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The pharmacology of 2-amino-4-methyl-6-phenylamino-1,3,5-triazine, a centrally acting muscle relaxant*

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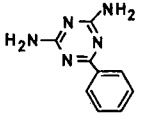
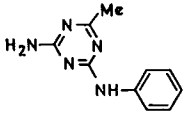
2-Amino-4-methyl-6-phenylamino-1,3,5-triazine (CB.2487) was found to resemble mephenesin both qualitatively and quantitatively in its actions in mice, and could be distinguished from meprobamate and pentobarbitone. In this species CB.2487 antagonised strychnine competitively but had no effect against leptazol or nicotine. In the anaesthetised cat, the most pronounced action of CB.2487 was to block multisynaptic crossed extensor and flexor reflexes in doses which did not suppress the monosynaptic patellar reflex. The compound also depressed selectively the crossed extensor reflex and not the patellar reflex in the spinal animal. CB.2487 had no effect on the myoneural junction. The results indicate that CB.2487 is a centrally acting muscle relaxant. Unfortunately the drug was poorly active by mouth and had a short duration of action. It is considered to have no advantage over mephenesin.

IT had been observed in these laboratories that 2,4-diamino-6-phenyl-1,3,5-triazine (I) caused paralysis in mice at levels well below the lethal dose (Brittain & Collier, unpublished observations). The precise mode of action was unknown but the properties of the drug were quickly shown to resemble those of mephenesin rather than those of curare. Previously, the synthesis of a series of related symmetrical triazines as potential antipyrimidines had been started by Dr. G. M. Timmis (Chester Beatty Research Institute). These structures were now investigated for their



I



II

effects on the central nervous system since they might have specific depressant properties of value as centrally acting muscle relaxants or as mild tranquillisers. One compound of particular interest in the series, 2-amino-4-methyl-6-phenylamino-1,3,5-triazine (CB.2487) (II), was submitted to detailed pharmacological analysis, the results of which are now described.

Experimental

METHODS

Paralysing activity and acute toxicity in mice. Paralysing activity was determined by the rotating drum technique of Collier, Hall & Fieller (1949). Groups of 10 animals (18–22 g) were used for each dose level

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and the drugs were given by oral, intraperitoneal and intravenous administration. The mean paralysing dose (ED50) for each compound was calculated by the method of Litchfield & Wilcoxon (1949). Acute toxicity (LD50) was determined after 7 days.

Anticonvulsant activity in mice. Compounds were investigated for their abilities to protect animals against the convulsive and lethal effects of leptazol and strychnine respectively and against tremor induced by nicotine. Groups of 10 animals received varying doses of drug intraperitoneally and were then challenged immediately with either leptazol, 100 mg/kg subcutaneously, or strychnine, 1·6 mg/kg intraperitoneally. Nicotine, 0·6 mg/kg intravenously, was injected 10 min after the administration of the test compound. The mean protective dose (PD50) was calculated for each drug. Antagonism to strychnine induced lethality was also used to find if a drug was active by mouth and, if so, to estimate the duration of activity. The nature of the antagonism between CB.2487 and strychnine in the mouse was investigated as follows. Using groups of 10 animals, LD50 values for strychnine were determined in the absence, or presence of, geometrically increasing doses of antagonist. The antagonist was injected intraperitoneally and immediately followed by strychnine injected by the same route.

Antagonism to maximal electric shock was determined using the method based on that described by Swinyard, Brown & Goodman (1952) and later modified by Cashin & Jackson (1962). A square wave stimulator (C. F. Palmer Model H.44) was connected through a time switch to ear electrodes of platinum wire. Rectangular pulses of 80 V, width 3 msec at a frequency of 100/sec for 0·3 sec, were found to induce a characteristic hind-leg tonic extensor seizure in all untreated mice. Drugs were administered orally in graded doses to groups of 10 animals 1 hr before the electric shock was applied. The median protective dose (PD50) was calculated for each drug. A similar test was used to estimate the duration of activity of the test compounds, by varying the interval between the administration of the compounds and the electric shock.

Antagonism of fighting behaviour in mice. The method used was based on that described by Tedeschi, Tedeschi, Mucha, Cook, Mattis & Fellows (1959). Drugs were administered orally to 5 pairs of animals at each of 3 dose levels of compound. Thirty min after the dose, each pair was subjected to 2 min continuous footshock and the number of fighting episodes were recorded. Three or more fighting episodes was taken as a positive response and 2 or less as negative so that each group could have a score of 0, 20, 40, 60, 80 or 100% response. Control groups of 5 pairs of non-drug-treated animals were also subjected to the footshock and had to achieve a 100% score before the test was considered to be valid. Drugs were compared on the basis of their ED50 values.

Antagonism of drug effects in mice by bemegride. It was of interest to determine whether the paralysis induced by CB.2487 was similar to that induced by barbiturates. Accordingly, varying doses of the compounds under test were injected intraperitoneally into groups of 10 mice, and the mean duration of paralysis or motor inactivation for each dosage group

was determined in the absence or in the presence of, 10 mg/kg intraperitoneally of bemegride. The bemegride was injected immediately after the onset of paralysis in each mouse. The duration of paralysis for each animal was determined using the rotating drum technique of Collier & others (1949) and was taken as the time interval from the bemegride injection to the time of recovery.

