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The effect of anti-Parkinsonian drugs on oxotremorine-induced analgesia in mice

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The antagonism of oxotremorine-induced analgesia in mice can be used to obtain a quantitative and objective assessment of the central anticholinergic activity of potential anti-Parkinsonian drugs.

The antagonism of the effects of tremorine (1,4-dipyrrolidino-but-2-yne) in laboratory animals is widely used in the examination of potential anti-Parkinsonian drugs. Since the introduction of tremorine by Everett (1956) it has been shown that it is converted to an active metabolite which is responsible for most of its pharmacological properties (Kocsis & Welch, 1960). Cho, Haslett & Jenden (1961) isolated and identified this metabolite as 1-(2-oxopyrrolidin-1-yl)-4-pyrrolidin-1-yl-but-2-yne (oxotremorine).

Cho, Haslett & Jenden (1962) have reported that whereas the effects of tremorine are delayed in onset and may be inhibited by substances such as SKF 525A [2-(diethylamino)ethyl-2,2-diphenylvalerate] and LILLY 18947 [2-(2,4-dichloro-6-phenylphenoxy)-*NN*-diethylethylamine hydrochloride] which are known to be inhibitors fo liver microsomal activity (Axelrod, Reichenthal & Brodie, 1954; Fouts & Brodie, 1955) the action of oxotremorine is immediate in onset, not inhibited by SKF 525A, and quantitatively some thousand times more effective than tremorine.

Leslie & Maxwell (1964) showed that a number of phenothiazine derivatives which were not themselves useful anti-Parkinsonian drugs were able to prevent the effects of tremorine but not the effects of oxotremorine. For these reasons oxotremorine is now replacing tremorine as a means of investigating anti-Parkinsonian activity.

A number of methods have been described for assessing the degree of tremor. Blockus & Everett (1957) used an electro-mechanical recording method. Halliwell, Quinton & Williams (1964) and Farquharson & Johnston (1959) used subjective scoring systems. The subjective methods are much the simpler to use and quantify, but have been criticized as being open to error on the part of the observer. Spencer (1965) compared the activity of a number of anti-Parkinsonian and other drugs against tremorine-induced tremor and hypothermia. He found that drugs with central anticholinergic activity prevented both tremor and hypothermia and that quaternary anticholinergics were inactive against the tremor but did to some extent reduce the hypothermia. However he found that sympathomimetic drugs like amphetamine would also antagonize the hypothermia and to a much lesser extent the tremor.

Chen (1958) reported that tremorine had analgesic properties in mice which could be detected at doses insufficient to cause tremor or parasympathomimetic effects. This analgesic action could be antagonized by anti-Parkinsonian drugs. Oxotremorine also possesses this analgesic (antinociceptive) action which can be antagonized by anti-Parkinsonian drugs, but not by sympathomimetic compounds or

by phenothiazine tranquillizers. When administered subcutaneously to mice, oxotremorine is approximately 3,000 times more potent than morphine as an antinociceptive agent.

EXPERIMENTAL

Male albino mice of the SAS TO strain, weighing 15–20 g, were used. Graded doses of test drugs were administered subcutaneously to groups of 10 mice 1 h before intraperitoneal injection of oxotremorine (50 μg/kg).

The mice were first tested to ensure that they would vocalize in response to electroshocks of 8 V applied at 1 s intervals to flat copper electrodes 1·5 cm apart placed on their tails. Animals which did not respond to five or fewer shocks were rejected. Groups of ten mice were used at each dose level. Forty min after administration of the test compound, the mice were again tested for their response to the electroshocks. Five min later, oxotremorine was administered and after a further 15 min the mice were tested once more. The dose of test compound reducing the antinociceptive effect of oxotremorine by 50% of control values was determined (ED50).

Following this, the mice were individually assessed also for the degree of tremor using a three point scale. This was done by an observer who did not know what drugs the animals had received. The dose of test compound reducing the oxotremorine tremor to 50% of that of the control values was found (ED50). ED50 values were determined on four occasions for both antitremor and antinociception.

RESULTS

The ED50 values for a number of anti-Parkinsonian and other drugs against oxotremorine tremor and analgesia are summarized in Table 1.

Table 1. *ED50 values for some anti-Parkinsonian and other drugs against oxotremorine analgesia and tremor*

Drug	*Subcutaneous dose to reduce by 50%	
	Analgesia ED50 (mg/kg) with s.d.	Tremor ED50 (mg/kg) with s.d.
Atropine (sulphate)	1·2 ± 0·2	1·8 ± 0·4
Benactyzine	14·8 ± 1·7	15·2 ± 1·9
Benzhexol	7·1 ± 0·8	5·3 ± 0·8
Benztropine	2·8 ± 0·4	3·2 ± 0·6
Caramiphen	11·2 ± 1·4	9·7 ± 1·2
Ethopropazine	6·2 ± 0·7	7·1 ± 1·1
Hyoscine	1·0 ± 0·2	1·8 ± 0·4
Promethazine	18·0 ± 2·3	15·4 ± 2·2
Atropine (methyl bromide)	>50	>50
Propantheline	>50	>50
Chlordiazepoxide	>100	40 ± 7
Chlorpromazine	>50	25 ± 6
Dexamphetamine	>40	15 ± 5·5
Diphenhydramine	29 ± 6	38 ± 8
Imipramine	45 ± 8	30 ± 7
Meprobamate	>100	>100
Pentobarbitone	>80	>80
Phentolamine	>10	>10
Phenytoin	>200	>200
Tranylcypromine	>50	>50

* Administered 60 min before the intraperitoneal injection of oxotremorine, 50 μg/kg.

It will be seen that all the centrally active anticholinergic drugs tested were active against both tremor and analgesia and that the correlation between the two series of results is very good.

Whereas chlorpromazine, chlordiazepoxide and dexamphetamine have weak activity against oxotremorine tremor, they are not active in preventing the analgesia.

DISCUSSION

The ability of a drug to prevent oxotremorine-induced analgesia (antinociception) in mice provides a useful objective measure of its central anticholinergic activity. Quaternary atropine-like drugs, e.g. atropine methyl bromide and propantheline, are not active nor are sympathomimetic drugs such as amphetamine, whereas they are active in the test of Spencer (1965) using hypothermia as a measure. The test is not liable to subjective errors in assessment as may occur in tests based on tremor scoring. Compounds which are analgesic would give false positives in this test but their antinociceptive properties would be seen before administration of oxotremorine.

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