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Effects of Organochlorine Compounds on Lipid Catabolism of Foetal Rat Liver Mitochondria and Microsomes

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The toxicity and action of organochlorines on intermediary metabolism is not well understood. Because of their lipid solubility, organochlorines in general are thought to interfere with lipid metabolism or membrane structure.

Effects may be rather variable; this is well illustrated in two recent studies. In one investigation it was shown that 3,4,5,3',4',5'-hexachlorbiphenyl increased the triacylglycerol content of adult rat liver 7-fold as compared to control animals but did not have any effect on the hepatic phospholipid concentration (KHOLI et al. 1981). On the other hand the ortho-substituted isomers 2,3,5,2',3',5'-hexachlorbiphenyl and 2,4,5,2',4',5'-hexachlorbiphenyl increased the hepatic phospholipid content but did not affect the triacylglycerol concentration (KOHLI et al. 1981). In another study, isolated leaf cell preparations obtained from kidney bean plants indicated that the aromatic organochlorine herbicides 2,4-dichlorophenoxyacetic acid and chloramben inhibit lipid synthesis as measured by acetate incorporation (ASHTON et al. 1977). This action of these two herbicides is not regarded to be related to their herbicidal action, but is considered as a separate effect on the intermediary metabolism of the cultured cells (ASHTON et al. 1977).

In an attempt to carry out a systematic study, three different types of organochlorines were selected to investigate their effect on lipid metabolism. The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was chosen, as in the classification by MATTHEWS & KATO (1980) it is the typical example of a type I halogenated aromatic compound. These are the most polar of the halogenated aromatic compounds and are therefore rapidly excreted. The insecticide and acaricide N'-(4-chloro-2-methyl-phenyl-N,N-dimethyl-foramidine-HCl (chlordimeform 80 SP) may be classed as a type II halogenated aromatic compound, having an intermediate polarity due to substituents other than organic halogens. Such compounds are eliminated at a slower rate than type I compounds and their excretion largely depends on the rate at which they are metabolized. The industrial solvent 1,1,1-trichlorethane was chosen for its extreme hydrophobicity and therefore its elimination is slower than that of the other two compounds.

The test-systems chosen for the present study were preparations of foetal rat liver mitochondria and microsomes. The catabolism of endogenous triglycerides in these organelles of foetal liver is

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markedly greater than that in liver mitochondria and microsomes of later developmental ages (POLLAK & HARSAS 1981). Comparisons were made between lipid catabolism of foetal rat liver mitochondria and microsomes after the pregnant rats had been exposed to 2,4,5-T or chlordimeform 80 SP or 1,1,1-trichloroethane. Pregnant rats injected with NaCl were used as controls.

MATERIALS AND METHODS

Animals. Random-bred Wistar rats from the University of Sydney Animal House were used throughout this investigation. Foetal rats of known age were obtained from overnight (16 hour) matings.

Injection of pregnant rats. Pregnant rats were injected subcutaneously on the 17th day of their gestation period, as well as on the three subsequent days (between the hours of 11 a.m. and 3 p.m.). The doses of the compounds that were injected were as follows: The free acid of 2,4,5-T (obtained from Ciba-Geigy Sydney, analytical reference standard of 98.3% purity was converted to the potassium salt) 50 mg/kg; chlordimeform 80 SP (Ciba-Geigy, Agrochemicals Div. Basel) 50 mg/kg (made up in 0.9% NaCl so that between 0.25-0.3 mL were injected); 1,1,1-trichloroethane A.R. (Ajax Chemicals, Sydney) 1.33g/kg (10mmol/kg injected without any further dilution); Na phenobarbital (Prosana Laboratories, Sydney) 60 mg/kg (made up in 0.9% NaCl so that 1 mL was injected); control rats were injected with 1 mL 0.9% NaCl.

Preparation of Mitochondria and Microsomes. The pregnant rats were killed by cervical fracture and the foetuses were dissected out on the 21st day of pregnancy and used for the preparation of liver mitochondria and microsomes. Mitochondria were isolated as described previously (POLLAK & SUTTON 1980). The isolation medium contained 250 mM-sucrose, 1mM-Hepes, pH 7.4, 1 mM EDTA adjusted to pH 7.4 with NaOH and 0.2% bovine serum albumin. Microsomes were isolated by centrifuging the supernatant obtained from the first mitochondrial sedimentation for 65 min, at 105,000g (av.) in a 42.1 rotor of a Beckman L2-65 centrifuge. The pellets were drained and the mitochondrial and microsomal pellets were resuspended in 0.1 M glycine buffer pH 9.0.

