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THE EFFECTS OF 2,2-BIS (PARA CHLORO-PHENYL) 1,1-DICHLOROETHANE (DDD) ON CHOLINE ACETYLASE OF THE THYMUS GLAND

A. S. GAWHARY*

Department of Pharmacology and General Therapeutics, Faculty of Medicine—University of Alexandria, Alexandria, U.A.R.

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Abstract—In the rabbit, the commercial grade 2,2-bis(parachloro-phenyl)1,1-dichloro-ethane (DDD), caused acute atrophy of the thymus gland and hypertrophy of the adrenals. The choline acetylase activity of these thymus glands showed significant difference from those treated with M,P′ isomer of DDD which caused hypertrophy of the thymus gland with increased choline acetylase (ChA) activity.

MATERIALS AND METHODS

(a) *Animals and materials*

Rabbits of a mixed breed 4–6 weeks old were used and when the animals were 49 days or more, they were separated as males and females in order to control the variability which occurs after the onset of sexual maturity. Chemical compounds used were: (a) Commercial grade DDD containing 90% P,P′ isomer and 7% O,P′ isomer (b) M,P′ isomer of DDD.

(b) *Methods*

(1) Treatment with 10% DDD in arachis oil containing Tween "80" on 4 consecutive days/week for 3 weeks, daily dose 40 mg/200 g body weight.[1] The control animals received a similar volume of arachis oil intramuscularly.

(2) Treatment with 10% M,P′ isomer in arachis oil containing Tween "80" on 4 consecutive days/week for 3 weeks, daily dose 20 mg/200 g body weight. The control animals were injected with arachis oil.

Preparation of the acetone-dried powder of thymus. At the end of each experiment the animals were given intravenous injections of sodium pentobarbitone (60 mg/ml). The subclavian vessels on both sides were cut and allowed to bleed freely in order to drain the blood as completely as possible from the thymus gland, to ensure that the (ChA) activity estimations were as far as possible due to the enzyme in the gland and not to a mixture with blood (ChA). The thymus gland was immediately dissected out and immersed in an ice-cold solution of acetone; the volume of the acetone was 100 times the tissue volume and the temperature was −10°. The acetone was filtered through a Buchner funnel fitted with a No. 1 Whatman paper. The powder was then dried over P_2O_5 in vacuum at 300 mm mercury for 4 hr.

* Present address: 57 Esmond Street, Liverpool L6 5AY, Lancs.

Choline acetylase estimations in thymus. A combination with a modification of the two methods previously used by Hebb and Smallmann,[2] and Feldberg and Mann[3] was adopted. Portions (188 mg) of the acetone dried powder of the tissue was carefully weighed into centrifuge tubes of 10 ml capacity, 2·5 ml.

Nachmansohn's buffer[4] were then added and the tubes which each contained a stirring rod were agitated in a shaking machine at room temperature for 10 min. The rods were removed and the tubes were centrifuged at 2800 rev/min for 3 min. The supernatant layer pipetted off and portions of 1 ml (equivalent to 75 mg acetone dry powder) were placed in the main compartments of Warburg flasks of 20 ml capacity. To the main compartment of each flask were then added 0·1 ml of each of the following solutions: choline chloride 30 mg/ml; sodium acetate 30 mg/ml; sodium citrate 67 mg/ml; sodium hydrogen phosphate 1·5 mg/ml; potassium chloride 40 mg/ml; sodium fluoride 30 mg/ml; eserine 5 mg/ml; the pH of the solution was adjusted to 7·0. The preparation of Co-A used contained 300 Lipmann units/mg. Kaplan and Lipmann,[5] therefore, 70 units were also transferred to the main compartment, with 0·3 ml of cysteine (30 mg/ml). Into the side arm of the flask 0·5 ml A.T.P. (10 mg/ml) was pipetted. The Warburg flasks were then connected to manometers and immersed in the bath after gassing with a mixture of carbon dioxide 95% and nitrogen 5% for 10 min. After thermal equilibration flasks were shaken for 5 min (15 min from the start of gassing) and the contents of the side arm tipped into the main chamber. The idea is to give enough time for the accumulation of adequate amounts of intermediate reaction products to be formed before mixing with A.T.P. Incubation in the flasks with continuous shaking was then carried on for 60 min at $38° \pm 0·2°$ the amount of ACh thus formed was estimated biologically on the frog rectus muscle preparation.

