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Effects of chlordane on conditioned avoidance response, brain seizure threshold and open-field performance of prenatally-treated mice

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Summary

1. Three groups of six pregnant albino mice at the third stage of gestation were given chlordane 1 or 2·5 mg/kg body weight or olive oil 10 ml/kg. They were dosed orally for seven consecutive days.

2. Ten young mice, regardless of sex, were randomly selected from the progeny of each group of treated mothers and tested for conditioned avoidance response, electroshock seizure threshold, and open-field performance.

3. Offspring of chlordane-treated mice made fewer conditioned avoidance responses than the controls on each day of training.

4. Electroshock seizure threshold was raised.

5. In the open-field test, progeny of mothers receiving the larger dose were more active than controls. A dose×days interaction indicated a complex response of the chlordane-treated mice to experience in the open-field.

6. The significance of these findings is discussed.

Introduction

Chlordane (1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane) is a polycyclic, polychlorinated hydrocarbon. It is a widely used insecticide for the control of flies, mosquitoes and field insects. It is fat-soluble ; thus it is absorbed from the digestive tract, skin and respiratory system (Thiens & Haley, 1964). It is toxic to man. Death has been reported to follow the ingestion of 104 mg/kg even though no solvent was involved. This high dose caused pathological changes such as congestion, oedema and scattered petechial haemorrhages in the lung, kidney and brain, and degenerative changes in the hepatic cells and renal tubules (Dreisbach, 1966).

Al-Hachim & Fink (1967) suggested that insecticides might have some effect on the development of the central nervous system of offspring of insecticide-treated mothers. Al-Hachim & Fink (1968a) also reported that progeny of female mice treated with DDT during pregnancy showed delayed acquisition of conditioned avoidance response (CAR). Al-Hachim (1971) showed that there were significant differences between the brain seizure threshold and body weight of progeny of aldrin-treated mothers and controls. Unpublished data (Al-Hachim) indicated that 40 to 50 mg/kg chlordane has a teratogenic effect in the offspring of chlordane-treated female mice. Thus it was suggested that chlordane might have an effect on the behaviour of offspring of chlordane-treated mothers if administered in small doses.

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Since CAR, electroshock seizure threshold (EST), and open-field (OF) tests are useful parameters in the investigation of the effects of drugs on behaviour (Al-Hachim & Fink, 1968b; Al-Hachim, 1971), these tests were used to examine the prenatal effects of chlordane on the postnatal behaviour of mice.

Methods

Three groups of six albino pregnant mice were used. These groups were given 1 or 2·5 mg/kg technical grade chlordane (obtained from Velsicol Chemical Corp., Chicago, Ill.) or 10 ml of olive oil/kg respectively by mouth. Each dose of chlordane was dissolved in 10 ml of olive oil. The drugs were administered during the third trimester and given for seven consecutive days. Each pregnant mouse received 5–7 doses. The doses used in the present experiment are much lower than those which cause marked pathological changes in man (Dreisbach, 1966).

Each member of these three groups of animals was caged separately and raised with its offspring. The average litter size for these groups of mice was 6·125, 6·00 and 7·75 respectively. Ten mice regardless of sex, were randomly selected from each group of litters and tested for CAR, EST and OF.

The test for CAR started when the offspring were weaned at the age of 30 days. The same groups of mice were tested daily until they were 37 days old. For avoidance training, a shuttle box (306×204×102 mm) was divided into two compartments by a low barrier. Each compartment had a grid floor through which a 45V shock could be delivered to the animals' feet. A mouse was placed in one compartment and the trial began with a warning buzzer for 5 seconds. A mouse which crossed to the opposite side of the barrier during the buzzer (conditioned response) avoided the electric shock that followed the buzzer. Buzzer and shock terminated with a cross-over to the opposite non-electrified compartment (non-conditioned response), and a 20 s rest followed before another trial began. Each mouse was subjected to 16 consecutive conditioned avoidance trials per day, and the number of conditioned responses were noted. This test continued for 7 consecutive days.

When the young mice were 38 days old, the EST was measured. The minimal electroshock seizure threshold was determined in the animal by measuring the intensity of unidirectional current (6 Hz, 0·2 ms pulse duration, 3 ms delay) required to evoke minimal seizure (stun response or 4–5 s of continued minimal clonic activity) in the animal. The electrical stimuli were delivered by a Grass stimulator (model 4SK) according to the parameters of Brown (1953). In the electric shock procedure ear clip electrodes were employed as indicated by Chen & Ensor (1954) with a long lead which was connected to the ear of the mouse after the ear canal had been moistened with 0·9% sodium chloride solution. Since brief restraint lowers seizure threshold, as reported by Swinyard, Radhakrishmen & Goodman (1962), care was exercised to measure seizure threshold only while the animals were unrestrained. They were tested 20 s after attachment of the electrodes.

