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ACTIVITY OF CENTRALLY ACTING AND OTHER DRUGS AGAINST TREMOR AND HYPOTHERMIA INDUCED IN MICE BY TREMORINE

BY

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Tremorine (1,4-dipyrrolidino-2-butyne) exerts a unique action in several species of laboratory animal, producing sustained tremors, miosis, salivation, diarrhoea and hypothermia (Everett, 1956). These effects can be antagonized by treatment with drugs active clinically against Parkinson's disease (Everett, 1956; Everett, Blockus & Shepperd, 1956; Frommel, 1958) and the antitremorine test is now used widely in the investigation of new compounds for potential antiParkinsonian activity. However, little attention has been paid to the hypothermia produced in these animals or to its modification by treatment with drugs. Although it is known that antiParkinsonian drugs will prevent or reverse the tremorine-induced hypothermia (Blockus & Everett, 1957; Farquharson & Johnston, 1959; Keranen, Zaratzian & Coleman, 1961) it is customary to measure antitremorine activity by reduction of the tremor.

In mice, the degree of tremor can be assessed on a quantal basis (Keranen *et al.*, 1961; Ahmed & Marshall, 1962), by electro-mechanical means (Blockus & Everett, 1957; Frommel, Gailland & Fleury, 1959) or by simple subjective scoring systems (Farquharson & Johnston, 1959; Halliwell, Quinton & Williams, 1964). These methods of assessing tremor are either time consuming or imprecise due to subjective error on the part of the experimenter. Recently it has become possible to measure rapidly the body temperature of individual small conscious laboratory animals using an oesophageal thermocouple and electric thermometer (Brittain & Spencer, 1964). Initial experiments with tremorine suggested that tremor and hypothermia developed simultaneously and the severity and duration of both these effects was dependent on the dose of tremorine used.

The object of the present work was to investigate in more detail the relationship between tremor and hypothermia induced by tremorine in mice, and to determine the activity of antiParkinsonian and other drugs on these two indices of tremorine activity.

METHODS

Animals. Male adult mice, weighing 17 to 22 g, of the Glaxo A_2G strain were used throughout. Animals were allowed free access to a cube diet (E. Dixon & Sons, No. 41B) and drinking water until the commencement of an experiment. Housing and laboratory temperatures were maintained at 18 to 22° C.

Measurement of tremor. Tremor was assessed visually and scored as follows: no tremor = 0, moderate or intermittent tremor = 1, pronounced continuous tremor = 2 marks. The mean individual score, or

" tremor index," was determined for each group of mice. The antitremor activity of a drug in either prevention or reversal experiments was assessed using three or more dose levels: the dose which reduced the tremor index to 50% of that in animals injected with tremorine alone, ED50 (tremor), was calculated by the usual graphical method.

Measurement of hypothermia. Body temperatures of individual mice were determined with a calibrated electric thermometer and thermocouple; the latter, mounted in an 18-gauge needle, was inserted briefly into the oesophagus until a constant reading was obtained (Brittain & Spencer, 1964). The temperatures of a group of ten mice could be measured and recorded within 1.5 to 2 min. The degree of hypothermia was the difference between the mean body temperature of a group of control untreated mice and the mean of the group under test. The antihypothermic activity of a drug in either prevention or reversal experiments was assessed using three or more dose levels: the dose which reduced the level of hypothermia to 50% of that in animals injected with tremorine alone, ED50 (hypothermia), was calculated by the usual graphical method.

Drugs and solutions. Tremorine hydrochloride is hygroscopic and was stored in a desiccator until required. Solutions of tremorine were prepared in 0.9% w/v saline immediately before use, and injected in a volume of 0.2 ml./20 g body weight. All test drugs were either dissolved, or suspended in 5% w/v acacia solution, and were administered orally either before or after the injection of tremorine. Doses were contained in a volume of 0.4 ml./20 g body weight. All doses in the text are expressed in terms of the free base or cation.

The following drugs were examined for their ability to antagonize the effects of tremorine: amitriptyline hydrochloride, amphetamine sulphate, amylobarbitone sodium, atropine methonitrate, atropine sulphate, bemegride, benzhexol hydrochloride, benztropine methanesulphonate, bretylium tosylate, caramiphen hydrochloride, chlordiazepoxide hydrochloride, chlorpromazine hydrochloride, (±)-3,4-dihydroxyphenylalanine (dopa), diphenhydramine hydrochloride, fencamfamin hydrochloride, guanethidine sulphate, haloperidol, hexamethonium bromide, hydrallazine, (−)-hyoscine hydrobromide, (−)-hyoscyamine alkaloid, imipramine hydrochloride, isoprenaline sulphate, (+)-lysergic acid diethylamide tartrate, mephenesin, meprobamate, mepyramine maleate, α-methyldopa, morphine hydrochloride, nikethamide, nortriptyline hydrochloride, pargyline hydrochloride, perphenazine hydrochloride, pethidine hydrochloride, phenelzine dihydrogen sulphate, phenindamine tartrate, phentolamine methanesulphonate, phenytoin sodium, promethazine hydrochloride, propantheline bromide and tranylcypromine sulphate.