CALCULATION OF RESULTS

The assay was performed by matching the response produced by alternate doses of standard and unknown solutions. Each dose was allowed to act for 1·5 min, the interval between the doses was 3·5 min. The activity of the unknown was expressed in terms of μg ACh/ml and then the ChA activity of the tissue was calculated and expressed as the amount of ACh produced by 1 g of acetone dried power per hour.

RESULTS

Table 1 shows that the commercial DDD is toxic for the rabbits as there was atrophy of the thymus gland compared with the control animals. The diminished rate of growth and loss of body weight of the treated animals can be taken as evidence of hypertrophy of the suprarenal glands as there was involution of the thymus gland. The mean weight of the thymus glands of the treated animals was significantly less than that of the control animals.

From Table 2 there was a substantial hypertrophy of the thymus glands in the treated animals with M,P′ isomer. The glands were large, soft in texture and were not surrounded by any fatty tissue, in contrast to those of the control group which were much firmer and surrounded by fat to a varying extent. In some of them pinkish patches of thymus tissue were scattered within a large mass of fatty and fibrous tissue.

The ChA activities ranged from 25 ACh μg/g/hr to 110 ACh μg/g/hr showing the correlation between the ChA activity and the weight of the thymus gland.

TABLE 1. EFFECT OF COMMERCIAL GRADE OF DDD ON THE WEIGHT OF THYMUS GLAND AND CHOLINE ACETYLASE ACTIVITY

	DDD treated group			Control		
	Wet wt. (mg)	ChA (ACh/g/hr. μg)	Dry wt. (mg)	Wet wt. (mg)	ChA (ACh/g/hr. μg)	Dry wt. (mg)
	705			3218		
	847	25	130	2598	79	621
	768					
mean	773·3			2908		
	1230			2607		
	1184	31	213	4634	76	541
	1839			2723		
mean	1326			3321·3		
	694			4983		
	574	21	154		90	808
mean	634			4983		

TABLE 2. EFFECT OF M,P′ DDD ON THE WEIGHT OF THYMUS GLAND AND CHOLINE ACETYLASE ACTIVITY

	DDD treated group			Control group		
	Wet wt. (mg)	ChA (ACh/g/hr. μg)	Dry wt. (mg)	Wet wt. (mg)	ChA (ACh/g/hr. μg)	Dry wt. (mg)
	2992			2602		
	2774	110	2147	2040	88	774
	3216					
	2911					
mean	2973·25			2321		
	3251			2108		
	2989	100	2068	2086	82	699
	3126					
	3051					
mean	3104·25			2097		

DISCUSSION

The results of this study show that there is a difference between the commercial preparation of DDD containing 90% P,P′–7% O,P′ and the pure M,P′ isomer of DDD. They differ of course, in their physicochemical properties but the chief interest in the present context lies in their different effects on the ChA activity and the weight of the thymus gland. Whereas rabbits treated with the former showed clear evidence of atrophy of the thymus gland with decreased ChA activity; those that received the M,P′ isomer appeared to be healthy animals; they showed hypertrophy of the thymus gland together with increased wet weight and the good yield of acetone dried powder due to absence of fat cells. The apparent increase in ChA activity could be attributed to the newly developed thymus tissue or to an activation

of the enzyme by M,P′ isomer. The effect of this compound on the adrenal glands was regarded as highly selective on the suppression of glucocorticosteroids as shown in the hypertrophy of the thymus gland.

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