Measurement of Lipolysis. All incubations were performed in flat-bottomed tubes shaken at 100 cycles/min at 37°C. The total incubation volume was 0.5 mL and contained 40 µmol $Ca(NO_3)_2$ and 0.1 mL of mitochondrial or microsomal suspension, containing 1-2 mg protein.

The reaction was stopped by withdrawing 0.1 mL from the incubation mixture and adding it to 3.0 mL 0.1 M glycine adjusted with H_2SO_4 to pH 2.7 for the assay of unesterified fatty acids (UFA) as described previously (POLLAK & HARSAS 1981). To the remaining 0.4 mL of the incubation mixture 8 mL chloroform-methanol (2:1) were added to extract phospholipids (FOLCH et al. 1957). After Folch-washing, portions of the extract were evaporated and total phosphate.P was determined (AMES 1966). Lysophospholipids were determined as described previously (POLLAK & HARSAS 1981).

The protein concentrations of mitochondria and microsomes were determined by the method of LOWRY et al. (1951), using bovine serum albumin (fraction V, Sigma Chemical Co.) as standard.

RESULTS AND DISCUSSION

When hepatic mitochondria and microsomes were isolated from 21-day foetal rats after injecting their mother on 4 consecutive days with 50 mg/kg 2,4,5-T, these organelles showed a statistically significant increase in the rate of phospholipid catabolism compared to mitochondrial and microsomal preparations from control rats (Table I). This was true if phospholipid degradation was measured in terms of phospholipid disappearance or if it was followed by the determination of newly released UFA (Table I). None of the other xenobiotic compounds tested had any statistically significant effect on lipid catabolism. Na phenobarbital acted as an additional control to show up any effects of a non-organochlorine inducing agent of microsomal membranes.

It was found in earlier work that foetal rat liver mitochondria and microsomes have a significantly greater triacylglycerol content than the corresponding adult organelles (POLLAK & HARSAS 1981). Furthermore it was found that foetal rat liver mitochondria, in contrast to adult rat liver mitochondria, contain a triacylglycerol lipase, which makes UFA available from the catabolism of neutral lipids. The released UFA may then presumably be utilized as substrates in the energy metabolism of the foetal rat liver.

In the present study it was found that 18 $\pm$ 2 nmol of lysophosphatides/mg protein were formed in 60 min by mitochondria obtained from control rats; the UFA derived from mitochondrial phospholipids therefore amount to 92 nmol/mg protein (37x2+18=92) (Table I). These UFA were released by phospholipase action. Since a total of 115 nmol UFA/mg protein were released by these mitochondria (Table I), 23 nmol/mg protein (115-92=23) of UFA may be assumed to be released by the action of triacylglycerol lipase of the control mitochondria (POLLAK & HARSAS 1981). Similar experiments with mitochondria isolated from 2,4,5-T treated rats indicated that 14 $\pm$ 1 nmol lysophosphatides/mg protein were formed in 60 min. Therefore, the formation of UFA from the phospholipids of these mitochondria amounts to 146 nmol/mg protein (66x2+14=146) (Table I). The actual amount of UFA formed during the 60 min incubation period was 146 nmol/mg protein (Table I). The results are therefore in complete agreement and suggest that 2,4,5-T inhibits UFA formation by the action of triacylglycerol lipase. This would diminish the availability of fatty acids which can either serve as substrates for energy metabolism or as building bricks for the synthesis of phospholipids. The large increase in phospholipase activity in the mitochondria isolated from 2,4,5-T-treated foetal rats may or may not be a mechanism to make more UFA available for energy metabolism. Whatever its cause, it results in the degradation of the mitochondrial membranes of foetal liver where mitochondrial membrane synthesis normally occurs at a rapid rate.

In microsomes isolated from foetal rat liver no lysophospha-

TABLE I. Loss of Phospholipids and Increases in Unesterified Fatty Acids in Foetal Rat Liver Mitochondria and Microsomes during *in vitro* Incubation.

