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ANTITETANUS ACTIVITY OF CENTRAL MUSCLE RELAXANTS

BY

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As early as 1854, Simpson observed that the clinical effects of tetanus toxin and strychnine hydrochloride are alike; Sherrington (1906) suggested that these agents convert central synaptic inhibition into excitation. More recent work has demonstrated that both tetanus toxin and strychnine have a specific blocking action in the central nervous system upon inhibitory synaptic transmission (Bradley, Easton & Eccles, 1953; Brooks, Curtis & Eccles, 1957; Purpura & Grundfest, 1957). Tetanus toxin presumably acts by preventing the release, rather than the action, of an inhibitor transmitter (Laurence & Webster, 1963). Experimentally, mephenesin produces a selective depression of the interneurones and exhibits powerful antagonism to strychnine. Clinically, mephenesin has proved to be very effective in controlling the convulsions of tetanus (Belfrage, 1947; Veronesi, 1956). The duration of action of mephenesin, however, is very short. Numerous central muscle relaxants have been tested for their antitetanus activity on experimental local tetanus in the hope of finding a more satisfactory drug than mephenesin (Webster, 1961). The mechanism of experimental local tetanus seems to be complicated by an additional peripheral action of the toxin (Harvey, 1939; Wright, 1955). Wright (1951) demonstrated that tetanus toxin administered by intracerebral or intrathecal (lumbar) routes was several hundred times more potent than the intravenous injection. Furthermore, tetanus toxin not only acts on the spinal cord but also at the brain stem level and "lockjaw" is often one of the earliest signs of clinical tetanus. In experimental studies with tetanus toxin, the polysynaptic reflexes are exaggerated more than the monosynaptic reflexes (Brooks *et al.*, 1957; Davies, Morgan, Wright & Wright, 1954). It was thought that the polysynaptic linguomandibular reflex (King & Unna, 1954), which is integrated at the brain stem level, might serve as a useful tool for the assessment of the antitetanus activity of mephenesin-like compounds.

In the present study a facilitation of the linguomandibular reflex was obtained by intraventricular injection of tetanus toxin or strychnine hydrochloride, and the inhibitory activity of intraventricularly administered central muscle relaxants was assessed on the facilitated linguomandibular reflex.

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METHODS

Thirty-seven cats of either sex, weighing between 2.5 to 4.0 kg, were used. All the animals were anaesthetized with α-chloralose (80 mg/kg, intravenously) and artificially ventilated.

An intraventricular cannula was introduced into a lateral cerebral ventricle according to the technique of Feldberg & Sherwood (1954). The linguomandibular (polysynaptic) reflex was recorded through a system of pulleys on kymograph paper as described by King & Unna (1954). The head of the cat was rigidly fixed in a head-holder in such a way that the lower jaw (mandible) could move freely. The movements of the lower jaw were elicited by stimulating the root of the tongue by means of needle electrodes. Rectangular wave pulses were obtained from a Grass Model S_4 electronic stimulator; the usual parameters of stimulation were 2 to 4 V, 1 shock/sec and 100 msec duration.

Carotid artery pressure was recorded by means of a mercury manometer on kymograph paper.

When uniform records of the linguomandibular reflex were obtained by suitable parameters of stimulation, intraventricular injection of strychnine or tetanus test toxin was given. A 50% facilitation of the linguomandibular reflex was obtained by intraventricular injection of strychnine (2 to 5 μg). Similar facilitation could be obtained with intraventricular injection of tetanus test toxin B.P. (0.1 to 0.2 ml. of 1 : 10 diluted solution). The facilitation was observed within 5 min of the injection and lasted for several hours. Occasionally when the reflex potentiation was associated with movements of the whole body or convulsions were seen after the administration of strychnine the preparation was rejected.

