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EFFECT OF PESTICIDES ON THE ACTIVITY OF MONOAMINE OXIDASE (MAO) IN RATS

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ABSTRACT

Male Wistar rats were orally administered with 20 different pesticides in equitoxic doses of the active ingredients (1/10 LD_{50}) daily for 6 days. MAO activity was determined in the brain and liver homogenates with kynuramine. A significant inhibition was found after the application of four insecticides (lindane, chlordimeform, kelthane, decis), six fungicides: imugan, karathane, benlate, nimrod, bayleton, zineb (in the liver) and two herbicides: atrazine and balan (in the brain). MAO activity in the brain increased as a result of the administration of unden, atrazine, zineb and in the liver as a result of the administration of balan. Behavioural manifestations in workers occupationally exposed to pesticides might prove to be related to some disturbances in biogenic amine metabolism.

Recent data have suggested the influence of certain pesticides on monoamine metabolism in experimental animals. Studies mainly on brain biogenic amines have been carried out after lindane[7], dieldrin[6,10], paraquat[3] and carbaryl[12] intoxications. Beeman and co-workers[2] have shown that chlordimeform inhibits MAO activity in the liver of rats. These data have been simultaneously but independently confirmed by Aziz and co-workers[1] *in vitro*.

The objective of the present study was to determine possible changes in MAO activity after repeated treatment with various widely used pesticides. Patterns of nearly all chemical groups were chosen.

MATERIAL AND METHODS

Male Wistar rats (180–220 g) were orally administered with equitoxic doses of the active ingredients – about 1/10 LD_{50}, daily for 6 days. The respective commercial formulations (20–50–75 per cent active substances), were used. The corresponding control rats were given the vehicle only. Every group consisted of 7 to 12 animals. The pesticides applied are shown in Table 1.

Two hours after the sixth application, MAO activity in the brain and liver tissues was determined by the method of Weissbach and co-workers[11] with kynuramine dihydrobromide (Sigma Chem. Ltd, USA), a substrate deaminated

TABLE 1
Pesticides applied for studying brain and liver MAO activity in rats.

Trade and/or common names	Active ingredients	Acute LD_{50} for rats (mg/kg)	Dose applied (mg/kg)
INSECTICIDES			
Lindane	gamma hexachlorocyclohexane	100 – 125	10
Actellic (pirimiphos-methyl)	0,0-dimethyl-0-(2-diethyl amino-6-pyrimidyl--4)-thiophosphate	2050	205
Polycron	0-ethyl-0-(2-chlor-4-bromphenyl)-8 (n)-propylphosphorothionate	100	10
Naled (Bromex, Dibrom)	0.0-dimethyl-0-(1,2-dibrom-2,2--dichloroethyl) phosphate	430	22
Unden (Baygon)	2-isopropoxy-phenyl-N-methyl carbamate	100 – 200	12
Padan (cartap)	1.3-bis (carbamoylthio/-2-/N,N-dimethyl) aminopropan hydrochloride	380	40
Decis (decamethrine)	(S)-alfa-cyano-m-phenoxybenzyl (1R, 3R)-3 -(2,2-dibromovinyl)-2,2-dimethylcyclopropan carboxylate	400	40
Chlordimeform (Galecron)	N-(4-chloro-2-methylphenyl)-N,N dimethyl formamidine	340 – 420	42
Kelthane (dicofol)	1,1-di (4-chlorphenyl)-2,2,2-trichloroethanol-1	730	75
FUNGICIDES			
Imugan	N-(2,2,2-trichlor-3,4-dichlorphenylamino) ethyl formamide	2500	250
Karathane (Mildex)	2,4-dinitro-6-F-octylphenyl-crotonate	766	76
Zineb	Zn-N,N-ethylene bisdithiocarbamate	5200	520
Benlate (Fundasol)	N-(1-butylcarbamidobenzimidazolyl-2-)--0-methylcarbamate	9950	900
Nimrod	5-butyl-2-ethylamino-6-methylpyrolidine-4-yl--dimethylsulfonate	4000	400
Bayleton (triadimeform)	1-chlorphenoxy (-3,3-dimethyl-1,2,3 triazolyl)--2-butanone	568	50
HERBICIDES			
Atrazine (Ceazine)	2-chlor-4-ethylamino-6-isopropyl amino symtriazine	2200	220
Propachlor (Ramrod)	N-isopropylanilide	1400	140
Balan (benefin)	4-trifluormethyl-2,4-dinitro-N-ethyl--N-buthyl aniline	1200	120
Paraquat (Gramoxone)	1,1-dimethyl-4,4-dipyridylium dichloride	100	10
Diquat (Reglone)	1,1-ethylene-2,2-dipyridylium dibromide	140	14

by both the A and B forms of mitochondrial MAO[8]. The incubation mixture contained kynuramine dihydrobromide $2.3 \cdot 10^{-4}$ M, potassium-sodium phosphate buffer pH 7.4 (0.09 M), and 13 times dilluted 1 : 4 tissue homogenate. The samples were exposed to oxygenated air and incubated continuously shaken for

