Brit. J. Pharmacol. (1959), **14**, 559.

ANTAGONISM OF THE EFFECTS OF TREMORINE BY TROPINE DERIVATIVES

BY

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(RECEIVED SEPTEMBER 7, 1959)

Methods of testing new drugs for anti-Parkinson activity are briefly reviewed. The production in animals of Parkinson-like effects by Tremorine (1,4-dipyrrolidin-1'-ylbut-2-yne), and the inhibition of these effects in mice by a number of tropine derivatives, are described. No correlation was found between the activity against tremor and the anticholinergic, antihistaminic or local anaesthetic properties of the compounds.

During the last twelve years a number of new synthetic drugs, chiefly antispasmodics and antihistaminics, have been found useful in the treatment of Parkinson's disease, but the selection of these drugs for clinical study has been largely empirical.

Until the introduction of synthetic compounds, the natural solanaceous alkaloids were the most reliable agents in the treatment of this disease (Critchley, 1958), but the use of these drugs was complicated by undesirable side-effects such as dry mouth, blurred vision, palpitations, and nausea. An exception was tigliodine, the tiglic ester of *pseudo*tropine, which Trautner and Noack (1951) tested in a small number of patients and considered to have a similar therapeutic action to atropine but without producing the same side-effects. In 1953 Maschovsky reported the diphenylacetic ester of tropine to protect against nicotine-induced tremors and to be effective in the treatment of Parkinson's disease, and subsequently benztropine methanesulphonate was found to be one of the most effective drugs in this condition (Doshay, 1956). Both these drugs, however, caused side-effects in some patients.

These reports led us to investigate the anti-tremor properties of other tropine derivatives in the hope of finding drugs possessing antitremor activity but little antiparasympathetic action. A series of esters, mainly substituted acetyltropeïnes, was therefore prepared (Table III).

Anti-Parkinson drugs have usually been evaluated by testing them against tremors produced in experimental animals by surgical or chemical means. Jenker and Ward (1953) obtained limb movements resembling tremor by stimulating the brain-stem reticular formation of anaesthetized monkeys. Vernier and Unna (1956) produced brain-stem lesions in monkeys which caused chronic postural tremor. In both cases anti-Parkinson drugs were effective in controlling the tremor, but the limitations of these methods lie in the cost and the necessity of operating on several monkeys to produce one with tremor. Previous workers, using the rabbit, found some correlation between the ability of a compound to protect against the hyperkinetic effects of nicotine and the parkinsonian tremor, but this protective effect was seen also with a wide variety of other agents (Bovet and Longo, 1951; Cahen and Lynes, 1951; Cahen, Thomas and Tvede, 1953).

The report by Everett (1956), that Tremorine, 1,4-dipyrrolidin-1'-ylbut-2-yne, produces, particularly in monkeys, effects closely resembling those seen in the Parkinson syndrome, and that currently used anti-Parkinson compounds protect against these effects (Everett, 1956; Everett, Blockus and Shepperd, 1956; Frommel, 1958), encouraged us to use anti-Tremorine activity as a guide to the possible therapeutic value of new compounds.

METHODS

Antagonism of Tremorine.—Albino mice of the same strain weighing 18 to 22 g. were used throughout. Tremorine was injected subcutaneously in a dose of 30 mg./kg. body weight and the mice were assessed at intervals for weakness, tremor, salivation, lachrymation, rigidity, diarrhoea and excessive micturition. The assessment was on an arbitrary scale ranging from ½ to 3. Very severe effects, such as tremors of the whole body or salivation so intense that it wet the face and neck, were scored as 3, while

slight effects, such as tremors which could be felt only in lifting the animal by the tail, or excessive saliva restricted to the mouth, were scored as ½.

Compounds under test were dissolved in water, and 0.5 ml./20 g. of body weight was injected subcutaneously 30 min. before Tremorine or given orally 1 hr. before. A group of control mice received water. Each compound was compared with caramiphen hydrochloride using ten mice at each of three dose levels. Successive doses were increased by a factor of two for compounds injected subcutaneously and three when given orally. Doses were arranged where possible to give effects varying from 0 to 100%. The score for each group of ten mice was determined for tremor and salivation and the % protection at each dose obtained from the expression 100−(score for group of treated mice/score for group of control mice)×100. The % protection obtained in this way for each dose was converted to its probit and plotted against the logarithm of the dose. Relative potency with respect to caramiphen was calculated by the method of Finney (1947).

Mydriatic Activity.—This was determined on the mouse pupil by the method of Ing, Dawes, and Wajda (1945), the diameter of the pupil being measured in groups of five mice 30 min. after intraperitoneal injection of the drug. Each compound was compared with atropine sulphate on the same day and the increase in pupil diameter plotted against the logarithm of the dose.