RESULTS

Relationship between tremor and hypothermia induced by tremorine in mice

Tremorine, 20 mg/kg, was administered to groups of ten mice either orally, subcutaneously, intraperitoneally or intravenously, and the intensity of tremor and hypothermia was determined at intervals up to 24 hr later (Fig. 1). Irrespective of the route of administration, tremorine quickly induced a severe, rapid and continuous tremor with episodes of violent whole-body shaking, muscular rigidity and weakness. Intense parasympathetic stimulation was also observed, characterized by lachrymation, salivation and diarrhoea. These effects were always accompanied by a marked hypothermia; the body temperature often fell by more than 6° C within 1 hr of injection of tremorine. After oral, intraperitoneal or intravenous injection, the tremor preceded any marked hypothermia by some 20 to 30 min. Although characterized by a slightly less intense tremor of slower onset, the subcutaneous administration of tremorine produced a tremor and a hypothermia which reached their maxima simultaneously.

Tremorine was most active when administered orally. It is possible that tremorine is converted to its active metabolite oxotremorine (Cho, Haslett & Jenden, 1961) in the liver. After oral administration, the tremorine would reach the liver quantitatively and be rapidly converted to oxotremorine, thereby giving it activity of quicker onset, greater intensity and

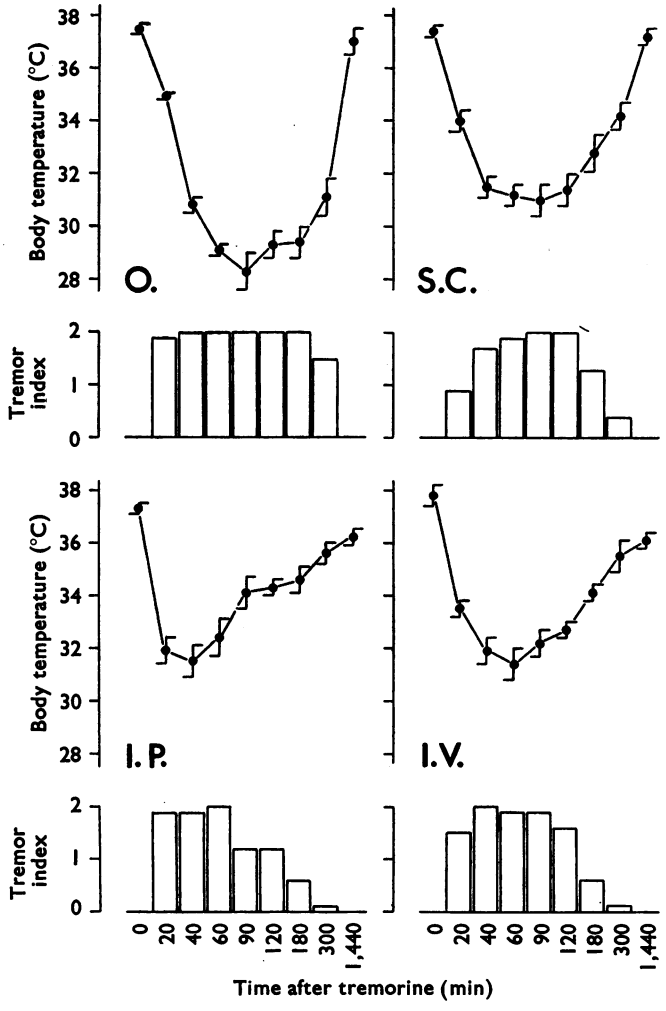

Fig. 1. Onset, severity and duration of tremor and hypothermia in mice: effect of different routes of administration of tremorine, 20 mg/kg. Routes: oral (O.); subcutaneous (S.C.); intraperitoneal (I.P.); intravenous (I.V.). Body temperatures are means with standard errors.

greater duration. Irrespective of the route of administration, all animals were devoid of tremor, hypothermia and other tremorine-induced symptoms 24 hr after injection.