The central muscle relaxants were injected intraventricularly (except where mentioned) in various doses to obtain inhibition of the facilitated linguomandibular reflex. At least three doses of each compound were tested to elicit reduction in the amplitude of the facilitated linguomandibular reflex. The ED50, standard error and 95% confidence limits were statistically calculated for each drug according to Finney (1952). The ED50 for mephenesin was taken as unity and the relative activity of other compounds was calculated. The onset and the duration of the inhibitory action was recorded for each drug.

The following drugs were used: propanediol derivatives—mephenesin (Myanesin), meprobamate (Miltown), mebutamate (W 583), 3-carbamoyloxy-2,2-dichloro-1-phenylpropanol (SQ 4909) and its *p*-chloro-derivative, 3-carbamoyloxy-2,2-dichloro-1-*p*-chlorophenylpropanol (SQ 10220); γ-aminobutyric acid and haloperidol (R 1625); phenothiazine derivatives—promethazine hydrochloride (Phenergan) and chlorpromazine (Largactil); the benzazole derivative—2-amino-4-methylbenzthiazole and orphenadrine hydrochloride (Disipal).

Drugs were dissolved in 0.9% saline. Meprobamate, mebutamate, SQ 4909 and SQ 10220 were first dissolved in a few drops of 90% alcohol and redistilled propylene glycol. The final solution was made up in 0.9% saline. Haloperidol was first treated with a few drops of 1% lactic acid and redistilled propylene glycol and then dissolved in warm 0.9% saline. The volume of the final drug solution for intraventricular injection did not exceed 0.2 ml. Control effects of the vehicle were observed in each experiment.

RESULTS

Typical records of the linguomandibular reflex from two experiments are shown in Fig. 1. (*a*) Shows the effects of mephenesin on the linguomandibular reflex facilitated by intraventricular injection of tetanus toxin (0.1 U). Intraventricular injection of mephenesin (5 mg) reduced the amplitude of the reflex to about 60% within 5 min. Recovery occurred within 20 min. (*b*) Shows the effect of SQ 10220 on the linguomandibular reflex facilitated by intraventricular injection of strychnine hydrochloride (2 μg). Intraventricular injection of SQ 10220 (0.25 mg) reduced the amplitude of the reflex to about 30% within 5 min. Recovery, however, occurred in 120 min. Mephenesin as well as SQ 10220 inhibited the facilitation of the reflex induced by strychnine hydrochloride in a manner similar to the facilitation induced by tetanus toxin.

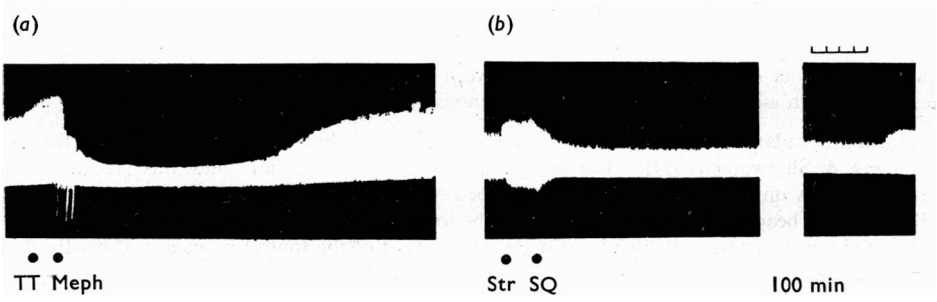

Fig. 1. Records of linguomandibular reflex in cats. (*a*) Facilitation of the reflex by intraventricular injection of tetanus test toxin (TT, 0.1 U) and its inhibition by mephenesin (Meph, 5.0 mg); note that recovery occurred in about 20 min. (*b*) Facilitation of the reflex in a different cat by intraventricular strychnine hydrochloride (Str, 2 μg) and its inhibition by SQ 10220 (SQ, 0.25 mg); note the prolonged duration of action. Time marks, 1 min.

