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RELATIONSHIP BETWEEN ANTI-ACETYLCHOLINE AND ANTI-TREMORINE ACTIVITY IN ANTI-PARKINSONIAN AND RELATED DRUGS

BY

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The anti-acetylcholine potency of a number of anti-Parkinsonism drugs and related phenothiazine compounds was determined using the isolated guinea-pig ileum. The antagonism was assessed by the difference between the pA_2 and pA_{10} values and by log concentration-response curves for acetylcholine in presence and absence of the antagonists. All compounds except chlorpromazine showed some evidence of competitive antagonism to acetylcholine. The anti-tremor potency of the compounds was assessed from suppression of Tremorine-induced tremors in mice. There was a relation between anti-acetylcholine and anti-Tremorine potency among the anti-Parkinsonism drugs, but not among the phenothiazine compounds. Some implications of the findings are discussed in relation to the mode of action of anti-Parkinsonism drugs.

To explain the effectiveness of atropine in the treatment of Parkinsonism, Feldberg (1945) suggested the possibility of an atropine-acetylcholine antagonism at central synapses. Since then a number of synthetic drugs have been introduced for the treatment of Parkinsonism, all having peripheral and central atropine-like properties. Jenkner & Ward (1953) have suggested that, in experimental lesions producing Parkinsonian-like tremors, the disconnected neurones in the medial reticular formation become hypersensitive to endogenous acetylcholine released from near-by uninvolved cells. If human Parkinsonian tremor results from central hypersensitivity to acetylcholine, then it is reasonable to expect that effective anti-Parkinsonism drugs would be specific antagonists of acetylcholine.

As no comprehensive quantitative assessment of the anti-acetylcholine potency of the anti-Parkinsonism drugs has yet been made, the present work was undertaken to measure anti-acetylcholine activity and its relation to anti-tremor potency in drug-induced tremor in mice.

METHODS

Measurement of anti-acetylcholine potency. Estimations of pA_2 and pA_{10} values were carried out essentially according to the method of Schild (1947). Pieces of distal ileum were removed from freshly killed guinea-pigs which had been fasted overnight. The organ bath,

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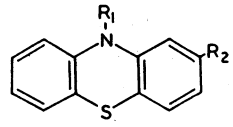
of 2.5 ml. capacity, was manually operated. Its contents were changed by upward displacement and overflow without exposing the preparation to air. Aerated Tyrode solution at 37° was used.

Assessment of type of antagonism. Two criteria were used to assess whether or not the antagonism to acetylcholine was competitive. First, when the difference between pA_2 and pA_{10} did not differ significantly from 0.95 at the 5% level, the antagonism was considered to be competitive. If the difference was significantly less than 0.95 ($P<0.05$), the antagonism was assumed to be non-competitive (Marshall, 1955a). Second, competitive antagonism was indicated by statistically significant parallelism between the log concentration-response curves plotted for acetylcholine alone and in the presence of the antagonist after conversion to linear form as described by Timms (1956) and the ability to achieve maximum contraction in the presence of the antagonist. The curves were plotted in the presence of two concentrations of each antagonist, approximating to the pA_2 and pA_{10} concentrations respectively. To test for parallelism, the curves were converted to linear form by plotting probits of the percentage of maximal contraction against log concentration of acetylcholine. The regression coefficients were calculated, and, if these did not differ significantly ($P<0.05$), the two curves were considered to be parallel.

Measurement of anti-tremor potency. Anti-tremor potency was measured by a mouse protection test against tremors produced by the compound Tremorine (1,4-dipyrrolidin-1′-ylbut-2-yne). Albino mice weighing 20 to 30 g were used. Food and water were removed from the cages 30 min before the experiments, which were carried out at room temperature. Groups of 5 or 10 mice were used and each received a dose of 30 mg/kg of Tremorine intraperitoneally. This dose caused severe tremor, together with profuse salivation and lacrimation. Aqueous solutions of the anti-tremor drugs were prepared so that 0.2 ml./25 g body weight could be given subcutaneously 30 min before the dose of Tremorine, and their potency was assessed by observing the percentage of mice in each group which showed tremor of the head, body, limbs and tail. For each drug, the dose which would reduce the incidence of Tremorine-induced tremor to 50% was calculated by the method of Miller & Tainter (1944). This dose was termed the ED50 value.