Anti-acetylcholine Activity.—This was measured on the isolated guinea-pig ileum. Each compound was compared with atropine sulphate (potency=1.0) on the same strip of guinea-pig ileum using a superfusion technique (Adam, Hardwick, and Spencer, 1954). The ileum was stimulated with acetylcholine (5×10^{-8}, w/v) in Tyrode solution for 15 sec. and then washed with Tyrode solution for 35 sec. Antagonists dissolved in Tyrode solution were applied to the gut manually during interruption of the flow of Tyrode solution. The % inhibitory effects of the compounds at various concentrations were plotted against the logarithm of the dose and the relative potencies of the two compounds estimated.

Acute Toxicity.—The toxicity of each compound was estimated using the same strain of mice as in the Tremorine test. Compounds were dissolved in water and injected subcutaneously into groups of five mice in a volume of 0.5 ml./20 g. of body weight. The % mortality recorded at 24 hr. for each dose was converted to the corresponding probit and plotted against the logarithm of the dose. The LD50 was estimated graphically.

Antihistamine Activity.—This was measured on the isolated guinea-pig ileum by the method used for determining anti-acetylcholine activity. The ileum was stimulated with histamine (10^{-8}, w/v) in Tyrode solution for 20 sec. and washed with Tyrode solution for 45 sec. Mepyramine maleate was used as the standard.

Local Anaesthetic Activity.—Anaesthetic potency was determined by the method of Chance and Lobstein (1944). A solution of the drug in saline was applied to the cornea of a guinea-pig and left there for 15 sec. The cornea was stimulated 45 sec. later and thereafter at 1 min. intervals for a total of 10 min., by touching it with a fine glass rod with a rounded end. Failure to blink after the stimulus counted as a positive response. Each compound was assayed against amethocaine hydrochloride using three concentrations varying by a factor of two. Both eyes of three guinea-pigs were used each day for six days and the experiment designed so that each drug solution was tested on each eye. The positive responses for each concentration of drug solution were added together at the end of the experiment, giving a maximum possible score of 60. The % effects were converted to their corresponding probits and plotted against the logarithm of the dose. Potencies were estimated graphically.

RESULTS

Tremorine in Intact Animals.—The effects of Tremorine, whether given by the oral, intravenous, intraperitoneal or subcutaneous route, lasted for several hours and, if death had not occurred, were often seen up to 24 hr. later. After an injection of Tremorine mice developed severe tremors with fits of shaking of the whole body as well as muscular rigidity and weakness. Rigidity was very noticeable since the animals walked with a high-stepping gait, the body held well clear of the ground. In addition, signs of intense parasympathetic stimulation were evident. These were characterized by profuse salivation spreading until the jaws and neck were soaked, lachrymation, diarrhoea, micturition, and sometimes miosis. Perhaps the most striking effect was a profound fall in body temperature, sometimes by as much as 14°.

Other species behaved like mice after Tremorine. The tremors in rats after a proportionate dose of Tremorine were usually less severe than in mice. Guinea-pigs showed marked tremors and were more susceptible to the lethal effects of Tremorine, dying in 1 to 2 hr. after an equivalent dose. In rabbits the most severe effect was profuse salivation which was difficult to control even with very large doses of atropine given intravenously or subcutaneously; tremors were barely noticeable. Intraperitoneal injection of Tremorine into young chickens produced effects similar to those seen in mice.

Protection Against the Effects of Tremorine.—Results obtained with recognized anti-Parkinson compounds are shown in Table I.

TABLE I

ANTI-TREMORINE TREMOR POTENCIES, TOXICITY, AND MYDRIATIC, ANTI-ACETYLCHOLINE AND LOCAL ANAESTHETIC POTENCIES OF SOME ANTI-PARKINSON DRUGS

Anti-Tremorine tremor potencies were determined in mice injected subcutaneously with 30 mg./kg. Tremorine either 30 min. after subcutaneous injection or 60 min. after oral administration of the compounds. Each compound was assayed against caramiphen hydrochloride; the limits of the assays are given in brackets below the potencies. Trade names are given in brackets.

