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A COMPARISON OF THE EFFECTS OF CHLORPROMAZINE AND SOME RELATED PHENOTHIAZINES IN REDUCING THE RIGIDITY OF THE DECEREBRATE CAT AND IN SOME OTHER CENTRAL ACTIONS

BY

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One of the many interesting properties of chlorpromazine is its ability to reduce skeletal muscle tone in experimental animals (Dasgupta & Werner, 1955; Sheatz, 1955). Henatsch & Ingvar (1956) and Busch, Henatsch & Schulte (1960) have shown that small doses of chlorpromazine abolish the rigidity of the intercollicular decerebrate cat and reduce the discharge of γ-motoneurones. These authors also found that much larger doses of chlorpromazine were required to depress the rigidity of the decerebrate cat prepared by the ischaemic method of Pollock & Davis (1930). It was suggested that chlorpromazine had a selective depressant effect on the γ- as opposed to the α-motoneurones at a supraspinal level. Chlorpromazine has been reputed to be of some value in reducing the muscle tonus in patients with spasticity (Basmajian & Szatmari, 1955).

Although the actions of chlorpromazine in reducing skeletal muscle tone and in affecting spinal reflexes have been extensively studied, little is known about the activity of related phenothiazine derivatives in this context. It thus appeared interesting to compare chlorpromazine with some related phenothiazine derivatives for their ability to reduce rigidity of the intercollicular decerebrate cat with the object of seeing to what extent there was correlation between activity in reducing skeletal muscle tone and other pharmacological actions.

The phenothiazine derivatives we have studied fall roughly into two groups, firstly those with central actions somewhat similar to chlorpromazine. It was thought that a study of these compounds might reveal to what extent there was correlation between the ability of the compound to reduce decerebrate rigidity and other central properties. The second group are phenothiazine derivatives with weak sedative properties, but with other pronounced pharmacological actions (anti-histaminic or anti-emetic activity). It was felt that a study of these compounds might lead to a substance effective in reducing rigidity at doses which did not produce marked central depression and sedation. It is also possible that a drug that possessed a specific action in reducing γ-motoneurone activity supraspinally without having significant sedative properties might be interesting in the treatment of spastic states (Rushworth, 1964).

As a first step to the comparative study of the phenothiazine derivatives it was essential to develop an objective method of assessing decerebrate rigidity and its reduction.

METHODS

Experiments in cats

Reduction of decerebrate rigidity. Cats were anaesthetized with ether, the carotid arteries were clamped and the mid-brain sectioned approximately between the colliculi. Ether was then discontinued and the clamps were removed from the carotids 5 min after decerebration. Recordings did not begin until at least 1 hr after decerebration. The animal was placed on one side and the left femur held in a vertical position by a steel pin in its lower end, the lower leg being allowed to hang freely from the knee. The electrical activity of the quadriceps femoris muscle was recorded by means of bipolar, stainless steel electrodes. These were inserted through a small incision in the skin, roughly midway along the length of the quadriceps and placed 0.5 cm apart. The resultant electrical activity was amplified and displayed on a pen recorder, the resultant maximum sensitivity being approximately 25 μV/cm.

The electrical activity recorded from the resting quadriceps muscle of the decerebrate cat was very variable and often absent. Good standard electromyographic recordings were obtained by gently extending the lower leg to its full length—that is, vertically—and lowering it gently until it was held by the tone of the quadriceps muscle (Fig. 1). Extensions of the lower leg ("stretch responses") were carried out at 5 min intervals for a control period of some 45–60 min. After this

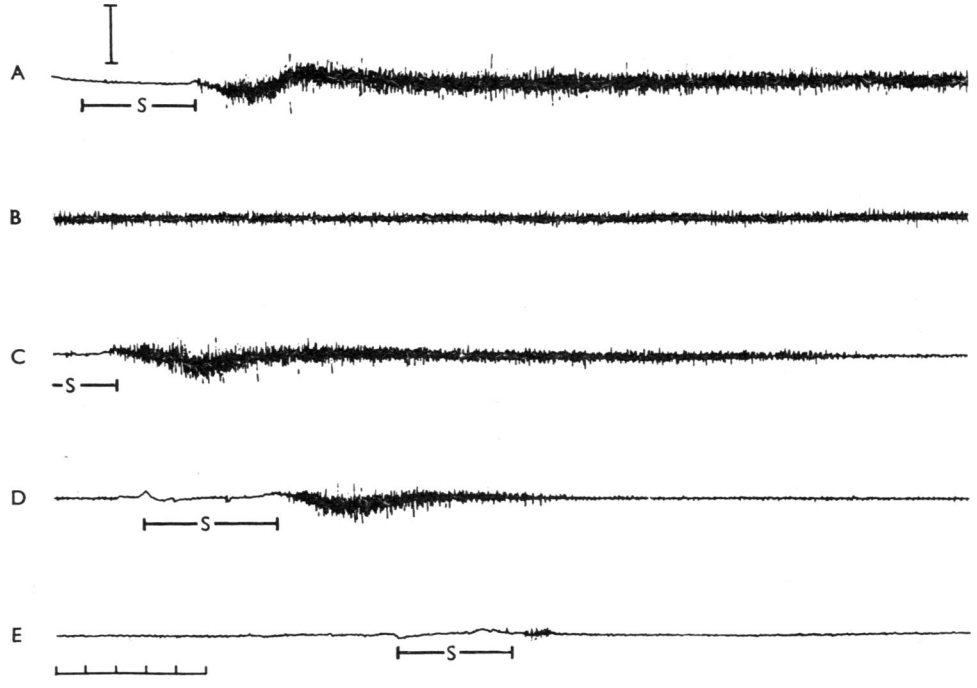


Fig. 1. Electromyographic response recorded from the quadriceps muscle of an intercollicular decerebrate cat (1.9 kg). At S the lower leg was fully extended and gently released. Tracings in descending order: A. Control response to stretch of the quadriceps femoris. B. Continuation of tracing A showing the prolonged response. C. Recording taken immediately following the intravenous infusion over two minutes of 0.5 mg/kg metopimazine hydrochloride. Note the shorter duration of electromyographic response in comparison with A and B. D. Recording taken 5 min after the subsequent administration of a further 0.5 mg/kg (i.e., 1 mg/kg total) 15 min after the first injection, showing the much reduced electromyographic responses. E. 5 min after the administration of a total of 3.75 mg/kg metopimazine hydrochloride. Abolition of the electromyographic response. Time marks 1 sec. Vertical scale 100 μv.

