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THE EFFECT OF DIELDRIN (HEOD) ON CHRONAXIE AND CONVULSION THRESHOLDS IN RATS AND MICE

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Dieldrin† is a chlorinated hydrocarbon insecticide widely used in crop protection and preservation. Cases of over-exposure to this compound have been reported in some operatives employed in its application by spraying. Among the diverse symptoms resulting from intoxication are muscular twitching increasing in severity to epileptiform convulsions with loss of consciousness (Hayes, 1957).

Khaïry (1960) studied the effect of dieldrin on the muscular efficiency of rats which had been exposed to diets containing 25 and 50 ppm dieldrin for a period of 1 hr each day for 60 days, and trained to pull various weights over a pulley through a fixed distance. The time taken to perform this exercise was used as a criterion of the muscular efficiency of the rat. Khaïry observed a progressive deterioration of muscular efficiency which was related to the amount of dieldrin administered, but the nature of this deterioration was not apparent.

The closely related chlorinated hydrocarbon insecticide, aldrin‡, is converted *in vivo* to dieldrin (Bann, De Cino, Earle & Sun, 1956). London & Pallade (1964) suggested the measurement of chronaxie to reveal intoxication by aldrin. In chronic experiments, rats received aldrin by oral intubation, 3 mg/kg/day, 6 days a week for 6 months, followed by 4.5 mg/kg/day for a further 7 months. Chronaxie was measured by applying an electric current to the tail of the rat, and measuring the pulse duration at varying current strengths which elicited a withdrawal response. It was found that the current strength-duration curve so described was displaced to the right—that is, a longer pulse duration was necessary—in the case of aldrin-exposed rats compared with control animals. London & Pallade also studied the effect of acute intoxication with aldrin on the same reflex. Rats received an oral dose of 97 mg/kg orally and were then tested at 2, 4 and 6 hr thereafter. In this instance the strength-duration curves were found to be displaced to the left, representing an increased excitability.

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† Dieldrin contains 85% w/w 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a,(−)octahydro-endo-1,4-exo-5,8-dimethano-naphthalene (HEOD).

‡ Aldrin contains 95% w/w of the endo-exo isomer of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethano-naphthalene (HHDN).

The preceding work on the effect of chronic exposure of laboratory animals to dieldrin and its closely related congener, aldrin, therefore suggests a deterioration of voluntary muscular efficiency in the intact animal. Nevertheless, the more recent recommendation of London & Pallade (1964) that an early diagnosis of aldrin intoxication may be made by measurements of chronaxie, prompted us to investigate whether or not chronic exposure of laboratory animals to dieldrin (HEOD) produced an effect on the passage of nervous impulses in somatic nervous pathways or in voluntary muscle itself by measuring chronaxie and related values, and to account for the alteration of the performance of chronically and acutely exposed animals by studying the effects of some convulsant drugs.

METHODS

Male rats, C.F.E. strain, and male mice, C.F. No. 1 strain, bred and maintained under specific pathogen-free conditions, were used throughout.

Analytically pure recrystallized HEOD was incorporated in the normal 86 diet and fed to the experimental animals at the concentration and for the period indicated below.

Strychnine hydrochloride (British Drug Houses, Ltd.) and pentylenetetrazole (leptazol, crystalline material kindly supplied by Dr. M. J. Davey, Pfizer Ltd., Kent) were used as convulsant agents. Diphenylhydantoin (Epanutin—Parke Davis and Co. Ltd.) was used as a reference anticonvulsant compound.

Isolated phrenic nerve-diaphragm preparation of rats

Rats were maintained for 26 weeks on a diet containing HEOD, 100 ppm. A similar group received a diet free of HEOD, and served as controls. Litter mates were evenly distributed between the test and control groups.

