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SITE OF ACTION OF CHLORPROMAZINE AND MEPHENESIN IN EXPERIMENTAL TETANUS

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The anti-tetanus activities of chlorpromazine and mephenesin have been determined quantitatively in intact, spinal and decerebrate rabbits with local tetanus. The activity of chlorpromazine in the spinal animal differed from that in the intact and decerebrate animal and depended on the level of brain-stem section. Mephenesin was equally effective in all preparations and probably suppressed tetanus by blocking transmission in motor pathways in the spinal cord.

Both chlorpromazine and mephenesin suppress the convulsion of tetanus in man without causing loss of consciousness or marked respiratory depression. This similar effect, even though chlorpromazine is considerably more potent than mephenesin (Laurence & Webster, 1958b), is perhaps somewhat surprising, because the two drugs are generally believed to have different actions within the central nervous system. An attempt has therefore been made to localize the site of action of chlorpromazine and of mephenesin in experimental tetanus in rabbits.

METHODS

Tetanus was induced in rabbits by injecting 1,250 mouse M.L.D. of toxin into the gastrocnemius muscle of one hind limb. Electromyograms were recorded with subcutaneous electrodes and integrated to give units of activity which could be displayed as deflexions on smoked paper or counted to give the level of activity per min (Laurence & Webster, 1958a). Fig. 1 shows a typical electromyogram before and Fig. 5 one after integration. Tetanus was activated at 1 min intervals by an electric current passed through the skin above the sacral vertebrae.

Spinal transections at the level of the lowest thoracic vertebrae were performed under aseptic conditions using thiopentone and ether anaesthesia. Animals recovered within 1 to 2 hr of surgery and generally required no elaborate post-operative care other than manual emptying of the bladder twice daily. Toxin was injected 2 to 24 hr after spinal section and the animals were generally killed after 5 to 6 days.

Decerebrations were performed at various levels in the corpora quadrigemina by blunt transection under thiopentone and ether anaesthesia. At the end of the experiment the brain tissue remaining intact was fixed in formalin so that the exact level of section could be confirmed.

The doses of mephenesin (28.8 mg/kg) and chlorpromazine (0.106 mg/kg) chosen for accurate comparison in the different preparations were those which had been found previously by Laurence & Webster (1958b) to produce a 50% reduction of tetanus activity in 2 dose assays.

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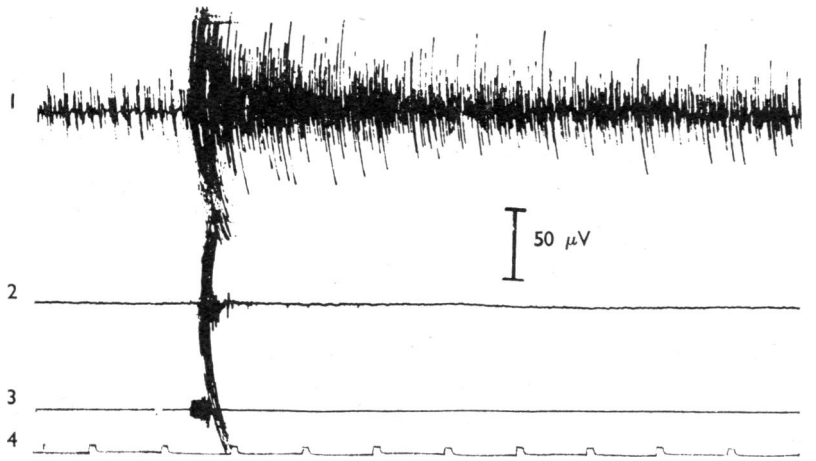

Fig. 1. Typical electromyogram from a rabbit with local tetanus. 1, Tetanus activity. 2, Control limb activity. 3, Afferent stimulus. 4, Time, sec. Note the increase in activity following the stimulus.

performed on rabbits, in which tetanus had been induced by half the present dose of toxin and activated by auditory and vibratory, instead of electrical, stimulation. As variable results were obtained with chlorpromazine in most instances, a number of different doses were tested.

Both drugs were given in aqueous solution into an ear vein and all drug effects, expressed as the percentage reduction in tetanus activity, measured for the 20 min immediately following injection.

RESULTS

Intact animals

The reductions in tetanus activity produced by chlorpromazine and mephenesin in 5 intact animals are given in Table 1 and the mean time courses of their action are plotted in Fig. 2.