period the drug under test was infused (Palmer slow injection apparatus) into a cannulated jugular vein at the rate of 0.5 ml./min. The electromyographic response to stretch of the quadriceps muscle was tested at intervals after infusion of graded doses of the compound. The dose of compound (see Fig. 1) which completely abolished the electromyographic response to leg extension was determined. After abolition of the stretch response drug infusion was stopped and the time required for a return of the control level of electromyographic response to leg extension was estimated. Throughout the experiments gross observations of the degree of rigidity were made. Some preliminary recordings were carried out in which the electromyogram was integrated by the use of a voltage discriminator and a decatron scaling unit. However, it was found that this added little to the decision as to when the electromyogram was abolished.

Blood pressure. In some experiments the arterial pressure of the decerebrate cat was recorded from a polyethylene cannula inserted into the femoral artery contralateral to the leg being used for electromygraphic recording. The polyethylene cannula led to a Statham P-23 G transducer and pen recorder. The wound around the incision in the leg was infiltrated with procaine.

Spinal reflexes. Experiments on spinal reflexes were carried out in cats anaesthetized with chloralose (70 mg/kg, intravenously) or spinalized at C_1 whilst under ether anaesthesia. The patellar reflex was elicited by means of a mechanical hammer as described by Schweitzer & Wright (1937).

The flexor reflex was elicited by electrical stimulation of the central end of the cut tibial nerve whilst recording twitches of the tibialis anterior muscle using a spring myograph. Bipolar, platinum, stimulating electrodes were placed on the tibial nerve about 2 cm distal to the point where the tibial and peroneal nerves separate. The stimulus consisted of a train of pulses of 0.5 msec width repeated at intervals of 10 msec for a 100 msec period. The nerve was thus stimulated every 10 sec.

Experiments in mice

The experiments were carried out on albino mice of either sex weighing between 18–25 g. All drugs were given by subcutaneous injection, the tests being carried out 1 hr after administration with the exception of experiments with mephenesin, which were carried out 15 and 30 min after drug administration.

Traction test. The experiments were a modification of that described by Courvoisier, Ducrot, Fournel & Julou (1958). Mice were suspended from a horizontal wire by their fore-paws, and the number of mice which were unable to draw themselves up to touch the wire with one posterior foot within 5 sec of being placed on the wire was determined. Groups of 10–20 mice per dose level were studied at a number of dose levels and the dose of compound (ED50) that reduced to 50% the number of animals which gave a positive response was determined.

Effect on pinna and corneal reflex. The method was based on that described by Witkin, Spitaletta & Plummer (1959). The pinna reflex, which involves the twitch tremor or laying back of the ear on stimulation of the external auditory meatus, was elicited by stimulation of each ear by stroking with a fine hair. The reflex was said to be negative when no reflex could be elicited from either ear.

The corneal reflex was elicited by touching the cornea and conjunctiva of both eyes with a hair and was considered abolished when the hair placed on the eye for 1 sec did not elicit a reflex in either eye.

Drugs

Drugs used were chlorpromazine hydrochloride; methotrimeprazine hydrochloride; promethazine hydrochloride; acepromazine maleate B.P.C.; thioproperazine methanesulphonate; chlorproethazine [2-chloro-10-(3′-diethylamino-n-propyl) phenothiazine] hydrochloride; dimethothiazine [10-2′-dimethylaminopropyl-2-dimethylsulphamoylphenothiazine] methanesulphonate; pericyazine [3-cyano-10-(3-4′-hydroxypiperidinopropyl) phenothiazine]; metopimazine [3-methylsulphonyl-10-(3,4′-carbamoyl-piperidinopropyl) phenothiazine]; mephenesin injection, B.P.; chlordiazepoxide hydrochloride; hyoscine hydrobromide; and butobarbitone sodium.

Solutions of pericyazine were prepared by dissolving the compound with an equivalent of tartaric acid and water. Metopimazine was dissolved in an equivalent of hydrochloric acid; the other

compounds were dissolved in distilled water. All solutions were freshly prepared, care being taken with the phenothiazine derivatives to keep them away from light. Doses quoted refer to the salts, except for pericyazine and metopimazine which refer to the base.

RESULTS

Effects on the rigidity of the decerebrate cat

The subjective assessment of the degree of rigidity in the limbs of a decerebrate cat was too variable and insufficiently objective to serve as a reliable method of drug evaluation. Similarly, the electrical activity recorded from the quadriceps femoris muscle in the resting state of the decerebrate cat was variable and often absent. However, by extending the lower limb at regular intervals and thus producing tonic extension of the quadriceps muscle (" stretch response ") due to the weight of the lower leg, reliable and reproducible electromyographic recordings were obtained.

Typical recordings obtained from the quadriceps muscle of the decerebrate cat are shown in Fig. 1, which illustrates the lack of activity in the resting state and the electromyographic response following leg extension. The duration of the electromyographic activity as the lower leg fell under its own weight varied from cat to cat, but was always longer than 30 sec in the decerebrate animal and often lasted for 3–5 min. Little electromyographic activity was recorded when the " stretch response " was elicited in cats anaesthetized with chloralose (70 mg/kg, intravenously) or in spinalized preparations, although a brisk patellar reflex was present.

