of efflux of chloride at concentrations 10^{-4} M (8%) and 10^{-3} M (10%) – see Table 3. Dichlorvos did not affect the efflux at all tested concentrations. Malathion caused enhanced efflux at 10^{-4} M (11%) and 10^{-5} M (13%). Methyl parathion produced a significant decrease in the efflux at all concentrations ranging from 12% at 10^{-3} to 24% at 10^{-5} M.

The obtained results testify to changes in transport of potassium, calcium and chloride ions caused by some organophosphorus compounds used as insecticides. The nature of the performed study does not allow to draw conclusions on the mechanism(s) which may underlie the observed changes.

There are a number of conflicting results of investigations dealing with insecticides and transport of ions. The organophosphorus insecticides parathion, azinphos and malathion proved to be activators of rabbit sarcoplasmic reticulum (Ca^{2+} + Mg^{2+})-ATPase and increased the ratio of translocated Ca^{2+} to hydrolyzed ATP [3]. On the other hand malathion was shown to be an inhibitor both of (Ca^{2+} + Mg^{2+})-ATPase activity and ATP-dependent calcium transport in brain synaptosome membrane vesicles [17]. It was also shown that some organophosphates had an inhibitory effect on the activity of (Na^{+}, K^{+})-ATPase and (Ca^{2+} + Mg^{2+})-ATPase of rat heart [8] and (Na^{+}, K^{+})-ATPase of erythrocyte membrane [15].

Table 3

Efflux of $^{36}Cl^{-}$ from lymphocytes under influence of organophosphorus insecticides (mean ± SD, n = 10, all exps.). For the explanation of the term „efflux" see Material and Methods

Insecticide	Concentration [M]	Efflux	p*
Methyl-bromphenvinphos	0	0.9408 ± 0.0834	
	10^{-5}	0.9343 ± 0.0863	>0.5
	10^{-4}	0.8673 ± 0.0613	<0.05
	10^{-3}	0.8492 ± 0.0787	<0.05
Dichlorvos	0	0.9432 ± 0.1125	
	10^{-5}	0.9404 ± 0.1099	>0.5
	10^{-4}	0.9302 ± 0.1274	>0.5
	10^{-3}	0.8492 ± 0.8883	>0.2
Malathion	0	1.0032 ± 0.0799	
	10^{-5}	1.0434 ± 0.0526	>0.2
	10^{-4}	1.1155 ± 0.0683	<0.005
	10^{-3}	1.1312 ± 0.1030	<0.01
Methyl parathion	0	1.0254 ± 0.1111	
	10^{-5}	0.7755 ± 0.0566	<0.001
	10^{-4}	0.8596 ± 0.0915	<0.002
	10^{-3}	0.9056 ± 0.1318	<0.05

* – Scheffe's multiple comparison method.

The insecticides malathion and parathion exert significant effects on the ionophore – induced permeability of liposome membranes for K^{+} and Ca^{2+} ions as a consequence of changes in the fluidity of membrane lipids [2].

The base of biological activity of insecticides should be considered on the molecular level. The structure of atoms P-O-C which is typical of organophosphates seems to be fundamental as far as the biological action of these compounds is considered. This structure is mainly responsible for interaction of organophosphate with nucleophiles of the cell. Nucleic acids and proteins have the largest amount of nucleophilic sites of the cell. A nucleophile can preferably attack a phosphorus atom causing the break of the -P-C- bond and, in turn, phosphorylation, or breaks -C-O- bond causing methylation. An inhibition of the active site of acetylcholinoesterase is the best known methylation reaction caused by insecticides [6, 16, 23]. Organophosphates can cause methylation of proteins [23] followed by inhibition of Na^{+}/Ca^{2+} exchange, being a component of the calcium transport system of biological membranes.

Due to the great importance of ion transport processes in the cell and lack of agreement in assessing the influence of organophosphorus agents on these processes the undertaken investigations need further study.

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WPŁYW INSEKTYCYDÓW FOSFOROORGANICZNYCH NA WYPŁYW WAPNIA, POTASU I CHLORU Z IZOLOWANYCH LIMFOCYTÓW ŚWINI

Badano, stosując technikę znakowania izotopowego, uwalnianie jonów potasu, wapnia i chloru z limfocytów świni *in vitro* pod wpływem związków fosforoorganicznych używanych jako insektycydy. Dwa spośród stosowanych związków, mianowicie metyloparation i metylobromfenwinfos powodowały znaczące (często przekraczające 20%) zmiany w wielkości wypływu, podczas gdy pozostałe dwa: malation i dichlorfos oddziaływały na wypływ w mniejszym stopniu. Fenomenologiczny charakter przeprowadzonego eksperymentu nie pozwalał na sformułowanie wniosków dotyczących mechanizmów leżących u podstaw obserwowanych zmian. Otrzymane rezultaty stanowią rozszerzenie o niektóre jony wcześniejszych stwierdzeń dotyczących zmian w transporcie kwasów nukleinowych i enzymów nukleolitycznych poprzez błony limfocytów pod wpływem insektycydów fosforoorganicznych.