TABLE 1

CHEMICAL FORMULAE OF PHENOTHIAZINE DERIVATIVES



Phenothiazine compound	Chemical formula				Optical isomers		
	R_1	R_2	Salt	Mol. wt.		Salt	Mol. wt.
Diethazine	$CH_2.CH_2.N(C_2H_5)_2$	H	Hydrochloride	334·9			
Ethopropazine	$CH_2.CH_2.CH_2.N(C_2H_5)_2$	H	Hydrochloride	348·9			
Promethazine	$CH_2.CH(CH_3).N(CH_3)_2$	H	Hydrochloride	320·9	RP 6415 (*dextro*) RP 6788 (*laevo*)	Hydrochloride	320·9
Chlorpromazine	$CH_2.CH_2.CH_2.N(CH_3)_2$	Cl	Hydrochloride	355·3			
RP 6549	$CH_2.CH(CH_3).CH_2.N(CH_3)_2$	H	Neutral tartrate	373·5	RP 6743 (*dextro*) RP 6778 (*laevo*)	Acid maleate	414·0
RP 6549 analogue	$CH_2.CH(CH_3).CH_2.N(CH_3)_2$	OCH_3			RP 7185 (*dextro*) RP 7044 (*laevo*)	Acid tartrate Hydrochloride	478·0 364·5

Drugs. The chemical formulae of the phenothiazine compounds investigated are shown in Table 1.

RESULTS

Anti-acetylcholine potency. Table 2 shows the pA_2 and pA_{10} values of the compounds against acetylcholine. The anti-Parkinsonian drugs are arranged in order of decreasing pA_2 values. Of the established anti-Parkinsonian drugs, hyoscine was the most potent and diethazine the least potent antagonist of acetylcholine.

TABLE 2

THE VALUES OF pA_2, pA_{10}, AND (pA_2–pA_{10}) FOR ANTI-PARKINSONIAN DRUGS AND PHENOTHIAZINE DERIVATIVES AGAINST ACETYLCHOLINE ON ISOLATED GUINEA-PIG ILEUM

The type of antagonism, competitive (+) or non-competitive (−), was deduced from (pA_2–pA_{10}) values and from concentration-response curves. The figures in parentheses denote the number of experiments upon which the estimates were based

				Type of antagonism		
					From concentration-response curves	
Drug	pA_2	pA_{10}	pA_2–pA_{10} ±s.e.	From (pA_2–pA_{10})	at pA_2	at pA_{10}
Hyoscine	8·96 (4)	8·39 (5)	0·57±0·052	−	+	+
Benztropine	8·94 (4)	8·20 (5)	0·74±0·063	+	+	+
Atropine	8·76 (4)	8·16 (4)	0·60±0·101	−	+	+
Cycrimine	8·30 (4)	7·43 (4)	0·87±0·020	+	+	+
Benzhexol	8·28 (4)	7·13 (4)	1·15±0·088	+	+	+
Procyclidine	8·17 (3)	7·34 (4)	0·83±0·130	+	+	+
Benactyzine	8·01 (4)	7·17 (4)	0·84±0·100	+	+	−
Promethazine	7·61 (5)	6·71 (4)	0·90±0·028	+	+	+
Ethopropazine	7·39 (4)	6·90 (4)	0·50±0·104	−	+	+
Caramiphen	7·15 (4)	6·43 (4)	0·72±0·078	+	+	+
Diphenhydramine	6·60 (3)	5·59 (4)	1·01±0·050	+	+	−
Diethazine	6·49 (4)	5·58 (4)	0·91±0·107	+	+	−
Chlorpromazine	6·13 (4)	5·61 (4)	0·52±0·026	−	−	−
RP 6415 (*dextro*)	7·59 (4)	6·86 (4)	0·73±0·117	+	+	−
RP 6788 (*laevo*)	7·05 (4)	6·22 (4)	0·83±0·068	+	+	−
RP 6549	6·39 (5)	5·80 (4)	0·59±0·020	−	+	−
RP 6743 (*dextro*)	6·66 (4)	6·10 (4)	0·56±0·122	−	+	−
RP 6778 (*laevo*)	7·0 (4)	6·51 (3)	0·49±0·055	−	+	−
RP 7185 (*dextro*)	6·96 (3)	6·41 (3)	0·55±0·150	−	+	−
RP 7044 (*laevo*)	6·76 (4)	6·22 (3)	0·54±0·132	−	+	−

