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THE EFFECT OF CINNAMOHYDROXAMIC ACID ON THE CENTRAL NERVOUS SYSTEM

BY

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Observations on the antibacterial and antifungal properties of hydroxamic acids have been published but their pharmacology has received little attention. Bernheim & Stoops (1960) have described the depressant properties of anthranilic and several other hydroxamic acid derivatives. The effectiveness of aliphatic and aromatic hydroxamates against sarin toxicity was investigated by Epstein & Freeman (1956). In this communication the pharmacological actions of an unsaturated hydroxamic acid derivative on the central nervous system are described.

METHODS

All experiments were carried out on male albino mice weighing 20 to 25 g except for the anticonvulsant experiments in which older mice weighing 25 to 30 g were used. In all experiments the sodium salt of cinnamohydroxamic acid was used except in the acute toxicity test when both the acid and the salt were used. All doses are given in terms of the acid.

Acute toxicity

This was determined in mice by the oral, subcutaneous and intravenous routes. All the animals were observed for 5 days.

Spontaneous activity

The effect was investigated by the photo-beam method of Dews (1953). Groups of five mice were placed in a Perspex chamber immediately after the drug administration and the effect was recorded at 10 min intervals for 30 min. By this method the depressant effect and the dose required to reduce activity by 50% were established. The duration of action was evaluated by determining the effect at 0, 10, 20, 40 and 80 min after drug administration. For each of these five intervals after drug administration the activity was recorded as for 30 min, and the results are expressed as a percentage reduction compared with the controls which were always tested at the same time as the treated mice. The effect of cinnamohydroxamic acid was compared with methylpentynol.

Effect against hyperactivity induced by amphetamine

Different doses of cinnamohydroxamic acid were given orally to mice; 10 min later they were injected subcutaneously with 15 mg/kg of amphetamine sulphate and immediately placed in activity cages. Activity was recorded as described above using five mice per dose.

Hypnotic effect

This was evaluated in mice by determining the oral, subcutaneous and intravenous dose required to abolish the righting reflex.

Analgesic activity

This was tested by the phenylquinone-writhing test described by Hendershot & Forsaith (1959) and the tail-clip method of Bianchi & Franceschini (1954). For the phenylquinone test groups of five mice were injected subcutaneously with different doses of cinnamohydroxamic acid and 20 min later were tested for analgesia.

Potentiation of hypnotic and analgesic drugs

The subcutaneous LD50, ED50 and duration of action were determined in mice for each analgesic and hypnotic drug alone. A similar experiment was carried out at the same time in which the animals were injected subcutaneously with either the analgesic or the hypnotic drug together with cinnamohydroxamic acid, and the above parameters were redetermined. The tail-clip method was used for assessing analgesia.

Effect of nalorphine against morphine and morphine plus cinnamohydroxamic acid

Groups of five mice were injected subcutaneously with either nalorphine and morphine, or nalorphine morphine and cinnamohydroxamic acid; 40 min later they were tested by the tail-clip method for analgesia.

Anticonvulsant activity

Mice were injected subcutaneously with different doses of cinnamohydroxamic acid (five mice per dose); 10 min later they were injected with 100 mg/kg of leptazol or 2 mg/kg of strychnine hydrochloride intraperitoneally. LD50s and ED50s were calculated by the method of Miller & Tainter (1944). All results are expressed as cinnamohydroxamic acid irrespective of whether the sodium or the meglumine (*N*-methylglucamine) salt was used.

RESULTS

Acute toxicity

The acute LD50s for cinnamohydroxamic acid and its salts are given in Table 1. The intravenous toxicity for the acid itself was not determined because of its poor solubility in a physiological acceptable solvent. The experiment showed that the intravenous toxicity was approximately twice the subcutaneous toxicity. Lethal doses caused respiratory depression followed by death within 5 hr after an intravenous injection and 30 hr after injection by the other two routes.

