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THE INFLUENCE OF DDT AND γ-CHLORDANE ON THE METABOLISM OF HEXOBARBITAL AND ZOXAZOALAMINE IN TWO MOUSE STRAINS*

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Abstract—The effect of pretreatment with two insecticides, γ-chlordane (50 mg/kg) and DDT (100 mg/kg), on zoxazolamine and hexobarbital disposition has been studied in two strains of male mice. In both Swiss-Webster and CF1 mouse strains, DDT and γ-chlordane stimulated hepatic oxidation of zoxazolamine *in vitro* and shortened zoxazolamine paralysis time. However, γ-chlordane stimulated metabolism of zoxazolamine to a greater degree than did DDT and shortened zoxazolamine paralysis time to a greater extent than did DDT. γ-Chlordane pretreatment stimulated oxidation of hexobarbital *in vitro* and shortened hexobarbital sleeping time in both mouse strains. γ-Chlordane also accelerated the rate of disappearance of a hypnotic dose of hexobarbital from the body of Swiss-Webster mice. However, DDT pretreatment affected neither hexobarbital sleeping time nor hexobarbital metabolism in either mouse strain. Upon arousal from hexobarbital-induced "sleep" the total body level of hexobarbital in DDT pretreated mice was the same as that in control mice, but γ-chlordane-pretreated mice awoke at a somewhat higher total body level of hexobarbital than did control or DDT-pretreated mice.

CHLORDANE and DDT have been reported to stimulate hepatic microsomal enzyme systems in rats, rabbits, and squirrel monkeys.[1–6] However in mice, DDT and chlordane appeared to act differently, since chlordane caused a shortening of hexobarbital sleeping times whereas DDT did not.[2] Phenobarbital will stimulate hepatic drug metabolism to a different extent in different strains of rabbits. Even within the same rabbit strain, different hepatic microsomal, enzymes can be stimulated to different degrees by phenobarbital, and some pathways do not respond to all.[7]

The aims of the present study were to investigate whether the apparent inactivity of DDT in mice (as contrasted to other species) was strain specific and whether a pathway other than that for hexobarbital metabolism might be affected by DDT in mice.

MATERIALS AND METHODS

Materials. Male Swiss-Webster mice were obtained from Arthur Sutter, Springfield, Mo., and male CF-1 mice were obtained from Carworth Farms, Portage, Mich. All mice weighed 24–30 g and were maintained on food and water *ad libitum*. γ-Chlordane, 99·8% pure, obtained from Velsicol Chemical Corp., Chicago, Ill., and 1,1,1 trichloro-2,2-bis-(*p*-chlorophenyl)-ethane (DDT), 95% pure, obtained from the University of Iowa Hospital Pharmacy, were used for pretreatment.

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Pretreatment procedure. Once daily for 3 days, groups of mice were injected i.p. either with corn oil (0·1 ml/10 g), or with an equal volume of a corn oil solution of either γ-chlordane, at a dose of 50 mg/kg, or DDT at a dose 100 mg/kg. All experiments *in vivo* and *in vitro* were performed on the fifth experimental day, 2 days after the last treatment.

Experiments in vitro. All animals were sacrificed by cervical dislocation. Livers were immediately excised and placed on ice and the gall bladders removed. The livers were weighed and homogenized in the cold in 1·15% KCl (2 ml KCl/g liver) with a Potter homogenizer (plastic pestle). This homogenate was then centrifuged at 1°–3° at 9000-*g* for 20 min, and the 9000-*g* supernatant fraction was used in studies of drug metabolism *in vitro*. The drug metabolic pathways assayed were the side-chain oxidation of hexobarbital[8] and the ring oxidation of zoxazolamine.[9] The conditions of incubation, cofactors and concentrations thereof were the same as those described previously.[9] Nitrogen determinations were made on the supernatant fractions by a modified Kjeldahl method.[9] Results were expressed as micromoles of substrate metabolized per gram of 9000-*g* supernatant fraction nitrogen per hr.