That the electromyographic response recorded was elicited reflexly was confirmed by experiments (Fig. 2A) in which the electrical activity of the muscle was reflexly inhibited by repetitive stimulation of the central end of the cut ipsilateral posterior tibial nerve. The electromyographic activity was also abolished when the lower leg was fully raised (Fig. 2B) so that there was no stretch of the quadriceps muscle. It appears likely that

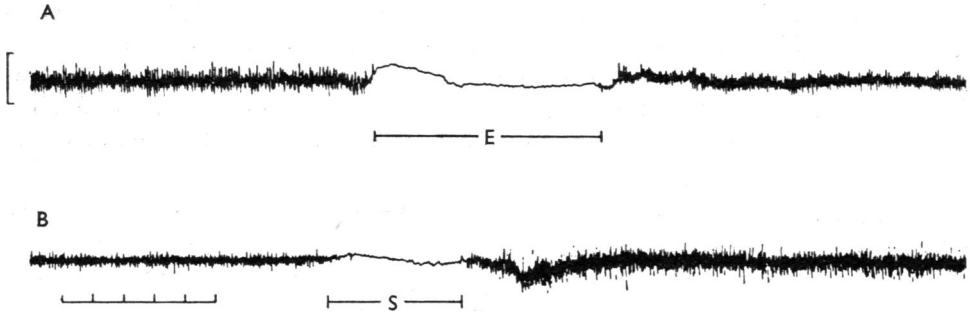


Fig. 2. A. Decerebrate cat (2.2 kg); electromyographic activity recorded from the quadriceps muscle being stretched by the lower limb. At E reflex inhibition of the activity produced by electrical stimulation of the central end of the ipsilateral posterior tibial nerve at 200/sec. Note inhibition of electromyographic activity with return, at a slightly lower level, on termination of the stimulus. B. Decerebrate cat (2 kg); inhibition of electromyographic activity from the quadriceps muscle produced by complete leg extension. At S the lower leg was extended fully so that the quadriceps was no longer being stretched. Note the electromyographic activity was abolished and returned to an enhanced degree when the leg was gently lowered. Time marks 1 sec. Vertical scale 100 μV.

the response being recorded was the tonic portion of the classical stretch reflex (Liddell & Sherrington, 1924) and could be taken as an objective indication of the degree of decerebrate rigidity. It is also possible that a combination of tonic and phasic reflexes was involved.

Figure 1 illustrates the method for assaying drug potency in reducing decerebrate rigidity. Tracings A and B are control recordings whereas the tracings C, D and E were taken short periods after intravenous infusion of increasing doses of metopimazine hydrochloride, showing virtually complete abolition of the electromyographic response after a dose of 3.75 mg/kg intravenously, this being taken as the minimum effective dose in this particular experiment.

The overall results obtained with a selection of phenothiazine derivatives and some reference compounds in reducing the decerebrate rigidity of the cat, together with the effects of these compounds in inhibiting spinal reflexes and in some other simple tests of central activity are set out in Tables 1 and 2. The figure quoted for reducing decerebrate rigidity in the cat is the mean minimum effective dose together with the indication of the duration of action of the compound. Estimations of this latter quantity were variable.

Of the phenothiazine derivatives examined, the most effective, dose for dose, was acepromazine, the mean intravenous effective dose in four experiments being 0.03 mg/kg as compared to 0.5 mg/kg for chlorpromazine. In a fifth experiment with acepromazine a dose of 13 mg/kg had no effect on the electromyographic response to stretch of the quadriceps. It is possible that the rigidity of this cat was not associated with increased γ-motoneurone activity, but may have been due to increased α-motoneurone activity (α-cat), possibly as a result of damage to the anterior cerebellum.

The next most effective drugs were methotrimeprazine and pericyazine, which were effective in doses of 0.25–0.3 mg/kg intravenously. Chlorpromazine and closely related chlorproethazine were effective in a dose of 0.5–0.6 mg/kg. The results obtained with promethazine were of interest as this compound possesses anti-apomorphine, anti-histaminic and anti-cholinergic properties. Promethazine is clinically useful in the treatment of Parkinsonian rigidity (Duvoisin, 1965) and it is interesting that it had a relatively high activity in reducing decerebrate rigidity, whereas hyoscine, also a drug effective in Parkinsonian rigidity, was ineffective. Chlordiazepoxide, mephenesin and butobarbitone are included for comparative purposes in view of their known centrally acting muscle relaxant properties.

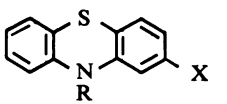
Some attempt was made to assess the duration of action of the phenothiazines. With the exception of mephenesin, with a duration of action of from 30–40 min, most of the compounds at the dose just sufficient to inhibit decerebrate rigidity had a duration of action of from 3–4 hr. Two exceptions to this were metopimazine and dimethothiazine, both of which had a relatively short duration of action of from 1–2 hr.

An attempt was made to assess whether tachyphylaxis occurred to the effects of these compounds in reducing decerebrate rigidity. This was not always possible, since often the experiments had to be terminated before the second or third dose of the compound could be administered. However, in two experiments with chlorpromazine where a second dose was administered some 4–5 hr after the first there was no indication of tachyphylaxis. Likewise, in the case of acepromazine, two experiments were carried

TABLE 1

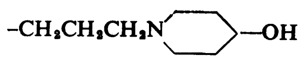
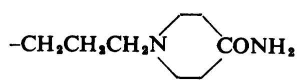
EFFECTIVENESS OF VARIOUS PHENOTHIAZINE DERIVATIVES AND SOME OTHER DRUGS IN REDUCING THE RIGIDITY OF THE INTERCOLLICULAR DECEREBRATE CAT AND IN INHIBITING SOME SPINAL REFLEXES IN THE CHLORALOSE CAT

Rigidity was objectified by the electromyographic response of the quadriceps muscle to extension of the lower leg. Figures in brackets refer to the number of experiments. All doses are mg/kg.