TABLE 1

ACUTE TOXICITY OF CINNAMOHYDROXAMIC ACID AND ITS SALTS IN MICE

Values are means and standard errors. The numbers in parentheses represent the numbers of mice used. Cinnamohydroxamic acid was given as a fine suspension in 5 or 20% tragacanth and was passed through a tissue grinder before use

Compound	LD50 (mg/kg) by route		
	Oral	Subcutaneous	Intravenous
Cinnamohydroxamic acid	1,350±72 (60)	86±84 (30)	—
Sodium cinnamohydroxamate	1,460±74 (45)	1,000±14 (20)	460±23 (20)
Meglumine hydroxamate	1,500±85 (35)	750±11 (30)	380±16 (22)

Spontaneous activity

Oral doses of 100 and 200 mg/kg produced within 10 min a considerable depressant effect. The animals tended to huddle together but could be disturbed by handling; when they moved they were slow and appeared to have little interest in their surroundings. No respiratory depression was observed at the doses studied. The effect was compared with methylpentynol and the results are shown in Figs. 1 and 2.

The dose reducing activity by 50% at 30 min was about 200 mg/kg of cinnamohydroxamic acid and about 400 mg/kg of methylpentynol. The depressant effect of cinnamo-

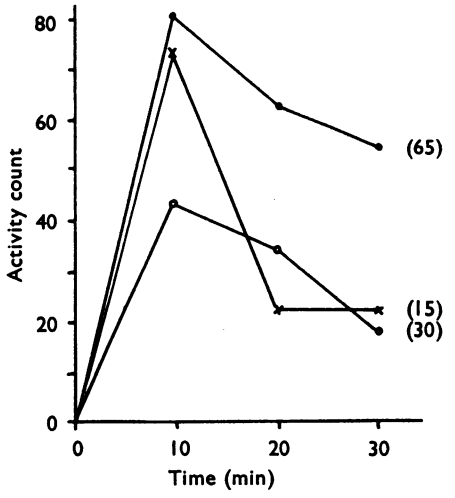

Fig. 1. The depressant effect on motor activity of 200 mg/kg of cinnamohydroxamic acid (○——○), 400 mg/kg of methylpentynol (×——×) and controls (●——●). Both compounds were administered to mice by gastric catheter. Figures in brackets represent the numbers of mice used. The ordinate represents the number of times the animal breaks the beam of light, expressed as an activity count.

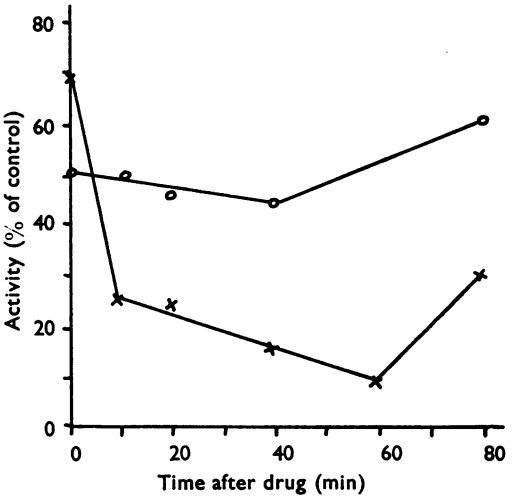

Fig. 2. The duration of action of 200 mg/kg of cinnamohydroxamic acid (○——○) and 400 mg/kg of methylpentynol (×——×) against motor activity in mice. Both compounds were administered by gastric catheter. Each point represents activity after a period of 30 min in the chamber and is the mean for fifteen mice.

hydroxamic acid came on very rapidly and lasted for 40 min, and after 80 min activity had only slightly increased over the peak effect of the drug. With methylpentynol onset was slower after which there was a sharp fall with the peak effect occurring at 60 min, and at the end of 80 min the depressant effect was beginning to wear off (Fig. 2).

Effect against hyperactivity due to amphetamine

Fig. 3 shows that 200 and 600 mg/kg suppressed the stimulant effect of amphetamine. If the total count for amphetamine alone is compared with amphetamine plus cinnamohydroxamic acid it will be observed that there is a 50 to 60% reduction in activity at the end of the 30 min recording period. The larger dose was initially more effective in antagonizing the stimulant effect.

Hypnotic effect in mice

The induction of hypnosis was not associated with ataxia, muscular incoordination or depression of respiration. The compound acted in the same way whether given orally, subcutaneously or intravenously, and the ED50s for all these routes are given in Table 2. Subcutaneous or oral doses of 500 mg/kg induced anaesthesia in some of the mice. The onset of action occurred within 15 min and the duration varied with the dose.