The test for OF began when the offspring were 6 weeks old. It was continued for 7 consecutive days. The apparatus for the test was a rectangular box with an open top. The internal dimensions were 534×534×254 mm. The floor was painted white and was divided into 76×76 squares by black lines. The lighting

was uniform and from the top. Each animal was placed in the open-field once on each of 7 consecutive days for 3 minutes. The number of squares traversed for each animal during 3 min was recorded.

Results

All data for the three tests, CAR, EST and OF are shown in Table 1. Three-way analysis of variance indicates the significance of these results. For CAR, the analysis of variance indicated that there were significant differences between days ($F=43{\cdot}3$, $df=6/162$), animals within treatments ($F=3{\cdot}62$, $df=27/162$) and between treatments ($F=17{\cdot}20$, $df=2/27$). All these were significant at $P<0{\cdot}001$.

TABLE 1. *Acquisition of conditioned avoidance response (CAR), minimal electroshock seizure threshold (EST) and number of squares traversed in open-field (OF) for offspring from chlordane or olive-oil treated mothers*

Days	No. of mice	Test	Mean of responses: 1 mg/kg chlordane	2·5 mg/kg chlordane	10 ml./kg olive oil
		CAR			
1	10		5	5	8
2	10		8	9	12
3	10		10	9	13
4	10		10	10	14
5	10		11	12	14
6	10		12	12	15
7	10		12	11	16
Treatment mean			9·7±2·9	9·7±2·4	13·1±2·6
Least significant difference 0·05 for treatment means=0·69					
		EST (Volts)			
1	10		108·6±19·7	134·9±26·8	90·1±27·8
Least significant difference 0.05 for treatment means=2·05					
		OF			
1	10		129	129	127
2	10		96	160	153
3	10		72	107	110
4	10		74	117	79
5	10		75	133	68
6	10		79	145	58
7	10		94	173	62
Treatment means			88·4±20·4	137·7±23·3	93·9±36·6
Least significant difference 0.05 for treatment means=12·94.					

On each day, the progeny of chlordane-treated mothers made fewer avoidance responses than controls. For EST, the analysis of variance indicated that there were significant differences among treatments ($F=10{\cdot}13$, $df=2/27$, $P<0{\cdot}001$). For OF, the statistical analysis also indicated differences between days ($F=7{\cdot}14$, $df=6/162$, $P<0{\cdot}001$), animals within treatments ($F=5{\cdot}31$, $df=27/162$, $P<0{\cdot}01$) and between treatments ($F=6{\cdot}42$, $df=2/27$, $P<0{\cdot}001$). Progeny of mothers given the larger dose showed greater activity than those of controls. There was also a dose × day interaction ($F=4{\cdot}13$, $df=12/162$, $P<0{\cdot}001$), indicating that the response of the treated mice changed with time or experience of the OF test.

Discussion

Since chlordane caused teratogenic effects in mice (Al-Hachim, unpublished observations), it seems reasonable to assume that it may pass the placental barrier and affect the brain of the foetus (Dreisbach, 1966; Thiens & Haley, 1964) or may have an action on the mother and/or the placenta. The data in Table 1 indicate that prenatal chlordane affects the foetal brain because it depressed the acquisition of avoidance response (showing poor learning ability or altered motivation), and raised seizure threshold (since a greater voltage was needed to induce seizure) and increased the exploratory activity as shown in the open-field. Because of the highly significant treatment effect in the CAR, it is possible that changes in EST and OF represent differential responses to the stress of CAR rather than direct effects of chlordane. The small dose (1 mg/kg) and high dose (2·5 mg/kg) of chlordane caused the same biochemical and/or physiological lesion in the brain of offspring from chlordane-treated mothers. The lesion was presumably prenatal, but may have been aggravated after birth, since chlordane can be excreted in milk (Boyd, 1970).

It is difficult to explain the type of chlordane-induced biochemical and/or physiological lesion in the brain because its mechanism of action is unknown. But it is known that chlordane inhibits the (Na-K)-activated Mg-dependent ATP-phosphohydrolase activity of brain microsomal fraction in rat and fish (Akera, Brody & Norman, 1971; Koch, Cutkomp & Yap, 1971). Thus it is possible to postulate that the effect of prenatal and/or postnatal chlordane on the postnatal development of the central nervous system might be caused by inhibition of Na-K-ATPase activity in the brain. These effects at relatively small doses should serve as a warning. Chlordane is a widely used insecticide and feeding is reported (Durham, 1965) to have cumulative effects. Observed residues of chlordane in food are far below the doses used here; it was detectable in at most 3·3% of grain samples tested, and even in these occurred at concentrations below 0·005 ppm (FAO-WHO, 1971). Nevertheless, prolonged exposure, particularly in pregnancy, could possibly have effects not easily recognizable as due to chlordane.

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