Compound	Potency of Tremor Inhibition (Caramiphen = 1·0)			Approx. Subcut. LD50 in Mice at 24 hr. (mg./kg.)	Mydriatic Potency in Mice 30 min. after i.p. Injection (Atropine = 1·0)	Anti-acetylcholine Potency on Guinea-pig Ileum (Atropine = 1·0)	Local Anaesthetic Potency on Guinea-pig Cornea (Amethocaine = 1·0)
	Subcutaneous Route		Oral Route				
	At 2 hr.	At 6 hr.	At 6 hr.				
Caramiphen hydrochloride (Parpanit)	1·0	1·0	1·0	400	0·01	0·9	0·1
Benzhexol hydrochloride (Artane)	8·6 (5·5–13·1)	8·0 (3·8–14·6)	2·8 (1·5–5·2)	380	0·7	0·5	<0·1
Benztropine methanesulfonate (Cogentin)	6·6 (2·8–8·4)	7·7 (4·2–9·4)	4·3 (2·3–7·7)	55	0·1	0·5	0·3
Atropine sulphate	3·2 (1·0–6·2)	2·4 (1·5–3·7)	3·0 (1·8–5·4)	610	1·0	1·0	<0·1
Ethopropazine hydrochloride (Lysivane, Parsidol)	0·8 (0·5–1·2)	1·3 (0·3–3·7)	1·2 (0·4–3·3)	670	<0·01	0·2	<0·1
Orphenadrine hydrochloride (Disipal)	0·6 (0·1–1·2)	0·4 (0·2–0·6)	0·25 (0·1–0·4)	125	0·02	0·2	0·15
Diethazine hydrochloride (Diparcol)	0·44 (0·15–1·1)	0·6 (0·5–0·9)	0·6 (0·2–2·0)	>1,000	<0·01	0·03	0·9
Diphenhydramine hydrochloride (Benadryl)	0·16 (0·03–0·3)	0·17 (0·1–0·24)	0·1 (0·07–0·17)	200	0·01	<0·01	0·1

These compounds, given orally or subcutaneously, all protected mice against Tremorine. After subcutaneous injection, maximum protection was obtained by 2 hr., and usually remained at the same level for at least a further 4 hr.; after oral administration, however, protective activity took up to 6 hr. to reach its peak. This was also found with the active tropine derivatives.

Benztropine and benzhexol were the most effective of the current anti-Parkinson compounds tested. Caramiphen, which was used as the standard throughout, had an antitremor ED50 (mean of all assays) of 7.5 mg./kg. at 2 hr. and 6.4 mg./kg. at 6 hr. after subcutaneous injection, while at 6 hr. after oral administration the ED50 was 6.8 mg./kg.

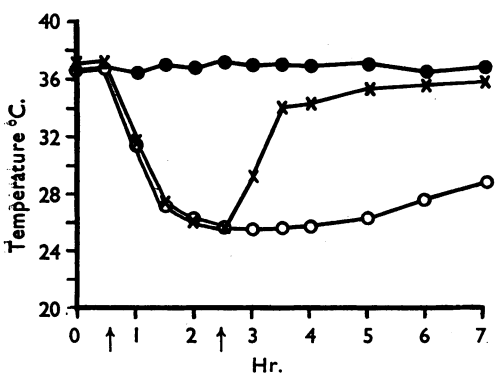

FIG. 1.—Hypothermic effect of Tremorine in mice and its reversal by caramiphen hydrochloride. Each point represents mean response of five mice. Room temperature was 22°. Arrows indicate times of injection. ●—●, saline 25 ml./kg. followed by caramiphen 20 mg./kg. X—X, Tremorine 30 mg./kg. followed by caramiphen 20 mg./kg. O—O, Tremorine 30 mg./kg. followed by saline 25 ml./kg.

On the whole the active compounds appeared to control tremor, rigidity, weakness, and parasympathomimetic effects simultaneously and to the same degree. Hypothermia was most difficult to control and, even with large doses of protective drugs, some fall in temperature could often be recorded after an injection of Tremorine. The action of caramiphen in reversing hypothermia due to Tremorine is illustrated in Fig. 1. Caramiphen alone had no effect on the temperature of normal mice.

Several compounds having different types of pharmacological properties (Table II) were found

TABLE II

COMPOUNDS WITHOUT ANTI-TREMORINE TREMOR ACTIVITY

Compounds were injected subcutaneously into mice 30 min. before Tremorine, 30 mg./kg. subcutaneously, and effects noted for up to 6 hr.

Compound	Greatest Dose with No Activity (mg./kg.)
Tropine hydrochloride	50
Homatropine ,, ...	50
Tigliodine hydrobromide	50
Tiglyltropeine ,, ...	50
Tubocurarine chloride	0·05
Mephenesin carbamate	50
Hexamethonium bromide	50
Morphine hydrochloride	50
Methylpentynol	20
Procaine hydrochloride	100
Lignocaine ,, ...	50

TABLE III

ANTI-TREMORINE TREMOR POTENCIES, TOXICITY, AND MYDRIATIC, ANTI-ACETYLCHOLINE AND LOCAL ANAESTHETIC POTENCIES OF SOME TROPINE DERIVATIVES

Anti-Tremorine tremor potencies were determined in mice injected subcutaneously with 30 mg./kg. Tremorine either 30 min. after subcutaneous injection or 60 min. after oral administration of the compounds. Each compound was assayed against caramiphen hydrochloride; the limits of the assays are given in brackets below the potencies. Asterisks indicate compounds which damaged the guinea-pig cornea during local anaesthetic test. Tr indicates tropane nucleus.