Experiments In vivo. Hexobarbital sleeping time (HST) was obtained in control and insecticide-pretreated mice after i.p. injection of hexobarbital sodium, at a dose of 125 mg/kg, by noting the time in minutes from the loss of the righting reflex until its recovery. Zoxazolamine paralysis time (ZPT) was obtained after i.p. injection of zoxazolamine, at a dose of 175 mg/kg, by noting the duration of the loss of the righting reflex.

Whole-body levels of hexobarbital were determined in control and insecticide-pretreated male Swiss-Webster mice at various times after i.p. injection of hexobarbital sodium at a dose of 125 mg/kg. Two different experimental designs were used. In both experiments, five mice from each pretreatment group were sacrificed 5 min after hexobarbital injection. But in one experiment additional mice from each insecticide-pretreated group were sacrificed 15 and 45 min after hexobarbital injection. In the other experiment, sacrifice time was not predetermined but depended on the duration of hexobarbital-induced loss of the righting reflex, as follows: (1) As each of five γ-chlordane pretreated mice regained its righting reflex, the arousal time was noted, it was sacrificed, and one DDT- and one corn oil-pretreated mouse were sacrificed after the same "sleeping time"; (2) as each of five corn oil-pretreated mice awoke, its sleeping time was noted, it was sacrificed and one γ-chlordane pretreated mouse was sacrificed at the same time; (3) as each of five DDT-pretreated mice awoke, its sleeping time was noted and it was sacrificed. Immediately after sacrifice each animal was homogenized in 1·15% KCl (2 ml/g mouse) in a Waring-Blendor, the resulting homogenate was made up to a total volume of 100 ml and filtered through cheese cloth, and an aliquot of this homogenate was taken and analyzed for hexobarbital content by the method of Cooper and Brodie.[8] The amount (mg) of hexobarbital sodium present per animal was expressed as mg hexobarbital/25 g mouse and was calculated by the use of the following formula: mg/25 g mouse = [(mg/mouse)/wt. of mouse] × 25.

Statistical analysis of data. The statistical methods used are described by Snedcor.[10] Student's *t* test was used to test the null hypothesis, and the level of significance chosen for all determinations was $P < 0{\cdot}05$. All values expressed in the tables are means plus or minus standard deviations.

RESULTS

Pretreatment of male mice of both the CF-1 and the Swiss–Webster strains with γ-chlordane significantly and markedly shortened hexobarbital sleeping time and zoxazolamine paralysis time (Table 1). In both mouse strains, DDT pretreatment produced a moderate and statistically significant shortening of ZPT from the control

TABLE 1. EFFECT OF DDT AND γ-CHLORDANE PRETREATMENT ON DURATION OF HEXOBARBITAL AND ZOXAZOLAMINE INDUCED LOSS OF THE RIGHTING REFLEX IN TWO MOUSE STRAINS

Parameter *in vivo*	Mouse strain	Pretreatment group: Corn oil (control)	DDT	γ-Chlordane
Hexobarbital sleeping time (min)	Swiss–Webster	46 ± 16*	51 ± 12	12 ± 4†
	Carworth CF-1	60 ± 12	55 ± 25	7 ± 5†
Zoxazolamine paralysis time (min)	Swiss-Webster	76 ± 23	55 ± 10‡	21 ± 13†
	Carworth CF-1	87 ± 28	52 ± 8‡	7 ± 4†

Mice were injected once daily for 3 days with γ-chlordane (50 mg/kg), DDT (100 mg/kg), or corn oil and tested for treatment effects 48 hr after the last dose.

* Values in the table indicate mean duration (in minutes) of loss of righting reflex after i.p. injection of either hexobarbital sodium (125 mg/kg) or zoxazolamine (175 mg/kg), expressed as mean ± standard deviation with n = 8–10 (8 to 10 mice in each group).

† Significantly different from control value ($P < 0.05$).

‡ Significantly different from control and γ-chlordane pretreatment values ($P < 0.05$).

level, but this effect on ZPT was less than that seen after γ-chlordane pretreatment. Pretreatment with DDT did not shorten HST in either mouse strain.