Because of the simultaneous appearance of tremor and hypothermia after subcutaneous administration, and the successful use of this route by other workers evaluating anti-Parkinsonian drugs (Frommel *et al.*, 1959; Farquharson & Johnston, 1959) it was decided to use this route for all subsequent work. In Fig. 2, the effects of four different doses of tremorine are compared. Using groups of ten mice, tremorine was injected subcutaneously at either 5, 10, 20 or 30 mg/kg and the tremor and hypothermia were determined at intervals up to 24 hr later. The intensity and duration of both tremor and hypothermia were dose-dependent, with maximal tremor and hypothermia occurring simultaneously at each

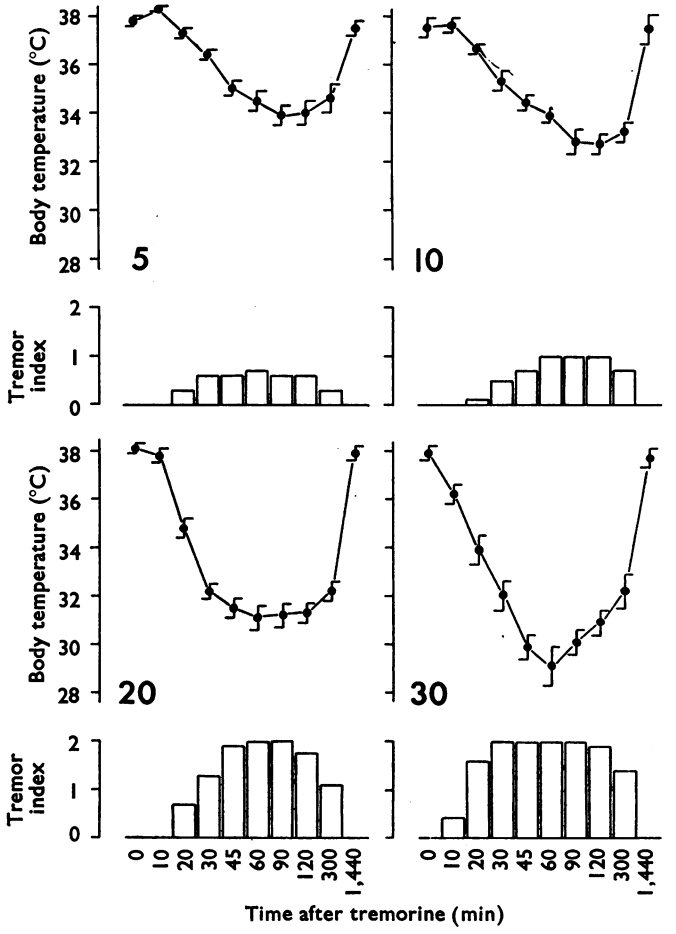

Fig. 2. Onset, severity and duration of tremor and hypothermia in mice: effect of different doses of tremorine administered subcutaneously; numerals represent doses in mg/kg. Body temperatures are means with standard errors.

dose level of tremorine investigated. 20 mg/kg was the smallest dose of tremorine investigated with which a period of maximal tremor was produced. This was accompanied by a marked hypothermia of approximately 7° C. Irrespective of the dose of tremorine injected, all animals were devoid of tremorine-induced symptoms 24 hr after injection.

From this initial work, it was decided that the maximal but relatively transient effects produced by the subcutaneous injection of tremorine, 20 mg/kg, provided the most suitable conditions for examining simultaneously the antitremor and antihypothermic effects of drugs. Accordingly, this dose and route of administration of tremorine were used throughout the rest of the investigation.

Prevention of tremor and hypothermia with atropine-like drugs

It is well established that tremorine-induced tremor is prevented in mice by treatment with atropine and similar alkaloids (Farquharson & Johnston, 1959) but little attention

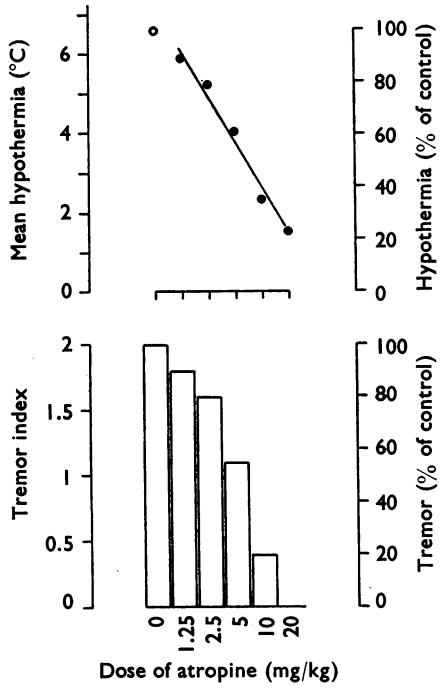

Fig. 3. Antitremor and antihypothermic activities of atropine in mice. Atropine was administered orally 2 hr before subcutaneous injection of tremorine, 20 mg/kg. Intensity of tremor and hypothermia were measured 60 min later.

