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THE ACTIVITY OF A VARIETY OF CHEMICAL COMPOUNDS AGAINST EXPERIMENTAL TETANUS

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A range of chemical compounds, mostly with central nervous system depressant activity, have been tested against experimental tetanus in the rabbit. A number of the more potent tested, including mephenesin, betanaphthoxyethanol, barbiturates and phenothiazine derivatives, have been accurately assayed by a method of quantitative electromyography. Phenothiazine derivatives were found to be the most potent anticonvulsants and of these acetylpromazine had the greatest activity. The difficulties of direct comparison of the potency of substances from different chemical groups is discussed.

Tetanus is a disease in which generalized convulsions, if uncontrolled, are an important, although by no means the sole, cause of death. The convulsions may be controlled by continuous neuromuscular block with artificial respiration or by sedation. Most sedative drugs, however, when used in adequate anticonvulsant dosage, cause either loss of consciousness or respiratory depression, or both. The ideal drug would arrest convulsions without these effects.

The survey of various central nervous depressants reported here was undertaken to find which series of compounds most closely approaches the ideal, and to obtain some quantitative comparison of the relative potency of the compounds tested. In this way it was hoped to discover drugs likely to be of use in clinical practice and to compare them with drugs in current use.

Choice of Drugs

The drugs chosen for accurate assay, together with the reasons for the choice, are given below.

Mephenesin.—The spinal cord and brain-stem depressant activity of mephenesin makes it theoretically an ideal drug for use in tetanus, and many examples of its use have been reported. It is, however, active for only a very short time and has other disadvantages.

Betanaphthoxyethanol.—This compound has central actions similar to mephenesin and may also have some additional anaesthetic effect which might enhance its anti-tetanus activity.

Barbiturates.—The barbiturates have had extensive use in the treatment of tetanus.

Phenothiazines.—Chlorpromazine is known to have marked anti-tetanus activity in both animals and man.

Other substances were chosen because they were known to have paralysing effects (for example benzimidazole derivatives), were related chemically to the above-mentioned drugs or had been tried or suggested for trial in clinical tetanus in the past.

METHOD

The method employed for producing and recording experimental local tetanus in the rabbit was that of Laurence and Webster (1958). Local tetanus was induced in rabbits by an injection of tetanus toxin into the gastrocnemius muscle of one hind limb. The electrical activity of this muscle was recorded with intramuscular electrodes and the potentials amplified and monitored on an oscillograph. These voltages were summed by an integrating circuit to a level at which an impulse was fed to a Dekatron counter. Every 100th pulse was then led off through a relay circuit to either a signal marker on a smoked drum, or an electromagnetic counter. The electrical activity of the tetanized musle was thus converted into arbitrary units and the anti-tetanus activity of a drug determined by a chosen criterion. The criterion originally used had a number of shortcomings that have been discussed (Laurence and Webster, 1958). In the present work, the % reduction of tetanus activity for a 20 or 30 min. period immediately after administration of the drug was used as the index of potency. A control count of the electrical activity from the tetanus muscle was made each minute for a 5 or 10 min. period, depending on the variability of the tetanus in the individual animal. An example of the derivation of the value for the % reduction of activity is shown in Fig. 1. All the drugs were given intravenously.

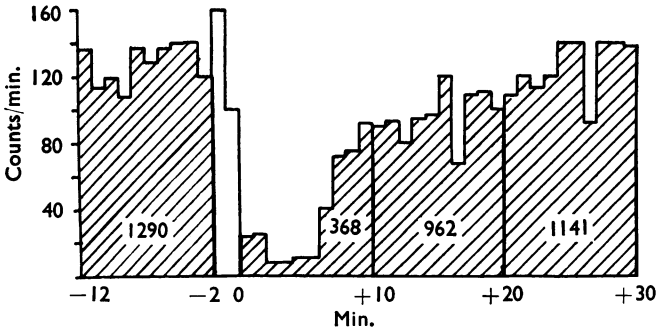


FIG. 1.—Derivation of the % reduction of tetanus activity produced by promazine. The ordinate is a count of the electrical activity /min. from the affected muscle of a rabbit with local tetanus. Total counts/10 min. periods are also shown. The 2-min. injection period is not included in the calculation. Between −2 and 0 min., 0·25 mg./kg. of promazine was injected intravenously. The % reduction of tetanus activity was calculated as follows. % Reduction = $100-\left(\frac{368+962+1,141}{3\times 1,290}\times 100\right)=36\%$.