DF No.	Chemical Name	$R_1R_2R_3C{\cdot}CO{\cdot}O{\cdot}Tr, HCl$			Potency of Tremor Inhibition (Caramiphen = 1·0)			Approx. Subcut. LD50 in Mice at 24 Hr. (mg./kg.)	Mydriatic Potency in Mice 30 min. after i.p. Injection (Atropine = 1·0)	Anti-acetylcholine Potency on Guinea-pig Ileum (Atropine = 1·0)	Local Anaesthetic Potency on Guinea-pig Cornea (Amethocaine = 1·0)
					Subcutaneous Route		Oral Route				
		R_1	R_2	R_3	At 2 Hr.	At 6 Hr.	At 6 Hr.				
258	Acetyltropeine	H–	H–	H–	<0·05	<0·05	<0·05	500	<0·01	<0·01	<0·1
256	Phenylacetyltropeine ..	H–	H–	C_6H_5–	<0·1	<0·1	—	255	0·01	<0·01	0·01
255	Diphenylacetyltropeine ..	H–	C_6H_5–	C_6H_5–	0·9 (0·3–1·8)	2·5 (1·9–3·4)	0·9 (0·4–1·1)	155	0·02	0·9	0·2
333	Diphenylacetyl*pseudo*tropeine	H–	C_6H_5–	C_6H_5–	0·3 (0·2–0·4)	0·2 (0·1–0·4)	—	200	0·01	0·1	0·2
286	Hexylphenylacetyltropeine ..	H–	C_6H_5–	CH_3–$[CH_2]_5$–	0·3 (0·2–0·5)	<0·1	<0·1	250	<0·01	<0·01	<0·1*
20	Cyclohexylphenylacetyl-tropeine	H–	C_6H_5–	Cyclohexyl	2·8 (0·9–6·1)	4·5 (2·0–9·2)	3·1 (1·7–6·0)	140	0·06	0·2	0·6
218	Naphth-1-ylphenylacetyl-tropeine	H–	C_6H_5–	Naphth-1-yl	<0·1	<0·1	—	170	<0·01	<0·01	0·2
303	*p*-Chlorodiphenylacetyl-tropeine	H–	C_6H_5–	*p*-Cl–C_6H_4–	1·0 (0·5–1·7)	0·9 (0·2–2·2)	0·7 (0·4–1·2)	310	<0·01	0·2	≏0·5*
458	*o*-Methoxydiphenylacetyl-tropeine	H–	C_6H_5–	*o*-CH_3O–C_6H_4–	0·3 (0·07–0·5)	0·5 (0·3–0·8)	—	185	<0·01	0·02	0·1
346	Phenyl-*p*-tolylacetyltropeine	H–	C_6H_5–	*p*-CH_3–C_6H_4–	<0·1	<0·1	—	190	<0·01	0·3	0·2
280	Cyclohexylnaphth-1-ylacetyl-tropeine	H–	Cyclohexyl	Naphth-1-yl	<0·2	<0·2	—	>500	<0·01	<0·01	<0·1*
287	Di(*p*-chlorophenyl)acetyl-tropeine	H–	*p*-Cl–C_6H_4–	*p*-Cl–C_6H_4–	<0·2	<0·2	—	>500	<0·01	<0·01	<0·1*
309	*aa*-Diphenylpropionyltropeine	CH_3–	C_6H_5–	C_6H_5–	1·4 (0·8–2·7)	1·9 (0·9–3·7)	0·7 (0·4–1·1)	220	0·08	0·5	0·5
459	*aa*-Diphenylbutyryltropeine..	C_2H_5–	C_6H_5–	C_6H_5–	<0·1	<0·1	—	>150	0·01	0·04	0·4
382	*aa*-Diphenylvaleryltropeine ..	CH_3–$[CH_2]_2$–	C_6H_5–	C_6H_5–	0·5 (0·2–1·0)	0·3 (0·2–0·5)	—	250	<0·01	<0·01	≏0·5*
462	*aa*-Diphenylisovaleryltropeine	$(CH_3)_2CH$–	C_6H_5–	C_6H_5–	<0·1	<0·1	—	215	<0·01	<0·01	—
254	Diphenylglycolloyltropeine ..	HO–	C_6H_5–	C_6H_5–	5·0 (2·9–9·1)	6·4 (4·2–11·0)	1·3 (0·8–2·0)	150	1·0	1·0	0·2
549	Methoxydiphenylacetyl-tropeine	CH_3O–	C_6H_5–	C_6H_5–	1·1 (0·6–1·7)	2·0 (1·5–2·6)	3·4 (1·0–10·5)	200	0·02	0·2	≏0·2
452	Ethoxydiphenylacetyl-tropeine	C_2H_5O–	C_6H_5–	C_6H_5–	3·7 (1·1–6·6)	3·1 (1·8–4·7)	5·8 (2·4–7·6)	170	0·07	0·04	0·3