Chlorpromazine was less effective than under the conditions of previous experiments (Laurence and Webster, 1958b), and 0.11 mg/kg, which had previously been as effective as 28.8 mg/kg mephenesin, only reduced tetanus activity by 28% compared with 46% for mephenesin (Table 1). Twice the dose of chlorpromazine, namely, 0.22 mg/kg, reduced activity by 50%, however, and also provided a more suitable dose for subsequent comparison with mephenesin in spinal and decerebrate animals.

Comparison of the time-course of action of these approximately equiactive doses of 28.8 mg/kg of mephenesin and 0.22 mg/kg of chlorpromazine (Fig. 2) over the 20 min recording period shows that, in contrast to the rapid and complete reduction in activity of short duration obtained with mephenesin, chlorpromazine had a weak but more prolonged effect. Higher doses of chlorpromazine (1 mg/kg) abolished activity almost completely for 1 to 2 hr. Dose-response relationships for chlorpromazine are plotted in Fig. 3.

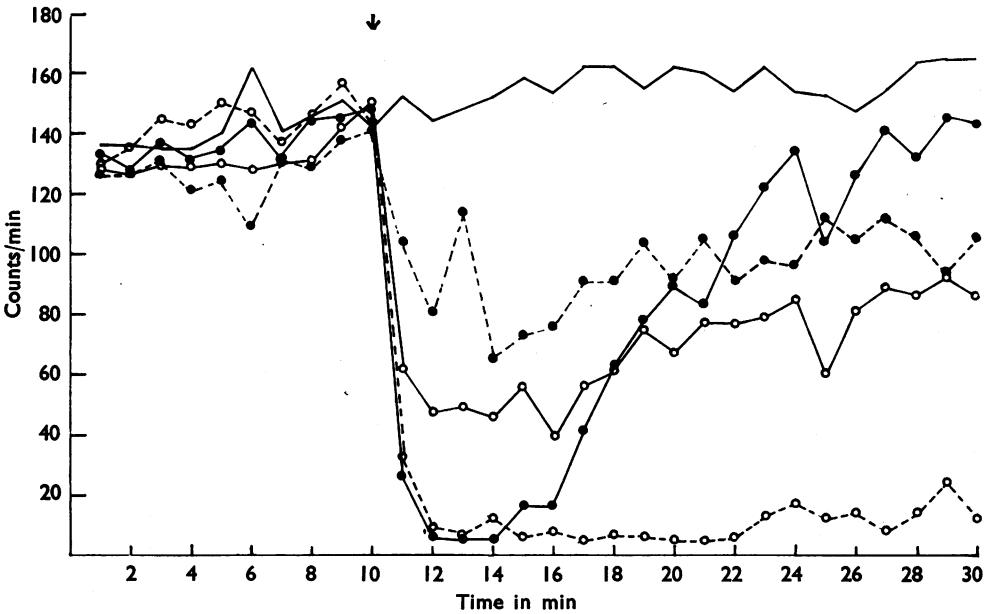

Fig. 2. Time course of the anti-tetanus activity of saline controls (——), mephenesin, 28.8 mg/kg (●——●) and chlorpromazine, 0.11 mg/kg (●---●), 0.22 mg/kg (○——○), 1.0 mg/kg (○---○) in intact rabbits. Tetanus is expressed as the count/min of integrated electromyogram activity. Mean values for 5 animals are plotted. All injections were performed at the arrow.

TABLE 1

PERCENTAGE REDUCTIONS IN TETANUS AND IN DECEREBRATE RIGIDITY PRODUCED BY MEPHENESIN AND CHLORPROMAZINE

Percentage reductions were calculated for a period of 20 min after each injection. All animals received the same dose of toxin (1,250 mouse M.L.D.) and were stimulated at 1 min intervals. Each value represents the reduction in activity recorded in one animal. In the case of the intact animals the same 5 animals were used for all observations, whilst in other instances mephenesin and chlorpromazine effects were not necessarily recorded in the same animals

