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Potentiation of oxotremorine lethality by antihistamines

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Antihistamines, representing the major chemical classes, administered intraperitoneally to mice in non-toxic doses potentiated the lethal effects of an LD10 dose (3·5 mg/kg) of oxotremorine in a dose related manner. Atropine and methylatropine were highly effective in blocking oxotremorine lethality alone and when it was potentiated by antihistamines. The antagonism by methylatropine suggests a peripheral site of toxicity. Antihistamines might enhance lethality by interfering with the inactivation of oxotremorine by liver microsomal drugs enzymes in a manner similar to SKF 525A.

Tremorine and its active metabolite oxotremorine [1-(2-oxopyrrolidino)-4-pyrrolindino-2-butyne] have proved useful in the evaluation of potential anti-Parkinsonian agents (Everett, 1964; Friedman & Everett, 1964) as well as in the study of chemical transmission in the central nervous system (Holmstedt, 1968). Both compounds produce peripheral and central cholinergic stimulation, resulting in tremors, muscle rigidity, akinesia, hypothermia, diarrhoea, salivation and lacrimation, all of which can be antagonized by tertiary cholinergic blocking agents like atropine (Everett, Blockus & Shepperd, 1956).

Several antihistamines, notably diphenhydramine, have been used successfully in the treatment of Parkinsonism, suggesting that an aberration in brain histamine may be involved in the aetiology of this disease (Barbeau, 1962; Friedman & Everett, 1964). Ungar & Witten (1963) found many antihistamines and atropine to antagonize the tremors and elevation in brain histamine induced by tremorine in the dog.

We have examined the interactions of antihistamines with oxotremorine, because it has a more rapid onset and intensive action than tremorine, also Leslie & Maxwell (1964) have suggested that compounds such as SKF 525A, which antagonize tremorine-induced tremors, inhibit the formation of oxotremorine, the agent responsible for the cholinergic stimulation observed (Cho, Haslett & Jenden, 1961).

EXPERIMENTAL

Materials and methods

Male Swiss albino mice, 20–25 g, were pretreated with antihistamine or atropine, or both, 10 min before oxotremorine.

All drugs were dissolved in distilled water in a concentration such that the volume injected (intraperitoneally) was 0·10 ml/10 g weight. Doses of atropine and the antihistamines were calculated as the salt; oxotremorine sesquifumarate as the base.

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Optimal dosages were determined in preliminary experiments, so too was the feasibility of terminating the experiment at 90 min, after which time we found there was no significant increase in the number of animals that died. All studies were made at a room temperature of 22 $\pm$ 1°.

RESULTS

A single dose of oxotremorine (3·5 mg/kg) in mice is approximately an LD10 value (Table 1). Pretreating the mice with SKF S25A had the expected effect of increasing the potency of the oxotremorine dose to an LD90. All of the other compounds tested (at two or more dose levels) also potentiated the lethal effects of oxotremorine. At the lowest doses tested, benztropine and cyproheptadine both appeared to have some slight protective effect, although at higher doses both drugs clearly potentiated the oxotremorine lethality. The survival time was also decreased as the potentiation increased. Before death, the animals showed clear signs of excessive cholinergic stimulation, i.e. tremors, salivation, lacrimation.

The apparent ability of benztropine, cyproheptadine and diphenhydramine to afford some protection was further explored by pretreating mice with the various antihistamines, then giving an LD50 dose of oxotremorine (5·0 mg/kg). Table 2 shows the results. Benztropine significantly reduced the number of deaths of animals given this higher dose of oxotremorine while diphenhydramine and cyproheptadine conferred partial protection. All other compounds tested were either without protective effect, or produced enhanced lethality.

The results obtained when atropine or methylatropine was given before the antihistamine-oxotremorine combination are shown in Table 3. These cholinergic blocking agents effectively prevented the potentiation of oxotremorine lethality by the antihistamine. In this, the quaternary methylatropine was as effective as its tertiary analogue, suggesting that the lethal effects of oxotremorine have strong peripheral components.

DISCUSSION

Similarities between the actions of histamine and acetylcholine, and between their respective antagonists have been discussed by Barlow (1955), Loew (1947), Marshall (1955) and Rocha e Silva (1955). Histamine and acetylcholine both produce vasodilatation of capillaries, stimulation of intestinal smooth muscle, release of adrenaline from the adrenal medulla and stimulation of gastric acid secretion (Douglas, 1965; Koelle, 1965). Administration of either of these compounds into the lateral hypothalamus stimulates water intake in water-satiated rats (Gerald & Stern, 1968).

