BIOELECTRICAL EFFECTS OF SOME PESTICIDES ON FROG'S STRIATED MUSCLE FIBERS

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Abstract: The bioelectrical effects induced by the Oleokalux insecticide, Oltisan Extra herbicide and Tilt fungicide, respectively, on frog's striated muscle fiber have been studied, the membrane potential being determined by the method of glass intracellular microelectrodes. Although the concentrations taken into study have been similar to those employed in farming practices, it was observed that such pesticides cause – as a function of their nature and concentration – obvious bioelectrical effects. In low concentrations, that is of 0.15 mg% and 0.75 mg% for Oeokalux and, respectively, of 0.25 mg% and 1 mg% for Tilt, they evidence a relatively, low effects while, in higher concentrations (1.5 mg% and 3 mg%, respectively), they cause ample membranary depolarizations. Instead, Oltisan provokes ample depolarizations at all concentration values taken into study (0.25, 1 and 2 mg%). Such effects induce modification of both supramolecular structure and cell membranes' permeability.

INTRODUCTION

The integrated combat of pests and weeds assumes controlled utilization of certain chemical agents. However, their positive action is accompanied by some negative effects, manifested equally on cultured plants, animals and humans (Căpăţână et al., 2002; Cîmpeanu and Cîmpeanu, 2004; Neacşu et al., 1989).

The present paper discusses the effects of such agents at the level of the animal cell, by the determination of the membrane potential - as correlated with the structure and normal functions of the cell membrane (Neacşu et al., 1996).

MATERIALS AND METHODS

The experiments performed assumed determination of the membrane potential on striated fibers of sartorius muscle, taken over from 5 different frog's, by the method of the glass intracellular microelectrodes.

The bioelectrical effect of three pesticides has been studied – three different concentrations being utilized for each substance, one similar to that usually applied in farming practices, a second, smaller one, and a third one, higher than the previous value, as follows: insecticide Oleokalux – 0.15, 0.75 and 1.5 mg%, herbicide Oltisan Extra – 0.25, 1 and 2 mg% and fungicide Tilt 250 EC – 0.25, 1 and 3 mg%, (Oltchim S.A.,1995) each of them dissolved in physiological Ringer solution.

The statistical evaluation of the results was made by the Student test.

RESULTS AND DISCUSSIONS

Treatment of the striated muscle fibers with the pesticides selected for the study causes modifications in the cell membranes' resting electrical charge, the directions and amplitudes of which depend on the nature and concentration of the substances – on one hand – and on the treatment's duration, on the other.

Thus, in low concentrations (0.15 and 0.75 mg%) similar to those currently applied in farming practices, the Oleokalux insecticide provokes a slight increase of the membrane potential (with 1.66% and respectively, 1.78% versus the initial normal value – NRP), after 10-60 minutes of treatment. Such effect is nevertheless reversible, the potential returning to its normal value through muscles' washing with normal physiological Ringer solution (NR), without any agents (Table 1).

At higher concentrations (1.5 mg%), Oleokalux provokes instead lowering of the membrane potential (depolarization), which gets intensified in time 10.70 mVat 60 minutes), yet again reversible when the muscles are brought back in NR without agent (Table 1).

The Oltisan Extra herbicide determines intense depolarizations of the membrane's muscle fibers for all the three concentrations considered (0.25, 1 and 2 mg%), although the first two concentrations are considered as inoffensive, being utilized in farming treatments. A 2 mg% concentration induces an ample depolarization as early as the first 10 minutes (a 30.70 mV amplitude), a slight intensification, up to 34.87 mV being noticed after 60 minutes of experiment (Table 1). However, the phenomenon is reversible on washing the muscle fibers with Ringer without agent, the recovery to the potential's initial value being nevertheless very low in experiments made with a 2 mg% concentration, which is indicative of a strong interaction of the agent with the membrane's structure, caused by their different chemical characteristics.

The Tilt fungicide evidences an apparently similar action to that of the Oleokalux insecticide (Table 1).

Thus when low concentrations are employed (0.25 mg% and 1 mg%), weak effects are recorded while, at higher concentration value (3 mg%), a quite ample depolarization occurs , i.e., of 10.39 mV at 30 minutes, and of 15.54 mV at 60 minutes, which is reversible when the muscle fibers are brought back in a normal Ringer solution without agent.

However certain differences are to be noticed between the effects of the two agents, probably determined by their different structure and chemical properties. Thus, when applied in lower concentrations, Tilt causes a slight depolarization, while Oleokalux a slight hyperpolarization (Table 1). Yet, on muscle fibers' washing with Ringer without agents, it is observed that the Tilt product had been tightly bond in the structure of the cell membrane, as a result of its lipophily and, with its release off the membrane, thus lower concentrations being attained it causes a slight membrane hyperpolarization , which is maintained for 60 minutes after muscles' transfer in normal Ringer. As a matter of fact, such effect is observable, too, at a concentration of 3 mg% when, after the disappearance of depolarization, an obvious hyperpolarization is installed (Table 1).