RESULTS

Comparison of Some Chemical Groups Known to Have Anti-tetanus Activity

The drugs used were mephenesin, thiopentone, chlorpromazine, and betanaphthoxyethanol.

The assay was designed to extend over four days, during which time each animal had two doses of all four compounds. The doses used (Fig. 2) were those found in preliminary experiments to give results that straddled the 50% reduction line. The reduction of activity was calculated for the 20 min. period following the injection. The dose order was randomized so that as far as possible any variation in the level of tetanus was nullified.

Not all the animals in which experimental tetanus was induced could be used in the assay, as in some cases a workable level of tetanus activity was never achieved.

The results of this assay are shown in Fig. 2. The plotted values for mephenesin and betanaphthoxyethanol are the mean results from nine animals. With chlorpromazine and thiopentone, however, the results from only six animals have been used. This is because the doses of these two compounds originally chosen, and used on the first three animals, were too high and gave results that were not comparable with those for the other drugs. Subsequently lower doses of chlorpromazine and thiopentone were used.

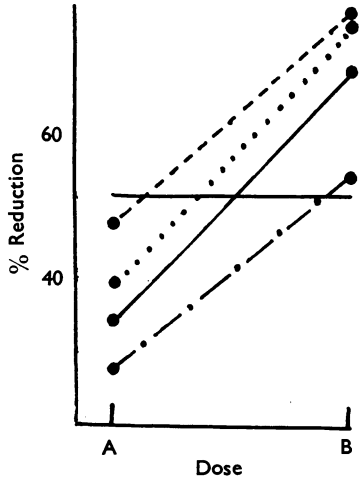


FIG. 2.—Mean results for the comparison of the anti-tetanus activity of .—.—. mephenesin (15 and 30 mg./kg.); ——— betanaphthoxyethanol (5 and 10 mg./kg.); — — — — thiopentone (5 and 10 mg./kg.); . . . . . chlorpromazine (0·08 and 0·16 mg./kg.). The % reduction of tetanus activity was calculated for the 20-min. period following the injection. A refers to the lower and B to the higher dose given.

TABLE I

A COMPARISON OF THE ANTI-TETANUS ACTIVITY OF SOME CENTRAL NERVOUS DEPRESSANTS

The dose producing a 50% reduction of tetanus activity over the 20-min. period following injection was calculated graphically (Fig. 2).

Compound	No. of Animals	50% Reduction Dose mg./kg.	Activity Ratio
Mephenesin	9	28·5	1
Betanaphthoxyethanol ..	9	7·5	3 8
Thiopentone	6	5·6	5 0
Chlorpromazine ..	6	0·11	266

The relative potencies were calculated by measuring the dose of each drug which produced a 50% reduction of tetanus activity. This dose, together with the activity of each compound, compared with mephenesin, is shown in Table I. From this it will be seen that chlorpromazine is over 200 times and thiopentone 5 times as active as mephenesin.

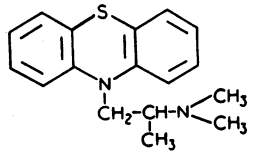
By "*t*" test calculations, all the lines were found to have a significant slope and not to deviate significantly from parallel. Since chlorpromazine was so much more active than the other compounds it was decided to investigate some other phenothiazines.

Comparison of the Anti-tetanus Activity of Some Phenothiazine Derivatives

The four most active derivatives of thirteen tested were chosen for accurate assay. They were: chlorpromazine, acetylpromazine, promazine, and

promethazine. Their formulae are shown in Fig. 3. The assay was arranged as before with the exception that the recording period following drug injection was 30 instead of 20 min., as phenothiazine compounds have the highly desirable property of long duration of action.

Promethazine



Promazine

Chlorpromazine

Acetylpromazine

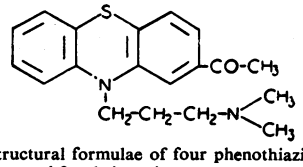


FIG. 3.—The structural formulae of four phenothiazine compounds assayed for their anti-tetanus potency.

The results of this assay for seven animals are shown in Table II and it will be seen that acetylpromazine has ten times the anti-tetanus activity of chlorpromazine. Again, "*t*" test calculations show that for each drug a significant regression line was obtained but that in no case did the lines deviate significantly from parallel.

TABLE II

A COMPARISON OF THE ANTI-TETANUS ACTIVITY OF SOME PHENOTHIAZINES

The dose producing a 50% reduction of tetanus activity over the 30-min. period following injection was calculated graphically. The graphical presentation of the results of this assay is similar to that shown in Fig. 2.