	Chlorpromazine				Mephenesin			
Animal	Dose mg/kg	Reduction in tetanus (%)	Mean	s.e.	Dose mg/kg	Reduction in tetanus (%)	Mean	s.e.
Intact	0·106	24 48 17 41 10	28·0	7·5	28·8	49 47 34 61 39	46·0	6·0
	0·212	49 65 29 60 49	50·4	6·2				
	0·424	— 93 24 80 59	64·0	15·0				
	1·00	— — 83 97 —	90·0					
Spinal	0·106	25 74 33 37 65 44 56 52	48·3	5·8	28·8	19 55 26 48 36 29 52 79 45	43·2	6·1
	0·212	79 47 52 83 34	59·0	9·46				
	1·00	88 76 62 76	75·5	5·3				
Decerebrate (rigid)	0·212	2 45 20 5	18		28·8	67 42 86	65	
Decerebrate (non-rigid)	0·212	49 60 43	57		28·8	32 75 32 88	57	
		Reduction in decerebrate rigidity (%)				Reduction in decerebrate rigidity (%)		
	0·212	08 −02 −17 −08 12	−1·3		28·8	93 22 94	70	

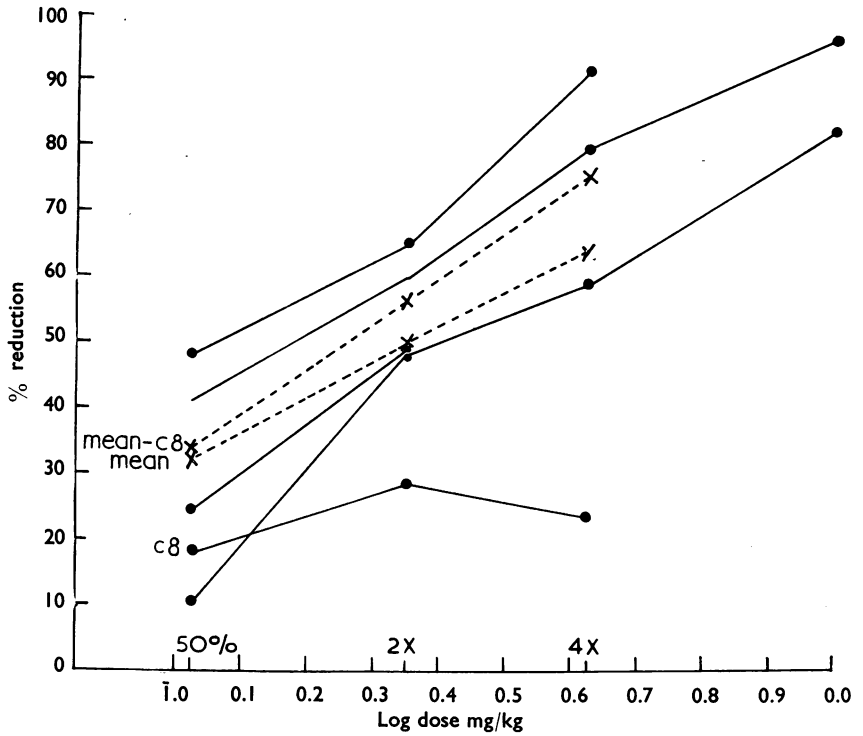

Fig. 3. Dose-response relation for the anti-tetanus activity of chlorpromazine. Individual results for 5 animals (full lines) are shown. Two mean curves (broken lines) are shown one with and one without the aberrant result for C8. Percentage reductions in activity were calculated for the 20 min period after each injection.

Spinal animals

A high level of tetanus activity was seldom obtained in spinal animals, and during periods of stimulation the degree of activity induced by successive stimuli gradually declined, so that all drug effects had to be measured with reference to a continuously falling baseline response. Control activity fell by as much as 20% in 20 min (Fig. 4) compared with an increase of 10% over the corresponding period in intact animals (Fig. 2). This decline in activity, which became even more marked in subsequent periods of stimulation, was due to a gradual decrease in the duration of activity after the stimulus without any noticeable reduction in the initial reflex response. Lengthening the period between cord section and the injection of toxin, to facilitate recovery from spinal shock, and increasing the dose of toxin failed to raise the level of activity. Typical integrated electromyograms from intact and spinal animals are shown in Fig. 5*a* and *b* respectively.