Assessment of type of antagonism. The (pA_2–pA_{10}) differences were calculated and the type of antagonism was assessed according to the method of Marshall (1955a). pA determinations of other workers for some anti-Parkinsonism drugs, summarized in Table 3, are in good agreement with our results.

The drugs could be divided into four groups when the type of antagonism was assessed on the basis of log concentration-response curves.

(1) Drugs which altered neither the slope nor the maximum contraction attainable at either the pA_2 or pA_{10} concentration, namely, hyoscine, atropine, benztropine, cycrimine, benzhexol, procyclidine, ethopropazine and caramiphen (Fig. 1 (1)).

(2) Drugs which, at pA_{10}, but not the pA_2, concentration, depressed the maximum contraction but not the slope between 20 and 70% of the maximal unantagonized contraction, namely, promethazine, RP 6415 and RP 6788 (Fig. 1 (2)).

TABLE 3

COMPARISON OF pA_2 AND pA_{10} VALUES OBTAINED BY VARIOUS WORKERS FOR ACETYLCHOLINE ANTAGONISTS ON THE ISOLATED GUINEA-PIG ILEUM

Drug	pA_2		pA_{10}		pA_2–pA_{10}
Atropine	8·61	Schild (1947)	8·05	Schild (1947)	0·56
	8·84	Marshall (1955b)	8·11	Marshall (1955b)	0·73
	8·74	Cambridge & Holgate (1955)	8·25	Cambridge & Holgate (1955)	0·49
	8·76	Present work	8·16	Present work	0·60
			8·59	Garven (1956)	
Diphenhydramine	6·57	Schild (1947)	5·40	Schild (1947)	1·17
	6·65	Marshall (unpublished observations)	5·78	Marshall (unpublished observations)	0·87
	6·60	Present work	5·59	Present work	1·01
Promethazine	7·81	Edge (1953)			
	7·61	Present work	6·71	Present work	0·90
Chlorpromazine	6·20	Ryall (1956)			
	6·13	Present work	5·61	Present work	0·52
Procyclidine			7·86	Garven (1956)	
	8·17	Present work	7·34	Present work	0·