General formula of phenothiazine derivatives 

	Structural formula of phenothiazine derivatives		Reduction of decerebrate rigidity (cat, intravenous)		Inhibition of reflex (chloralose cat, intravenous)	
Compound	X	R	Minimum effective dose intravenous	Duration of action (hr)	Dose causing 20–40% depression of patellar reflex	Effect on flexor reflex
Chlorpromazine	–Cl	$-CH_2CH_2CH_2N(CH_3)_2$	0·5 (4)	3–4	0·75–1·0 (6). No further effect on increasing dose	20–40% depression at 0·75–1·0 (3). No further effect on increasing dose. No effect to 40 (1)
Chlorproethazine	–Cl	$-CH_2CH_2CH_2N(C_2H_5)_2$	0·6 (3)	2–4	0·5–5·0 (5). No further effect on increasing dose	20–40% depression at 0·5–5·0 (3). No further effect on increasing dose. No effect to 20 (1)
Acepromazine	$-COCH_3$	$-CH_2CH_2CH_2N(CH_3)_2$	0·03 (4)	2–4	0·1 (2)	Slight depression at 0·1. No greater effect to 45 (2)

Table 1 (continued)

Methotrimeprazine	$-OCH_3$	$-CH_2CH(CH_3)CH_2N(CH_3)_2$	0·3 (2)	4	0·05–0·1 (2)	Slight depression at 1·0 (1). No depression to 35 (1)
Promethazine	–H	$-CH_2CH(CH_3)-N(CH_3)_2$	2·8 (2)	2–4	Not tested	Not tested
Dimethothiazine	$-SO_2N(CH_3)_2$	$-CH_2CH(CH_3)N(CH_3)_2$	2·2 (2)	1–2	3–8 (4)	Inactive to 40 (2). Slight depression in 1 experiment
Pericyazine	–CN		0·25 (3)	3–4	0·02–0·4 (2)	20–40% depression at 0·5. No greater effect to 16 (2)
Metopimazine	$-SO_2CH_3$		2·2 (4)	1–2	No effect to 40 (4)	No effect to 40 (4)
Thioproperazine	$-SO_2N(CH_3)_2$		>8	—	Not tested	Not tested
Mephenesin	Not phenothiazines		25 (3)	0·5	Inactive at 50 (4)	Inhibition at 25 (4)
Bhlordiazepoxide			5 (2)	3–4	Inactive at 5 (3)	Inhibition at 4·5 (2)
Butobarbitone			6 (2)	—	Depression at 5 (1)	Inhibition at 5 (1)
Hyoscine			>6 (3)	—	Not tested	Not tested

out in an attempt to assess tachyphylaxis. In one of these experiments (Fig. 3) three doses (of 0.025 mg/kg intravenously) were given at intervals of 2–3 hr, with no obvious indication of tachyphylaxis. The possible occurrence of tachyphylaxis was also studied with pericyazine and methotrimeprazine and in one experiment in each case there was no reduction of drug response on the second administration.

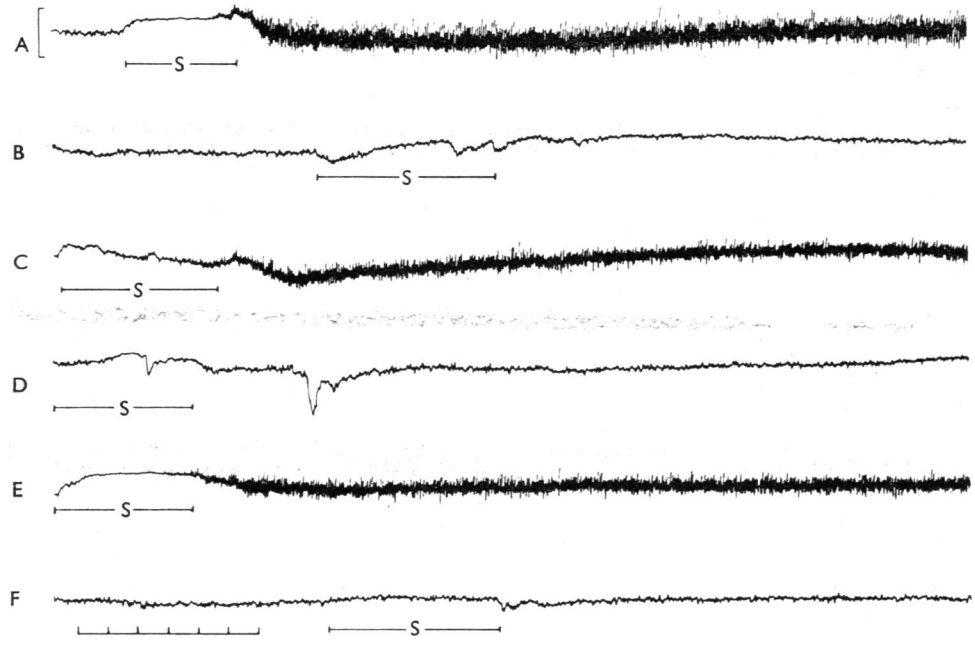


Fig. 3. Electromyographic recordings from the quadriceps femoris of a decerebrate cat (2 kg). The effect of repeated administration of acepromazine. During the period indicated by S the lower leg was fully extended and gently released. Tracings in descending order: A. Control electromyographic response to leg extension. B. Abolition of response following intravenous administration of 0.02 mg/kg acepromazine maleate. C. 2 hr after recording B showing recovery of electromyographic response. D. Abolition of stretch response following the second administration of 0.02 mg/kg intravenously of acepromazine. E. 2 hr after D showing recovery of electromyographic response to stretch. F. Immediately after the administration of further 0.014 mg/kg acepromazine. Note lack of tachyphylaxis to the action of acepromazine. Time marks 1 sec. Vertical scale 100 μv.

In four out of six experiments with metopimazine low frequency spike electromyographic activity was recorded from the resting muscle after abolition of the electromyographic activity to " stretch response," that is—following doses of 2–4 mg/kg intravenously. The cause of this effect has not been analysed further.

Effects on arterial pressure

In some experiments the arterial blood pressure was recorded from the decerebrate cat and the effect of the phenothiazine derivatives on both the arterial pressure and the electromyographic response to stretch of the quadriceps was studied. In three experi-

ments with acepromazine, in which the electromyographic activity was abolished by doses of 0.01–0.05 mg/kg intravenously the abolition of the muscular activity was accompanied by little significant change in arterial pressure. Similarly, abolition by dimethothiazine of the electromyographic response to stretch of the quadriceps was accompanied by little or no change in arterial pressure (Fig. 4). In two experiments with metopimazine, abolition of electromyographic activity occurred with a fall in mean blood pressure of 20–40 mm Hg.