TABLE 2

Monoamine oxidase activity in brain and liver homogenates (μM/min/g tissue-A) or μM/min/g protein-B) in control (upper line) and experimental (lower line) group ($\bar{X} \pm$ S.E.$\bar{X}$).

	Brain		Liver	
	A	B	A	B
Lindane	0.198 ± 0.014^{d}	3.01 ± 0.38^{c}	0.548 ± 0.041^{d}	4.05 ± 0.48^{c}
	0.107 ± 0.029	1.38 ± 0.33	0.260 ± 0.023	1.67 ± 0.17
Actellic	0.198 ± 0.014	3.01 ± 0.38	0.548 ± 0.041	4.05 ± 0.48
	0.233 ± 0.028	2.78 ± 0.42	0.423 ± 0.029	3.11 ± 1.27
Polycron	0.198 ± 0.014	3.01 ± 0.38	0.548 ± 0.041	4.05 ± 0.48
	0.211 ± 0.008	2.75 ± 0.27	0.490 ± 0.033	3.37 ± 0.37
Naled	0.204 ± 0.020	2.76 ± 0.26	0.610 ± 0.040	4.33 ± 0.31
	0.244 ± 0.020	2.56 ± 0.02	0.699 ± 0.030	5.10 ± 0.30
Unden	0.198 ± 0.014	3.01 ± 0.38^{a}	0.548 ± 0.041^{c}	4.05 ± 0.48^{a}
	0.214 ± 0.024	3.65 ± 0.51	0.783 ± 0.086	5.47 ± 0.56
Padan	0.198 ± 0.014	3.01 ± 0.38	0.548 ± 0.041	4.05 ± 0.48
	0.197 ± 0.006	2.78 ± 0.17	0.603 ± 0.054	4.39 ± 0.04
Decis	0.356 ± 0.039^{a}	3.71 ± 0.59	0.535 ± 0.058^{a}	3.83 ± 0.42
	0.235 ± 0.012	3.21 ± 0.28	0.428 ± 0.038	3.73 ± 0.33
Chlordimeform	0.204 ± 0.020	2.76 ± 0.26	0.610 ± 0.040^{d}	4.33 ± 0.31^{d}
	0.187 ± 0.020	2.19 ± 0.22	0.312 ± 0.030	2.13 ± 0.20
Kelthane	0.204 ± 0.020	2.76 ± 0.26	0.610 ± 0.040^{b}	4.33 ± 0.31^{b}
	0.170 ± 0.010	3.48 ± 0.44	0.372 ± 0.020	2.72 ± 0.25
Imugan	0.204 ± 0.020	2.76 ± 0.26	0.610 ± 0.040	4.33 ± 0.31^{a}
	0.200 ± 0.080	2.56 ± 0.48	0.500 ± 0.040	3.29 ± 0.26
Karathane	0.204 ± 0.020	2.76 ± 0.26^{a}	0.610 ± 0.040	4.33 ± 0.31
	0.176 ± 0.021	1.85 ± 0.18	0.616 ± 0.060	3.82 ± 0.36
Zineb	0.204 ± 0.020	2.76 ± 0.26^{b}	0.610 ± 0.040^{a}	4.33 ± 0.31^{a}
	0.242 ± 0.050	4.02 ± 0.28	0.430 ± 0.020	3.31 ± 0.20
Benlate	0.204 ± 0.020^{a}	2.76 ± 0.26	0.610 ± 0.040^{d}	4.33 ± 0.31^{b}
	0.140 ± 0.020	2.21 ± 0.27	0.306 ± 0.040	2.43 ± 0.44
Nimrod	0.356 ± 0.039	3.71 ± 0.59	0.480 ± 0.058	3.56 ± 0.42^{a}
	0.253 ± 0.035	2.60 ± 0.39	0.437 ± 0.017	2.18 ± 0.20
Bayletqn	0.356 ± 0.038^{b}	3.71 ± 0.59^{a}	0.535 ± 0.058^{a}	3.83 ± 0.42^{b}
	0.257 ± 0.036	2.84 ± 0.50	0.480 ± 0.058	3.02 ± 0.40
Atrazine	0.356 ± 0.039	3.71 ± 0.59^{b}	0.480 ± 0.058^{b}	3.56 ± 0.42^{a}
	0.317 ± 0.020	3.11 ± 0.41	0.668 ± 0.016	4.46 ± 0.20
Propachlor	0.198 ± 0.014	3.01 ± 0.38	0.548 ± 0.041	4.05 ± 0.48
	0.191 ± 0.017	3.51 ± 0.52	0.481 ± 0.243	4.02 ± 0.24
Balan	0.356 ± 0.039^{a}	3.71 ± 0.59	0.480 ± 0.058^{b}	3.56 ± 0.42
	0.274 ± 0.020	2.61 ± 0.24	0.760 ± 0.052	4.56 ± 0.25
Paraquat	0.198 ± 0.014	3.01 ± 0.38	0.548 ± 0.041	4.05 ± 0.48
	0.154 ± 0.014	2.40 ± 0.16	0.434 ± 0.033	3.75 ± 0.28
Diquat	0.356 ± 0.039	3.71 ± 0.59	0.480 ± 0.058	3.56 ± 0.42
	0.322 ± 0.048	3.83 ± 0.59	0.535 ± 0.100	4.20 ± 0.83