The pattern of effects of DDT and γ-chlordane on hepatic microsomal oxidation *in vitro* of hexobarbital and zoxazolamine (Table 2) qualitatively paralleled the effects

TABLE 2. EFFECTS OF DDT AND γ-CHLORDANE PRETREATMENT ON METABOLISM OF HEXOBARBITAL AND ZOXAZOLAMINE BY HEPATIC 9000-*g* FRACTIONS FROM TWO MOUSE STRAINS

Drug substrate	Mouse strain	Drug metabolized*/hr by 9000-*g* fraction from mice treated† with: Corn oil (μmoles/g N)	DDT (μmoles/g M)	γ-chlordane (μmoles/g N)
Hexobarbital	Swiss–Webster	148 ± 31	162 ± 17	237 ± 22‡
	CF1	155 ± 25	137 ± 35	215 ± 28‡
Zoxazolamine	Swiss–Webster	171 ± 19	222 ± 39§	268 ± 30‡
	CF1	200 ± 41	256 ± 14§	339 ± 11‡

* Values in the table are mean (± S.D.) μmoles drug metabolized/hr per g of 9000*g* supernatant nitrogen. There were four mice in each group.

† Mice were injected i.p. once daily for 3 days with corn oil, DDT (100 mg/kg), or chlordane (50 mg/kg). Enzyme assays were run 48 hr after the last injection.

‡ Significantly different from control value ($P < 0.05$).

§ Significantly different from control and γ-chlordane-pretreated values ($P < 0.05$).

of these insecticides on HST and ZPT. γ-Chlordane significantly stimulated oxidation of hexobarbital and zoxazolamine *in vitro* by hepatic 9000-*g* supernatant fraction from both mouse strains. DDT-Pretreatment did not affect oxidation of hexobarbital *in vitro* in either mouse strain, but it significantly ($P < 0.05$) enhanced hydroxylation of zoxazolamine *in vitro* to a level intermediate between that of the control and that after γ-chlordane pretreatment.

The experiments involving whole-body hexobarbital determinations were performed in only the Swiss–Webster mouse strain. From Table 3 it can be seen that γ-chlordane

TABLE 3. EFFECT OF γ-CHLORDANE PRETREATMENT ON RATE OF DISAPPEARANCE OF HEXOBARBITAL FROM SWISS–WEBSTER MICE

Pretreatment group	Hexobarbital concentration at various sacrifice times*			Hexobarbital half-life
	5 min	15 min	45 min	(min)
Corn oil (controls)	2·6 ± 0·2	2·3 ± 0·2	1·6 ± 0·2	50
γ-Chlordane	2·5 ± 0·4	1·9 ± 0·2†	0·7 ± 0·2†	30

Mice were injected once daily for 3 days with γ-chlordane (50 mg/kg), DDT (100 mg/kg), or corn oil and tested for treatment effects 48 hr after the last dose.

* Values are average concentrations of unchanged hexobarbital present at various time intervals after i.p. injection of hexobarbital sodium (125 mg/kg or 3·1 mg/25 g mouse), expressed as mean mg hexobarbital/25 g mouse ± standard deviation. All values are for groups of five mice.

† Significantly lower than control value at that time interval ($P < 0.05$).

pretreated Swiss-Webster mice disposed of hexobarbital more rapidly than control mice; 15 and 43 min after injection of a hypnotic dose of hexobarbital, significantly less hexobarbital remained in γ-chlordane-pretreated mice than in control mice. The data in Table 4 show that when γ-chlordane-pretreated Swiss–Webster mice recovered from hexobarbital-induced loss of the righting reflex, they had less hexobarbital in their bodies than did concomitantly injected, but still sleeping, control or DDT-pretreated mice. DDT-Pretreated mice regained their righting reflex at the same time as did control mice, and had similar body levels of hexobarbital. However, γ-chlordane-pretreated mice awoke at a significantly higher total-body level of hexobarbital (1·7 mg/25 g) than did control or DDT-pretreated mice (1·1–1·3 mg/25 g). This may be related to the stimulatory effects of high doses of chlordane on the central nervous system.[11]

DISCUSSION

Our attention in this paper has been focused on an apparent species variation in response to DDT vs. chlordane. In all other species studied (i.e. rats, rabbits, and squirrel monkeys), both DDT and chlordane stimulate several hepatic microsomal drug-metabolizing enzyme systems, including that oxidizing hexobarbital.[1–6] Previous work[2] suggested that DDT had no stimulating effect on hexobarbital metabolism in mice, whereas chlordane stimulated hexobarbital metabolism in this species as well as in all other species studied. In most species, DDT is less potent as a stimulator of heptatic drug metabolism than is chlordane.[2, 6]