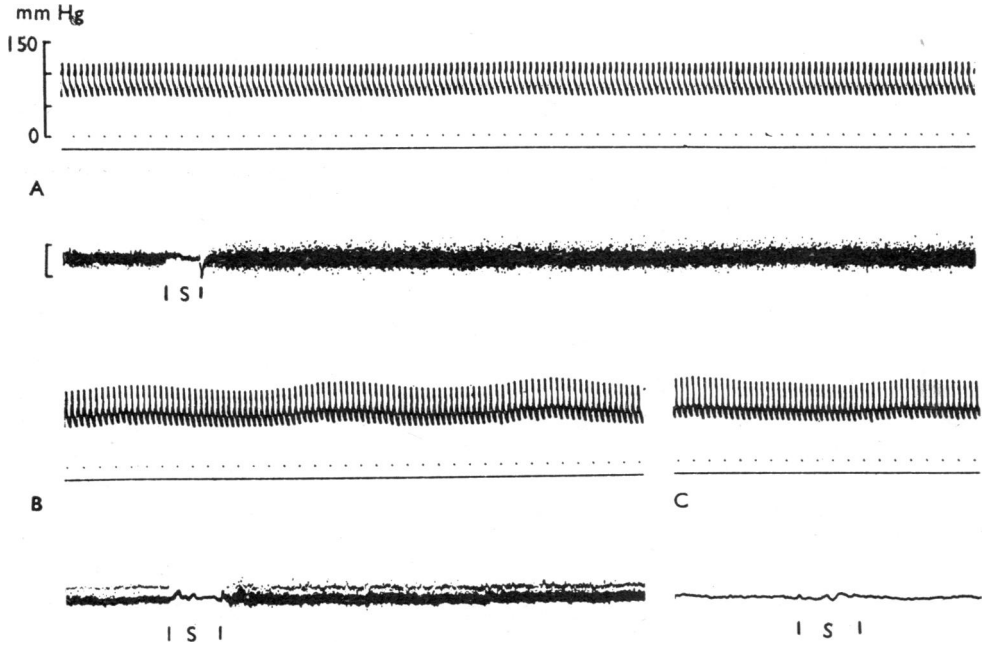


Fig. 4. Decerebrate cat (2.4 kg). Effect of dimethothiazine on femoral arterial pressure (upper trace) and electromyographic response to stretch of quadriceps (lower trace). During the period indicated by S the lower leg was fully extended and gently released. A. Control response. B. Response after intravenous infusion of 2 mg/kg of dimethothiazine. Note reduction in electromygraphic response, but no reduction in arterial pressure. C. After total of 3.5 mg/kg dimethothiazine. Abolition of electromyographic activity, but no significant reduction in arterial pressure. Time marks 1 sec.

Effect on spinal reflexes

Experiments carried out on the patellar and flexor reflexes of the chloralose cat showed that mephenesin, chlordiazepoxide and pentobarbitone depressed the flexor reflex at a dose similar to that required to abolish the electromyographic response to stretch of the quadriceps muscle in the decerebrate cat (Table 1). At this concentration neither mephenesin nor chlordiazepoxide had any depressant effect on the patellar reflex.

In the case of the phenothiazine derivatives, however, there was only a very slight depression of reflexes in the chloralose cat at concentrations which abolished the electromyographic activity to the stretch of the quadriceps muscle in the decerebrate preparation.

The depression of the spinal reflexes obtained on administration of the phenothiazines was interesting in that it appeared to be greater and more consistent on the patellar reflex than on the flexor reflex.

Chlorpromazine in doses of 0.75–1.0 mg/kg intravenously produced a 20–40% depression of the patellar reflex. This depression was not increased by further increase in dosage. This effect on the patellar reflex was accompanied by a slight depression of the flexor reflex, which increased only slightly with increasing dosage. Chlorproethazine was qualitatively and quantitatively similar to chlorpromazine. Doses between 0.5–5.0 mg/kg produced depression of the patellar reflex in five out of five experiments. This dosage of chlorproethazine produced slight depression of the flexor reflex in three out of four experiments.

A similar depression of the patellar reflex was also recorded in two out of three experiments with methotrimeprazine; and in one out of two experiments with pericyazine. The drug dimethothiazine appeared to have a more specific effect on the patellar reflex than chlorpromazine, in that in four experiments doses of 3 to 8 mg/kg intravenously produced a 20–40% depression of the patellar reflex with little or no effect on the flexor reflex (Fig. 5). No attempt was made, however, to compare quantitatively dimethothiazine and chlorpromazine in detail in this respect. In the four experiments carried out with metopimazine this compound had no effect on either the flexor or patellar reflex in doses up to 40 mg/kg.

The effects of chlorpromazine, dimethothiazine and chlorproethazine were examined for their effect on spinal reflexes on the spinal cat. In contrast to their depressant action on reflexes in the intact cat under chloralose, no depressant effect on reflexes was recorded in spinalized animals.

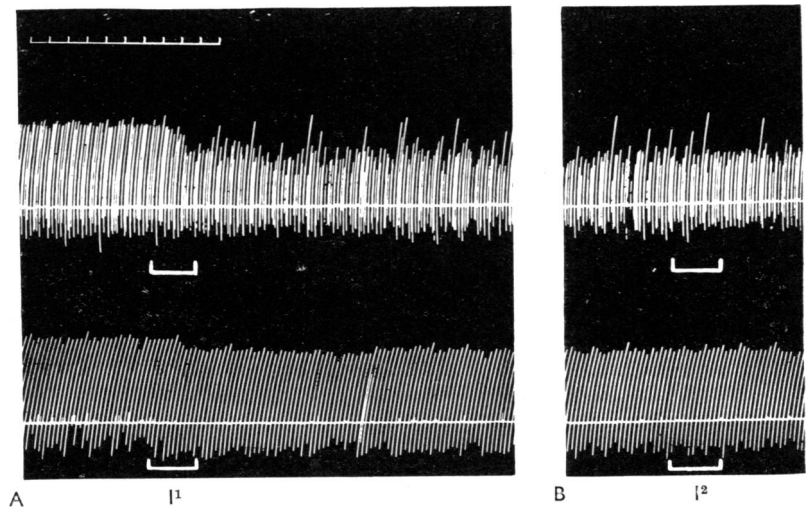


Fig. 5. Cat (3 kg). Chloralose anaesthesia. Effect of dimethothiazine on the patellar (upper trace) and flexor (lower trace) reflexes. At I_1 dimethothiazine 5 mg/kg was infused intravenously and at I_2 a further dose of 15 mg/kg was infused. The time interval between A and B was 30 min. Note partial reduction of the patellar reflex by the low dose of dimethothiazine with little effect on the flexor reflex. A higher dose had no significant further action. Time marks 1 min.