has been given to the fact that these alkaloids also antagonize the hypothermia. Consequently the antitremor and antihypothermic activities of atropine were examined simultaneously. Groups of ten mice were treated orally with atropine, either 1.25, 2.5, 5, 10 or 20 mg/kg, followed 2 hr later by a subcutaneous injection of tremorine, 20 mg/kg. By this route and at this dose level, maximal tremor and hypothermia occur 60 min after the

TABLE 1

ABILITY OF ATROPINE, HYOSCYAMINE AND HYOSCINE TO PREVENT TREMORINE-INDUCED TREMOR AND HYPOTHERMIA IN MICE

The alkaloids were administered 2 hr before a subcutaneous injection of tremorine, 20 mg/kg. Tremor and hypothermia were determined 60 min later. * Illustrated in Fig. 3

	Dose to prevent by 50%	
Alkaloid	Tremor (mg/kg)	Hypothermia (mg/kg)
Atropine	4·90*	6·80*
	7·00	6·05
	8·92	6·65
	11·10	7·30
(−)-Hyoscyamine	3·45	3·70
	4·25	4·00
(−)-Hyoscine	3·20	3·90
	3·95	3·30
	4·50	4·00
	2·60	3·45

injection of tremorine. Therefore, tremor and hypothermia were measured at this time (Fig. 3). Atropine clearly reduced the extent of both tremor and hypothermia; this was more marked as the dose of atropine was increased. An approximately linear relationship was observed between the dose of atropine and the degree of antagonism of both tremor and hypothermia. Although greatly reduced, the tremorine-induced tremor and hypothermia were maximal at 60 min and decreased in intensity thereafter. Thus the effect of atropine is to reduce quantitatively tremor and hypothermia throughout the period of tremorine activity. It was observed that the peripheral parasympathomimetic effects are more easily antagonized (that is, with lower doses of atropine) than are tremor and hypothermia. It was also noticed that it was very difficult to completely prevent the hypothermia. It is likely, however, that, with these higher doses of atropine, the low level of hypothermia persisting was more easily detected and measured than would be low levels of spasmodic tremor.

Similar experiments were performed with (−)-hyoscyamine and (−)-hyoscine and the doses reducing tremor or hypothermia by 50% were calculated graphically. The results of several experiments are summarized in Table 1. The two laevo-alkaloids are clearly more active than the racemic atropine, suggesting that the dextro-isomer possesses little or no antitremor or antihypothermic activity. The relative inactivity of the dextro-isomers has also been reported recently by Buckett & Haining (1965).

Effect of centrally acting and other drugs on tremor and hypothermia

Many compounds were examined in preliminary experiments to determine their effect on tremor and hypothermia induced by tremorine. Where activity was detected in a preliminary experiment, the drug was reinvestigated to obtain a quantitative estimate of its activity. The results with this series of compounds, grouped according to their general pharmacological activity, are summarized in Table 2. The synthetic drugs with central anticholinergic activity and of known clinical usefulness against Parkinsonism—benzhexol, caramiphen and benztropine—showed considerable activity against both tremor and hypothermia, as well as the other effects of tremorine injection, such as salivation, micturition, sweating and diarrhoea. The tremor and hypothermia were equally antagonized by these drugs. On the other hand, propantheline bromide and atropine methonitrate which do not gain access readily to the central nervous system blocked only the peripheral effects and were almost inactive against tremor and hypothermia. These compounds did produce a marginal antihypothermic effect at 50 mg/kg but this was probably due to a reduction in the rate of heat loss through less-intense sweating, salivation and diarrhoea. Two antihistamine drugs, diphenhydramine and mepyramine, with known atropine-like properties, showed definite antitremor and antihypothermic activity, besides activity against the peripheral effects of tremorine.

