ACETYLCHOLINESTERASE ACTIVITY OF RAT LYMPHOSIDES DURING PESTICIDE POISONING

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The extensive use of pesticides in agriculture has led to the emergence of the problem of the harmful action of these substances on human health. Among mechanisms of the lowering of resistance and immunodepression in human subjects in contact with pesticides, the toxic action of these substances on internal organs, disturbance of metabolism, and disturbance of the mechanisms of neurohumoral regulation of the protective responses of the body are regarded as particularly important [1, 3]. It has recently been discovered that human lymphocytes contain, not only receptors for acetylcholine on their membrane, but also the enzyme acetylcholinesterase AChE [8]. These observations indicate that acetylcholine, released by nerve endings in the microenvironment of lymphoid cells, is a natural factor regulating activity of lymphoid cells, and that the AChE of lymphocytes is a factor limiting the immunomodulating action of the neurotransmitter. As a result of this, the question has arisen of the possible connection between the immunosuppressive effect of pesticides and their acetylcholinesterase activity and with disturbance of the physiological mechanisms of cholinergic regulation of functions of cells belonging to the immune system of the body.

The aim of this investigation was to study the effect of chronic administration of the widely used organophosphorus pesticide Anthio (formothion) on AChE activity of rat lymphocytes in the primary and secondary lymphoid organs.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats (from Rappolovo nursery) weighing 180-240 g. For 2 months the animals were given perorally by means of a tube a solution of the pesticide Anthio in doses of 3.5 mg/kg daily and 17.5 mg/kg daily (0.01 and 0.005 LD_{50}, respectively). The control animals were given an equal volume of the solvent at the same time. The structure of Anthio is as follows:

In the original form the substance does not possess acetylcholinesterase activity, but in the body it readily undergoes oxidative desulfuration and, when converted into a P—O derivative, it becomes a powerful AChE inhibitor [3].

At the end of the period of poisoning, the animals were killed and the thymus and spleen removed. Suspensions of lymphocytes were obtained by the usual methods (mild pressing of the organ, filtration through a Kapron filter, centrifugation in a Ficoll—Verografin density gradient with relative density 1.090, washing the suspension in cold phosphate-buffered saline, pH 7.2). The number of lymphocytes in the suspension was counted. The suspensions of lymphocytes from the experimental and

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TABLE 1. Acetylcholinesterase (AChE) and Nonspecific Esterase (NSE) Activity of Lymphocytes from Rat Thymus and Spleen following Chronic Administration of Anthio (ncat/10^9 cells, $M \pm m$)

Preparation and dose	Thymocytes		Splenocytes	
	AChE	NSE	AChE	NSE
Control	1,30±0,30 (n=10)	15,5±4,1 (n=10)	29,7±5,3 (n=11)	67,3±8,5 (n=11)
Anthio 3.5 mg/kg/day	0,90±0,30 (n=9)	10,8±2,8 (n=9)	15,2±2,2* (n=9)	44,4±9,1 (n=9)
17.5 mg/kg/day	0,74±0,18 (n=9)	10,6±1,3 (n=9)	11,9±1,6* (n=9)	48,3±4,9 (n=9)

Legend. *) Statistically significant ($p < 0.05$) difference from control, n) number of animals tested.

control animals were then homogenized in Potter's homogenizers. Activity of AChE in the lymphocyte homogenate was determined separately, using acetylcholine as the substrate [5], from that of nonspecific esterases (NSE), including carboxylesterase, for which the substrate was *p*-nitrophenyl acetate [4]. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Chronic poisoning of the rats with the organophosphorus pesticide Anthio in doses of 0.01 and 0.05 LD_{50} had no effect on the general state or behavior of the animals, and the experimental rats gained weight just like the control animals. All the rats taken in the experiment survived until its end. Determination of AChE in lymphocyte homogenates revealed a significant difference in the activity of this enzyme in cells of the thymus and spleen, for AChE activity of the splenocytes was 15-20 times greater than that of the thymocytes. Determination of lymphocyte NSE revealed smaller differences between the organs, for NSE activity of the splenocytes on average was 4 times higher than in thymocytes (Table 1).

The causes of the difference in AChE activity of lymphocytes from the thymus and spleen are not yet clear. Higher enzyme activity in spleen cell homogenates evidently has little to do with contamination by erythrocytes, for calculations showed that this contamination in the suspensions could give rise only to an increase in AChE activity not exceeding 1.5%. Most probably the difference was due to the fact that the primary and secondary organs of immunogenesis, namely the thymus and spleen, contain different lymphocyte populations. We know that 95% of thymocytes are immature T lymphocytes, and that splenocytes are a mixture of mature T- and B-lymphocytes [2]. It can accordingly be postulated that high AChE activity in spleen cell homogenates is due to the presence of B-lymphocytes in the suspension. On the other hand, splenic T lymphocytes, unlike thymocytes, are mature immunocompetent cells, and in the spleen they established contact with antigen and become activated. The possibility cannot therefore be ruled out that both maturation and activation of T cells may be accompanied by an increase in AChE activity on the membrane of these cells. The need to take this possibility into account is demonstrated by data showing that maturation or activation of T lymphocyte by mitogens or antigens leads to an increase in the number of muscarinic acetylcholine receptors on their membrane [6, 7]. Thus the available evidence suggests that the higher AChE activity of splenocytes may be connected with the presence of E lymphocytes,of mature T lymphocytes, or of activated T lymphocytes in the spleen, evidence of differences in ACh activity in different lymphocyte subpopulations. This problem requires appropriate investigation. Under the influence of Anthio AChE activity of the splenocytes was reduced, whereas for thymocytes only a tendency for the activity of this enzyme to decrease was observed (Table 1). Incidentally, higher doses of the preparation caused more marked inhibition of AChE of the splenic lymphocytes. As regards NSE, poisoning with the pesticide depressed activity of these enzymes a little (not statistically significantly).

The experiments thus revealed acetylcholinesterase activity in rat lymphocytes and showed that AChE activity differs significantly in lymphoid cells of different populations. The dose-dependent inhibition of AChE in splenic lymphocytes observed during chronic administration of the organophosphorus pesticide Anthio suggests the existence of a hitherto unknown mechanism of the immunodepressive effect of pesticides, realized through involvement of these preparations in the processes of neurotransmitter control over functions of lymphoid cells.

LITERATURE CITED

1. F. Kaloyanova-Simeonova, Pesticides. Toxic Action and Prophylaxis [in Russian], Moscow (1980).

2. R. V. Petrov, Immunology and Immunogenetics [in Russian], Moscow (1976).
3. V. I. Rozengart and O. E. Sherstobitov, Selective Toxicity of Organophosphorus Insecticides and Acaricides. Comparative Biochemical Aspects [in Russian], A. P. Brestin (ed.), Leningrad (1978).
4. Z. Telyabaev, Biokhimiya, **44**, 2083 (1979).
5. G. M. Ellman, K. D. Courtney, and V. Anders, Biochem. Pharm., **7**, No. 1, 88 (1961).
6. W. Maslinski, M. Kullberg, O. Nordström, and T. Bartfai, J. Neuroimmunol., **17**, 265 (1988).
7. T. B. Strom, M. A. Lane, and K. George, J. Immunol., **127**, 705 (1981).
8. J. Szelenyi, P. Paldy-Haris, and S. Hollan, Immunol. Lett., **16**, 49 (1987).