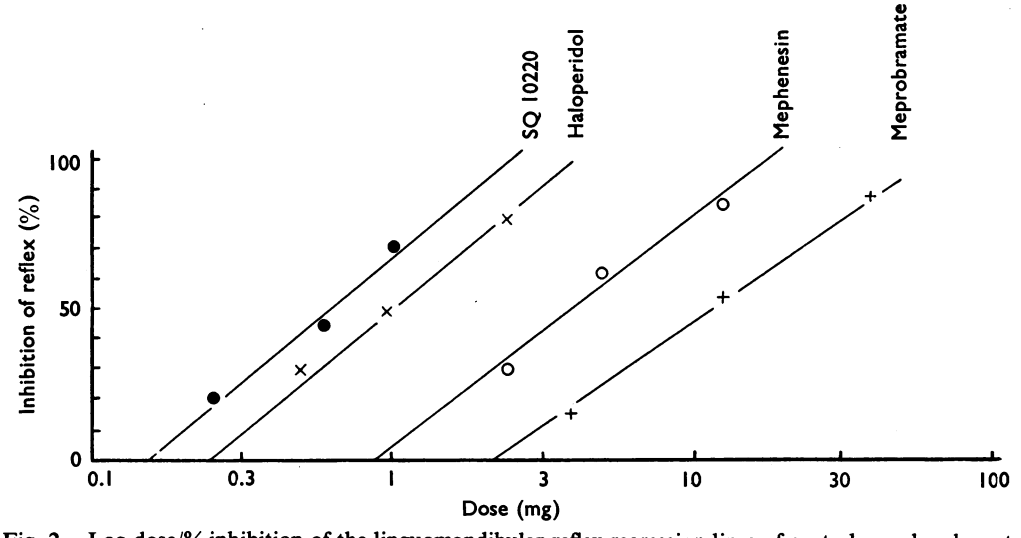

Fig. 2. Log dose/% inhibition of the linguomandibular reflex regression lines of central muscle relaxants.

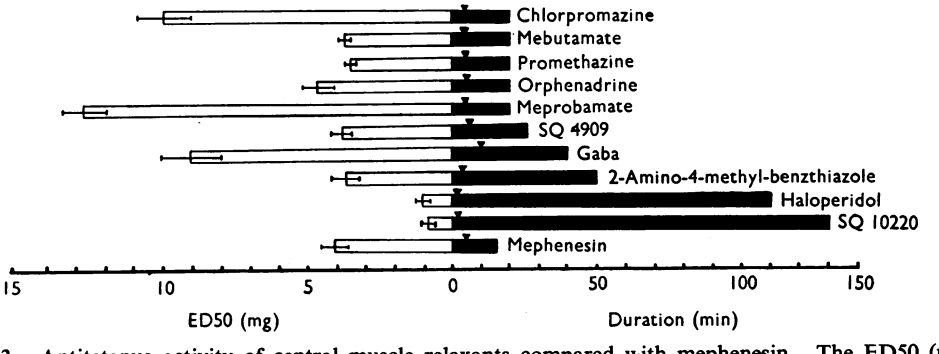

Fig. 3. Antitetanus activity of central muscle relaxants compared with mephenesin. The ED50 (mg) and standard error are shown on the left and the duration of action (min) is shown on right. The time of onset of maximum effect is marked by the triangles on the bars. Note that SQ 10220 was most potent and had the longest duration of action. Mephenesin had the shortest duration of action.

