EFFECT OF DIPYROXIME ON CONTENT OF NICOTINAMIDE COENZYMES AND ADENINE NUCLEOTIDES IN THE MYOCARDIUM AND LIVER OF RATS POISONED WITH PHTHALAPHOS

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Experiments on rats showed that phthalaphos inhibits the blood cholinesterase activity by 68.6% and reduces the concentration of nicotinamide coenzymes in the myocardium on account of a decrease in the content of oxidized forms. In the liver of these animals phthalaphos reduces the concentration of both oxidized and reduced forms of nicotinamide coenzyme and lowers the level of adenine nucleotides chiefly on account of ATP. Dipyroxine prevents the changes produced by phthalaphos and reactivates the blood cholinesterase up to 47.5%. It is suggested that the ability of dipyroxime to restore the normal action of oxidative processes in the cells through its action on the nicotinamide coenzymes and adenine nucleotides is a feature of the mechanism of its antidotal action.

KEY WORDS: phthalaphos; dipyroxime; antidote.

Much evidence has recently accumulated to show that the antidotal effect of cholinesterase reactivators in poisoning by organophosphorus compounds is a complex phenomenon and is due not only to restoration of the enzyme activity [4].

There is evidence of absence of correlation between the degree of restoration of the inhibited cholinesterase and the state of function of individual organs [3].

The nicotinamide coenzymes play an important roles in the transport of electrons and protons from oxidation substrates to oxygen in the respiratory chain, and also in ATP synthesis [5, 7, 9, 10]. Many drugs and also certain poisons affect the level of these components of the respiratory chain in the tissues of animals [1, 6, 8, 11, 12].

TABLE 1. Changes in Blood Cholinesterase Activity and Content of Nicotinamide Coenzymes in Myocardium and Liver of Rats 24 h after Poisoning with Phthalaphos

Conditions	Number of rats	Statistical index	Blood cholinesterase activity, %	Content of coenzymes, μg/g wet weight of tissue						$K=\frac{NAD+NADP}{NADH+NADPH}$	
				NAD+NADP		NADH+NADPH		total of oxidized and reduced forms			
				myocardium	liver	myocardium	liver	myocardium	liver	myocardium	liver
Control	12	M	100,0	354	345	226	255	580	600	1,56	1,35
		$\pm m$	9,6	15,2	21,0	10,0	13,0	9,0	15,0	0,10	0,09
Dipyroxime	12	M	100,8	411	367	199	260	610	627	2,06	1,41
		$\pm m$	7,8	19,0	10,1	8,7	17,0	21,6	23,7	0,10	0,07
		P	>0,1	<0,05	>0,1	<0,05	>0,1	>0,1	>0,1	<0,01	>0,1
Phthalaphos	10	M	31,4	267	285	229	209	496	494	1,16	1,36
		$\pm m$	5,6	13,7	15,0	12,0	5,1	8,7	16,8	0,12	0,10
		P	<0,001	<0,01	<0,01	>0,1	<0,05	<0,01	<0,01	<0,05	>0,1
Phthalaphos+ dipyroxime	12	M	47,5	363	326	218	225	581	551	1,66	1,45
		$\pm m$	7,1	10,6	18,6	9,0	12,0	17,0	24,0	0,08	0,09
		P	<0,01	>0,1	>0,1	>0,1	>0,1	>0,1	>0,1	>0,1	>0,1

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The object of this investigation was first, to study the character of action of the cholinesterase inhibitor phthalaphos and of the cholinesterase reactivator dipyroxime on the content of oxidized and reduced forms of the nicotinamide coenzymes and adenine nucleotides in the myocardium and liver of animals, and second, to discover the correlation between the degree of reactivation of cholinesterase by dipyroxime and restoration of the level of the above-mentioned components of the respiratory chain in animals poisoned with phthalaphos.

EXPERIMENTAL METHOD

Experiments were carried out on 82 male albino rats weighing 180-250 g. Phthalaphos (70 mg/kg), in the form of a 2% emulsion in sunflower oil, was injected by gastric tube in a single dose. Dipyroxime (10 mg/kg), as a 1% aqueous solution, was injected into the thigh muscle 10-15 min after poisoning.

All the rats were decapitated 24 h after poisoning and the content of the nicotinamide coenzymes (NAD + NADP and NADH + NADPH) in the myocardium and liver [13] was determined. The total content of the coenzymes was calculated as the sum of the oxidized and reduced forms, and the ratio between them was expressed as the coefficient K. The content of adenine nucleotides (ATP, ADP, and AMP) in the organs frozen in liquid nitrogen, and ground into a powder, was determined by electrophoresis in paper followed by spectrophotometry at wavelengths of 260 and 290 nm and expressed in μmoles/g wet weight of tissue [2]. Cholinesterase activity was determined by Hestrin's method in blood obtained from the rats at decapitation. A group of intact animals was used as the control.

