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A comparative study of the effect of some centrally acting skeletal muscle relaxants in mice

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The possibility of differentiating between sedative-hypnotics and centrally acting muscle relaxants has been investigated in mice. The ED50 values for loss of muscle tone and loss of righting reflex were determined for 2-(3′,6′-dioxaheptyl) aminomethyl-1,4-benzodioxane (ambenoxan), 2-(4′,7′-dioxaoctyl) aminomethyl-1,4-benzodioxane (WB 4123) and compared with mephenesin, meprobamate, methocarbamol and the sedative-hypnotic, sodium pentobarbitone. Comparison of the slopes of the regression lines for hypotonia and loss of the righting reflex for the compounds suggests that ambenoxan offers a greater separation between the two responses than clinically used muscle relaxants.

Green, Shapero & Wilson (1969) have reported on the adrenolytic and central nervous system depressant properties of 2-substituted aminomethyl-1,4-benzodioxanes. It was observed that 2-(3′,6′-dioxaheptyl) aminomethyl-1,4-benzodioxane (ambenoxan) and 2-(4′,7′-dioxaoctyl) aminomethyl-1,4-benzodioxane (WB 4123) were the two most potent muscle relaxants of the series.

The experiments now reported were made with the aim of investigating the potency of the two compounds, ambenoxan and WB 4123, relative to other centrally acting skeletal muscle relaxants in mice. We have therefore compared the results from two tests of the activities of three skeletal muscle relaxants used clinically: mephenesin, meprobamate and methocarbamol, the two most active members of the benzodioxane series (WB 4123 and ambenoxan), and a sedative-hypnotic compound, sodium pentobarbitone.

METHODS

Two tests were used. The first was based on the inclined screen method (Randall, Scheckel & Banziger, 1965) modified by the use of a vertical ladder. This test was designed to measure drug induced impairment of muscle tone (Roszkowski, 1964). The second was for assessing the compound's ability to cause loss of the righting reflex.

Male albino mice of the TT strain in groups of eight were injected subcutaneously with graded doses of test compound. Fifteen min after injection, the animals were tested for (i) ability to remain on a vertical ladder (VLT) for 1 min, and (ii) loss of righting reflex (LRR) lasting at least 30 s.

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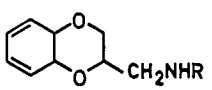
The acute toxicity was determined by injecting subcutaneously different doses of each compound into groups of mice and observing them for seven days. The ED50 and LD50 values were determined by the method of Litchfield & Wilcoxon (1949).

The effect of pre-dosing with neostigmine on the activity of ambenoxan, WB 4123 and tubocurarine was investigated in groups of mice injected subcutaneously with neostigmine (20 μg kg^{-1}) or with water. Five min later each group was given either ambenoxan, WB 4123 or tubocurarine and the effect on duration was recorded.

Drugs used: 2-(3′,6′-dioxaheptyl) aminomethyl-1, 4-benzodioxane hydrochloride (ambenoxan), 2-(4′,7′-dioxaoctyl) aminomethyl-1, 4-benzodioxane hydrochloride (WB 4123), mephenesin, meprobamate, methocarbamol (Robaxin Injectable), sodium pentobarbitone (Nembutal Veterinary), (+)-tubocurarine and neostigmine methylsulphate.

Ambenoxan, WB 4123 and neostigmine were dissolved in water; mephenesin was used as a suspension in 0·5% tragacanth; and meprobamate was dissolved with warming in a 50% mixture of PEG 300 and water.



$R=(CH_2)_2OCH_2CH_2OCH_3$ (Ambenoxan)

$R=(CH_2)_3OCH_2CH_2OCH_3$ (WB 4123)

RESULTS

The findings based on the criterion of the ratio of the ED50 for loss of the righting reflex to that for the vertical ladder test (Table 1) showed sodium pentobarbitone to produce the most selective action of the compounds studied. It has an ED50 paralysing dose approximately three times that of the ED50 VLT. Meprobamate, with an index of 2·03, was the next most selective showing the best separation of effects amongst the skeletal muscle relaxants, although the range for all the compounds, excluding sodium pentobarbitone, extended only from 1·65 to 2·03.

Reference to the ratio of LD50/ED50 VLT indicates that, of the three clinically used skeletal muscle relaxants, mephenesin would appear to have the largest therapeutic index at 5·6, while both WB 4123 and especially ambenoxan show improved indices (7·9 and 9·0 respectively) indicating a theoretically greater safety margin.

The comparisons given in Table 1 are based on relative activities of the various compounds at ED50 doses. In Fig. 1, complete dose-response curves have been

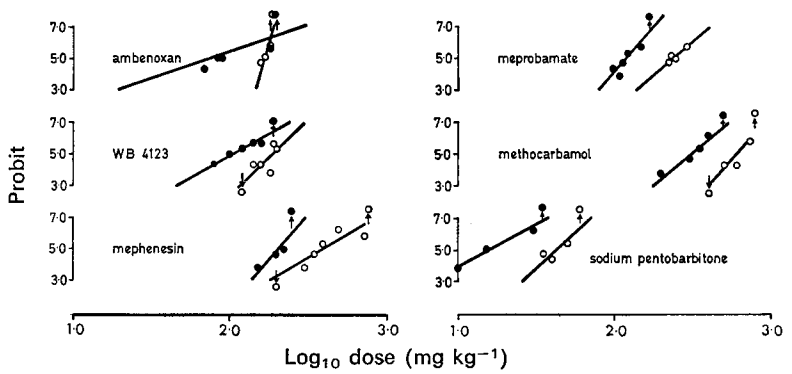


FIG. 1. Probit-log dose responses for muscle relaxation (MR) and loss of the righting reflex (LRR) in mice: ●- - - - ●, muscle relaxation; ○ - - - - ○, loss of righting reflex.