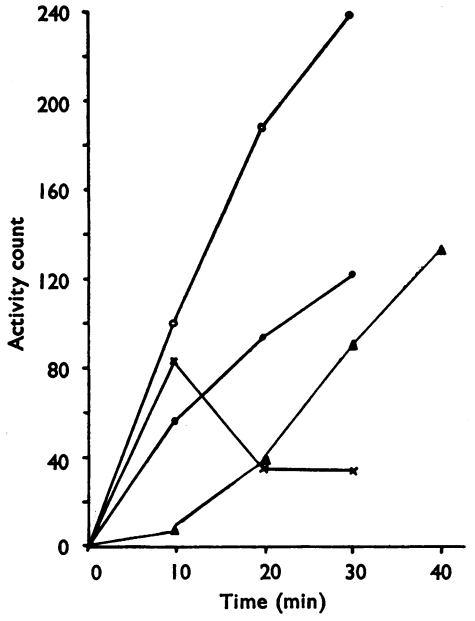

Fig. 3. The inhibitory effect of cinnamohydroxamic acid against amphetamine-induced hyperactivity in mice. Controls (×——×); amphetamine only (○——○); 200 mg/kg (●——●) and 600 mg/kg (▲——▲) of cinnamohydroxamic acid orally. 10 min later the mice were injected subcutaneously with 15 mg/kg of amphetamine. The ordinate represents the number of times the animal breaks the beam of light, expressed as an activity count.

Analgesia

Cinnamohydroxamic acid abolished the writhing induced in mice by phenylquinone, and the ED50 was 75 mg/kg when given subcutaneously and 119 mg/kg given orally. In a similar test with acetylsalicylic acid the subcutaneous and oral ED50s were 50 and 123 mg/kg respectively. Analgesic action could not be demonstrated with the tail-clip method after the subcutaneous injection of 200 mg/kg.

TABLE 2

THE HYPNOTIC EFFECT OF CINNAMOHYDROXAMIC ACID

For hypnotic action pairs of numbers give mice responding and mice tested. Values for ED50s are means and standard errors

Dose (mg/kg)	Route	Hypnotic action	ED50 (mg/kg)
250	Oral	0/10	
500	Oral	4/10	540±33
700	Oral	8/10	
900	Oral	10/10	
300	Subcutaneous	1/5	
400	Subcutaneous	3/5	390±35
500	Subcutaneous	8/10	
250	Intravenous	2/10	
350	Intravenous	6/10	310±14
400	Intravenous	7/10	

TABLE 3

THE POTENTIATION OF SLEEPING TIME DUE TO HEXOBARBITONE

Ten mice were used in each group. 100 mg/kg of cinnamohydroxamic acid, subcutaneously, and 100 mg/kg of hexobarbitone sodium, intraperitoneally, both compounds injected simultaneously. Sleeping time values are means and standard errors

Drug	Duration of sleeping time (min)	*P*
Hexobarbitone sodium	26.8 ± 4.3	—
Cinnamohydroxamic acid	0	—
Hexobarbitone sodium + cinnamohydroxamic acid	42.1 ± 5.43	<0.05

Potentiation of hypnotic and analgesic drugs

Table 3 shows that a nonhypnotic dose of cinnamohydroxamic acid significantly prolonged the sleeping time induced by hexobarbitone. Further experiments demonstrated that the hypnotic ED50 for barbiturates was lowered when the drugs were injected together with cinnamohydroxamic acid. With the exception of thiopentone there was no significant change in toxicity (Table 4).

Table 5 shows that the duration of analgesia was significantly prolonged by cinnamohydroxamic acid when tested by the tail-clip method. Pethidine, 50 mg/kg, doubled the analgesic time; this dose had no effect on the action of the other three analgesics. The minimum dose which increased the duration of action of morphine and diamorphine was 100 mg/kg, and for codeine it was 200 mg/kg.

In the presence of cinnamohydroxamic acid the ED50 for each analgesic was reduced without a corresponding change in the toxicity (Table 6). It is interesting to note that the ED50 for morphine plus cinnamohydroxamic acid was approximately four times less than that required for morphine alone.

