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δ-Aminolevulinic Acid Synthetase and Heme Oxygenase Activity in Lung and Liver of Rats Given DDT and Endosulfan Intratracheally

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Cytochrome P-450, a heme protein is the terminal oxidase of drug metabolizing enzyme system and its levels are regulated by the δ-aminolevulinic acid(ALA)Synthetase and heme oxygenase (Omura et al. 1965, Cooper et al. 1965). The functional capacity of these enzyme systems is affected by a variety of environmental and physiological factors(Maines and Kappas 1977, Campbell and Hayes 1974). A major fraction of the heme synthesized in the liver is utilized for the formation of microsomal cytochrome P-450 (Estabrook et al. 1970). ALA synthetase is the first and the rate limiting enzyme for heme biosynthesis (Granick and Urata, 1963). The ultimate fate of synthesized heme is its degradation by heme oxygenase into bilirubin and other degradation products which are then eliminated from the liver (Tenhunen et al. 1969). In an earlier report it was shown that intratracheal administration of DDT significantly enhanced cytochrome P-450 levels in lung and liver microsomes whereas endosulfan did not (Satya Narayan et al. 1985). To understand the mechanism by which intratracheally administered endosulfan and DDT affect cytochrome P-450 levels of liver and lung, the activities of ALA Synthetase and heme oxygenase have been determined and the results are being communicated.

MATERIALS AND METHODS

Adult male Wistar strain rats were used in the present investigation. They were fed Hind Lever diet(Hindustan Lever Ltd. Bombay, India)ad libitum and had free access of water all the times. The rats were divided into three groups. Group one and two were given DDT (5 mg/100 g body weight) and endosulfan (1 mg/100g body weight) respectively through intratracheal route for three consecutive days. The insecticides were dissolved in minimum volume of ground nut oil. The third group was

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the control and was given only vehicle solution(0.1 ml/ 200 g body weight) intratracheally (Satya Narayan et al. 1985) for the same period. Twenty four hours after last exposure of insecticides rats were fasted over night. They were lightly anaesthetized with diethyl ether and were secured on a board with rubber bands.Their abdominal and thoracic cavities were opened. Lungs were perfused through right ventricle with cold physiological saline. The lung and liver were removed, washed, wiped and weighed. Tissues were homogenized in 3 volumes of ice-cold 0.25 M sucrose separately. The homogenate was centrifuged at 8000 x g for 15 min in a Sorvall refrigerated centrifuge, model RC-5B. The pellet was washed with ice-cold 1.15% KCl, 0.1 M phosphate buffer, pH 7.4 and was used for the assay of ALA Synthetase activity as described by Sassa et al. (1979). The supernatant was centrifuged at 18,000 x g for one hour. The pellet was discarded. The supernatant was diluted 3 times to its original tissue weight and was used for the assay of heme oxygenase according to the method of Tenhunen et al. (1969). The protein was estimated by the method of Lowry et al. (1951) using crystalline bovine serum albumin as standard.

RESULTS AND DISCUSSION

The effects of intratracheal administration of DDT and endosulfan on body weight and organ weight were similar to those reported earlier (Satya Narayan et al. 1985). Intratracheal administration of DDT significantly increased the activity of ALA synthetase in both lung and liver as compared to the untreated controls. In contrast endosulfan administration did not show any effect on the activity of the enzyme, either in lung or in liver (Table 1). These results thus show that the observed increase in the levels of cytochrome P-450 in DDT administered rats (Satya Narayan et al. 1985) appears to be due to its enhanced synthesis by ALA synthetase. Heme oxygenase, which is responsible for the degradation of heme moiety of cytochrome P-450 was significantly reduced by both DDT and endosulfan in lung but in liver its activity was not affected either by DDT or by endosulfan (Table 2). These results thus show that intratracheally administered DDT increased cytochrome P-450 levels in lung by enhancing the activity of ALA Synthetase(Table 1)and inhibiting that of heme oxygenase (Table 2). In liver the increase observed in cytochrome P-450 levels in DDT treated rats appear to arise by increase in the activity of ALA Synthetase (Table 1). Endosulfan,which did not affect the levels of cytochrome

Table 1. Effect of intratracheal administration of DDT and endosulfan on δ-aminolevulinic acid synthetase activity in rat.

	nmol ALA/hr/mg protein	nmol ALA/hr/ organ
	LUNG	
Control (4)	0.031±0.003	0.863±0.085
DDT (6)	0.052±0.004*	1.405±0.117*
Endosulfan (6)	0.025±0.002	1.007±0.070
	LIVER	
Control (5)	0.018±0.002	7.965±0.919
DDT (6)	0.026±0.002*	12.923±0.741*
Endosulfan (5)	0.015±0.001	6.429±0.754

Results are expressed as Mean±SE. The number of samples assayed in each group are given in parenthesis. The lung and liver pellets of 8000 x g were suspended in 2 and 3 volumes of reaction mixture respectively. The reaction mixture contained in micromoles/ml, of glycine, 75; citrate, 75; Tris buffer, pH 7.4, 37.5; phosphate buffer, pH 7.4, 37.5; $MgCl_2$, 15; EDTA, 7.5 and pyridoxal 5'-phosphate, 2.2. Duplicates of 0.6 ml aliquots were incubated at 37°C for 60 min.

*Values significantly different from controls ($P \leq 0.05$).

P-450 either in lung or in liver, also did not affect the activity of ALA synthetase (Table 1),but it decreased the activity of heme oxygenase in lung (Table 2). From these results it can be concluded that endosulfan maintains the levels of cytochrome P-450 by inhibiting its degradation through inhibition of heme oxygenase.DDT, on the other hand, increases cytochrome P-450 levels by both enhancing synthesis and preventing its metabolism. Vila and Deviale (1982) also did not find any change in ALA Synthetase activity in endosulfan-treated chick embryo liver.

Table 2. Effect of intratracheal administration of DDT and endosulfan on heme oxygenase activity in rat.

	nmol bilirubin/ hr/mg protein	nmol bilirubin/ hr/organ
	LUNG	
Control (4)	2.192±0.006	118.178±9.878
DDT (5)	1.558±0.244*	78.946±10.278*
Endosulfan (5)	0.980±0.058*	44.450±1.490*
	LIVER	
Control (4)	0.506±0.040	328.146±3.140
DDT (5)	0.344±0.064	307.140±64.624
Endosulfan (5)	0.452±0.084	279.946±51.276

Results are expressed as Mean±SE. The number of samples assayed in each group are given in parenthesis. The reaction mixture (3.0ml) contained 18000 x g supernatant; hemin, 17 uM; potassium phosphate buffer, pH 7.4, 90mM and NADPH, 180 uM. The samples were incubated at 37°C for 30 min in a metabolic shaker. Absorbance was read spectrophotometrically against the sample blank at 468 nm.

* Values significantly different from controls ($p \leq 0.05$).

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