Compound	No. of Animals	50% Reduction Dose mg./kg.	Activity Ratio
Acetylpromazine ..	7	0·019	10·5
Chlorpromazine ..	7	0·20	1·0
Promazine	7	0·44	0·46
Promethazine	7	0·60	0·33

Other Compounds

In addition to those assayed above, the following compounds were tested qualitatively for anti-tetanus activity. These were not assayed accurately as they were obviously ineffective either in comparison with other members of their chemical group as with some phenothiazines; or the group as tested was impotent, for example benzimidazole derivatives. It was not thought worth while to devote to them the time that an accurate assay would have demanded.

The *phenothiazine derivatives* were: diethazine, 10-(2-2-diethylaminoethyl)phenothiazine; ethopropazine, 10-(2-diethylaminopropyl)phenothiazine; pecazine (Pacatal), 10-(1-methylpiperid-3-ylmethyl)-phenothiazine; prochlorperazine (Stemetil), 2-chloro-10-{3-(4-methylpiperazin-1-yl)propyl}-phenothiazine; trimeprazine (6549 RP), 10-(3-dimethylamino-2-methylpropyl)phenothiazine; methotrimeprazine (7044 RP), 10-(3-dimethylamino-2-methylpropyl)-2-methoxyphenothiazine; thiopropazate (SC 7105, Dartal), 2-chloro-10[3-{4-(2-acetoxyethyl)piperazin-1-yl}propyl]phenothiazine; SC 8723, 2-chloro-10-[3-{4-(3-oxobutyl)-piperazin-1-yl}propyl]phenothiazine; SC 8950, 2-chloro-10-{3-(4-methylcarbamoylpiperidino)-propyl} phenothiazine.

Two *benzimidazole derivatives* have been reported to have marked central depressant effects (Domino, Unna, and Kerwin, 1952). They are 2-amino-6-methylbenzthiazole (SKF 1045A) and 2-amino-6-chlorobenzthiazole (I.C.I. 5502). Both of these compounds are less active than mephenesin. Other compounds also tested were: pentobarbitone, hyoscine, benzhexol, benactyzine, caramiphen, diphenhydramine, procaine, morphine, and amphetamine.

DISCUSSION

In evaluating the activity of any drug both the duration and intensity of its effect have to be considered. Whilst giving a comparison which is considered to be sufficiently accurate for screening for possible clinical use, the criterion used in this work is, in effect, a combination of these two factors. It is, however, important to distinguish between these two factors when comparing drugs of different chemical constitution.

Consider two hypothetical substances A and B both exhibiting anti-tetanus activity. They produce, at a certain dosage, a 50% reduction of tetanus activity over a 20 min. period following their injection. Drug A produces total abolition for 10 min. followed by normal tetanus activity for the remaining time, whilst drug B produces a

50% reduction in activity for each of the 20 min. observed. Thus whilst the net result is the same, the means by which it is derived are different and strict comparison of their activities is impractical. To judge the importance of this difference in action in these experiments the 20 min. recording period was divided into 5 min. intervals and the % of tetanus activity, compared with the untreated tetanus, calculated in each instance. The time course of action of the drugs was then compared.

Histograms of the results of these calculations are shown in Fig. 4. From these it will be seen that the type of action of the drugs tested does in fact vary. Chlorpromazine *at the doses used* has a weak prolonged effect (like substance B) whilst the action of mephenesin, although of short duration, is intense, producing sometimes total abolition of tetanus. These graphs also demonstrate the delay in the onset of activity of chlorpromazine.

With the doses used in these experiments, it appears that strict comparison of different chemical compounds by the method employed is not completely practical or fully valid. Mephenesin is apparently only active, particularly at the lower dose level, for about 10 min. and it might be suggested that in order to appreciate the full effect of mephenesin one should compare the drugs over this period only. If, however, the dose of chlorpromazine is increased to produce an effect, in this same time interval, comparable with that of mephenesin, then the action of chlorpromazine is not only evident during this time but extends for