Structural similarities exist between antihistamine and anticholinergic agents with a transition from the predominance of one action to the other following chemical modifications (Barlow, 1955; Rocha e Silva, 1955). Marshall (1955) has shown indirectly the attraction of certain antihistamines to both histamine and acetylcholine receptors. Atropine, and antihistamines representative of the major chemical classes, decrease thirst-induced water consumption in a dose-related manner and have mutually parallel log dose-response curves (Gerald, 1968; Gerald & Stern, 1968). Ungar & Witten (1963) reported antagonism of tremorine-induced tremors and a rise in brain histamine in the dog with many antihistamines and atropine.

It was originally thought that the relative anticholinergic potency of the antihistamines tested would be found to be related to their effectiveness in blocking oxotremorine-induced lethality in mice; Leslie (1969) had related central anticholinergic

Table 1. *Antihistamine-potentiation of oxotremorine (OTMN) lethality.* Mice were pretreated with the antihistamine 10 min before oxotremorine (OTMN), i.p. Doses of antihistamines were calculated as the salt; oxotremorine as the base. Values are expressed as the mean survival time (min) ± s.e. after oxotremorine administration. Extreme values are given in parentheses. Number dead were counted at 90 min. Statistical comparisons were determined by the "u"-test (Li, 1964).

Treatment	Dose of antihistamine (mg/kg)	No. dead/ total	% Dead	"u" Test *P* values	Survival time (min ± s.e.)
Saline + OTMN 3·5	—	6/70	8·6		18·5 ± 0·7
Benztropine	16	0/10	0		—
+ OTMN 3·5	4	0/10	0		—
	8	3/10	30	0·5	18·0 ± 2·2
	16	10/10	100	0·01	3·9 ± 0·5
Bromodiphenhydramine	50	0/10	0		—
+ OTMN 3·5	12·5	3/10	30	0·5	23·0 ± 1·9
	25	4/10	40	0·1	22·8 ± 2·7
	50	9/10	90	0·01	9·0 ± 1·4
Chlorcyclizine	50	0/10	0		—
+ OTMN 3·5	12·5	2/10	20		(16, 34)
	25	8/10	80	0·01	11·4 ± 0·7
	50	10/10	100	0·01	5·2 ± 0·3
Chlorpheniramine	64	0/10	0		—
+ OTMN 3·5	4	3/10	30	0·5	17·0 ± 0·3
	8	6/10	60	0·01	12·8 ± 0·7
	16	8/10	80	0·01	9·6 ± 0·5
Chlorpromazine	40	0/10	0		—
+ PTMN 3·5	10	2/10	20		(11, 31)
	20	4/10	40	0·1	20·0 ± 3·9
	40	10/10	100	0·01	12·7 ± 0·9
Cyproheptadine	64	0/10	0		—
+ OTMN 3·5	4	0/10	0		—
	8	3/10	30	0·5	13·3 ± 1·4
	16	10/10	100	0·01	8·8 ± 1·9
Diphenhydramine	50	0/10	0		—
+ OTMN 3·5	12·5	1/10	10		16
	25	4/10	40	0·1	18·8 ± 2·3
	50	10/10	100	0·01	7·7 ± 0·6
Methapyrilene	40	0/10	0		—
+ OTMN 3·5	10	3/10	30	0·5	31·0 ± 1·5
	20	5/10	50	0·01	16·4 ± 2·5
	40	9/10	90	0·01	15·4 ± 1·9
Promazine	40	0/10	0		—
+ OTMN 3·5	20	4/10	40	0·1	13·3 ± 2·3
	30	7/10	70	0·01	13·7 ± 2·4
	40	10/10	100	0·01	8·5 ± 0·9
SKF 525A	40	0/10	0		—
+ OTMN 3·5	40	9/10	90	0·01	5·7 ± 0·9
Tripelennamine	50	3/13	23		(5, 7, 69)
+ OTMN 3	12·5	4/10	40		24·3 ± 2·9
	25	6/10	60	0·01	12·5 ± 2·2
	50	9/9	100	0·01	4·0 ± 0·8

activity to antagonism of oxotremorine-induced analgesia. Although certain antihistamines and anticholinergic agents are effective in the treatment of motion sickness, Brand & Perry (1966) were unable to correlate *in vitro* antihistamine and cholinergic blocking potency with efficacy as anti-Parkinsonian agents or in motion sickness.