Reductions in activity produced by mephenesin and chlorpromazine in spinal animals are given in Table 1 and the mean time-course of action of each dose plotted in Fig. 4. Mephenesin had an action similar to that seen in intact animals. However, chlorpromazine, although effective, had an entirely different type of action in that

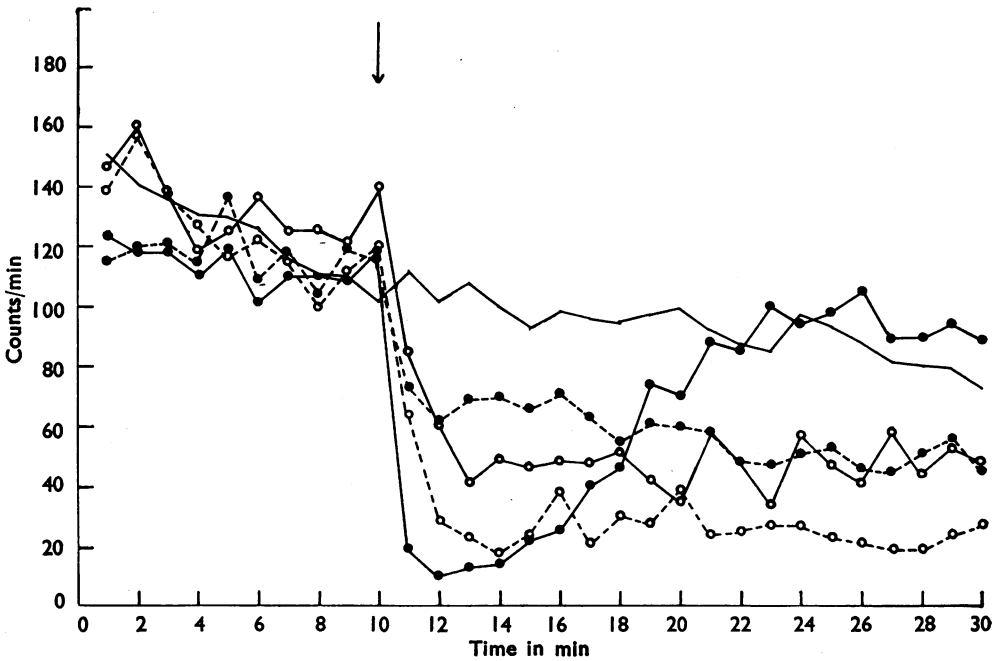

Fig. 4. Time-course of the anti-tetanus activity of saline controls (——), mephenesin, 28.8 mg/kg (●——●) and chlorpromazine, 0.11 mg/kg (●- - -●), 0.22 mg/kg (○——○), 1.0 mg/kg (○- - -○) in spinal animals. Tetanus is expressed as the counts/min of integrated electromyogram. Mean results for 2 to 5 animals are plotted. All injections were performed at the arrow.

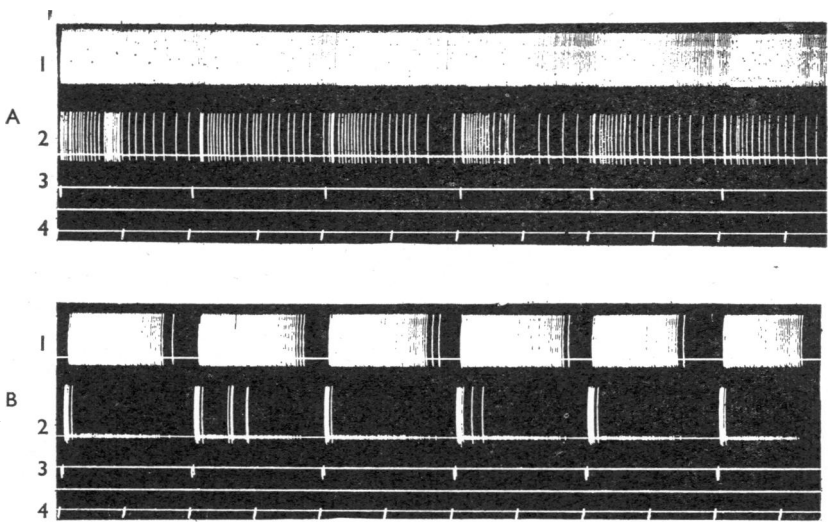

Fig. 5. Integrated electromyograms of untreated tetanus activity in intact and spinal animals. In each record, 1 shows affected limb activity; 2, control limb activity; 3, stimuli; and 4, time, 30 sec. The marked reflex response, rapid decline in activity and quiescent period before the next stimulus are typical of the spinal animal (B). This contrasts with the more well-maintained activity obtained in the intact animal (A).

activity continued to decline after chlorpromazine almost in parallel with control tetanus activity but at a lower level, as if chlorpromazine had shifted activity from one plane to another.