556	Diphenylpropoxyacetyltropeïne	CH_3-$[CH_2]_{12}$-O-	C_6H_5-	C_6H_5-	3·4 (2·0–8·0)	3·7 (2·1–10·5)	2·3 (1·1–4·3)	170	0·04	<0·01	0·5
550	Phenoxydiphenylacetyltropeïne	C_6H_5-O-	C_6H_5-	C_6H_5-	1·1 (0·3–2·0)	2·7 (1·7–4·1)	1·2 (0·2–3·5)	300	<0·01	<0·01	0·3
555	Benzyloxydiphenylacetyltropeïne	C_6H_5-CH_2-O-	C_6H_5-	C_6H_5-	2·8 (1·0–6·6)	4·2 (2·0–10·9)	7·8 (5·8–9·0)	>500	<0·01	<0·01	—*
		n									
272	1-Phenylcyclopropane-1-carbonyltropeïne	2			0·2 (0·1–0·4)	0·3 (0·2–0·5)	—	135	<0·01	0·5	0·06
263	1-Phenylcyclopentane-1-carbonyltropeïne	4			4·5 (2·0–9·2)	5·0 (3·0–8·4)	5·0 (3·6–7·0)	230	0·08	0·5	⩽0·5*
288	1-Phenylcyclohexane-1-carbonyltropeïne	5			4·1 (1·9–7·0)	4·4 (1·8–9·9)	17·0 (12·2–25·6)	300	0·05	0·2	0·3
721	3-o-Methyldiphenylmethoxytropane				5·1 (3·2–8·1)	4·4 (2·7–6·5)	1·7 (0·5–5·5)	43	0·03	0·3	0·6

to be ineffective in antagonizing the action of Tremorine, thus confirming the results of Everett (1956). Hexobarbitone and ether in doses causing anaesthesia did control tremors and rigidity, though some twitching, mainly of the tail, was seen even when reflex movements to a painful stimulus were absent.

Structure/Activity Relationships. — The results of modification of the structure of acetyltropeïne can be seen in Table III. Acetyltropeïne (DF258) did not control tremors. Substitution of one hydrogen atom of the acyl radical by a phenyl group still gave an inactive compound (DF256), but substitution of two hydrogen atoms by two phenyl groups (DF255) gave moderate activity against tremors. There was a fall in activity with the isomeric *pseudo*tropine ester (DF333, diphenylacetyl*pseudo*tropeïne). If one phenyl group was present then replacement of the second hydrogen atom by an aliphatic radical (DF286, hexylphenylacetyltropeïne) gave slight activity, but the presence of a suitably located hydroxyl group as in atropine (Table I) raised it still further. Replacement of one phenyl group in diphenylacetyltropeïne by a cyclohexyl group (DF20) substantially increased activity although a similar replacement by an α-naphthyl group (DF218), or replacement of both phenyl groups by a cyclohexyl group and an α-naphthyl group (DF280, cyclohexylnaphth-1-ylacetyltropeïne), was disadvantageous. In general monosubstitution in one or both phenyl groups (DF303, *p*-chlorodiphenylacetyltropeïne; DF458, *o*-methoxydiphenylacetyltropeïne; DF346, phenyl-*p*-tolylacetyltropeïne; and DF287, di(*p*-chlorophenyl)-acetyltropeïne) also considerably depressed activity.

There was no advantage in replacing the third hydrogen atom in the acyl group of diphenylacetyltropeïne by a straight-chain or branched-chain aliphatic residue (DF309, $\alpha\alpha$-diphenylpropionyltropeïne; DF459, $\alpha\alpha$-diphenylbutyryltropeïne; DF382, $\alpha\alpha$-diphenylvaleryltropeïne; and DF462, diphenylisovaleryltropeïne), but introduction of a hydroxyl group at this point enhanced potency considerably (DF254, diphenylglycolloyltropeïne). The effect of substitution in this tertiary hydroxyl group by an alkyl, aryl or aralkyl group was to reduce

TABLE IV

ANTI-TREMORINE TREMOR POTENCIES, TOXICITY, AND MYDRIATIC, ANTI-ACETYLCHOLINE AND LOCAL ANAESTHETIC POTENCIES OF A SERIES OF KNOWN ALIPHATIC AMINOALCOHOLS

Anti-Tremorine tremor potencies were determined in mice injected subcutaneously with 30 mg./kg. Tremorine either 30 min. after subcutaneous injection or 60 min. after oral administration of the compounds. Each compound was assayed against caramiphen hydrochloride; the limits of the assays are given in brackets below the potencies.