EXPERIMENTAL RESULTS

On the first day after injection of phthalaphos a clinical picture of acute poisoning developed and some of the animals (16.6%) died; the blood cholinesterase activity fell by 68.6%. Phthalaphos also changed the content of nicotinamide coenzymes in the tissues (Table 1). The level of oxidized forms in the myocardium was decreased by 24.6%, the total oxidized and reduced forms fell by 14.5%, and the coefficient K fell by 25.7%. In the liver, under the influence of phthalaphos, there was a decrease in the content of both oxidized (by 17.4%) and reduced forms (by 18.1%), and also in the total content of nicotinamide enzymes (by 17.7%).

All the rats treated with dipyroxime after poisoning survived. The content of nicotinamide coenzymes in the organs of these animals did not differ significantly from the corresponding values in intact rats. Meanwhile, the blood cholinesterase activity of these animals was reduced by more than 50%.

It is an interesting fact that dipyroxime led to an increase in the content of oxidized forms of nicotinamide coenzymes in the myocardium and that a tendency also was observed toward an increase in the oxidized and reduced forms in the liver of the intact animals. Phthalaphos lowered the ATP level in the liver of the animals by 39.7% and the total adeninenucleotides by 20%. Dipyroxime had a normalizing effect on the content of adenine nucleotides in the liver of rats poisoned with phthalaphos.

The experimental results described above are evidence of the ability of the organophosphorus cholinesterase inhibitor phthalaphos to influence not only the content of NAD + NADP and of NADH + NADPH, but also the ratio between them. As a result, evidently, ATP synthesis is disturbed.

The antidotal effect of dipyroxime in this form of poisoning is due to restoration of normal oxidation–reduction processes in the body. This is confirmed by the increased level of nicotinamide coenzymes in the tissues of the intact rats and also by the more marked normalizing action of dipyroxime on the content of nicotinamide coenzymes and adeninenucleotides in the tissues of the animals compared with its cholinesterase reactivation.

The facts obtained add to existing information on the mechanism of the toxic action of organophosphorus compounds on the body and they justify the search for new pharmacological agents for the treatment of poisoning by these compounds among substances capable of normalizing oxidation–reduction processes in the body and, in particular, substances with a beneficial effect on the components of these respiratory chains. The results of these experiments also add further details to the mechanism of the antidotal effect of cholinesterase reactivators. With their proven ability to normalize the key stages of energy metabolism, dipyroxime and other substances of this group may prove useful in acute poisoning by other chemical compounds accompanied by a disturbance of oxidation–reduction processes.

LITERATURE CITED

1. S. M. Alekhina, "Metabolism of nicotinamide coenzymes, activity of NAD-dependent drhydrogenases and their isozyme spectrum during exposure to organochlorine pesticides," Author's Abstract of Candidate's Dissertation, Kiev (1975).
2. G. V. Voskoboinikova, Biokhimiya, No. 5, 1041 (1966).
3. E. V. Gembitskii, E. A. Moshkin, and G. V. Maksimov, Voen. Med. Zh., No. 10, 49 (1970).
4. S. N. Golikov and S. D. Zaugol'nikov, Cholinesterase Reactivators [in Russian], Leningrad (1970).
5. A. Lehninger, Biochemistry, Worth, New York (1970) [Russian translation; Moscow (1974)].
6. M. V. Natsyuk and I. S. Chekman, Byull. Éksp. Biol. Med., No. 4, 58 (1975).
7. V. L. Nemchinskaya, V. M. Bozhkov, and V. P. Kushner, Tsitologiya, No. 7, 799 (1971).
8. I. I. Pavlova, "Special features of metabolism of nicotinamide coenzymes (NAD and NADP) in cellular structures of the tissues of warm-blooded animals under the influence of DDT and Sevin (the mechanism of action of DDT and Sevin)," Candidate's Dissertation, Kiev (1970).
9. S. E. Severin, V. I. Telepneva, and L. A. Tseitlin, in: Chemical factors in the regulation of activity and biosynthesis of enzymes [in Russian], Moscow (1969), p. 118.
10. L. A. Tseitlin, in: Progress in Biological Chemistry [in Russian], Vol. 8, Moscow (1967), p. 249.
11. I. S. Chekman, N. N. Potemkina, and V. A. Tumanov, Farmakol. Toksikol., No. 1, 113 (1977).
12. J. G. Farekas and B. Reingei, Enzymologia, 35, 263 (1968).
13. J. W. Huff and W. A. Perlzweig, J. Biol. Chem., 167, 157 (1947).