a = $p < 0.05$; b = $p < 0.01$; c = $p < 0.0025$; d = $p < 0.001$

10 min at 37°C. The reaction was stopped by a deproteinizing solution (mixed after thorough solvation of 1 l of bidistilled water with 23 ml of concentrated sulfuric acid p.a., and 2.5 ml of 80 per cent orthophosphoric acid p.a. and 1 l of bidistilled water containing 50 g sodium tungstate, p.a.) in a 1 : 3 ratio of the total mixture. The samples were mixed, centrifuged, and the optical density was read at 360 nm and compared with its own blanks incubated without substrate (added after deproteinization). The protein was determined according to the method of Lowry and co-workers[5]. The calculations in μM kynuramine/min/g tissues or protein were done by calibration with standard solutions of kynuramine dihydrobromide. Statistical analyses were made using the Student's t-test.

RESULTS

The results showing the pesticide influence on MAO activity are presented in Table 2. A significant MAO activity inhibiton was found in both organs tested after lindane and decamethrin administration. Liver homogenate MAO activities were decreased after chlordimeform and kelthane treatment. Repeated administration of unden brought about increased brain and liver MAO activities. No statistically significant changes were revealed after the application of other insecticides involved in the screening test. The fungicide examination suggested that nearly all active ingredients, with the exception of karathane, decreased MAO activity in the liver. As far as MAO activity in the brain was concerned, karathane, benlate, and bayleton showed marked inhibitory properties. MAO activity in brain was enhanced only in rats administered with zineb. Herbicide studies established the same inhibition in the brain of rats given atrazine and balan. Atrazine and balan increased the MAO activity of the liver. In the administered doses the dipyridylium herbicides paraquat and diquat did not affect MAO activity.

DISCUSSION

The problem of the psychical state of workers chronically exposed to pesticides but not seeking medical attendance has given rise to various implications. The behavioural manifestations of organophosphate (OP) toxicity are generally attributed to the decrease of acetylcholinesterase (AChE) activity and the imbalance of the central adrenergic and cholinergic factors. Levin and co-workers[4] have studied psychical manifestations in workers without any obvious signs of OP poisoning. Commercial spraymen demonstrated elevated levels of anxiety, which showed to be occupationally dependent. Cholinesterase has been the only enzyme studied in similar epidemiological investigations. Soboleva[9] has found behavioural deviations, disturbed biogenic amine metabolism and functionally changed arterioles in agricultural workers exposed to chlororganic insecticides and TMTD. The significant deviations of brain and liver MAO activities with substrate kynuramine, referring to the A and B enzyme forms, suggest a possible involvement in the mechanism of anxiety reported among workers using pesticides.

An epidemiological study of greenhouse workers exposed to fungicides and insecticides revealed a lack of deviations in serum ChE, transaminases (GOT, GPT), gamma-glutamiltranspeptidase activities and liver functional tests. The serum MAO activity of persons growing pinks and vegetables proved to be significantly inhibited in comparison with the respective controls. Serum MAO activity determined with substrate kynuramine may be used as an exposure test for pesticides decreasing enzyme activity in experimental conditions[13]. Compilation of more data from workers highly exposed to pesticides in greenhouses would be of importance for practical occupational hygiene.

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