Compound	$R_1R_2R_3C{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}N[C_2H_5]_2$			Potency of Tremor Inhibition (Caramiphen = 1·0)			Approx. Subcut. LD50 in Mice at 24 hr. (mg./kg.)	Mydriatic Potency in Mice 30 min. after i.p. Injection (Atropine = 1·0)	Anti-acetylcholine Potency on Guinea-pig Ileum (Atropine = 1·0)	Local Anaesthetic Potency on Guinea-pig Cornea (Amethocaine = 1·0)
				Subcutaneous Route		Oral Route				
	R_1	R_2	R_3	At 2 Hr.	At 6 Hr.	At 6 Hr.				
Adiphenine	H–	C_6H_5–	C_6H_5–	< 0·05	< 0·05	< 0·05	400	0·01	0·01	< 0·1
Trasentin 6H	H–	C_6H_5–	Cyclohexyl	0·7 (0·4–1·1)	1·1 (0·5–2·9)	0·6 (0·3–1·0)	500	0·01	0·01	0·1
Benactyzine	HO–	C_6H_5–	C_6H_5–	1·2 (0·7–1·6)	0·9 (0·3–1·4)	0·9 (0·3–1·1)	250	0·08	1·0	< 0·1

greatly the peripheral anti-acetylcholine activity whilst maintaining to a large extent the antitremor activity (DF549, methoxydiphenylacetyltropeïne; DF452, ethoxydiphenylacetyltropeïne; DF556, diphenylpropoxyacetyltropeïne; DF550, phenoxydiphenylacetyltropeïne; and DF555, benzyloxydiphenylacetyltropeïne).

A few tropine esters of 1-phenylcycloalkane-1-carboxylic acids were also examined. The size of the cycloalkyl ring was of importance in determining antitremor activity since the cyclopentane (DF263) and cyclohexane (DF288) derivatives were considerably more active than the cyclopropane analogue (DF272).

It was of interest to determine whether replacement of the diethylaminoethyl group by a tropyl radical in compounds of the type used in Parkinson's disease would have advantages. Results obtained with adiphenine (Trasentin), Trasentin 6H and benactyzine are shown in Table IV. Comparison of their properties with those of the corresponding tropine analogues DF255, DF20, and DF254 shows that the use of tropine increases potency against tremors but that the toxicity is also increased, though not to the same extent. Similar comparison between compounds derived from tropine and those derived from aliphatic aminoalcohols may be made between diphenhydramine and benztropine (Table I), caramiphen (Table I) and DF263 (Table III), and orphenadrine (Table I) and DF721 (3-*o*-methyldiphenylmethoxytropane) (Table III). In each of these instances the tropane analogue is both more potent and more toxic.

Effects of Quaternization.—A number of the compounds containing a tertiary nitrogen atom were converted to quaternary salts, such as the methiodide or methobromide, and tested against Tremorine. With these compounds inhibition of tremor was slow to appear and even 6 hr. after subcutaneous injection was usually not as great as that seen with a corresponding dose of the tertiary compound (Table V). However, these compounds readily controlled the parasympathomimetic effects and a dose could be found which completely inhibited these effects whilst having no action on the tremors.

TABLE V

COMPARISON AS TREMORINE ANTAGONISTS OF PAIRS OF COMPOUNDS CONTAINING TERTIARY OR QUATERNARY NITROGEN ATOMS

Compounds injected subcutaneously into groups of five or ten mice 30 min. before Tremorine, 30 mg./kg. subcutaneously. Pairs of compounds were tested on different days. Anti-acetylcholine potency was measured on guinea-pig ileum relative to atropine sulphate (= 1·0).

Compound	DF No.	Salt	Approx. ED50 for Tremorine Tremor Inhibition (mg./kg.)		Anti-acetylcholine Potency
			At 2 hr.	At 6 hr.	
Atropine	—	Sulphate	2·0	1·5	1·0
	80	Methiodide	10·0	1·5	1·3
Cyclohexylphenylacetyltropeïne	20	HCl	2·0	1·0	0·2
	261	Methobromide	20·0	7·0	0·1
p-Chlorodiphenylacetyltropeïne	303	HCl	8·0	4·5	0·2
	302	Methobromide	30·0	12·0	0·4
αα-Diphenylpropionyltropeïne	309	HCl	9·0	7·0	0·5
	310	Methobromide	50·0	50·0	0·03
Diphenylglycolloyltropeïne	254	HCl	2·0	2·0	1·0
	262	Methobromide	4·0	1·0	1·5
Ethoxydiphenylacetyltropeïne	452	HCl	3·0	2·0	0·04
	451	Methobromide	45·0	10·0	0·07
1-Phenylcyclopentane-1-carbonyltropeïne	263	HCl	3·0	1·5	0·5
	264	Methobromide	12·0	12·0	1·8
Methanthelinium ..	—	Bromide	50·0	26·0	4·0

Anti-acetylcholine Activity.—There was no correlation between the anti-acetylcholine and the antitremor activities of the compounds (Table III) whether measured against acetylcholine contractions of the guinea-pig ileum or by mydriasis of the mouse pupil.

