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The central and peripheral effectiveness of two oxotremorine-antagonists determined using oxotremorine-induced tremor and salivation

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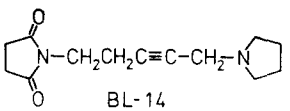
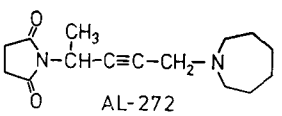
Two oxotremorine antagonists have been examined using oxotremorine as the tremor and salivation inducer and the ratios between the central and peripheral nervous systems determined. Under these conditions these compounds are still more effective antagonists centrally than peripherally. Differences in distribution to the various muscarinic receptors involved is believed to be the major factor in determining this separation of central and peripheral activities.

The *N*-(4-*t*-amino-2-butynyl) subsitututed succinimides have been reported to have a blocking action on the motor effects of oxotremorine (OTMN), but were less active on the peripheral cholinergic effects (Cho & Jenden, 1964; Dahlbom, Karlén & others, 1966a, b; Karlén & Jenden, 1970; Lindqvist, Lindgren & others, 1970). Recently, Inch & Brimblecombe (1974) stated that one of the possible reasons a favourable central-peripheral nervous system (cns-pns) was obtained for these compounds was the methodology employed to determine the antitremor and mydriatic potencies. The disadvantages in the methodology, as they point out, are that the antitremor (cns) potency depends on the use of an exogenous agonist (OTMN) while the mydriasis (pns) potency depends presumably on antagonism of endogenous acetylcholine. Therefore, because these compounds are more specific antagonists of OTMN than acetylcholine, the favourable cns:pns ratios had to be obtained.

We have examined two representative OTMN antagonist compounds, BL-14 (I) and AL-272 (II)



BL-14



AL-272

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using the methods suggested by Brimblecombe, Green & others, (1971) and Inch, Green & Thompson (1973).

METHODS

The antagonists were injected intraperitoneally or intravenously into male mice, 18–25 g at selected times before the intravenous administration of OTMN (100 μg kg^{-1}). Animals were examined at 5 min after OTMN for occurrence of tremors and salivation. The mice were placed on their backs and the hind limbs observed for the presence or absence of tremors. Presence or absence of salivation was determined by lightly pressing the mouth of the animal to a paper tissue. The size of the salivary stain on the paper was graded using a scale of 0 (absence), 1, 2, 3, 4 (hypersalivation). The scores from 5 animals were accumulated per dose of antagonist. Effective doses of the antagonists were also determined using an equipotent dose of OTMN to induce salivation (54 μg kg^{-1} or 1·5 × ED90).

Antitremor and antisalivation potencies of the antagonists were also determined using arecoline as the tremorgen. Arecoline, intravenously 1·5 mg kg^{-1} (1·5 × ED90) was used for tremor and salivation and additional studies were done with AL-272 using an equipotent dose of arecoline 0·75 mg kg^{-1} to induce salivation. Arecoline was administered 10 min after the antagonists in these studies.

All drug solutions were prepared daily in distilled water for intraperitoneal administration and saline for intravenous injection. Volumes injected were 10 ml kg^{-1} (i.p.) and 5 ml kg^{-1} (i.v.). All drug doses are expressed in terms of their respective salts.

ED50 and 95% confidence limits were calculated through the use of a computer program.

RESULTS

I. *Antagonism of OTMN-induced tremors and salivation in mice*

a. Antagonism by intraperitoneal administration at various time intervals (Table 1). BL–14 has a peak blocking effect on tremor and salivation between 5–10 min, with the antagonist activity diminishing equally for both effects as indicated by the similar cns : pns at 30 min to those obtained for the earlier times. An antagonist dose for salivation was computed with BL-14 even though a weak salivation response was seen when doses over 100 mg kg^{-1} were administered. The separation of cns from pns effects is still maintained for BL-14 when OTMN is used as the central and peripheral agonist. AL-272, a more potent antagonist of OTMN tremor and salivation, was an equally effective antagonist at 2·5, 5, 10 min, however, the blocking activity diminished at 30 min equally for both tremor and salivation. Even though no salivation response was detected with AL-272 at the doses used before OTMN injection, it has a more favourable separation of central from peripheral effectiveness than BL-14.

Table 1. *Antagonism of OTMN-induced tremors, and salivation in mice.*

Time min	Central*	Peripheral†	Ratio cns : pns
	Atropine sulphate (i.p.)		
15	2·71 (1·91–3·82)	0·15 (0·15–0·19)	18·1
	BL-14 i.p.)		
2·5	10·3 (8·7–12·1)	324·6 (260·6–401·4)	0·032
5·0	6·7 (5·0–9·1)	155·9 (150·9–200·9)	0·043
10·0	7·2 (5·2–9·9)	177·7 (133·6–233·6)	0·043
30·0	15·4 (12·4–19·2)	321·5 (258·2–399·1)	0·048
	AL-272 (i.p.)		
2·5	0·23 (0·20–0·30)	21·8 (17·6–26·8)	0·011
5·0	0·20 (0·17–0·23)	15·3 (10·6–22·2)	0·013
10·0	0·23 (0·17–0·33)	15·3 (10·6–22·2)	0·015
30·0	0·63 (0·40–1·07)	29·4 (23·4–36·9)	0·022
	Atropine sulphate (i.v.)		
15	1·15 (0·79–1·67)	0·02 (0·01–0·03)	53·3
	BL-14 (i.v.)		
2·5	9·08 (6·5–12·7)		
5·0	6·55 (5·1–8·4)	(See Fig. 2)	
10·0	12·65 (10·2–14·4)		
20·0	15·09 (13·4–15·1)		
	AL-272 (i.v.)		
2·5	0·13 (0·10–0·20)	2·6 (1·8 –3·9)	0·050
5·0	0·13 (0·10–0·50)	4·1 (2·9–5·9)	0·032
10·0	0·13 (0·07–0·23)	4·1 (2·9–5·9)	0·032
20·0	0·60 (0·30–1·17)	16·3 (13·4–18·1)	0·037