TABLE 5

THE EFFECT OF CINNAMOHYDROXAMIC ACID ON THE DURATION OF ANALGESIA

Codeine was used as the phosphate, the other drugs as hydrochlorides. Pairs of numbers for response to analgesic drugs are mice responding and mice tested. Durations of analgesia are means. N.S. = not significant

Analgesic	Cinnamohydroxamic acid (mg/kg)	Analgesia		
		Response	Duration (min)	*P*
Pethidine, 60 mg/kg	0	7/10	23	—
	200	10/10	50	<0.001
	100	10/10	43	<0.001
	50	9/10	43	<0.01
Morphine, 80 mg/kg	0	9/10	80	—
	200	10/10	124	<0.001
	100	10/10	105	<0.02
	50	10/10	82	N.S.
Diamorphine, 6 mg/kg	0	10/10	36	—
	200	10/10	65	<0.001
	100	10/10	49	<0.05
	50	10/10	37	N.S.
Codeine, 140 mg/kg	0	7/10	41	—
	200	10/10	91	<0.001
	100	8/10	54	<0.2
	50	8/10	41	N.S.

TABLE 4

THE EFFECT OF CINNAMOHYDROXAMIC ACID ON THE ED50s (HYPNOTIC ACTION) AND LD50s OF BARBITURATES

Both compounds were injected simultaneously. 100 mg/kg of cinnamohydroxamic acid (CHA) was injected with sodium hexobarbitone and sodium amylobarbitone, 200 mg/kg with phenobarbitone and sodium thiopentone. Values are means and standard errors. Numbers in parentheses refer to the numbers of animals used. N.S.=not significant

Barbiturate	ED50			LD50		
	Barbiturate only (mg/kg)	Barbiturate +CHA (mg/kg)	*P*	Barbiturate only (mg/kg)	Barbiturate +CHA (mg/kg)	*P*
Sodium hexobarbitone	93±6 (35)	62.4±4 (20)	<0.01	560±33 (20)	420±24 (20)	N.S.
Sodium amylobarbitone	79±1.7 (20)	48.7±7.6 (20)	<0.01	286±14 (35)	270±11 (30)	N.S.
Phenobarbitone	61±4.1 (35)	31 ±4.1 (25)	<0.01	140±8.7 (30)	135±8.2 (25)	N.S.
Sodium thiopentone	54±3.5 (40)	12 ±5.4 (25)	<0.001	100±4.4 (15)	30±1 (15)	<0.001

TABLE 6

THE EFFECT OF CINNAMOHYDROXAMIC ACID (CHA) ON THE ED50s AND LD50s OF ANALGESICS

Both compounds were injected simultaneously. Values are means and standard errors. Numbers in parentheses refer to the numbers of mice used. N.S.=not significant

Analgesic	ED 50				LD 50			
	Dose of CHA (mg/kg)	Analgesic alone (mg/kg)	Analgesic + CHA (mg/kg)	*P*	Dose of CHA (mg/kg)	Analgesic alone (mg/kg)	Analgesic + CHA (mg/kg)	*P*
Pethidine	100	48 ±13 (20)	23.5±2.2 (20)	<0.002	200	200±12.4 (20)	140±21 (30)	N.S.
Diamorphine	100	3.4±0.3 (20)	1.7±0.3 (25)	<0.01	200	170±25 (45)	205±32 (25)	N.S.
Morphine	100	41.5±0.5 (20)	11.2±2.4 (30)	<0.001	100	520±89 (25)	430±79 (30)	N.S.
Codeine	100	118 ±10 (25)	57 ±9 (30)	<0.001	200	300±30 (35)	215±11 (30)	N.S.

The times taken for morphine and codeine to reach their maximum effects were 40 and 30 min respectively. The injection of these compounds with 50 mg/kg of cinnamohydroxamic acid resulted in the peak effect being reached within 10 min.

The stimulant effect and the Straub tail phenomenon associated with morphine and diamorphine were abolished by cinnamohydroxamic acid without altering the analgesic properties of the drugs.

Reversal of morphine-cinnamohydroxamic acid analgesia by nalorphine

The subcutaneous injection of 0.25 mg/kg of nalorphine effectively antagonized the analgesic effect of morphine and morphine plus cinnamohydroxamic acid. No effect on analgesia was found if the nalorphine was injected 10 min before but the peak analgesic effect was not reached for 40 min. The analgesic effect was reversed if the nalorphine was injected 10 min after the morphine plus cinnamohydroxamic acid.