Treatment	MITOCHONDRIA		MICROSOMES	
	Phospholipid loss nmol/mg protein	UFA release nmol/mg protein	Phospholipid loss nmol/mg protein	UFA release nmol/mg protein
Control (NaCl)	37 ± 5 (5)	115 ± 12 (5)	43 ± 7 (7)	120 ± 22 (7)
Na phenobarbital	30 ± 10(6)	98 (2)	56 ± 11(5)	137 (2)
Chlordimeform 80 SP	42 ± 2 (3)	108 ± 16 (3)	25 ± 12(3)	115 ± 7 (3)
2,4,5-T K-salt	66 ± 6*(5)	146 ± 19***(4)	70 ± 6**(5)	180 ± 35***(5)
1,1,1-trichloroethane	47 ± 8 (3)	140 ± 32 (3)	41 ± 12(3)	89 ± 32 (3)

Incubation conditions were stated in the Material and Methods section. The data represent mean values ± S.E.M. of the changes in lipid concentration after 60 min. incubation. The number of experiments are given in parentheses.

The results of the rats injected with xenobiotic compounds were compared with the control group by using an independent t test: * $P < 0.005$; ** $P < 0.01$; *** $P < 0.05$. UFA = unesterified fatty acids.

tides were formed (present investigation and POLLAK & HARSAS 1981). From the results presented in Table 1 it can be shown that the microsomal triacylglycerol lipase is not inhibited by 2,4,5-T.

TABLE 2. Phospholipid and Protein Content of Mitochondrial and Microsomal Fractions Isolated from Foetal Rat Liver

	MITOCHONDRIA		MICROSOMES	
Treatment	Phospholipid nmol/mg protein	Protein mg/g liver	Phospholipid nmol/mg protein	Protein mg/g liver
Control	197±8 (5)	6.8±0.8 (5)	191±10 (7)	5.5±0.7 (7)
2,4,5-T	251±11 (5)	3.1±0.4 (5)	229±12 (5)	3.5±0.5 (5)
K-salt	$P < 0.005$	$P < 0.01$	$P < 0.05$	P = n.s.

The results of the rats injected with 2,4,5-T were compared with the control group by using an independent t test; n.s. = not significant.

Paradoxically, the results of this study appear to indicate an increase in the phospholipid/protein ratio in both the mitochondrial and microsomal fractions of foetal liver from rats treated with 2,4,5-T as compared to those isolated from control rats (Table 2). This increase in the endogeneous (0 min) phospholipid/protein ratio encountered in organelles isolated from 2,4,5-T-treated rats may be explained in terms of lowered mitochondrial and microsomal protein concentrations (Table 2). It is however difficult to argue on this basis, as the lower protein content of the mitochondrial fractions isolated from the foetal livers of the 2,4,5-T injected rats could depend on a decreased recovery of mitochondria caused by the 2,4,5-T treatment. The possibility that the decrease of mitochondrial protein/g liver is due to 2,4,5-T injections, is supported by results obtained in our laboratory which indicate that 2,4,5-T significantly inhibits amino acid incorporation into foetal and neo-natal rat liver mitochondria and microsomes (S.R. Murdoch & J.K. Pollak, unpublished work). This additional evidence tends to sustain the hypothesis that the increased phospholipid/protein ratio encountered in 2,4,5-T-treated rats is due to a decreased protein content, rather than to an increase of phospholipid synthesis in foetal rat liver.

As pointed out in the introduction, the effects of organochlorines on intermediary metabolism are variable (KHOLI et al.1981; ASHTON et al. 1977). The results of the present study imply that a rapid rate of excretion of an organochlorine compound does not ensure non-interference in enzyme action. The two organochlorines which are excreted more slowly, chlordimeform 80 SP and 1,1,1-trichloroethane had no effect on lipid catabolism, while 2,4,5-T which is thought to be more rapidly excreted (MATTHEWS & KATO 1980) inhibited triacylglycerol lipase. Another enzyme which has been shown to be specifically inhibited by 2,4,5-T is ribonucleotide reductase (MILLARD et al. 1973). On the other hand 2,4-D and not

2,4,5-T has been shown to inhibit Mg^{2+} and Na^{+}, K^{+} stimulated ATPases (SEILER, 1971). In mice the absorption of glucose by the small intestine is diminished by a single oral does of 2,4,5-T (25-250 mg/kg) (MADGE 1977).

The present investigation as well as the other five studies cited above illustrate the variability and unpredictability of the effects that organochlorine compounds may exert on the lipid content and the intermediary metabolism of cellular organelles, cells and tissues.

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