TABLE 1

THE ANTITETANUS ACTIVITY OF CENTRAL MUSCLE RELAXANTS

Values are means and standard errors of the ED50s. Activity ratios are based on mephenesin=1

Drug	ED50 (mg)	95% Confidence limits	Activity ratio
Mephenesin	4·05±0·48	4·99– 3·11	1·00
SQ 10220	0·78±0·24	1·25– 0·31	5·19
Haloperidol	0·94±0·24	1·45– 0·43	4·31
Promethazine	3·46±0·23	3·91– 3·01	1·17
Mebutamate	3·63±0·21	4·04– 3·22	1·11
2-Amino-4-methylbenzthiazole	3·65±0·52	4·67– 2·63	1·11
SQ 4909	3·80±0·32	4·43– 3·17	1·06
Orphenadrine	4·64±0·56	5·73– 3·55	0·87
γ-Aminobutyric acid	9·06±0·97	10·96– 7·16	0·45
Chlorpromazine	9·88±0·84	11·52– 8·24	0·41
Meprobamate	12·85±0·64	14·00–11·70	0·31

In three experiments facilitation of the linguomandibular reflex was obtained by intravenous injection of strychnine hydrochloride in a subconvulsive dose of 0.04 mg/kg, and intravenous injection of mephenesin (15 to 30 mg/kg) inhibited the reflex within 5 min. Recovery in these experiments also occurred in 20 min.

In similar experiments facilitation of the linguomandibular reflex induced by intraventricular strychnine hydrochloride was inhibited by various doses of centrally acting drugs and their log dose/% inhibition regression lines were plotted (Fig. 2). The ED50 of mephenesin was 4.05 mg. The compounds, with their ED50s and activity ratios, relative to mephenesin, are listed in Table 1.

It may be noted that SQ 10220 and haloperidol were more potent than mephenesin. SQ 4909, mebutamate, 2-amino-4-methylbenzthiazole and promethazine were slightly more potent than mephenesin. Meprobamate, orphenadrine, γ-aminobutyric acid and chlorpromazine were less active than mephenesin.

Fig. 3 is a graphical representation of the ED50, time of onset and duration of the inhibition of the linguomandibular reflex by different compounds. The onset of action of all the compounds was less than 10 min. Earliest to act were SQ 10220 and haloperidol and the slowest onset of action was observed with γ-aminobutyric acid. SQ 10220 and haloperidol had the longest duration of action. Compounds in decreasing order of duration of action were 2-amino-4-methylbenzthiazole, γ-aminobutyric acid and SQ 4909. Mebutamate, promethazine, chlorpromazine and orphenadrine had the same duration of action. Mephenesin had the shortest duration of action.

DISCUSSION

The linguomandibular polysynaptic reflex was found to be a suitable preparation for the quantitative assessment of antitetanus activity. The facilitation of the reflex could be quickly induced by intraventricular injection of strychnine hydrochloride or tetanus toxin. Essentially no difference was found in the facilitation produced either by strychnine or by tetanus toxin. In our earlier studies tetanus toxin was used but uniformly reproducible facilitation could not be obtained with the same dose of the toxin. The toxin is likely to deteriorate on storage and its potency may vary from preparation to preparation. Reproducibly uniform facilitation of the linguomandibular reflex was, however, obtained with intraventricular injection of strychnine (2 to 5 μg).

Mephenesin, mebutamate and meprobamate are closely related chemically. Prior administration of mebutamate was found to be more effective than mephenesin against strychnine convulsions in mice by Berger, Douglas, Kletzkin, Ludarg & Margolin (1961). In the present study mebutamate was only slightly more active than mephenesin. Meprobamate has potent anticonvulsant activity against metrazol and is clinically useful in *petit mal* epilepsy (Berger, 1954; Perlstein, 1956). Against strychnine convulsions, de Salva, Clementis & Nrcoli (1959) reported a more potent action of meprobamate when compared with mephenesin. However, Berger (1954) found poor antistrychnine activity of meprobamate. Very large doses of meprobamate were required to inhibit the linguomandibular reflex (Domino, 1962). The activity ratio of mephenesin and meprobamate on experimental local tetanus was found to be 1 : 0.43 (Webster, 1961), which does not significantly differ from the activity ratio of 1 : 0.31 obtained in the present study. The value of meprobamate in clinical tetanus is doubtful (Laurence & Webster, 1963). Of the two new compounds chemically related to mephenesin (SQ 10220 and SQ 4909), the *p*-chloro-derivative (SQ 10220) was more potent. This agent has about twice the muscle relaxant activity of mephenesin and has a long duration of action (Burke, Papandrianos, Brannick & Hassert, 1961). In the present study, when SQ 10220 was given by the intraventricular route the activity compared with that of mephenesin was about five times and it had a prolonged duration of action. The compound should prove clinically more useful in the management of tetanus. SQ 4909 was slightly more potent than mephenesin.