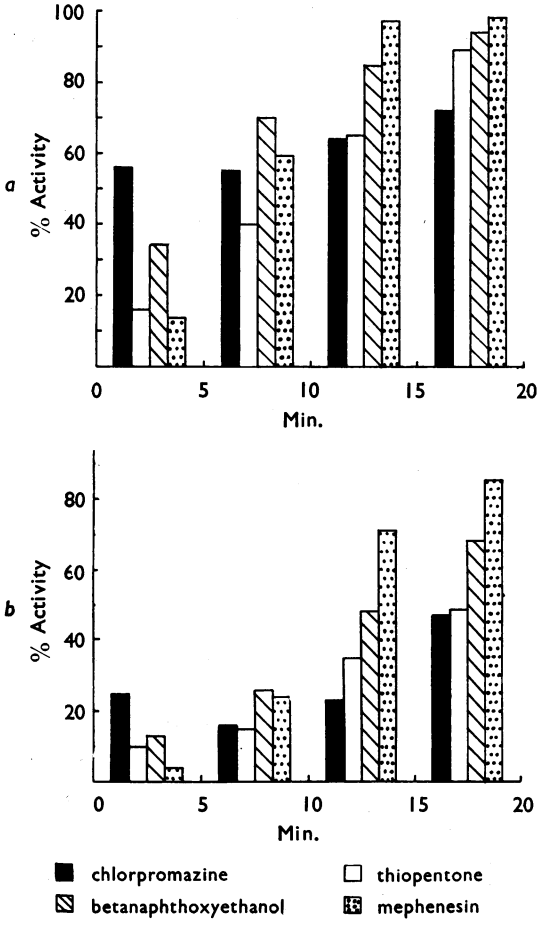


FIG. 4.—Mean time course for the reduction in tetanus activity produced by chlorpromazine (6 rabbits), thiopentone (6), betanaphthoxyethanol (9), and mephenesin (9). The % activity for each 5-min. period following the injection was calculated by comparison with the activity recorded in the control period. In *a*, 0·08 mg./kg. chlorpromazine, 5 mg./kg. thiopentone, 5 mg./kg. betanaphthoxyethanol, and 15 mg./kg. mephenesin were used. In *b*, twice the dose of each compound was given.

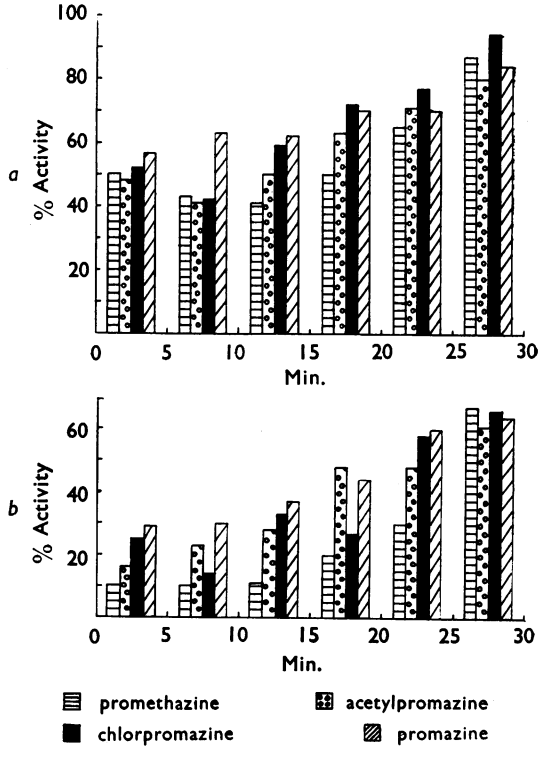


FIG. 5.—Mean time course of the reduction in tetanus activity, in 7 rabbits, produced by promethazine, acetylpromazine, chlorpromazine, and promazine. The % activity for each 5 min. period following the injection was calculated in comparison with the activity recorded in the period immediately before. In *a* 0·5 mg./kg. promethazine, 0·0125 mg./kg. acetylpromazine, 0·125 mg./kg. chlorpromazine, and 0·25 mg./kg. promazine were used. In *b*, twice the dose of each compound was given.

a further 10 min. or more. In fact we would then be failing to record the full effect of chlorpromazine. Bearing these facts in mind the criteria used are a compromise, yet are adequate for the purpose of seeking drugs for clinical use provided the type of action is noted in addition to the simple potency figure.

In Fig. 5 the analysis of the results for the comparative assay of the phenothiazine derivatives is shown. The types of action of all four compounds are sufficiently alike to make strict comparison possible. The delay in onset of action is noticeable.

Phenothiazine compounds are clearly the most effective drugs available at present against tetanus. Chlorpromazine has been used extensively in the treatment of clinical tetanus (Laurence, Berman, Scragg, and Adams, 1958). It has some manifest disadvantages including occasional inability to control severe convulsions.

Arrangements have been made for the clinical trial of acetylpromazine.

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