Effects in mice

A distinct difference was also noted between the phenothiazines examined and mephenesin, chlordiazepoxide and butobarbitone in their efforts on the pinna and corneal reflexes in mice (Table 2). With the exception of thioproperazine and metopimazine, which were inactive, the phenothiazines examined produced a depression of the pinna reflex in mice at relatively low doses with no effects on the corneal reflex to toxic doses. A high dose (160 mg/kg) of mephenesin was required to depress the pinna reflex and there was no effect on the corneal reflex up to 320 mg/kg. Chlordiazepoxide was inactive in both tests up to 300 mg/kg. In the case of butobarbitone, the dose required to depress the pinna reflex was only half that required to depress the corneal reflex. This

TABLE 2

ACTIVITY OF PHENOTHIAZINE DERIVATIVES AND SOME OTHER DRUGS IN INHIBITING THE PINNA OR CORNEAL REFLEXES OR TRACTION RESPONSE IN THE MOUSE

Figures refer to the mean effective dose (mg/kg) subcutaneously together with the standard deviation. Figures in brackets are the number of experiments.

Compound	Effective dose for inhibition of reflex (mg/kg. subcutaneously)		Effective dose for inhibition of traction response (mg/kg. subcutaneously)
	Corneal	Pinna	
Chlorpromazine	Inactive to 40 (4)	4·6±0·4 (2)	3·1±1·0 (3)
Chlorproethazine	Inactive to 80 (3)	48·5±5 (2)	11·7±0·9 (3)
Acepromazine	Inactive to 160 (1)	1·3±0·2 (2)	1·7 (2)
Methotrimeprazine	Inactive to 160 (2)	2·6±0·1 (3)	2·2±0·4 (3)
Promethazine	Inactive to 80 (2)	40·5±2 (2)	29±2 (4)
Dimethothiazine	Inactive to 160 (3)	Slight activity at 160 (3)	53±11 (3)
Pericyazine	Inactive to 150 (1)	1·2±0·2 (3)	0·8±0·2 (3)
Metopimazine	Inactive to 100 (2)	Inactive to 100 (2)	Inactive to 100 (2)
Thioproperazine	Inactive to 320 (3)	Inactive to 320 (3)	Inactive to 320 (3)
Mephenesin	Inactive to 320 (2)	160 (2)	160 (2)
Chlordiazepoxide	Inactive to 300 (2)	Inactive to 300 (2)	55±7 (2)
Butobarbitone	83±4 (2)	47±2 (2)	32±18 (2)
Hyoscine	Not tested	Not tested	Not tested

TABLE 3

COMPARISON OF THE APPROXIMATE RELATIVE POTENCIES OF PHENOTHIAZINE DERIVATIVES AND OTHER DRUGS IN REDUCING DECEREBRATE RIGIDITY IN THE CAT AND ACTIVITY IN INHIBITING THE PINNA RELFEX AND TRACTION RESPONSE IN THE MOUSE

Relative potency (chlorpromazine=1) is expressed as the ratio of effective dose of chlorpromazine to that of test compound. Phenothiazines are listed in rank order of potency in reducing decerebrate rigidity

Compound	Reduction of rigidity (cat: intravenous)	Inhibition of pinna reflex (mouse: subcutaneous)	Inhibition of traction response (mouse: subcutaneous)
Acepromazine	17	3·5	1·8
Pericyazine	2	3·8	3·9
Methotrimeprazine	1·7	1·8	1·4
Chlorpromazine	1·0	1·0	1·0
Chlorproethazine	0·83	0·1	0·27
Promethazine	0·18	0·11	0·11
Dimethothiazine	0·23	<0·02	0·06
Metopimazine	0·23	<0·01	<0·06
Thioproperazine	<0·063	<0·01	<0·01
Chlordiazepoxide	0·1	<0·01	0·06
Mephenesin	0·02	0·02	0·02
Butobarbitone	0·25	0·1	0·1

is in contrast to the large separation of effective dose for the two reflexes with the phenothiazines. Metopimazine and thioproperazine were inactive in both tests to 300 mg/kg subcutaneously.

Pericyazine was the most effective compound in depressing the ability of mice to pull themselves on to a horizontal wire in the traction test, being some three times as potent as chlorpromazine (Tables 2 and 3). No significant activity was found in the phenothiazines promethazine, metopimazine and thioproperazine. Chlorproethazine had approximately one quarter the activity of chlorpromazine in these tests in contrast to the similar potency in reducing decerebrate rigidity.

DISCUSSION

The primary purpose of our experiments was to compare a series of phenothiazine derivatives with chlorpromazine for their ability to decrease the rigidity of the intercollicular decerebrate cat. There is substantial evidence that the state of enhanced reflex extensor tonus of the intercollicular decerebrate cat is due to increased sensitivity of the stretch reflex produced by an increase in the rate of firing of γ-motoneurones (Eldred, Granit & Merton, 1953 ; Granit, 1955 ; Granit, Holmgren & Merton, 1955 ; Jansen, 1966). This is in contrast to the rigidity produced by the method of ischaemic decerebration described by Pollock & Davis (1930) which is thought to be due to enhanced activity of α-motoneurones.

In the intercollicular decerebate cat the well-known stretch reflex (Liddell & Sherrington, 1924) is thought to depend upon excitation of the primary endings of the muscle spindle by stretch. In our experiments we have studied the stretch reflex in the quadriceps femoris muscle. The reflex was elicited by the weight of the lower leg, and the degree of the activation of the stretch reflex was measured by electromyographic activity of the quadriceps muscle. It is likely that it was the tonic portion of the reflex that was primarily involved in the response being studied.