Chlorpromazine did not abolish the initial reflex response to stimulation as it did in the intact animal, and its dose-response relationship differed in the two preparations (Fig. 6).

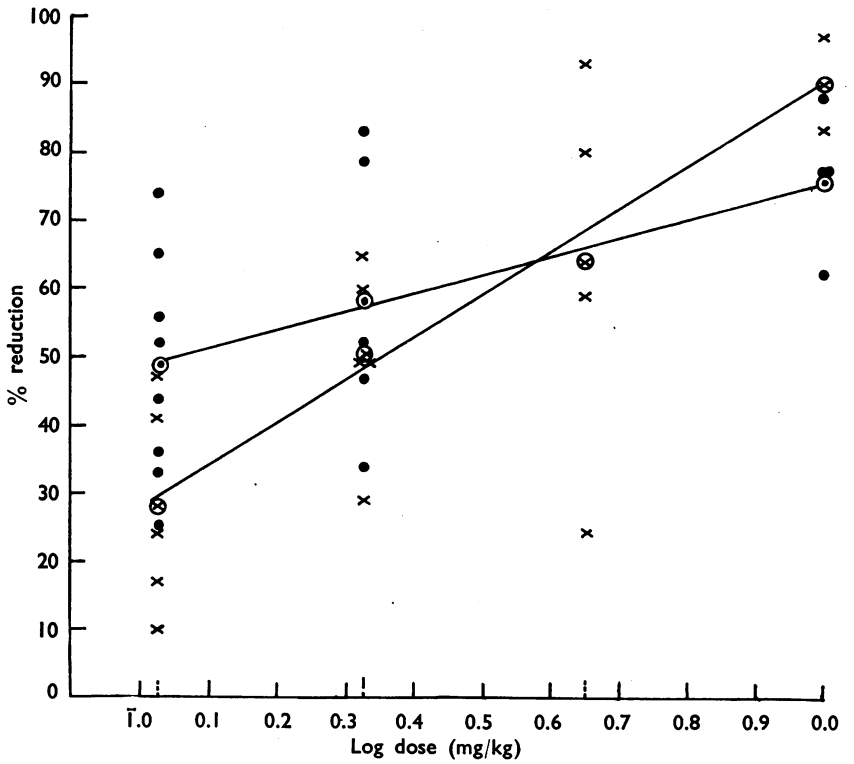

Fig. 6. Comparison of the dose-response relationship for chlorpromazine in intact and spinal animals. Percentage reductions in tetanus activity were calculated for the 20 min following each injection. Results for intact (crosses) and spinal (dots) animals are shown. Mean values are encircled.

Decerebrate animals

Tetanus activity varied considerably in the decerebrate animals and could not be readily related with the level of brain-stem section. The preparations were therefore divided into two groups depending on the degree of activity in the control limb. In those animals in which the brain stem was sectioned at the rostral border of the pons, rigidity developed in the control limb either immediately or some time after decerebration. Rigidity was less marked and often absent after more cephalic sections, but tremors sometimes occurred. These two groups, in which there were 5 and 7 animals respectively, will be referred to subsequently as " rigid " and " non-rigid " animals. Rigidity and tremors seldom occurred in the affected limb.

It was possible to distinguish between the effect of mephenesin and chlorpromazine against (1) tetanus and rigidity and (2) tetanus alone in two distinct groups of decerebrate animals. Reductions in activity effected by both compounds in a number of animals are given in Table 1.

The time-courses of action for mephenesin (Fig. 7) against rigidity and tetanus show it to be effective in all preparations irrespective of the level of brain-stem section or the type of resulting activity.