γ-Aminobutyric acid has nonspecific depressant actions on the central synapses (Bhargava & Srivastava, 1964). Intraventricular injection of this drug was less effective in inhibiting the strychnine-induced facilitation of the linguomandibular reflex compared with mephenesin. Haloperidol is a substituted butyrophenone with a structure resembling γ-aminobutyric acid and actions similar to chlorpromazine (Janssen, Neimegeers & Scheliekens, 1960). Haloperidol has actions on the extrapyramidal system which are responsible for its beneficial effects in the treatment of Parkinsonism and allied disorders (Nodine, Bodi, Levy, Siegler, Slap, Mapp & Khorsandian, 1962). Haloperidol, in the present study, was a very promising antitetanus drug. It was about 4.3-times more potent than mephenesin on strychnine—as well as tetanus toxin-potentiated linguomandibular reflex. The duration of action was about six times longer than mephenesin.

Several 2-aminobenzazole derivatives were studied by Funderburk, King, Domino & Unna (1953) for their central muscle relaxant and antistrychnine properties. The 4- and 7-position substituted compounds were the most potent as spinal interneurone depressants. Webster (1961) reported a potent antitetanus activity of the compound substituted in the 6-position. The 2-amino-4-methylbenzothiazole studied by us was slightly more potent than mephenesin and had almost twice the duration of action.

Of the two phenothiazines studied for their inhibitory activity on the facilitated linguomandibular reflex, promethazine was more potent than chlorpromazine. From the results of our comparative study, chlorpromazine does not seem to be a good antitetanus drug. Clinically also this agent has not proved useful in the management of tetanus.

Orphenadrine is chemically 2-dimethylaminoethyl-2-methyldiphenylmethyl ether and has central muscle relaxant properties (Bijlasma, Harms, Funcke, Tersteege & Nauta, 1956). In the present study, orphenadrine was less active than mephenesin and the duration of action was not appreciably greater than that with mephenesin.

Finally, it may be stated that in this experimental study on cats, several central muscle relaxants were tested for their inhibitory action on the linguomandibular reflex and drugs were given by the intraventricular route. The activity of the compounds when given by the intravenous or intramuscular routes is likely to differ depending upon how much of the drug crosses the blood brain barrier. A controlled evaluation of these agents in clinical tetanus is warranted.

SUMMARY

1. Ten centrally acting muscle relaxants were compared with mephenesin for their antitetanus activity in cats.

2. The polysynaptic linguomandibular reflex elicited by electrical stimulation of the root of the tongue was recorded on a kymograph. Facilitation of the reflex was induced by intraventricular injection of tetanus toxin or strychnine. Inhibition of the reflex was observed after intraventricular injection of different doses of the muscle relaxants. The ED50, standard error and 95% confidence limits of all the compounds were calculated. The ED50 of mephenesin was taken as unity to obtain the relative activity of other agents.

3. 3-Carbamoyloxy-2,2-dichloro-1-*p*-chlorophenylpropanol (SQ 10220) was the most potent agent and had the longest duration of action. Haloperidol was next in order of potency and its duration of action was prolonged. 3-Carbamoyloxy-2,2-dichloro-1-phenylpropanol (SQ 4909), promethazine, mebutamate and 2-amino-4-methylbenzthiazole were slightly more potent than mephenesin. Orphenadrine, γ-aminobutyric acid, chlorpromazine and meprobamate were less active than mephenesin.

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