Antagonism of oxotremorine (100 μg kg^{-1}, i.v.) induced * tremors or † salivation in mice ED50 μmol kg^{-1} (95% confidence limits).

b. Antagonism by intravenous injection at various times (Table 1). BL-14 has a peak activity at 5 min after intravenous administration at a dose similar to that obtained after intraperitoneal injection. BL-14 produced more salivation after intravenous injection to interfere with the determination of an anti-salivation potency. Therefore, in Fig. 1, BL-14 is injected intravenously followed by OTMN 100 μg kg^{-1} five min later and the salivation response graded. As the dose of BL-14 is raised, it caused salivation at 50 mg kg^{-1} in all animals but suppressed the OTMN hypersalivation at about the same dose level. AL-272 has a lower effective anti-tremor dose following intravenous injection with a peak activity at 2·5 min for both tremor and salivation. When the cns : pns ratios are computed using the antisalivation doses obtained for AL-272 using an equipotent dose of OTMN, the ratios are closer to unity than seen after intraperitoneal injection but they are still much less than one.

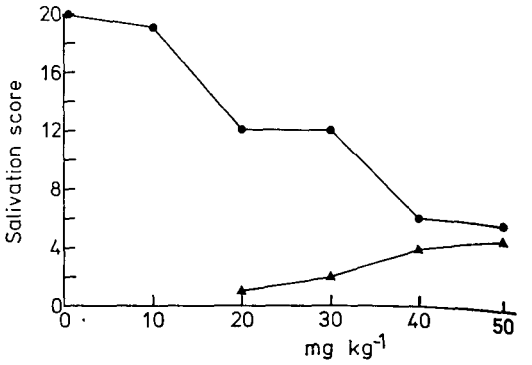

FIG. 1. Salivation score cumulated for 5 mice per dose: ●—● BL-14, intravenously followed by OTMN (100 μg kg^{-1}, i.v.); ▲—▲ salivation produced by BL- 14 before OTMN.

II: *Antagonism of arecoline-induced tremors and salivation in mice (Table 2)*

a. Antagonism by intraperitoneal administration. The effective antitremor and antisalivation doses, using the same dose of arecoline to determine each, gives cns : pns ratios for both BL-14 and AL-272, that are still less than one.

Table 2. *Antagonism of arecoline-induced tremor and salivation in mice.*

Compound	Cns Antitremor ED50 (μmol kg^{-1}) (95% confidence limits)	Pns Antisalivation ED50 (μmol kg^{-1}) (95% confidence limits)	cns : pns ratio
Atropine SO_4	2·24 (1·54– 3·27)	0·37 (0·23–0·60)	6·01
AL-272	0·73 (0·50– 1·44)	10·5 (7·2 –15·2)	0·07
BL-14	9·3 (6·4 –13·6)	126·5 (78·8 –181·4)	0·07
AL-272*	0·22 (0·15– 0·32)	1·18 (0·77–1·77)	0·19

* AL-272 intravenous equipotent dose of arecoline used to produce salivation. 1·5 mg kg^{-1} dose of arecoline to induce tremor and salivation, 10 min after antagonist.

b. Antagonism by intravenous administration. To fully evaluate AL-272, as it did not cause salivation following intravenous injection, it was further studied by obtaining an antisalivation effective dose using an equipotent dose of arecoline. When this dose was used to compute the cns : pns ratio a value of 0·19 was obtained.

DISCUSSION

Previously BL-14 was reported to have an antitremor ED50 of 4·5 μmol kg^{-1} with a mydriatic dose of 13·0 μmol kg^{-1} in mice (Karlén & Jenden, 1970) and AL-272 had an antitremor ED50 of 0·45 μmol kg^{-1} with a mydriatic dose of 5·6 μmol kg^{-1} also in mice (Lindqvist & others, 1970). The resulting cns : pns ratios using these data are 0·35 for BL-14 and 0·08 for AL-272. In the present studies, when OTMN was used as the tremor and salivation inducer, the ED50 blocking doses for BL-14 and AL-272 show an even greater separation between central and peripheral effectiveness.

When the pA_2 determinations were made with BL-14, it was found that low bath concentrations (10^{-8}M) of it potentiated the acetylcholine-induced contraction while at higher concentrations (10^{-5}M) it competively blocked acetylcholine (Karlén, 1970). AL-272 was found to be a pure competitive antagonist of acetylcholine (unpublished results). The weak agonist activity of BL-14 appears to play a significant role in determining the separation of antitremor and antisalivation effects seen with BL-14 and a minor role in determining the separation between antitremor and mydriatic effects. It was surprising that AL-272 had an even larger separation of antitremor and antisalivation effects than BL-14, as AL-272 did not exhibit any production of salivation by itself. A more favourable distribution to the cns and a more specific blockade of OTMN are felt to be the major factors involved in AL-272 having this separation of effects. When the OTMN bias is removed, as is evident from the arecoline data, both compounds have approximately the same cns : pns ratios and the AL-272 ratio is more in line with that ratio obtained when mydriasis and antitremor are compared. However, because salivation is used as an endpoint, the ratio for BL-14 is distorted due to its weak agonist activity.

These studies have shown that the OTMN antagonists maintain the separation of central from peripheral effects even when the same agonist is employed to produce both effects. If blockade of salivation is used as a measure of peripheral activity for these compounds the obtained cns : pns ratios would have been distorted by the agonist properties of one of these compounds.

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