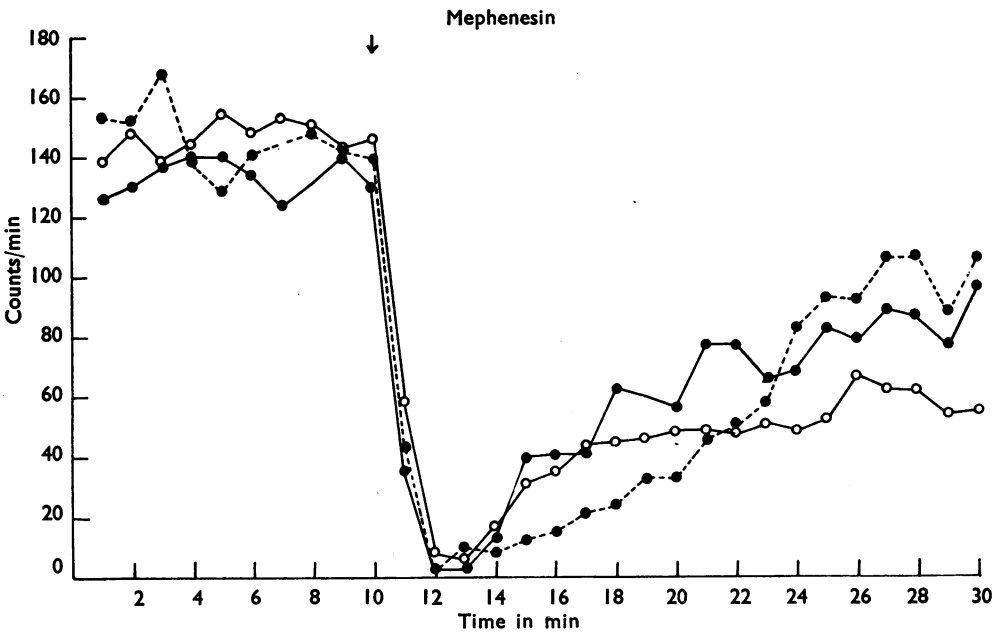

Fig. 7. Comparison of the mean time-course of action of mephenesin against tetanus and extensor rigidity in the decerebrate rabbit. Tetanus activity is expressed as count/min of integrated electromyogram. All injections were performed at the arrow. Each point represents the mean from at least 3 animals. ●——●, tetanus muscle activity in non-rigid decerebrate animal. ●- - -●, tetanus muscle activity in rigid decerebrate animal. ○——○, control muscle activity in rigid decerebrate animal.

Variable results were obtained with chlorpromazine (Fig. 8). In the rigid animal, in which the brain stem was sectioned at the pre-pontine level, the standard dose only reduced tetanus by 18%, and was totally ineffective against decerebrate rigidity. Larger doses were more effective, however, and both rigidity and tetanus were invariably abolished by doses of 3 mg/kg or more. Chlorpromazine was as active in the "non-rigid" decerebrate preparation as in the intact animal and was particularly effective after section at the inferior colliculus. The difference between the anti-tetanus activity of chlorpromazine in the rigid and non-rigid decerebrate preparations ($t=4.14$) is statistically significant ($P=0.01$–0.001). Large doses of chlorpromazine, after an initial depression, actually stimulated activity in animals with more rostral sections. Table 2 gives the results with chlorpromazine over a wide dose range.