The rats were killed by a blow on the head, and the phrenic nerve-diaphragm preparation was removed as described by Bülbring (1946). The preparations were placed on a Perspex electrode for phrenic nerve (C. F. Palmer (London) Ltd.) and attached to an isotonic lever recording on a smoked drum. They were maintained at 37° C in Krebs solution, gassed with a mixture of 95% O_2–5% CO_2 in a 100 ml. bath. Stimuli to either the nerve or the muscle were delivered from a Grass square-wave stimulator. Voltage-duration curves were constructed for each individual tissue, from which rheobase and chronaxie (Winton & Bayliss, 1949) were determined. Shocks were delivered at a rate of 1/sec with a fixed duration of 0.01 msec. The voltage was gradually raised until a threshold value eliciting a contraction was achieved. Duration of the shock was then gradually increased and the threshold voltage redetermined for each duration. The asymptote of the curves so described corresponded to the rheobase. Chronaxie was taken as the duration of the threshold stimulus at twice the rheobase.

Tetanic frequency was determined by stimulating either the nerve or the muscle at their respective chronaxie durations with four times the rheobase voltage and gradually increasing the frequency until tetanus occurred.

Threshold convulsive doses of leptazol and of strychnine hydrochloride in mice

Mice were used to determine the amounts of either leptazol or strychnine hydrochloride required to produce tonic extension of the hind limbs. In some mice a solution of leptazol in physiological saline (20 mg/ml.) was infused into the tail vein at a rate of 0.82 mg/min using a continuous slow injection apparatus (C. F. Palmer (London) Ltd.). In other mice a solution of strychnine hydrochloride in physiological saline (250 μg/ml.) was similarly infused at a rate of 6.87 μg/min. The duration of the infusion was measured to the nearest 0.05 min, and the total amount of the convulsant agent was calculated and expressed in terms of weight/kg body weight of the individual mouse. This procedure is based on the method described by Orloff, Williams & Pfeiffer (1949) in which solutions of either leptazol or strychnine are injected intravenously in mice at a rate of 0.05 ml. every 10 sec.

The effect of diphenylhydantoin on the threshold convulsive doses of strychnine and of leptazol was examined by the oral administration of a solution of this anticonvulsant in distilled water to groups of 5 mice at dose levels of 4, 8, and 16 mg/kg. The dose volume was 10 ml./kg body weight. The mice were challenged with the convulsant agents 3 hr after receiving the diphenylhydantoin.

The effect of HEOD on the threshold convulsive doses of strychnine and of leptazol was examined following both chronic and acute administration of the chlorinated hydrocarbon. In chronic experiments, mice were divided into four groups throughout which the litter mates were evenly distributed. Individual animals were allocated to the appropriate group in a statistically randomized manner. One group was maintained on normal 86 diet and served as controls, while the remaining three groups received diets containing 5, 10 and 20 ppm HEOD respectively. After 10 weeks' exposure, the threshold convulsive doses to strychnine hydrochloride and to leptazol were determined. In acute experiments, groups of 10 mice received a solution of HEOD in dimethylsulphoxide by oral intubation. HEOD was given at 15, 30 and 60 mg/kg in a dose volume of 10 ml./kg. One control group received dimethylsulphoxide alone, and another control group received distilled water. The threshold convulsive doses of strychnine hydrochloride and of leptazol were determined 18–24 hr later.

The concentration of HEOD in the livers of rats and mice at the time of the experimental observations was determined by electron capture-gas chromatography, as described by de Faubert Maunder, Egan, Godly, Hammond, Roburn & Thomson (1964).

RESULTS

Isolated phrenic nerve-diaphragm preparation of rats

The exposure of rats to diets containing HEOD, 100 ppm for 26 weeks, did not significantly affect rheobase, chronaxie or tetanic frequency in phrenic nerve-diaphragm preparations stimulated through either the nerve or the muscle. These values, together with the liver content of HEOD at the time of the observations, are given in Table 1.

TABLE 1

RHEOBASE, CHRONAXIE AND TETANUS VALUES OBTAINED *IN VITRO* BY STIMULATION OF THE PHRENIC NERVES AND DIAPHRAGM MUSCLES OF RATS EXPOSED TO HEOD (100 PPM FOR 26 WEEKS) AND OF CONTROLS

Mean values±S.E. (number of observations).