Definite activity was also observed amongst the sympathomimetic agents studied. Amphetamine and fencamfamin, which have considerable central stimulant activity, abolished the effects of tremorine, including the tremor and hypothermia; in fact, the hypothermia was more easily antagonized by these drugs than was the tremor. Isoprenaline, with only limited access to the brain, was far less active, whilst dopa—which does gain access to the brain—was virtually inactive. Definite activity against both the tremor and hypothermia was also observed amongst imipramine and its analogues; as with the more

TABLE 2

ABILITY OF CENTRALLY ACTING AND OTHER DRUGS TO PREVENT TREMORINE-INDUCED TREMOR AND HYPOTHERMIA

All drugs were given orally 2 hr before the subcutaneous administration of tremorine, 20 mg/kg. The levels of tremor and hypothermia were determined 1 hr after injection of tremorine. The figures in parentheses after the word inactive indicate the maximum dose of drug examined. * Marginal activity

Drug	Dose to prevent by 50%	
	Tremor (mg/kg)	Hypothermia (mg/kg)
Central and peripheral anticholinergics		
Atropine methonitrate	Inactive (50)	10% at 50*
Benzhexol	5·30	5·85
Benztropine	3·00	2·50
Caramiphen	9·80	10·30
Propantheline	Inactive (50)	15% at 50*
Antihistamines		
Diphenhydramine	≏25	≏30
Mepyramine	>50	>50
Phenindamine	Inactive (50)	Inactive (50)
Promethazine	Inactive (100)	Inactive (100)
Sympathomimetics		
(±)-Amphetamine	15	6·50
Dopa	Inactive (200)	≏150
Fencamfamin	25	10
Isoprenaline	≏100	≏25
Antidepressants and monoamine oxidase inhibitors		
Amitriptyline	>50	35
Imipramine	≏50	≏20
Nortriptyline	40	20
Pargyline	Inactive (200)	Inactive (200)
Phenelzine	≏50	≏20
Tranylcypromine	>50	12
Major tranquillizers and depressants		
Chlorpromazine	2·50	Inactive (25)
Haloperidol	≏5·0	Inactive (10)
Perphenazine	>5	Inactive (5)
Miscellaneous		
Amylobarbitone	Inactive (100)	Inactive (100)
Bretylium	Inactive (75)	Inactive (75)
Chlordiazepoxide	≏25	Inactive (150)
Guanethidine	Inactive (200)	≏200
Hexamethonium	Inactive (200)	Inactive (200)
Hydrallazine	≏25	Inactive (50)
Lysergic acid diethylamide	Inactive (100 μg)	Inactive (100 μg)
Mephenesin	Inactive (200)	Inactive (200)
Meprobamate	Inactive (100)	Inactive (100)
α-Methyldopa	Inactive (50)	Inactive (50)
Morphine	≏200	≏200
Pethidine	>100	>100
Phentolamine	Inactive (25)	Inactive (25)
Phenytoin	Inactive (200)	Inactive (200)

directly acting sympathomimetics, hypothermia was more easily antagonized by these drugs than was the tremor. The monoamine oxidase inhibitor tranylcypromine also showed considerable activity, although again this could be related to its high sympathomimetic activity; thus, other monoamine oxidase inhibitors were either completely inactive (pargyline) or far less active (phenelzine).

Among the other drugs examined, only chlorpromazine and haloperidol showed clear

activity. In these, no antihypothermic activity was seen. It is possible that at least some of the activity of chlorpromazine was due to an inhibition of tremorine-oxotremorine conversion in the liver (Leslie & Maxwell, 1964); in addition, the persistent hypothermia was probably in response to the chlorpromazine rather than the tremorine.