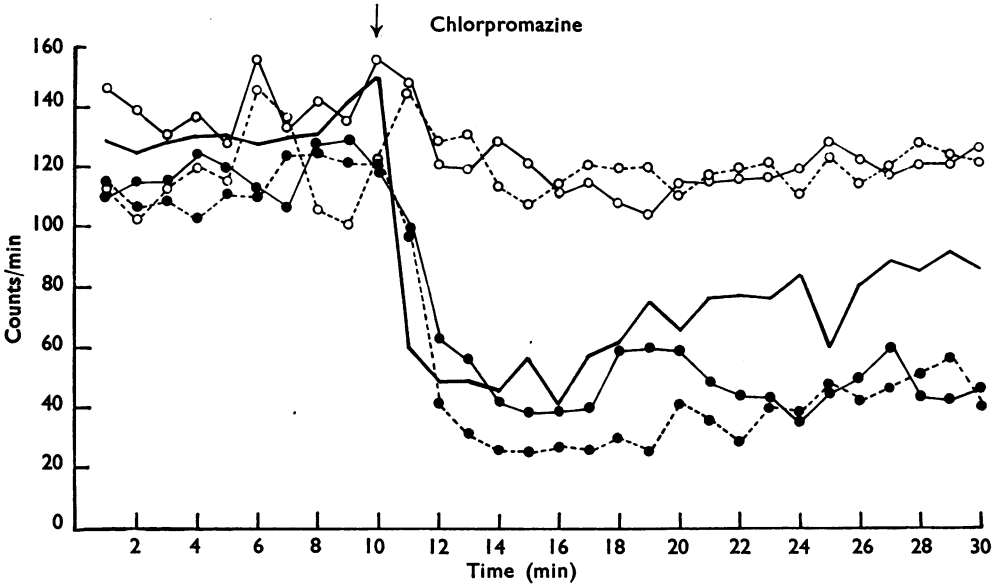

Fig. 8. Comparison of the mean time-course of action of chlorpromazine against tetanus and extensor rigidity in decerebrate animals. Tetanus activity is expressed as count/min of integrated electromyogram. All injections were performed at the arrow. Mean values for at least 4 animals are given in each instance. Although chlorpromazine (0.2 mg/kg) is ineffective against extensor rigidity (control limb, ○- - -○) and tetanus (affected limb, ○——○) in the rigid pre-pontine animal, it reduces tetanus in the non-rigid intercollicular animal (●—— ●). This effect is comparable to that recorded in two groups of intact animals (●- - -●) and (——). In one of these (●- - -●) the activity of chlorpromazine before decerebration is shown.

TABLE 2

THE EFFECT OF REPEATED DOSES OF CHLORPROMAZINE ON TETANUS AND CONTROL LIMB ACTIVITY IN THE DECEREBRATE ANIMAL

Percentage reductions in affected (A) and control limb activity (C) (rigidity tremors or slight toxic activity) are shown for 20 min after 1 mg/kg injection of chlorpromazine spaced at various time intervals. The mean count of activity per min during this period (control level in brackets) is given for B94 and B100. Negative values indicate stimulation. Up to 4 mg/kg of chlorpromazine was needed to abolish both tetanus and rigidity (control limb) in animals with the brain stem sectioned at the pre-pontine level (Group I). When the section was at the inferior colliculus an immediate abolition was obtained (Group II). Stimulation occurred in some instances after more rostral sections (Group III)

		Total dose (mg/kg)									
		1		2		3		4		5	
	Animal	(A)	(C)	(A)	(C)	(A)	(C)	(A)	(C)	(A)	(C)
I	C1	31	78	31	78	54	98	94	98	100	100
	C4	65	44	88	87	95	96	100	100	—	—
	C12	—	22	—	35	—	57	—	81	—	100
	B86	65	42	75	75	90	93				
	B99	—	33	—	33	—	81				
II	C15	100	100								
	C16	100	100								
III	B87	45	82	80	92	100	100				
	B85	1	−25								
	B100	13 (109)	13 (11)	109	69	112	140	77	140	83	175
	B94	24 (51)	69 (27)	34	138	34	150	68	180		

The activity of chlorpromazine in the decerebrate animal may be summarized as follows: Small doses (0.2 mg/kg) readily abolish tetanus and other activity, such as tremors or slight rigidity, in animals in which the brain stem has been sectioned at the inferior colliculus. Much larger doses (3 to 5 mg/kg) are needed to reduce either tetanus or rigidity if the section is more caudal, whilst, if it is more rostral, stimulation may be obtained after an initial transient depression.

DISCUSSION

Comparison of the activity of chlorpromazine and mephenesin in intact, spinal and decerebrate animals showed that, whilst the effectiveness of mephenesin was almost identical in all three preparations, that of chlorpromazine varied considerably. In addition to a difference in the potency of chlorpromazine in the spinal and intact animal its type of activity also differed.

This may be related to the difference in untreated tetanus activity in these two preparations. In the intact animal the afferent stimulus produced a reflex withdrawal of the hind limbs followed by sustained contraction of the affected muscle, presumably due to the abolition by toxin of central inhibition. In the spinal animal reflex activity was relatively low and the electromyogram which followed each stimulus did not persist as long as in the intact animal. Also the response to successive stimuli gradually declined in this preparation until a low but constant level of activity was reached. The steepness of decline in activity and the level at which a constant response was obtained were both variable.

Despite these differences in tetanus activity in intact and spinal animals, the time-course of action of mephenesin remained similar in the two preparations, and it seemed likely that mephenesin suppressed tetanus primarily by an action on the spinal cord.