Although all the three substances under consideration are lipophilic, their chemical structure is different (Oltchim S.A.,1995; Worthing and Walder, 1983) which explains the differences between their membranary effects. Thus, the Oleokalux insecticide contains Quinalphos, an organophosphoric product (phosphorothyonat), which penetrates the cells easily and inhibits some enzymes,(Huma et al.,2003; Oltchim S.A.,1995), while fungicide Tilt contains Propiconazol – a substance interacting in a specific manner with the sterols from the cell membranes' structure (Oltchim S.A., 1995) The herbicide Oltisan Extra contains two substances which act in a complementary way (Oltchim S.A.,1995), namely Dicamba and 2,4 D (phenoxyacetic derivative) Both substances, which are rapidly absorbed in the organism, show stimulating or inhibiting effect – as depending on the applied dose – on, different biological processes (Neacşu et al.,1989; Oltchim S.A.,1995).

TABLE 1 – Bioelectrical effects of pesticides: mean amplitude (- mV) ± ES and percent values from normal resting potential (NRP), (+) = hyperpolarization, (-) = depolarization, (*) = significant (n = 25).

AGENT	CONC (mg%)	NRP±ES (- mV)	EFECT	AGENT			NORMAL RINGER		
				MINUTES			MINUTES		
				10	30	60	10	30	60
OLEO-KALUX	0.15	90.50 ± 0.61	± mV	+ 1.75	+ 1.25	+ 1.50	+ 0.42	+ 0.41	- 0.38
			%NRP	1.93	1.38	1.66	0.46	0.45	0.41
	0.75	91.25 ± 0.39	± mV	- 1.67	+ 1.58	+ 1.63	+ 0.25	0	- 0.05
			%NRP	1.83	1.73	1.78	0.27	0	0.05
	1.50	89.70 ± 0.63	± mV	- 0.12	- 2.62*	- 10.70*	-1.70	- 0.20	- 0.37
			%NRP	0.13	2.92	11.93	1.89	0.22	0.41
OLTI-SAN	0.25	90.10 ± 0.77	± mV	- 0.10	- 5.52*	- 15.22*	- 0.27	- 0.18	+ 0.20
			%NRP	0.11	6.13	17.23	0.30	0.20	0.22
	1	89.50 ± 0.65	± mV	- 2.15	- 9.94*	- 13.62*	- 5.48*	1.80	+ 1.10
			%NRP	2.40	11.10	15.21	6.12	2.01	1.22
	2	90.10 ± 0.97	± mV	- 30.70*	- 33.68*	- 34.87*	- 17.02*	- 12.52*	- 5.54*
			%NRP	33.41	37.38	38.70	18.89	13.89	6.15
TILT	0.25	89.35 ± 0.85	± mV	- 1.10	- 0.35	- 0.94	+0.98	+ 3.15*	+ 1.52
			%NRP	1.23	0.39	1.05	0.99	3.52	1.70
	1	89.20 ± 0.77	± mV	+ 1.35	- 0.30	- 1.70	+ 0.30	+ 1.30	+ 2.00
			%NRP	1.52	0.34	1.90	0.34	1.45	2.24
	3	92.05 ± 0.55	± mV	- 0.55	- 10.39*	- 15.54*	- 5.97*	- 3.39*	+ 4.41*
			%NRP	0.59	11.29	16.88	6.48	3.68	4.46

Our experimental results put into evidence the fact that the membranotropic action specific to all the three pesticides consists of the depolarization of the striated muscle fibers' membrane, especially at higher concentrations of the substance. The depolarizing effect involves a series of correlations between the agents and the structure of the cell membrane – the passive and active transmembranary transport, and the activity of the Na^+-K^+ pump (Neacşu et al.,1996).

Thus membrane's depolarization result from the decrease of the packing degree of the membrane structure, concomitantly with the increase of membrane's fluidity and labilization, which induces increase of passive permeability, lowering of the ionic gradients and membrane's depolarization. As a result, modification of Na^+-K^+-ATP-ase's activity and that of the active ionic transport occurs. Elucidation of the inner mechanism of action of such pesticides at the cell level assumes additional, extended investigations for the establishment of some suitable protection methods, in parallels with the reduction of their toxicity.

CONCLUSIONS

The pesticides considered in the study evidence pronounced membranotropic actions at the level of striated muscular cells, although the concentrations applied had been similar to those currently utilized in farming practices.

The characteristic membranary effect consists in the depolarization of the striated muscle fibers' membrane, its amplitudes depending on the substance's nature and concentration. The Oltisan Extra herbicide evidences the strongest effects, while the fungicide Tilt and the insecticide Oleokalux have weaker, although obvious effects – especially at high concentrations.

Membrane's depolarization involves the influence of pesticides on the structure and on the passive and active membrane permeability, too, which may be a component of their specific action currently utilized in practice – as well as of their toxic effects on the animal organism.

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