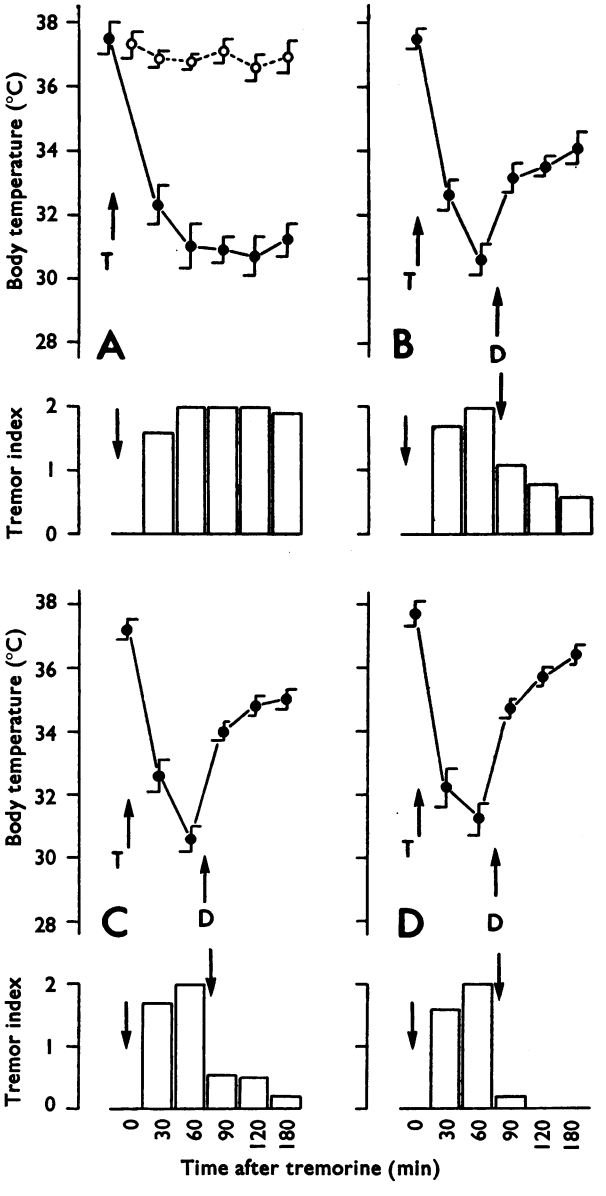

Fig. 4. Ability of atropine to reverse established tremor and hypothermia in mice. Tremorine, 20 mg/kg, was administered subcutaneously, followed 60 min later by atropine, orally. Doses of atropine: none, controls (A); 5 mg/kg (B); 10 mg/kg (C); and 20 mg/kg (D). Administration of drugs: at T, tremorine; at D, atropine. Body temperatures are means with standard errors.

Reversal of established tremorine-induced tremor and hypothermia

Previous workers have shown that it is possible to reverse established tremor (Everett *et al.*, 1956) and hypothermia (Farquharson & Johnston, 1959) by giving specific anti-Parkinsonian drugs some time after the injection of tremorine. Accordingly, it was decided to investigate simultaneously the effects of different doses of atropine on established tremor and hypothermia. At 60 min after a subcutaneous injection of tremorine, 20 mg/kg, mice were given orally a dose of atropine. Tremor and hypothermia were measured at intervals over the succeeding 2 hr (Fig. 4). Tremorine produced marked tremor and hypothermia which were maximal at 60 min. These effects were rapidly reversed by atropine and at higher doses they were completely abolished. By converting to percentage inhibition the residual tremor and hypothermia present 60 min after administration of the atropine (that is, 120 min after the tremorine), and plotting this percentage against the dose of atropine administered, the ED50-(tremor) and ED50-(hypothermia) for atropine were respectively 4.30 and 5.60 mg/kg. Hyoscyamine, hyoscine and the synthetic anticholinergic drugs were examined in a similar way. The results with this group are summarized in Table 3. The order of potency of these drugs is identical to that found in the prevention experiments (see Tables 1 and 2).

TABLE 3

ABILITY OF ANTICHOLINERGIC DRUGS TO REVERSE ESTABLISHED TREMORINE-INDUCED TREMOR AND HYPOTHERMIA IN MICE

The drugs were administered 60 min after a subcutaneous injection of tremorine, 20 mg/kg. Tremor and hypothermia were determined a further 60 min later. The figures in parentheses after the word "inactive" indicate the maximum dose investigated

Test drug	Dose to reverse by 50%	
	Tremor (mg/kg)	Hypothermia (mg/kg)
Atropine	4·30	5·60
(−)-Hyoscyamine	2·00	1·50
(−)-Hyoscine	1·52	1·45
Benzhexol	7·50	8·05
Caramiphen	12·0	12·4
Benztropine	1·65	1·35
Propantheline	Inactive (50)	Inactive (50)
Atropine methonitrate	Inactive (50)	Inactive (50)

A criticism often made of the tremorine test is that too often drugs with no clinical anti-Parkinsonian activity prevent the appearance of tremorine-induced effects. If this is due to a reduction in the liver's ability to convert tremorine to oxotremorine (Kocsis & Welch, 1960; Leslie & Maxwell, 1964), drugs of this type should prove unable to reverse established tremor and hypothermia, although the duration of these effects might be affected. Accordingly, many of the drugs examined previously (see Table 2) were re-examined for their ability to reverse the effects of tremorine (Table 4). As in the prevention experiments, drugs with either direct or indirect central sympathomimetic activity were active in abolishing tremor and particularly hypothermia. On the other hand, the phenothiazines were either less active (chlorpromazine) or inactive (perphenazine), suggesting that much of their activity in prevention experiments could be due to inhibition in the liver of tremorine metabolism (Leslie & Maxwell, 1964). Under these conditions, the phenothiazines have no antihypothermic activity at all. The remaining antitremor activity of chlorpromazine is most likely mediated through inhibition in the spinal cord.