The present work has extended that done previously by showing that DDT is indeed unable to stimulate hexobarbital metabolism measured either *in vitro* or *in vivo* in two strains of mice. However DDT can stimulate the metabolism of at least one drug—zoxazolamine—in mice. The effects of DDT on zoxazolamine metabolism are of less magnitude than those of chlordane, but are seen both *in vivo* (zoxazolamine paralysis time) and *in vitro* (metabolism of zoxazolamine by hepatic 9000-*g* fractions).

TABLE 4. EFFECT OF γ-CHLORDANE AND DDT PRETREATMENT ON WHOLE BODY CONCENTRATION OF HEXOBARBITAL IN MICE AT VARIOUS TIME INTERVALS AFTER HEXOBARBITAL-INDUCED LOSS OF THE Rrighting REFLEX

Sacrifice time (mean ± S.D.)	Total body hexobarbital in mice pretreated with*		
	Corn oil	DDT	γ Chlordane
5 min after i.p. hexobarbital (5)	2·6 ± 0·2	2·6 ± 0·4	2·5 ± 0·2
Arousal of γ-chlordane-pretreated mice (15 ± 6 min)	2·5 ± 0·4	2·1 ± 0·3	1·7 ± 0·3†‡
Arousal of DDT-pretreated mice (54 ± 19 min)		1·1 ± 0·2†	
Arousal of control mice (64 ± 14 min)	1·3 ± 0·2†		0·4 ± 0·3§

Mice were injected once daily for 3 days with γ-chlordane (50 mg/kg), DDT (100 mg/kg), or corn oil and tested for treatment effects 48 hr after the last dose.

* All values in the table are average concentrations of unchanged hexobarbital remaining at various time intervals after i.p. injection of hexobarbital sodium (125 mg/kg, or 3·1 mg/25 g mouse), expressed as mean mg hexobarbital/25 g mouse ± standard deviation. All values are for groups of five mice.

† Values represent average whole-body hexobarbital concentration at time of recovery from hexobarbital-induced loss of the righting reflex.

‡ Total-body hexobarbital level of γ-chlordane-pretreated mice at arousal significantly differs (a) from that of concomitantly sacrificed (sleeping) control and DDT-pretreated mice. (b) from that of control mice at arousal, and (c) from hexobarbital cencentration in DDT-pretreated mice at arousal ($P < 0·05$).

§ Value significantly differs from that for concomitantly sacrificed control mice ($P < 0·05$).

Chlordane affects both hexobarbital and zoxazolamine metabolism in both strains of mice studied. These effects are manifested on drug action (ZPT and HST) and drug metabolism (by hepatic 9000-*g* fractions). Whole-body levels of hexobarbital fall more rapidly in chlordane-pretreated mice than in control mice.

At very high doses (e.g. above 100 mg/kg), DDT can apparently inhibit the metabolism of hexobarbital in mice. This is suggested by the increased duration of hexobarbital sleeping time in mice pretreated with DDT as compared with controls (unpublished observations).

These results suggest that DDT is a less potent and perhaps more selective stimulator of hepatic drug-metabolizing enzymes than is chlordane. In most species studied, DDT can stimulate the same hepatic enzymes as chlordane and to nearly the same extent if the dose of DDT used is raised high enough. In mice however, DDT apparently cannot mimic the effects of chlordane on hexobarbital metabolism, although its effects on zoxazolamine metabolism are similar but of a lesser magnitude. Therefore, it would seem that enzymes metabolizing hexobarbital and zoxazolamine may be different in the mouse, and that DDT and chlordane may enhance hepatic drug metabolism in mice

by slightly different mechanisms. In practical terms this suggests that the use of hexobarbital sleeping time, or hexobarbital metabolism by liver fractions *in vitro* as a routine "screen" for chemicals and drugs which stimulate hepatic microsomal enzymes, might cause one to miss inducers like DDT.

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