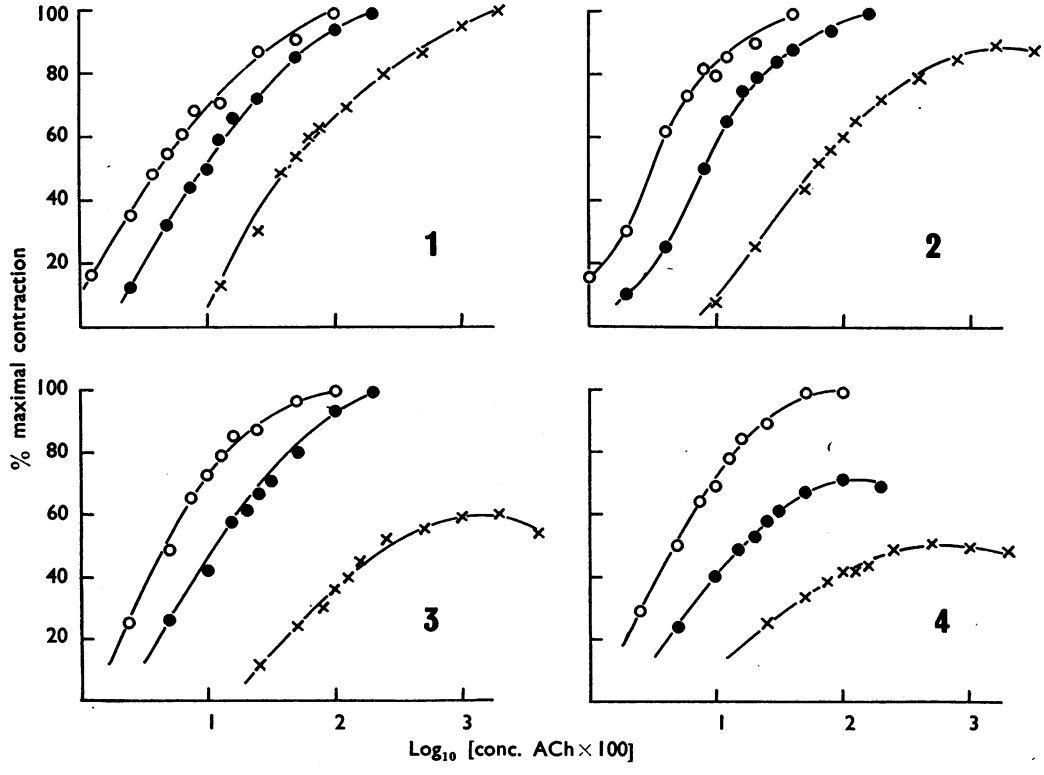


Fig. 1. Concentration-effect curves for acetylcholine alone (○ —— ○), and acetylcholine in the presence of antagonist at pA_2 (● —— ●) and pA_{10} (X —— X) concentrations. Four types of antagonism are illustrated (see text). (1) No change in slope or maximum (hyoscine). (2) Depression of the maximum but not the slope at pA_{10} concentration (promethazine). (3) Depression of the slope and maximum at pA_{10} concentration (diethazine). (4) Depression of the slope and maximum at pA_2 and pA_{10} concentration (chlorpromazine).

(3) Drugs which reduced both the slope and the maximum contraction at the pA_{10}, but not at the pA_2, concentration, namely, diphenhydramine, diethazine, benactyzine, RP 6549, RP 7185, RP 7044, RP 6778 and RP 6743 (Fig. 1 (3)).

(4) Drugs which reduced the slope and the maximum contractions at both pA_2 and pA_{10} concentrations, namely, chlorpromazine (Fig. 1 (4)).

The type of antagonism as derived from statistically significant or non-significant parallelism (at the 5% level) between the unantagonized acetylcholine curve and the curves in presence of pA_2 and pA_{10} concentrations after conversion to the linear form is also given in the last column in Table 2.

Anti-tremor potency. The anti-tremor potencies of the drugs against Tremorine-induced tremors in mice are given in Table 4. Comparison of Tables 4 and 2 shows that, when anti-Parkinsonian drugs are arranged in order of decreasing anti-tremor

TABLE 4
ANTI-TREMOR POTENCY AGAINST TREMORINE IN MICE

Drug	Anti-tremor potency ED50 (mg/kg)
Hyoscine	0·64
Atropine	0·89
Benactyzine	1·0
Benztropine	1·2
Cycrimine	2·0
Procyclidine	2·9
Benzhexol	3·55
Caramiphen	3·63
Promethazine	8·7
Diethazine	9·32
Ethopropazine	10·96
Diphenhydramine	13·2
Chlorpromazine	3·5
RP 6415 (*dextro*)	5·4
RP 6788 (*laevo*)	9·0
RP 6549	4·5
RP 6743 (*dextro*)	18·2
RP 6778 (*laevo*)	22·8
RP 7185 (*dextro*)	15·0
RP 7044 (*laevo*)	2·2