The method we have described for assaying the ability of drugs to reduce the electromyographic response of the quadriceps femoris muscle in the decerebrate cat appears to be a reliable and objective method which produces reproducible results. It would appear to be less cumbersome than that described by Smith & Murayama (1964). The method measures the minimum effective dose of the compound and in addition some measure of the duration of action of a drug can be obtained.

Of the phenothiazine derivatives we have studied the most effective was acepromazine, which was seventeen times as effective as chlorpromazine in reducing decerebrate rigidity (Table 3). This finding is interesting since Webster (1961) found acepromazine 9.4 times more potent than chlorpromazine in reducing experimental tetanus in the rabbit. However, this author also described the development of tachyphylaxis to acepromazine in the rabbit (Laurence & Webster, 1961). In our experiments, however, we have been unable to detect any indication of tachyphylaxis following three repetitive administrations of acepromazine to the cat. This difference may be due to the difference in species being studied.

Pericyazine and methotrimeprazine were both approximately twice as active as chlorpromazine in reducing the rigidity of the decerebrate cat. Both compounds have central

actions qualitatively similar to those of chlorpromazine whilst being quantitatively more potent (Julou, Courvoisier, Ducrot, Fournel, Bardone, Leau & Myon, 1963; Courvoisier, Ducrot, Fournel & Julou, 1957). The diethylamino analogue of chlorpromazine (chlorproethazine) is of interest since although the compound is equipotent to chlorpromazine in reducing the decerebate rigidity of the cat it has less pronounced sedative action and had one quarter the potency of chlorpromazine in the traction test in the mouse. Chlorproethazine has been found to some extent effective (Sigwald, Bouttier & Raymondeaud, 1961; Matthews, 1965) in the treatment of clinical spasticity, when administered parenterally. Some data on the muscle relaxant properties of this compound have already been published (Meidinger, 1962). Thus, if one can compare potency intravenously in the cat with subcutaneous activity in the mouse there would appear to be a separation between effectiveness in reducing decerebrate rigidity and sedation or muscular weakness (as measured by the traction test) with these two compounds, chlorproethazine being less sedative in relation to equi-effective muscle relaxant dose. Promethazine had approximately $\frac{1}{16}$ the potency of chlorpromazine in the decerebrate cat. In addition to its well-known anti-histaminic properties, promethazine has potent anti-cholinergic activity and is clinically useful in the treatment of Parkinsonian rigidity (Ahmed & Marshall, 1962; Duvoisin, 1965). We thus wondered to what extent the activity of promethazine was related to its anti-cholinergic property which is thought to be related to the anti-Parkinsonian action.

Hyoscine, however, was ineffective in reducing decerebrate rigidity in the conditions of our experiments, suggesting that the activity of promethazine in reducing the rigidity of the intercollicular decerebrate cat was probably unrelated to a central anti-cholinergic action. The inactivity of hyoscine in our experiments is of interest, since Chin & Smith (1962) found that some depression of the tonic portion of the stretch reflex in the intercollicular decerebrate cat could be obtained with hyoscine although they found the effect to be variable and to have an atypical dose response relationship. Dimethothiazine and metopimazine showed approximately $\frac{1}{4}$ the potency of chlorpromazine in reducing the rigidity of the decerebrate cat. Both compounds are interesting, since they have weak sedative actions (Julou, Ducrot, Bardone, Detaille, Feo, Guyonnet, Loiseau & Pasquet, 1966), for example, metopimazine was ineffective up to 100 mg/kg subcutaneously in the traction test in the mouse, while dimethothiazine showed activity at 53 mg/kg. This again indicates that the ability of phenothiazine derivatives to reduce decerebrate rigidity may be dissociated from their central sedative effects. Thioproperazine is a compound with weak sedative actions in comparison with chlorpromazine, but with potent anti-apomorphine activity and produces an increase in muscle tonus in some species (Leslie & Maxwell, 1964). This compound was ineffective in the decerebrate cat up to doses of 8 mg/kg. Of the other drugs studied for comparative purposes chlordiazepoxide was approximately 1/10 as potent as chlorpromazine whilst mephenesin was weakly active in doses of 20–25 mg/kg. The relatively low activity of mephenesin may be related to its short duration of action, together with the fact that the drug was being given by intravenous infusion over a period of 4–6 min.

There are many ways in which a compound can reduce the rigidity of the decerebrate cat. These include an effect at the neuromuscular junction, an effect directly on skeletal muscle, a depressant action on the muscle spindle, depression of nerve conduction,

depression of spinal interneurones, or possibly a marked effect on arterial pressure. The experiments carried out with phenothiazine derivatives on spinal reflexes in the chloralose cat indicate that the phenothiazine drugs are not acting either at the neuromuscular junction or in depressing peripheral nerve conduction. A comparison of the effects of phenothiazine derivatives with, for example, chlordiazepoxide or mephenesin, indicates that whereas the phenothiazine derivatives tend to have a more marked effect on the patellar reflex and a variable effect on the flexor reflex, interneuronal blocking agents such as chlordiazepoxide and mephenesin, depress the polysynaptic flexor reflex at doses having little effect on the monosynaptic patellar response. It thus appears unlikely that depression of spinal interneurones is an important factor in the reduction of decerebrate rigidity produced by the phenothiazine derivatives.

The effect of chlorpromazine on motor reflexes is very complex and has been extensively studied (Silvestrini & Maffii, 1959; Hudson & Domino, 1963; Hudson, 1966). Most authors suggest that chlorpromazine has a depressant action on the descending reticular activating system. In marked contrast to the effects of chlorpromazine in intact or decerebrate animals, the compound is ineffective in depressing the spinal reflexes in the spinal animal (Preston, 1956; Henatsch & Ingvar, 1956; Silvestrini & Maffii, 1959). Hudson (1966) suggests that in addition to a supraspinal action, chlorpromazine may have a depressant action at the spinal cord level inhibiting a flow of impulses originating from supraspinal structures.