Acute Toxicity.—Subcutaneous administration to mice of large doses of derivatives of acetyltropeïne produced hypotonia as well as tremors and convulsions, but these latter effects were reduced or abolished when two of the hydrogen atoms of the acyl group were replaced by two phenyl groups (DF255), a phenyl and a *p*-chlorphenyl group (DF303), two *p*-chlorophenyl groups (DF287), a phenyl and a hexyl group (DF286), or a phenyl and a cyclohexyl group (DF20), and when three of the hydrogen atoms are replaced by a methyl and two phenyl groups (DF309) or a hydroxyl and two phenyl groups (DF254).

Toxic doses of most of the compounds caused the appearance of red urine which varied from pale pink to bright red; this was also found with some of the known anti-Parkinson drugs, for example, orphenadrine and benztropine. This colour was shown both by chemical and spectrophotometric tests to be due to haemoglobin, but few blood cells were seen on microscopic examination. The plasma of heparinized blood removed from animals showing haemoglobinuria was distinctly red while that from control animals was colourless.

Local Anaesthetic Activity.—A considerable number of tropine derivatives were found to possess surface anaesthetic activity. Some of the compounds damaged the cornea at concentrations below or about anaesthetic level (Table III). There was no correlation between antitremor and local anaesthetic activity.

Antihistamine Activity.—Very little antihistamine activity was seen with any of the tropine derivatives with the exception of diphenylacetyltropeïne, which was about one-tenth as active as mepyramine maleate.

Discussion

All methods of testing drugs in animals for possible anti-Parkinson activity have limitations, and the results often show discrepancies from those found clinically. Bovet and Longo (1951) found some correlation between antagonism of nicotine-induced tremors and clinical effectiveness of anti-Parkinson drugs, but atropine, which is useful clinically, was found to be ineffective against nicotine-induced tremors. Cahen *et al.* (1953), on the other hand, found that some anti-adrenaline agents inhibited nicotine-induced tremors in the rabbit. The experiments of Jenker and Ward (1953) on anaesthetized monkeys also showed certain discrepancies from clinical experience; scopolamine, diethazine, amyl nitrite and some quaternary nitrogen derivatives of phenothiazine were effective in monkeys although only the first two are useful clinically. Vernier and Unna (1956) found that the technique of assaying drugs in monkeys with chronic tremor induced by brain-stem lesions gave extremely good qualitative agreement with clinical testing of anti-Parkinson compounds as far as tremor was concerned, but this method did not measure the effect on rigidity. Another disadvantage was that active compounds often caused sleep or depression in these monkeys.

The use of Tremorine suggests a much easier method of screening compounds for anti-Parkinson activity. The present work shows that the recognized anti-Parkinson drugs are all highly effective against Tremorine-induced tremors. This confirms results by Everett (1956), Everett *et al.* (1956) and Frommel (1958). The chief advantage of the Tremorine technique is that it consistently produces effects in small animals similar to those seen clinically in Parkinson's disease. Doubt has been cast on the validity of the Tremorine test by Trautner and Gershon (1959), based mainly on the fact that tigliodine is ineffective in this test. Trautner and Gershon (1959) also confirm the report by Everett (1956) that quaternary aminoalkyl esters such as methanthelinium antagonize only the parasympathomimetic effects of Tremorine without affecting the tremor, and they therefore agree with Everett (1956) that the central and parasympathomimetic effects of Tremorine can be separated. The results in Table V show that quaternary compounds can in fact antagonize tremor though doses can be found which show preferential antagonism of the parasympathetic effects. These compounds take longer to act than the corresponding tertiary compounds and greater doses are required for the same protective effect. These results suggest that the tremors and parasympathomimetic effects of Tremorine may both be centrally mediated, but that the latter actions can be antagonized peripherally as well as centrally.

Although the symptoms of Parkinsonism are believed to be of purely central origin most of the drugs commonly used in this disease have considerable peripheral anti-acetylcholine activity (Table I). Thus the difficulty in employing such drugs clinically is reflected in the frequent criticism

TABLE VI

RATIO (R) OF ANTI-TREMORINE ACTIVITY TO PERIPHERAL ANTI-ACETYLCHOLINE ACTIVITY

Anti-Tremorine tremor activity was measured in mice 6 hr. after oral administration of compounds. 30 mg./kg. Tremorine was injected subcutaneously 1 hr. after compounds. Caramiphen hydrochloride (=1·0) was used as standard. Anti-acetylcholine activity was measured on guinea-pig ileum using atropine sulphate (=1·0) as standard. See text for ratio R.