potency or of decreasing anti-acetylcholine potency, there are but few differences in the orders. In Fig. 2, anti-acetylcholine potency (Table 2, pA_2) is plotted against anti-tremor potency (taking only the anti-Parkinsonian drugs in Table 4), and calculation of the correlation coefficient shows a significant correlation coefficient, $r=0.67$, between these two functions ($P<0.01$). Although some of the RP compounds showed anti-tremor potency comparable with that of the anti-Parkinsonian drugs, this activity, except in the case of the optical isomers of promethazine (RP 6415 and 6788), appeared to be due to a general sedative effect rather than to a specific anti-tremor action. Furthermore, these compounds did not inhibit the peripheral cholinergic symptoms which accompanied Tremorine-induced tremors. In this group of drugs there was found to be no significant correlation between anti-acetylcholine and anti-tremor potencies.

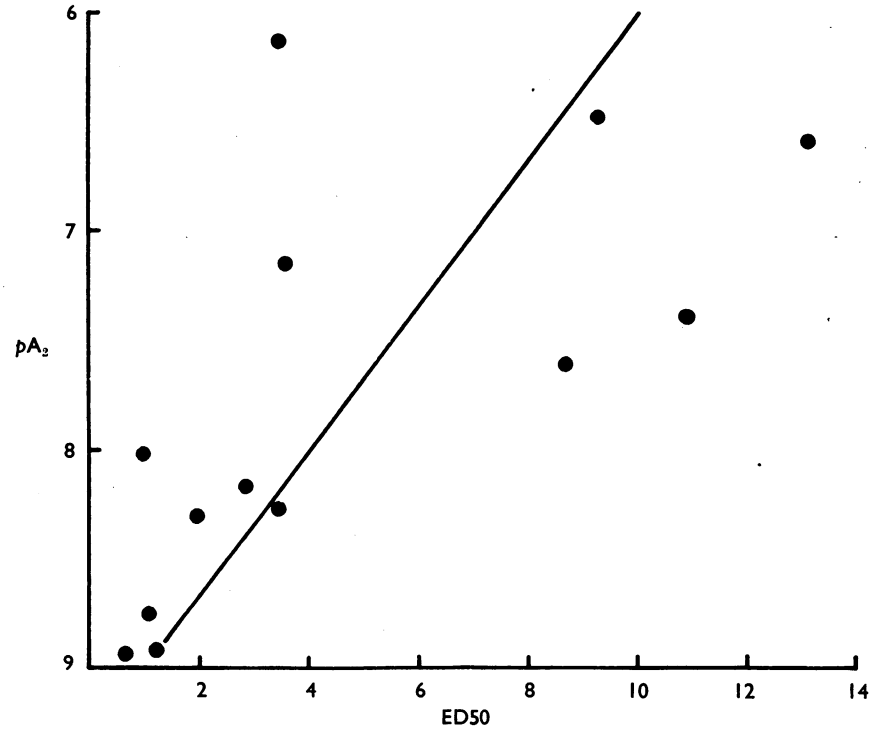


Fig. 2. Significant correlation between anti-acetylcholine and anti-Tremorine potency in thirteen anti-Parkinsonian drugs, with calculated regression line. Ordinate: anti-acetylcholine potency, pA_2 units (from Table 2). Abscissa: anti-Tremorine potency, ED50 (mg/kg) (from Table 4). Correlation coefficient, $r=0.67$, $P<0.01$.

DISCUSSION

Investigations of the kinetics of drug antagonism serve only to emphasize the difficulty of determining precisely whether or not an antagonist is acting competitively. We have used two criteria, the concentration-response curve and the (pA_2–pA_{10}) difference. Study of the curves shows that there is no sharp dividing-line between competitive and non-competitive antagonist action. Some drugs may act competitively at lower concentrations, but become non-competitive at higher concentrations. This is not surprising, since most competitive antagonists of plain muscle agonists such as acetylcholine also have a direct spasmolytic effect at higher concentrations. A transition from competitive to non-competitive would therefore be expected as antagonist concentration is increased, being especially apparent in the less potent compounds in which the margin between the two types of action is narrow. Such an effect might account for the discrepancies between the (pA_2–pA_{10}) method and the concentration-response curve method for assessing type of antagonism in the new phenothiazine compounds. On the other hand, the discrepancy between results with the (pA_2–pA_{10}) method and concentration-response curves in the case of hyoscine, atropine and ethopropazine, in which there is a wide margin between specific anti-acetylcholine and general spasmolytic potencies, cannot be so explained. The apparent non-competitive nature of atropine when assessed