Most of the phenothiazine derivatives studied resembled chlorpromazine in their action on spinal reflexes, producing a small depression of the patellar reflex at low doses followed by a gradual depression of the polysynaptic flexor reflex. The similarity is also seen in that chlorproethazine and dimethothiazine resembled chlorpromazine in being ineffective in depressing spinal reflexes in the spinal animal.

Our experiments on reflexes were not designed to study in detail the mechanism of action of the phenothiazines, but rather to differentiate them from drugs such as mephenesin which depress spinal interneurones, and thus inhibit the polysynaptic flexor reflex without depressing the patellar reflex. The drug metopimazine is of note for although it was effective in doses of 2.2 mg/kg intravenously in reducing decerebrate rigidity, it was ineffective in doses up to 40 mg/kg in spinal reflexes in the chloralose cat.

In view of the chemical relationship between the phenothiazine derivatives we have studied and chlorpromazine, it is tempting to think that the mechanism of action of these compounds may be similar to that of chlorpromazine, that is, in reducing the enhanced activity of γ-motoneurones in the intercollicular decerebrate cat. We have, however, no direct evidence on this point.

Chlorpromazine and related phenothiazines such as pericyazine and methotrimeprazine have hypotensive properties in the anaesthetized cat and it is difficult to assess clearly to what extent any effect on arterial pressure might influence the reduction of decerebrate rigidity. Hudson & Domino (1963) have shown that the reduction of the patellar reflex produced by chlorpromazine in the chloralose cat is not due to an effect on arterial pressure. It is likely that this also applies to the reduction of decerebrate rigidity since chlorpromazine does not readily reduce the rigidity of the ischaemic decerebrate cat (Henatsch & Ingvar, 1956). A detailed analysis with the phenothiazine derivatives of the

possible contributions of a general hypotensive action to the reduction of decerebrate rigidity has not been carried out. However, with both acepromazine and dimethothiazine, reduction of decerebrate rigidity was not accompanied by any marked reduction in arterial pressure. In the case of metopimazine reduction in arterial pressure did occur at muscle relaxant doses in the decerebrate cat.

The experiments in mice on the pinna and corneal reflex were carried out to see to what extent these simple experiments might be correlated with the ability of a new compound to reduce decerebate rigidity in the cat and thus serve as a screening method (Table 3). Our results are in general agreement with those of Witkin *et al.* (1959) in that chlorpromazine was more effective in depressing the pinna reflex than the corneal reflex, whereas separation between effective dose on both reflexes is much less with a barbiturate or with mephenesin. The result obtained with chlordiazepoxide is interesting since this compound has interneuroneal blocking actions (Randall, 1960) but was inactive in depressing both the pinna and corneal reflexes up to very high doses. In general terms there was a poor correlation between the ability of the phenothiazine derivatives to depress the pinna reflex and their ability to reduce the decerebrate rigidity although some correlation is seen with the more potent drugs.

Of interest again is that metopimazine was without action on either the corneal or pinna reflex in the mouse. Thus, although this compound is effective in reducing decerebrate rigidity in the intercollicular decerebrate cat, it appears to be without significant effect on spinal reflexes in the cat under chloralose anaesthesia or in the conscious mouse.

Dimethothiazine also appears to be an interesting compound in this context for it is quite active in the decerebrate cat with little effect in the tests carried out in mice.

The traction test was carried out primarily as a means of possibly determining the effect of these drugs on voluntary power. This is a property difficult to assess in experimental animals, and it was felt that this test, involving the ability of a mouse to pull itself onto a horizontal wire, might afford some measure of this action. It is interesting, therefore, that there was not a direct correlation between the ability of the phenothiazines to reduce decerebrate rigidity and the activity in the traction test, suggesting (if the latter is a reliable measure of effect on voluntary power) that it is possible to separate the ability of phenothiazine derivatives to reduce the enhanced muscle tonus of the decerebrate animal from their effects on voluntary power. For example, although acepromazine was seventeen times as potent as chlorpromazine in the decerebrate cat, it was only twice as effective in the traction test. In contrast, pericyazine, which is only twice as effective as chlorpromazine in reducing decerebrate rigidity, was four times as effective in the traction test. It is probably unwise to take such comparisons too far, however, in view of the differences in species and routes of administration used in the two methods. Nevertheless, with drugs such as dimethothiazine, metopimazine and chlorproethazine, it appears possible to differentiate reduction of decerebrate rigidity from other central actions and a fuller analysis on the mechanism by which these drugs reduce decerebrate rigidity would appear worthwhile.

SUMMARY

1. A group of phenothiazine derivatives related to chlorpromazine have been compared for their ability to reduce the rigidity of the intercollicular decerebrate cat, for their action on spinal reflexes and in some other tests.

2. The method of assessing decerebrate rigidity involved recording the electromyographic response to stretch of the quadriceps femoris muscle.

3. Of the compounds tested, acepromazine was most potent, being 17 times as potent as chlorpromazine.

4. Chlorproethazine was approximately equi-active to chlorpromazine in reducing decerebrate rigidity, but had less depressant activity in mice. Dimethothiazine and metopimazine had one quarter the potency of chlorpromazine, but were both considerably less sedative.

5. At doses comparable to that required to abolish decerebrate rigidity most of the phenothiazine derivatives produced 20–40% depression of the patellar reflex in the chloralose cat with, in some cases, a less regular depressant effect on the flexor reflex. Mephenesin, chlordiazepoxide and butobarbitone depressed the flexor reflex with little action on the patellar reflex. Metopimazine had no effect on spinal reflexes.

6. None of the phenothiazine derivatives tested depressed spinal reflexes in the cat.

7. With the exception of metopimazine, and thioproperazine, which were inactive, the phenothiazine derivatives abolished the pinna reflex in mice at low doses while not inhibiting the corneal reflex to toxic levels. In contrast, butobarbitone and mephenesin reduced the corneal reflex at a dose comparable to that reducing the pinna reflex while chlordiazepoxide had no effect on either reflex.

8. It is concluded that it is possible to separate the skeletal muscle relaxant activity of phenothiazines from their sedative activity and that tests involving the pinna and corneal reflexes in the mouse are of poor predictive value of the effect on decerebrate rigidity in the cat.

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