DF No.	Compound	Ratio R
—	Benactyzine hydrochloride	0·9
255	Diphenylacetyltropeïne hydrochloride	1·0
—	Caramiphen hydrochloride	1·1
—	Orphenadrine ,,	1·3
254	Diphenylglycolloyltropeïne hydrochloride	1·3
309	αα-Diphenylpropionyltropeïne ,,	1·4
—	Atropine sulphate	3·0
303	*p*-Chlorodiphenylacetyltropeïne hydrochloride	3·5
—	Benzhexol hydrochloride	5·6
721	3-*o*-Methyldiphenylmethoxytropane hydrochloride	5·7
—	Ethopropazine hydrochloride	6·0
—	Benztropine methanesulphonate	8·6
—	Diphenylhydramine hydrochloride	>10·0
263	1-Phenylcyclopentane-1-carbonyltropeïne hydrochloride	10·0
20	Cyclohexylphenylacetyltropeïne hydrochloride	15·5
549	Methoxydiphenylacetyltropeïne ,,	17·0
—	Diethazine hydrochloride	21·0
—	Trasentin 6H	60·0
288	1-Phenylcyclohexane-1-carbonyltropeïne hydrochloride	85·0
550	Phenoxydiphenylacetyltropeïne hydrochloride	>120·0
452	Ethoxydiphenylacetyltropeïne ,,	145·0
556	Diphenylpropoxyacetyltropeïne ,,	>230·0
555	Benzyloxydiphenylacetyltropeïne ,,	>780·0

that salivation is controlled more readily than tremor or rigidity, or, alternatively, that in order to exercise reasonable control over the latter symptoms, undesirable side-effects become prominent. One possibility of improving these compounds was to reduce selectively this peripheral anti-acetylcholine action. With several compounds it was possible to do this and at the same time increase the antitremor potency by replacing the aliphatic aminoalcohol radical by tropine. The results of modification to the acetyltropeïne structure indicate that anti-Tremorine activity may be obtained with very little associated peripheral anti-acetylcholine activity. It is evident from consideration of Tables I, III, and IV that peripheral anti-acetylcholine activity is not a reliable guide to central activity as measured by inhibition of tremors produced by Tremorine. If attention is confined to the most potent drugs, and if the arbitrary ratio R (tremor inhibition 6 hr. after an oral dose/the anti-acetylcholine activity on the guinea-pig ileum, both expressed as ratios of controls using standard drugs) is calculated, some interesting variations are seen (Table VI). At the low end of the scale, ratios between 0.9 and 3 are given by benactyzine, diphenylacetyltropeïne (DF255), caramiphen, orphenadrine, diphenylglycolloyltropeïne (DF254), αα-diphenylpropionyltropeïne (DF309) and atropine, and at the high end of the scale, ratios of over 100 are given by the ethers of benziloyltropeïne with the exception of the methyl ether. The value of 85 for the tropine ester of 1-phenylcyclohexane-1-carboxylic acid (DF288) is not far behind. From this point of view it seems that some of the compounds having a low peripheral effect (high value of R) are worthy of clinical trial in patients with Parkinson's disease.

We are indebted to Miss A. L. G. Hall, Miss V. M. Hutton and Miss I. J. Palmer for technical assistance. We wish to thank Mr. J. M. Smith for preparing the ethers of diphenylglycolloyltropeïne, and Mr. H. J. F. Angus and Dr. R. P. Paton for making several of the remaining esters. We also wish to thank Messrs. N. V. Koninklijke Pharmaceutische Fabrieken v/h Brocades-Stheeman and Pharmacia, Amsterdam, for the sample of orphenadrine hydrochloride.

REFERENCES

Adam, H. M., Hardwick, D. C., and Spencer, K. E. V. (1954). *Brit. J. Pharmacol.*, **9**, 360.
Bovet, D., and Longo, V. G. (1951). *J. Pharmacol.*, **102**, 22.
Cahen, R. L., and Lynes, T. E. (1951). Ibid., **103**, 44.
—— Thomas, J. M., and Tvede, K. M. (1953). Ibid., **107**, 424.
Chance, M. R. A., and Lobstein, N. M. (1944). Ibid., **82**, 203.
Critchley, M. (1958). *Brit. med. J.*, **2**, 1214.
Doshay, L. S. (1956). *J. Amer. med. Ass.*, **162**, 1031.
Everett, G. M. (1956). *Nature, Lond.*, **177**, 1238.
—— Blockus, L. E., and Shepperd, I. M. (1956). *Science*, **124**, 79.
Finney, D. F. (1947). *Probit Analysis.* London: Cambridge University Press.
Frommel, E. (1958). *Pr. méd.*, **66**, 1745.
Ing, H. R., Dawes, G. S., and Wajda, I. (1945). *J. Pharmacol.*, **85**, 85.
Jenker, F. L., and Ward, A. A. (1953). *Arch. Neurol. Psychiat., Chicago*, **70**, 489.
Maschkovsky, M. D. (1953). *Pharm. & Toxic.*, **16**, 3.
Trautner, E. M., and Gershon, S. (1959). *Nature, Lond.*, **183**, 1462.
—— and Noack, C. H. (1951). *Med. J. Aust.*, **1**, 751.
Vernier, V. G., and Unna, K. R. (1956). *Ann. N.Y. Acad. Sci.*, **64**, 690.