Table 1. *Comparison of LD50's and ED50's with 95% confidence limits for hypotonia (VLT) and loss of righting reflex (LRR) with slopes.*

Compound	LD50 (mg kg^{-1})	ED50 VLT (mg kg^{-1})	ED50 LRR (mg kg^{-1})	Ratio of ED50 LRR / ED50 VLT	T.I. LD50 / ED50 VLT	Slope probit $\log_{10}$ dose VLT	Slope probit $\log_{10}$ dose LRR
Ambenoxan	920 (829–1021)	103 (73·6–144)	170 (159–182)	1·65	9·0	4·03	33·15*
WB 4123	790 (699–893)	100 (80–125)	185 (162–211)	1·85	7·9	5·67	9·46
Mephenesin	1230 (116–1290)	225 (184–275)	400 (333–480)	1·78	5·6	11·02	5·64
Meprobamate	520 (444–608)	123 (100–151)	250 (172–363)	2·03	4·1	11·28	8·10
Methocarbamol	1100 (1040–1170)	300 (256–351)	590 (502–693)	1·97	3·6	8·20	10·95
Pentobarbitone sodium	160 (150–171)	15 (11·6–19·4)	44·5 (37·1–53·4)	2·97	10·6	5·32	8·86

* $P<0{\cdot}001$.

plotted for each compound in linear form as probit-log dose regression lines. The lines were fitted by the method of maximum likelihood (Finney, 1962).

It can be seen that the relations between the regression lines for VLT and for LRR for the various compounds show a variety of patterns.

Numerical values were calculated for the slopes of each line. The results are shown in Table 1. For ambenoxan the regression line for VLT has a value of only 4·03 and is the flattest slope recorded in the series. The slope for LRR is 33·15 and is the steepest in the series.

For each compound the probability has been estimated on the null hypothesis of obtaining a significant difference between the slopes of VLT and LRR regression lines. The results in Table 1 show that at the 5% level no significant difference is apparent for any compound other than ambenoxan. For ambenoxan the calculation confirms the graphical impression given by Fig. 1 that there is a real difference ($P<0{\cdot}001$) between the slopes.

Tests carried out to determine whether prior administration of neostigmine influenced the duration of hypotonia induced by the two benzodioxanes showed that before neostigmine the duration for ambenoxan (100 mg kg^{-1}) and WB 4123 (120 mg kg^{-1}) was 97·5 $\pm$ 7·2 min and 131·3 $\pm$ 8·9 min respectively. Pre-treatment with neostigmine did not significantly influence the duration of either compound which then gave values of 122·8 $\pm$ 12·7 min and 126·6 $\pm$ 14·4 min respectively ($P>0{\cdot}05$).

DISCUSSION

The evaluation of centrally acting muscle relaxants was questioned by Domino (1956, 1957). Though it is customary to determine potency of centrally acting muscle relaxants by their paralysing actions, such an end-point is unrealistic as the usefulness of this class of drugs in the clinical situation is to reduce or abolish hypertonia without loss of mobility.

In an attempt to improve the assessment of these agents, Roszkowski (1960) has used a variety of tests involving several species. In our experiments we have com-

pared the dose producing hypotonia (VLT) with the paralysing dose (LRR) in mice for the test compounds. We have compared the results with those of three established centrally acting skeletal muscle relaxants and a sedative/hypnotic compound.

The regression lines obtained by plotting the probit-log dose responses show one group of compounds (mephenesin, meprobamate, methocarbamol and sodium pentobarbitone), the slopes of whose regression lines do not significantly diverge from parallelism, while for ambenoxan alone the regression lines for VLT and LRR depart significantly from parallelism. Although WB 4123 falls into the first group, it is not typical since its dose lines strongly resemble those of ambenoxan in converging with increase of dose. It does not however fulfil the statistical criterion for being placed with ambenoxan.

That ambenoxan may differ in its mode of action from many skeletal muscle relaxants in common use is seen from the present work and is suggested by other findings. Shapero & Southgate (1970) showed that in the rabbit loss of muscle tone after ambenoxan was characterized by splaying of the limbs, which differed from the effect of mephenesin.

The same authors have also demonstrated that ambenoxan and WB 4123 (unpublished data) exhibit no anticonvulsant activity against leptazol and strychnine. In contrast, mephenesin, meprobamate and methocarbamol antagonize strychnine and, to a lesser extent, leptazol (Berger & Bradley, 1946; Roszkowski, 1960).

Experiments by Shapero & Southgate (1970) indicated that 5 mg kg^{-1} ambenoxan intravenously did not affect the response of the rabbit gastrocnemius muscle to electrical stimulation of the sciatic nerve. This result showed that ambenoxan did not produce muscle relaxation by virtue of peripheral neuromuscular blockade. The failure of neostigmine, in the present study, to influence the muscle relaxation produced by ambenoxan and WB 4123 in mice confirmed that neither of these compounds has curare-like properties.

Berger & Bradley (1946) stated that recovery from mephenesin paralysis was accelerated by neostigmine, although they did suggest that the curare-like effect did not significantly contribute to the production of muscle relaxation. However, the complete lack of influence shown by neostigmine on the responses of the two benzodioxanes in the present study accentuates the difference between the properties of the two classes of compounds.

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