	Nerve stimulation		Muscle stimulation	
	HEOD	Control	HEOD	Control
Rheobase (V)	0·231±0·015 (11)	0·213±0·019 (6)	3·70 ±0·30 (11)	4·50±0·52 (6)
Chronaxie (m sec)	0·072±0·003 (11)	0·067±0·005 (6)	0·125±0·016 (11)	0·10±0·012 (6)
Tetanus (impulses/sec)	24·59 ±1·08 (11)	25·08 ±1·13 (6)	25·95 ±0·91 (11)	26·92±1·14 (6)
Liver concn of HEOD μg/g	13·97 ±0·84 (11)	0·015±0·002 (6)	13·97 ±0·84 (11)	0·015±0·002 (6)

Threshold convulsive doses of leptazol and strychnine in mice

The effect of the oral administration of graded doses of diphenylhydantoin 3 hr previously on the threshold convulsive doses of leptazol and of strychnine hydrochloride are presented in Table 2. Diphenylhydantoin, 4 mg/kg completely abolished tonic hind limb extension in 3/5 mice infused with leptazol, while this end-point was abolished in all mice receiving diphenylhydantoin, 8 or 16 mg/kg. In these instances, the infusion was continued until respiratory arrest. The threshold convulsive dose of strychnine hydrochloride was unaffected by prior treatment of the mice with diphenylhydantoin.

TABLE 2

EFFECT OF PRETREATMENT OF MICE WITH DIPHENYLHYDANTOIN ON THE AMOUNT OF LEPTAZOL AND OF STRYCHNINE HYDROCHLORIDE REQUIRED TO PRODUCE TONIC EXTENSION OF THE HIND LIMBS

Mean values±S.E. (number of mice).

Diphenylhydantoin (mg/kg p.o.)	Leptazol (mg/kg)	Strychnine hydrochloride (μg/kg)
Control	97·22±3·98 (5)	618·11±47·25 (5)
4	149·80* 109·60 ±21·08 (2) (3)	639·63±95·29 (5)
8	184·92*±7·48 (5)	614·40±46·91 (4)
16	134·03*±12·19 (5)	642·19±58·68 (5)

* Mean dose to produce respiratory arrest in mice not showing extension of the hind limbs. These values are significantly greater than those of the control mice ($P<0·02$).

Chronic exposure of mice to HEOD for 10 weeks did not affect the threshold convulsive doses of strychnine hydrochloride or of leptazol. The mean threshold convulsive doses of strychnine hydrochloride and of leptazol in mice exposed for 10 weeks to diets containing HEOD, 5, 10 and 20 ppm, and their corresponding controls are shown in Table 3. The mean liver concentrations of HEOD in each dose group at the time of estimating the convulsive doses are also shown.

TABLE 3

THRESHOLD CONVULSIVE DOSES OF LEPTAZOL AND OF STRYCHNINE HYDROCHLORIDE IN MICE MAINTAINED ON HEOD FOR 10 WEEKS

Mean values±S.E. (number of mice).

	Leptazol		Strychnine hydrochloride	
Dietary HEOD ppm	Threshold convulsive dose (mg/kg)	Bulked liver HEOD (μg/g)	Threshold convulsive dose (μg/kg)	Bulked liver HEOD (μg/g)
0	96·06± 3·15 (20)	0·045	832·18±51·77 (18)	0·064
5	92·74± 5·15 (18)	5·18	7975·0±35·81 (20)	4·77
10	86·75± 6·50 (20)	6·98	847·90±50·60 (18)	6·51
20	86·06±10·59 (15)	13·69	832·16±50·11 (15)	16·05

The threshold convulsive doses of leptazol and of strychnine hydrochloride in mice 18–24 hr after oral dosing with HEOD dissolved in dimethylsulphoxide are shown in Table 4. The inclusion of a control group dosed with distilled water reveals an acute effect of dimethylsulphoxide on the sensitivity of mice to strychnine, and to a lesser extent, to leptazol.