TABLE 4

ABILITY OF CENTRALLY ACTING AND OTHER DRUGS TO REVERSE ESTABLISHED TREMORINE-INDUCED TREMOR AND HYPOTHERMIA IN MICE

The drugs were administered 60 min after a subcutaneous injection of tremorine, 20 mg/kg. Tremor and hypothermia were determined a further 60 min later. The figures in parentheses after the word "inactive" indicate the maximum dose investigated

Test drug	Dose to reverse by 50%: Tremor (mg/kg)	Dose to reverse by 50%: Hypothermia (mg/kg)
(±)-Amphetamine	20·6	8·05
Dopa	Inactive (200)	>200
Fencamfamin	24·5	11·0
Isoprenaline	>100	>100
α-Methyldopa	Inactive (100)	Inactive (100)
Tranylcypromine	11·5	7·30
Amitriptyline	≏40	≏30
Imipramine	20·5	15·4
Nortriptyline	22·0	10·6
Diphenhydramine	≏30	25·3
Haloperidol	Inactive (10)	Inactive (10)
Chlorpromazine	5·50	Inactive (25)
Perphenazine	Inactive (5)	Inactive (5)
Chlordiazepoxide	≏25	Inactive (150)
Megimide	Inactive (15)	Inactive (15)
Mephenesin	≏120	Inactive (200)
Meprobamate	≏180	Inactive (200)
Nikethamide	Inactive (10)	Inactive (10)
Pethidine	Inactive (50)	Inactive (50)

Activity amongst the miscellaneous drugs was similar to that observed previously, with two notable exceptions: the central muscle relaxants, mephenesin and meprobamate (as well as chlordiazepoxide), have definite antitremor activity without any effect on the hypothermia. It is likely that this action also is mediated through an effect on the spinal cord which, because of short duration of action, was not effective after a 2-hr treatment period in the prevention experiments.

The central nervous stimulants bemegride and nikethamide were inactive, confirming that stimulants without central sympathomimetic activity are inactive against tremorine.

DISCUSSION

In the present paper, a comparison is made of the effects of a series of centrally acting and other drugs on the tremor and hypothermia produced in mice by tremorine. From the simultaneous measurement of both tremor and hypothermia, several facts emerge which allow differentiation between central and peripheral actions, and also between anticholinergic, sympathomimetic and other types of activity.

Drugs which have central anticholinergic activity prevent and abolish both the tremor and hypothermia as well as the peripheral parasympathetic effects of tremorine. On the other hand, quaternary anticholinergics are inactive against the tremor and only marginally reduce the hypothermia.

Central sympathomimetics also antagonize tremorine in both prevention and reversal experiments. Amphetamine, fencamfamin and tranylcypromine show considerable

activity against the hypothermia, and to a lesser extent the tremor and peripheral effects. In all experiments, the hypothermia was more easily antagonized than the other effects. This action is likely to be a central one since fencamfamin, which has few peripheral effects (Brittain, Jack & Spencer, 1964), is almost as active as amphetamine; furthermore, isoprenaline, which does not readily gain access to the central nervous system, is far less active. On the other hand, dopa—which should reach the brain—is almost inactive by mouth. Preliminary experiments suggest this activity of dopa can be increased by previous treatment with the monoamine oxidase inhibitor, pargyline. The action of tranylcypromine is most probably due to its known " amphetamine-like " sympathomimetic action since other monoamine oxidase inhibitors are either far less active (phenelzine) or completely inactive (pargyline) when given alone.

Some very interesting results were obtained in both prevention and reversal experiments with imipramine, amitriptyline and nortriptyline. Halliwell *et al.* (1964) recently found that neither imipramine nor desmethylimipramine have significant antitremor activity although amitriptyline is active. In the present investigation, definite but weak antitremor activity was found in each of the three imipramine-like compounds examined. However, these compounds have a far greater antihypothermic action, the order of activity in this respect being similar to that found clinically as antidepressants. Imipramine and its analogues are also active in reversing established tremor and particularly hypothermia, confirming that the effect is a central one rather than an effect on liver microsomes (Kato, Chiesara & Vassanelli, 1963; Løvtrup, 1963). Because imipramine, amitriptyline and nortriptyline have a more potent antihypothermic action than an antitremor one, they more closely resemble the true sympathomimetic drugs than the central anticholinergics. Fink (1959) and Benešová & Trinerová (1964) have suggested that an important part of the antidepressant actions of imipramine-like compounds is a central anticholinergic component. If these compounds do have a central anticholinergic action the present experiments suggest that it is well masked by a more potent sympathomimetic action.