The action of chlorpromazine differed in intact and spinal animals. In the former low doses (0.2 mg/kg) produced a marked reduction in activity of relatively short duration (10 to 15 min), whilst higher doses completely abolished all activity, including the initial reflex withdrawal to afferent stimulation, as did mephenesin. In the spinal animal low doses had a less intense but more prolonged effect, so that the percentage reduction in activity over a 30-min period appeared greater than in the intact animal. Activity could not be completely abolished in the spinal animal, however, even with high doses (1 mg/kg) of chlorpromazine. The slope of the dose-response curve for chlorpromazine is consequently much steeper in the intact than in the spinal animal.

In the spinal animal chlorpromazine appears to decrease excitability so that a steady state is reached in which the level of activity is below that in the untreated animal, the level to which the activity falls depending on the dose. This suggests that chlorpromazine may accelerate the process normally responsible for the decrease in reflex activity in the spinal animal. It may do this by interfering with the production or maintenance of central excitation. Possible actions are a depletion of an essential metabolite or excitatory substance, or interference with the production of such substances. The antiadrenaline activity of chlorpromazine is of interest in this connexion.

The decerebrate animals used in the present work were divided into " rigid " and " non-rigid " animals. Chlorpromazine readily abolished tetanus in the latter but was relatively ineffective in the former. This may be due to the different neural pathways being concerned with the maintenance of motor activity in the two preparations. If decerebration is performed by transection of the brain stem in the intercollicular region, then there is an augmented γ discharge to muscle spindles and extensor hypertonus is maintained by reflex activation of spinal motorneurones. This preparation may be called a γ animal. After anaemic decerebration (Pollock & Davis, 1930), the γ discharge is relatively low and rigidity is maintained by discharges in α motor nerves (α animal). This condition can be produced in the intercollicular animal by asphyxiation or if the anterior lobe of the cerebellum is damaged or cooled (Granit, Holmgren & Merton, 1955).

Since in three out of the five rigid animals the anterior lobe of the cerebellum was slightly damaged during decerebration, whilst in the remaining two rigidity did not develop until several hours after decerebration when the condition had presumably deteriorated, it seems probable that the rigid and non-rigid animal are α and γ preparations respectively.

Chlorpromazine has been found to abolish γ activity in the intercollicular and the intact anaesthetized animal (Henatsch & Ingvar, 1956) but to be without effect on the rigidity produced by anaemic decerebration, namely, α activity. This might account for the fact that chlorpromazine is much more effective against tetanus in the non-rigid than in the rigid animal.

Tetanus and reflex excitability were reduced or abolished by chlorpromazine in doses below 1 mg/kg in animals in which the brain stem was sectioned at the intercollicular level, but 3 to 4 mg/kg was required to produce the same effect after pre-pontine section. A similar limitation of the effect of chlorpromazine to the intercollicular level was observed by Silvestrini & Maffii (1959), who found that the ability of chlorpromazine to inhibit the patellar reflex was lost after pontine or pre-pontine sections. In contrast to the varied effectiveness of chlorpromazine in the decerebrate animals, mephenesin reduced or abolished tetanus and rigidity in all decerebrate preparations, irrespective of the level of brain-stem section. In two animals in which the brain stem was sectioned at or just above the intercollicular level relatively large doses of chlorpromazine produced some stimulation, similar to that observed in intact animals (Laurence & Webster, 1961). The effect of adrenaline on spinal activity in the decerebrate animal also depends on the level of brain-stem section (Dell, Bonvallet & Hugelin, 1954). Spinal activity is facilitated by adrenaline in animals with pre-collicular section but inhibited after pre-pontine section. In view of the marked antiadrenaline activity of chlorpromazine and the opposite effects of adrenaline and chlorpromazine in these preparations, further investigations into the effect of chlorpromazine on brain-stem facilitation and inhibition are indicated.

It is concluded, since mephenesin has a similar anti-tetanus activity in intact, spinal and decerebrate animals, that it probably suppresses tetanus by depressing transmission in motor pathways at the spinal level. Chlorpromazine, however, although effective in the spinal animal, has a different type of activity to that in the

intact animal, and this may only become apparent under the changed conditions of excitability existing in the spinal animal. Consequently, since the action of chlorpromazine in certain decerebrate preparations is similar to that observed in the intact animal, it seems probable that chlorpromazine reduces tetanus primarily, if not wholly, by an action on the reticular system. In addition, the tranquillizing properties of chlorpromazine, derived presumably from its reticular action, will enhance its effectiveness as an anti-tetanus agent by reducing considerably the impact of afferent stimuli.

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