Table 2. *Reduction in oxotremorine lethality by some antihistamines*

Treatment	Dose of antihistamine (mg/kg)	No. dead/ total	% Dead	Survival time (min ± s.e.)
OTMN 5·0	—	30/50	60	18·8 ± 1·3
OTMN 5·0 +				
Benztropine	4·0	1/10	10	13
Bromodiphenhydramine	12·5	5/5	100	11·2 ± 1·9
Chlorcyclizine	12·5	4/5	80	14·5 ± 3·6
Chlorpheniramine	4·0	3/5	60	13·6 ± 1·2
Cyproheptadine	4·0	4/10	40	15·3 ± 0·7
Diphenhydramine	12·5	3/10	30	17·0 ± 1·1
Methapyrilene	10·0	3/5	60	12·3 ± 0·7
Tripelennamine	12·5	4/5	80	13·3 ± 1·7

Mice were treated as described in Table 1.

Table 3. *Cholinergic blockade and potentiation of oxotremorine lethality by antihistamines.* Atropine or methylatropine, 10 mg/kg, was administered i.p. with the antihistamine 10 min before oxotremorine (OTMN), given i.p. Values are expressed as number of mice dead at 90 min.

Treatment	Dose anti-histamine mg/kg	Controls	Anti-histamine + OTMN 3·5	OTMN 3·5 + antihistamine with: Atropine 10 mg/kg	Me-atropine 10 mg/kg
Saline +					
OTMN 3·5		6/70			
Atropine 10		0/10			
Methylatropine 10		0/10			
Antihistamine:					
Chlorcyclizine	25·0		8/10	0/5	1/5
Chlorpheniramine	8·0		6/10	0/5	0/5
Chlorpromazine	20·0		4/10	0/5	0/5
Cyproheptadine	8·0		3/10	0/5	0/5
Diphenhydramine	25·0		4/10	0/5	0/5
Methapyrilene	20·0		5/10	0/5	1/5
Tripelennamine	25·0		6/10	1/5	2/5

A comparison of the results from those groups of mice that were given atropine with the pooled results from the non-atropine treated animals, indicates that atropine yields significant protection with $\chi^2 < 0{\cdot}001$.

We found that there was no apparent relation between protection against oxotremorine lethality and the relative anticholinergic potency of the antihistamines. Rather, whereas all higher doses of antihistamines tested potentiated oxotremorine lethality, atropine and methylatropine were effective in antagonizing this effect, presumably through their cholinergic blocking actions. The results with methylatropine suggest a strong peripheral toxicity component, since this quaternary compound does not cross the blood-brain barrier appreciably (Herz, Teschemacher & others, 1965; Khavari & Maickel, 1967).

The potentiation of oxotremorine by antihistamines can be reconciled with the antihistamine antagonism of tremorine observed by Ungar & Witten (1963), if it is assumed that the antihistamines are acting as liver microsomal enzyme inhibitors. Thus Ungar & Witten (1963) found these drugs reduce the conversion of tremorine to oxotremorine, whereas we found the metabolism of oxotremorine to be inhibited.

SKF 525A possesses antihistamine activity and is an effective microsomal enzyme inhibitor (Sjöqvist, Hammer & others, 1968). These workers demonstrated enhanced

hypothermia when SKF 525A was administered before oxotremorine in rats. We too found potentiation of oxotremorine by SKF 525A. The similarity of survival times of the SKF 525A + oxotremorine combination with the antihistamines + oxotremorine combinations supports the likelihood of enzyme inhibition.

No satisfactory explanation is available for the enhanced toxicity seen when benztropine is combined with a low dose of oxotremorine; with a higher dose of oxotremorine, benztropine, as expected, proved to be an effective antagonist. This clinically well-established anti-Parkinsonian agent possesses both anticholinergic and antihistamine activity.

The antihistamines tested possess anticholinergic, sympatholytic, antiserotonergic and local anaesthetic properties (Barlow, 1955; Douglas, 1965); antagonism of histamine is the only action common to all these agents. It is suggested that these antihistamines enhanced oxotremorine lethality by interference with the inactivation of oxotremorine by liver microsomal enzymes.

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