by the (pA_2–pA_{10}) method has been described previously by Marshall (1955b) and was ascribed to the racemic nature of atropine, being a mixture of (+)- and (−)-hyoscyamine, the former appearing to be non-competitive and the latter competitive by the (pA_2–pA_{10}) method. The results of Timms (1956) and those in the present work using the concentration-response curve method suggest that the action of atropine over a wide range of concentrations is competitive, and this is also true of (+)-hyoscyamine (Marshall, unpublished observations). An alternative explanation of this discrepancy might lie in the suggestions of Ariëns & van Rossum (1957) that the difference between pA_2 and pA_{10} can also depend upon the ratio of the number of molecules of receptor and antagonist combining together.

Of the compounds studied, only chlorpromazine has shown a consistently non-competitive anti-acetylcholine action by both methods of assessment. All the other compounds exhibited competitive antagonism to acetylcholine action over some, at least, of the range of concentrations used. The most potent acetylcholine antagonists were those drugs which are used for Parkinsonism in man, and, within this group, there was a significant correlation ($P<0.01$) between anti-acetylcholine potency and ability to antagonize experimental tremors in mice produced by Tremorine. This relationship does not necessarily hold for other tremor-producing drugs. For instance, atropine, hyoscine, diethazine, benzhexol and caramiphen, in doses up to 20 mg/kg in mice, had no effect on tremors induced by the amino-alcohol 3-amino-1,1,3-triphenylpropan-1-ol (compound no. 6, Ahmed, Marshall & Shepherd, 1958). Again, when anti-tremor potencies were determined against harmine-induced tremors (Zetler, 1957), these differed in order of potency from

TABLE 5

COMPARISON BETWEEN ANTI-ACETYLCHOLINE POTENCY AND ANTI-PARKINSONIAN POTENCY EXPRESSED AS THE HUMAN MAXIMAL ORAL DAILY DOSE (*Extra Pharmacopoeia*, Martindale, 24th ed., 1958)

Drug	Anti-acetylcholine potency (pA_2)	Anti-Parkinsonian potency oral dose (mg)
Hyoscine	8·96	0·6
Benztropine	8·94	1·0
Atropine	8·76	1·0
Cycrimine	8·30	45·0
Benzhexol	8·28	2·0
Procyclidine	8·17	7·5
Benactyzine	8·01	4·0
Promethazine	7·61	75·0
Ethopropazine	7·39	100·0
Caramiphen	7·15	37·5
Diphenhydramine	6·60	100·0
Diethazine	6·49	150·0

the anti-Tremorine values and there was no significant correlation ($P>0.05$) with pA_2 values against acetylcholine. On the other hand, the anti-acetylcholine pA_2 values seem to be related to the maximum human therapeutic dosage of the anti-Parkinsonism drugs (Table 5).

There appears to be a relation between anti-acetylcholine potency and anti-Parkinsonian activity in human beings on the one hand and between anti-acetylcholine potency and anti-Tremorine action in mice on the other. That this latter

relationship applies only when tremor is induced with Tremorine confirms the previous conclusions of Everett, Blockus, Shepperd & Toman (1956) that this agent is particularly suitable for the experimental evaluation of drugs for anti-Parkinsonian action. The correlation of anti-tremor and anti-Parkinsonian potency with anti-acetylcholine potency provides further support for the view that the lesion in Parkinsonism is associated in some way with acetylcholine, possibly in the rôle of a central nervous system transmitter.

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