Several facts suggest that tremorine induces hypothermia, as well as tremor (Everett *et al.*, 1956; George, Haslett & Jenden, 1962), by a direct central effect. For example, only anticholinergics and sympathomimetics (including the imipramine-like drugs) with a central action antagonize the hypothermia. Also, abolition of the peripheral parasympathetic effects only marginally reduces the severity of the hypothermia. In addition, the rapidity of onset and depth of hypothermia are too great to be explained by central nervous depression alone, and instead point to a more specific action similar to that of chlorpromazine and haloperidol. Thermoregulation in animals is mediated by two hypothalamic centres (Ingram, 1960; Ström, 1960). A rostral (preoptic) area is associated with promoting heat loss (parasympathetic) and a second caudal area with heat conservation (sympathetic) (Elliott, 1963). Reciprocal inhibition between these two zones is likely. Patten, Sakamoto, Van Woert, Papavasiliou & Cotzias (1964) have suggested that a probable site of action of tremorine is the hypothalamus and not, as thought previously, the basal ganglia. It is possible therefore that tremorine causes a profound hypothermia by stimulation of the rostral hypothalamus, with simultaneous inhibition of the caudal heat-conserving area. Such an action would explain why, in the continued presence of tremorine, relatively small doses of a centrally acting sympathomimetic reverse the hypothermia.

Although both tremor and hypothermia appear to be of central origin there is ample evidence that they are two separate effects and are not produced as a result of one another. Blockus & Everett (1957) showed that if mice were placed in an oven at 38° C no hypothermia developed but the tremor symptoms were fully developed. In the present experiments it was observed that the sympathomimetics antagonize the hypothermia at doses which do not detectably affect the tremor. On the other hand tremor could be abolished without affecting the hypothermia; in reversal experiments, centrally acting muscle relaxants such as mephenesin, meprobamate and chlordiazepoxide abolished established tremor but had no effect on the hypothermia. At similar dose levels, none of these drugs alone produced a fall in body temperature.

Use of tremorine as a pharmacological tool

Of the drugs so far examined for antitremorine activity, only central anticholinergics, central sympathomimetics and drugs of the imipramine type are consistently active against hypothermia as well as the tremor. This clearly contrasts with the poor predictive value of antitremor activity. An antihypothermic action is therefore of more predictive value in assessing possible antiParkinsonian activity than is antitremor activity alone. In addition, the test differentiates qualitatively the pharmacological actions of imipramine and its analogues from those of chlorpromazine. This is in contrast to the suggestion of Domenjoz & Theobald (1959), Costa, Garattini & Valzelli (1960) and Metyšová, Metyš & Votava (1963) that the differences between chlorpromazine and imipramine are mainly quantitative.

SUMMARY

1. Besides tremor and peripheral parasympathetic stimulation, tremorine induces in mice a considerable hypothermia. After subcutaneous injection, maximal tremor and hypothermia coincide; therefore, this route of administration was chosen to determine the action of drugs on tremor and hypothermia.

2. The severity of the hypothermia and its antagonism only by centrally acting drugs points to a central mechanism by which hypothermia as well as tremor is induced. However, both effects are separate entities and each can exist in the absence of the other.

3. Both tremor and hypothermia are prevented by treatment with centrally acting anticholinergic drugs. If already established, both effects are readily reversed by such drugs. Quaternary anticholinergic drugs are inactive against tremor and hypothermia although they readily abolish peripheral parasympathetic effects.

4. Tremor and hypothermia are also prevented or reversed by centrally acting sympathomimetic drugs. However, the hypothermia is more sensitive to antagonism than is the tremor, and therefore this test differentiates central sympathomimetics from anticholinergics.

5. Imipramine-like compounds are active in both prevention and reversal experiments, abolishing tremor and hypothermia. They have a greater antihypothermic than antitremor action; in this respect they more closely resemble the sympathomimetics than the anticholinergics. It is suggested that this is an example of a qualitative difference between the pharmacological properties of imipramine-like and chlorpromazine-like drugs.

6. Antihypothermic activity has so far only been observed in drugs with central anticholinergic or sympathomimetic activity. This action therefore is of greater value in predicting antiParkinsonian activity than is antagonism of tremor.

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