Anticonvulsant properties

The subcutaneous injection of 200 to 600 mg/kg gave no protection against convulsions due to strychnine. Some of the mice injected with 400 and 600 mg/kg showed only slight convulsive activity after leptazol.

The higher doses of cinnamohydroxamic acid protected against the lethal effects of strychnine and leptazol, and the approximate ED50s were 400 to 600 mg/kg.

DISCUSSION

The results show that cinnamohydroxamic acid depresses the central nervous system. Sedation and sleep occurred without the ataxia and stimulation which is associated with barbiturates. A rapid reduction in motor activity after an oral dose indicates a rapid absorption of the compound by this route. Within 10 min activity was reduced by about 50% compared with 15 min required for methylpentynol to produce a comparable effect. After 30 min both compounds had reduced activity to approximately the same level.

Cinnamohydroxamic acid in common with many sedatives was able to reduce the increased motor activity induced by amphetamine. None of the doses studied showed a prolonged effect.

Cinnamohydroxamic acid did not significantly alter the toxicity of the other compounds, with the exception of sodium thiopentone, suggesting a comparison of the therapeutic indices (ED50 and LD50) with and without the potentiating agent. The greatest effect was found with morphine and diamorphine in which the index was increased by 300 and 200% respectively, pethidine by 150%, sodium hexobarbitone by 116% and sodium amylobarbitone by 130%. A dose of 100 mg/kg had no effect on phenobarbitone and sodium thiopentone; however, the index was increased by 187 and 160% respectively by 200 mg/kg of cinnamohydroxamic acid.

In addition to a potentiating effect on potency, cinnamohydroxamic acid prolonged the duration of action of sodium hexobarbitone and of the analgesic drugs. This effect cannot be attributed to the dose used as it has no hypnotic or morphine-like properties. Furthermore it is unlikely that the weak aspirin-like action of cinnamohydroxamic acid could contribute to the total effect. From the evidence available it appears that, in this respect,

cinnamohydroxamic acid shows properties similar to chlorpromazine (Courvoisier, Fournel, Ducrot, Kolsky & Koetschet, 1953).

The mode of action of cinnamohydroxamic acid in prolonging and potentiating the action of analgesic and hypnotic drugs without significantly altering their LD50s is not known. It is unlikely that absorption or delayed excretion were involved as a change in the toxicity of the drugs would be expected. A possible explanation may well be that cinnamohydroxamic acid potentiates and prolongs drug action by inhibiting liver microsomal enzymes in a similar way to SKF 525A (2-diethylaminoethyl αα-diphenylvalerate hydrochloride) (Axelrod, Reichenthal & Brodie, 1954; Cooper, Axelrod & Brodie, 1954; Brodie, 1965). However, other workers have suggested that the site of action of SKF 525A may be the central nervous system as well as the liver (Cook, Narvis, Tonner & Fellows, 1953; Swinyard, Madsen & Goodman, 1954; Herken, Neubert & Timmler, 1959; Medaković & Bavić, 1963). The fact that cinnamohydroxamic acid significantly prolonged drug action suggests that the metabolic breakdown of hypnotics and analgesics is decreased, though this would not explain the increase in potency. It is possible that, in addition to inhibiting liver microsomes, cinnamohydroxamic acid acts on some specific mechanism involving the brain. Reversal of analgesia induced by morphine plus cinnamohydroxamic acid by nalorphine supports the hypothesis that the compound acts on the same receptors as morphine.

The experiment investigating the effect of nalorphine injected 10 min before morphine plus cinnamohydroxamic acid is of particular interest. In this test analgesia was not abolished but the effect of cinnamohydroxamic acid was antagonized, as shown by the time taken to reach the peak effect, and in this respect the drug behaved similarly to morphine alone. This adds support to the view that the receptors involved are common to all these compounds.

SUMMARY

1. The effect of cinnamohydroxamic acid on the central nervous system has been investigated.

2. The intravenous, subcutaneous and oral toxicity was evaluated in mice.

3. The depressant effect on motor activity and the antagonism of amphetamine-induced hyperactivity were demonstrated by means of activity cages. The hypnotic effect was assessed by the loss of the righting reflex.