TABLE 4

EFFECT OF ACUTE ORAL ADMINISTRATION OF HEOD TO MICE ON THE THRESHOLD CONVULSIVE DOSES OF LEPTAZOL AND OF STRYCHNINE HYDROCHLORIDE

HEOD was dissolved in dimethylsulphoxide (DMSO) and administered orally 18–24 hr before challenging with the convulsant.

Mean threshold convulsive doses ± S.E. (number of observations)

Pretreatment	Bulked liver HEOD content (μg/g)	Leptazol (mg/kg)	Strychnine hydrochloride (μg/kg)
Distilled water	0·045	100·22 ± 8·93 (8)	584·71 ± 18·2·8* (8)
DMSO	0·050	112·65 ± 4·11 (8)	638·90 ± 17·07 (8)
HEOD/DMSO (15 mg/kg p.o.)	6·48	82·29 ± 5·87† (10)	581·40 ± 16·65* (9)
HEOD/DMSO (30 mg/kg p.o.)	11·95	64·58 ± 4·84† (10)	594·77 ± 34·34 (9)
HEOD/DMSO (60 mg/kg p.o.)	17·40	58·29 ± 8·79† (12)	597·55 ± 31·32 (11)

Significantly different from DMSO control values: $*P<0.05$; $\dagger P<0.001$.

A significant increase in sensitivity to the tonic convulsant activity of leptazol was apparent with increasing doses of HEOD, cf. both distilled water and dimethylsulphoxide controls. This effect was not seen in the case of strychnine. The convulsive dose of strychnine hydrochloride in the group of mice pretreated with HEOD, 15 mg/kg, while significantly lower than that of the dimethylsulphoxide control group, did not differ from that of the distilled water control group.

DISCUSSION

Acute intoxication of rats with aldrin increases the excitability of their withdrawal reflex, whereas chronic exposure to this chlorinated hydrocarbon insecticide has the reverse effect (London & Pallade, 1964). Chronic exposure of rats to the closely related epoxide, dieldrin, has been suggested to reduce their muscular efficiency in performing a work exercise (Khaïry, 1960). Ibrahim (1964) showed this treatment to reduce the period over which a tetanic state could be maintained in the gastrocnemius muscle following high-frequency stimulation of the sciatic nerve, and suggested this to indicate a rapid onset of fatigue, which might account for Khaïry's observation.

We have studied the effect of exposure of rats and mice to HEOD (dieldrin) from the point of view of attempting to define the locus at which it exerts its effect. No effect has been found on the motor nerve or voluntary muscle of the phrenic nerve-diaphragm preparation of chronically exposed rats, nor in the nervous pathway between the site of action of leptazol and the hind limbs, or the pathway between the site of action of strychnine hydrochloride and the hind limbs in chronically exposed mice. Strychnine, which has a spinal locus of activity, causes tonic hind limb extension in mice, which is thought to be due to a removal of the effect of inhibitory interneurones on the nervous pathway to extensor muscles (Goodman & Gilman, 1965). Leptazol, on the other hand,

produces a similar tonic extension by an excitatory action predominantly on cerebral structures (Lewin & Esplin, 1961). The use of these two convulsive agents was, therefore, envisaged to provide a means of determining the site of any effect on nervous transmission between the brain and the spinal cord. This idea was supported by the finding that diphenylhydantoin selectively elevated the threshold convulsive dose of leptazol but not that of strychnine hydrochloride, indicating an anticonvulsant activity on the nervous pathway between the predominant locus of activity of leptazol and the hind limbs.

The reduction of the threshold convulsive dose of leptazol observed in mice acutely intoxicated wth HEOD but not that of strychnine hydrochloride suggests a facilitation of the transmission of nervous impulses from the higher motor centres and the cerebrospinal axis by the chlorinated hydrocarbon insecticide. This agrees with the observations of London & Pallade (1964) on the effect of acute intoxication with aldrin on chronaxie measured by the tail withdrawal response in rats. The observations that chronic exposure to HEOD did not raise the threshold convulsive dose of either leptazol or of strychnine hydrochloride in mice whereas the chlorinated hydrocarbon insecticides have been found to reduce the motor responsiveness of intact animals (Khaïry, 1960; London & Pallade, 1964) may lead to some confusion. However, the recent work of Ryan & Shankland (1967) into the effect of HEOD on the giant axons of the American cockroach, *Periplaneta americana* (L.), provides a tentative explanation for this phenomenon. Perfusion of the isolated giant axon with HEOD, 2×10^{-6}M, revealed a multiphasic effect on the membrane action potentials. Within 10 min of its application, HEOD initiated an increase in the negative after-potential which gradually became more pronounced. After 70 min, 3 to 4 repetitive action potentials resulted in the fibres in response to a single stimulus. These repetitive action potentials regressed until only single action potentials were again seen. Ultimately, complete and irreversible blockage of the response resulted. The whole sequence required some $4\frac{1}{2}$ hr perfusion. In agreement with this, Gowdey, Graham, Seguin, Stavraky & Waud (1952) found the injection of 4–8 mg aldrin into the spinal arteries to increase the excitability of the spinal centres of decerebrate cats to acetylcholine and to leptazol, but repeated administration ultimately reduce the excitability of the spinal cord. By analogy, therefore, HEOD (dieldrin) may initially facilitate the response of the nerve membrane to stimuli arising in the higher centres, which would account for the decreased chronaxie durations reported by London & Pallade (1964) in rats acutely intoxicated with aldrin and the lowered threshold convulsive dose of leptazol seen in the present experiments on mice acutely intoxicated with HEOD. Ultimately the chronic exposure to HEOD would be evident as apparently normal axonal membrane responses and therefore normal threshold convulsive doses to leptazol would be observed.

In the present experiments no elevation in threshold convulsive doses to leptazol or strychnine hydrochloride was evident in mice chronically exposed to HEOD. These convulsive agents excite innumerable neurones in the intact animal, some of which may be paralyzed while others may be in the phase of repetitive discharge. Hence the overall effect may result from the degree of exposure of each individual neurone to HEOD.

The results of this investigation do not support the claim of London & Pallade (1964) that chronaximetric observations indicate incipient or toxic exposure to chlorinated hydrocarbon insecticides. The results of a limited study in human volunteers who

ingested HEOD each day for 18 months similarly revealed no obvious difference in chronaxie between test and control groups (Natoff, 1967), although, in animals and man, the presence of HEOD has been demonstrated both qualitatively and quantitatively.

The multiphasic effects of dieldrin on individual neurones (Ryan & Shankland, 1967) may account for the effects observed by Khaïry (1960) and by London & Pallade (1964) on the behavioural responses of the intact animal. Acute intoxication with dieldrin (HEOD) facilitates nervous excitability emanating from the higher motor centres rostral to the site of action of strychnine. Chronic intoxication does not produce any quantitative difference in the sensitivity of the intact animal to convulsant agents. Mice surviving chronic exposure to HEOD may therefore be in a state of submaximal intoxication, at which stage single action potentials result from single stimuli (Ryan & Shankland, 1967). Maximal intoxication would coincide with paralysis of nervous transmission, which would be lethal in the intact animal.

SUMMARY

1. Chronic exposure of rats to diets containing dieldrin (HEOD), 100 ppm for 26 weeks, did not affect the rheobase, chronaxie or tetanic frequency values of phrenic nerve-diaphragm preparations on direct or indirect stimulation.

2. Chronic exposure of mice to diets containing dieldrin (HEOD), 5, 10 and 20 ppm for 10 weeks did not affect the threshold convulsive doses of leptazol or strychnine hydrochloride.

3. Acute oral administration of dieldrin (HEOD), 15, 30 and 60 mg/kg to mice, and challenging them with the convulsant 18–24 hr later, significantly increased the sensitivity to leptazol, but not that to strychnine hydrochloride.

4. Dieldrin (HEOD) is thought to have a facilitatory action on cerebral structures at the site of action of leptazol, or on the nervous pathway between the site of action of leptazol and the hind limbs. This is apparent on acute administration only. It does not influence transmission between the site of action of strychnine and the hind limbs. The possible mechanism of this effect is discussed.

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