4. Morphine-like analgesia could not be detected by the tail-clip method. The phenylquinone-writhing test showed that the compound had an action similar to aspirin.

5. The interaction of the drug with barbiturates and analgesics, and the effect on toxicity, potency and duration of action are described. Cinnamohydroxamic acid increased the potency and duration of morphine, diamorphine, pethidine and codeine without significantly altering toxicity. A similar effect was found with hexobarbitone and amylobarbitone with 100 mg/kg of cinnamohydroxamic acid; 200 mg/kg was necessary to achieve a similar effect with phenobarbitone and thiopentone.

6. Anticonvulsant properties against strychnine and leptazol were determined. There was no protection against convulsions due to strychnine but there was some against leptazol; higher doses protected against the lethal effects of the convulsant drugs.

7. The mode of action is discussed and it is suggested that cinnamohydroxamic acid may influence potency and duration of analgesic drugs and barbiturates by acting on the central nervous system as well as on liver microsomes.

REFERENCES

AXELROD, J., REICHENTHAL, J. & BRODIE, B. B. (1954). Mechanism of the potentiating action of β-diethylaminoethyl diphenylpropylacetate. *J. Pharmacol. exp. Ther.*, **112**, 49–54.

BERNHEIM, F. & STOOPS, J. K. (1960). Pharmacological effects of anthranilic hydroxamic acid and certain other hydroxamic acids. *Proc. Soc. exp. Biol. (N.Y.)*, **105**, 542–543.

BIANCHI, C. & FRANCESCHINI, J. (1954). Experimental observations on Haffner's method for testing analgesic drugs. *Brit. J. Pharmacol.*, **9**, 280–284.

BRODIE, B. B. (1956). Pathways of drug metabolism. *J. Pharm. Pharmacol.*, **8**, 1–17.

COOK, L., NARVIS, G. G., TONNER, L. & FELLOWS, E. G. (1953). Potentiation of certain analgesic drugs by β-diethylaminoethyl diphenylpropylacetate hydrochloride (S.K.F. 525A). *Fed. Proc.*, **12**, 313.

COOPER, J. R., AXELROD, J. & BRODIE, B. B. (1954). Inhibitory effects of β-diethylaminoethyl diphenylpropylacetate on a variety of drug metabolic pathways *in vitro*. *J. Pharmacol. exp. Ther.*, **112**, 55–63.

COURVOISIER S., FOURNEL, J., DUCROT, R., KOLSKY, M. & KOETSCHET, P. (1953). Propriétés pharmacodynamiques du chlorhydrate de chloro-3-(dimethylamino-3-propyl)-10-phénothiazine (4,560 R.P.). *Arch. int. Pharmacodyn.*, **92**, 305–361.

DEWS, P. B. (1953). The measurement of the influence of drugs on voluntary activity in mice. *Brit. J. Pharmacol.*, **8**, 46–48.

EPSTEIN, M. A. & FREEMAN, G. (1956). Toxicity of hydroxamic acid analogues: prophylactic and therapeutic efficacy against nerve gas poisoning in mice. *Proc. Soc. exp. Biol. (N.Y.)*, **92**, 660–662.

HENDERSHOT, L. C. & FORSAITH, J. (1959). Antagonism of the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and non-analgesics. *J. Pharmacol. exp. Ther.*, **125**, 237–240.

HERKEN, H., NEUBERT, D. & TIMMLER, R. (1959). Die enzymatische N-Demethylierung durch Leber-Mikrozomes bei der Morphin-Gewohung. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **237**, 319–333.

MEDAKOVIĆ, M. & BAVIĆ, B. (1963). The effects of two phenylacetic acid derivatives on the analgesic action of morphine in mice. *J. Pharm. Pharmacol.*, **15**, 660–665.

MILLER, L. C. & TAINTER, M. L. (1944). Estimation of the ED50 and its error by means of logarithmic-Probit graph paper. *Proc. Soc. exp. Biol. (N.Y.)*, **57**, 261–264.

SWINYARD, E. A., MADSEN, J. A. & GOODMAN, L. S. (1954). The effect of β-diethylaminoethyl diphenylpropylacetate (S.K.F. 525A) on the anticonvulsant properties of antiepileptic drugs. *J. Pharmacol. exp. Ther.*, **111**, 54–63.