Action on spinal reflexes. Cats were lightly anaesthetised with chloralose (70–80 mg/kg intraperitoneally) or spinalised under ether anaesthesia and then maintained under artificial respiration. Reflex contractions of the quadriceps femoris or of the tibialis anterior muscles were recorded kymographically; in some experiments contractions of the same quadriceps were elicited by alternately tapping the patellar tendon (Schweitzer & Wright, 1937) and by stimulating the cut contralateral sciatic nerve with rectangular pulses of 5 msec duration and strength 2–5 V at a frequency of 20–40/sec for 0·2 sec. Flexor contractions of the tibialis anterior muscle were elicited by stimulating the ipsilateral superficial peroneal nerve with rectangular pulses of 10 msec duration and strength 2–8 V at a frequency of 20–30/sec for 0·2 sec, the nerve being ligated peripherally to the electrodes. Blood pressure was recorded from the carotid artery with a mercury manometer. Drugs were injected intravenously through a cannula in an external jugular vein.

Action on the myoneural junction. Twitches of the tibialis anterior muscle were elicited by supramaximal rectangular shocks of 0·4 msec duration applied to the peripheral end of the cut sciatic nerves in chloralose anaesthetised cats. Recordings were made kymographically. Blood pressure was recorded from the carotid artery with a mercury manometer. Drugs were injected intravenously through a cannula in a jugular or a femoral vein.

DRUGS AND SOLUTIONS

The following drugs were used: 2-amino-4-methyl-6-phenylamino-1,3,5-triazine methane sulphonate, mephenesin, meprobamate, phenytoin sodium, pentobarbitone sodium, leptazol, strychnine hydrochloride, nicotine tartrate, bemegride. All drugs were either dissolved in physiological saline or suspended in 5% solution of gum acacia in physiological saline. For basic and acidic drugs the doses given in the text refer to the free bases and acids respectively.

Results

Paralysing activity and acute toxicity in mice. The effectiveness of CB.2487 and the reference drugs mephenesin and meprobamate in paralysing and killing animals following oral, intraperitoneal and intravenous administration is summarised in Table 1.

The paralysis caused by CB.2487, mephenesin and meprobamate was neither preceded nor followed by excitation, nor was there evidence of respiratory distress at paralysing doses. However, if the dose was increased, death resulted from respiratory failure. It is clear that the

TABLE 1. PARALYSING ACTIVITIES OF CB.2487, MEPHENESIN AND MEPROBAMATE ADMINISTERED ORALLY, INTRAPERITONEALLY AND INTRAVENOUSLY IN MICE

Compound	Route	Paralysing activity		Acute toxicity
		ED50 (95% Fiducial limits) mg/kg	Approx. duration of paralysis at ED50 (min)	LD50 (95% Fiducial limits) mg/kg
CB.2487	Oral	134·3 (97·68–184·7)	10–15	>500
	i.p.	74·1 (65·0–84·5)	2–3	358·1 (326·5–392·0)
	i.v.	41·7 (36·7–48·6)	0·5–1	208·9 (198·6–219·8)
Mephenesin ..	Oral	162·2 (132·9–197·9)	8–12	>500
	i.p.	112·2 (106·9–117·9)	1·5–3	464·5 (441·1–489·5)
	i.v.	30·9 (28·3–34·6)	0·5–1	186·2 (166·3–203·7)
Meprobamate ..	Oral	>200	–	>500
	i.p.	174·2 (151·7–206·2)	20–35	>500

paralysing activities and acute toxicities of CB.2487 and mephenesin are quantitatively similar following oral and parenteral administration to mice.

Anticonvulsant activity in mice. Compound CB.2487, 200 mg/kg intraperitoneally, showed little or no antagonism to leptazol- or nicotine-induced convulsions, indicating that the drug did not exert a prime action of a known type on the higher centres of the central nervous system. However, CB.2487, in doses below its paralysing dose and well below its lethal dose, did antagonise strychnine. The intraperitoneal PD50 values for CB.2487 and mephenesin in preventing strychnine induced lethality were 33·1 (27·2–40·3) and 144·5 (110·6–187·0) mg/kg respectively. The

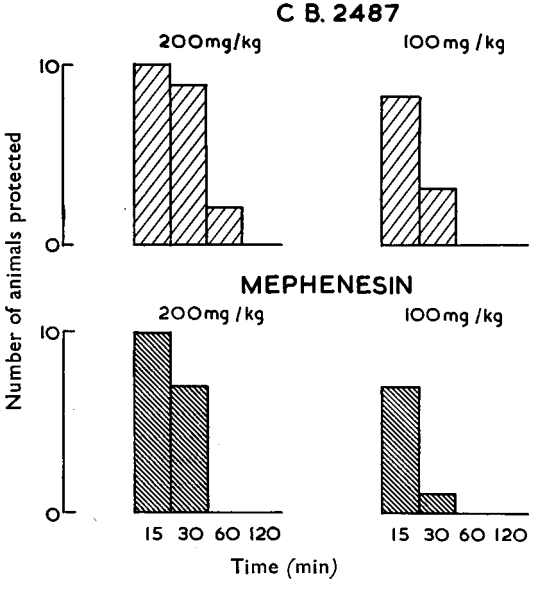

FIG. 1. Effects of CB.2487 and mephenesin, administered orally to groups of 10 mice, in preventing death induced by the intraperitoneal injection of 1·6mg/kg strychnine. The ordinate gives the interval between the administration of drug and the injection of strychnine.

potency and duration of antagonism to strychnine of CB.2487 following oral administration were marginally greater than mephenesin, but both compounds exhibited only brief activity by this route (Fig. 1).

Because of the low oral activity it was decided to revert to the intraperitoneal route to examine the nature of the strychnine antagonism. The effect of geometrically increasing doses of CB.2487 on the intraperitoneal lethality of strychnine was determined and the resulting log dose/probit mortality plots are illustrated in Fig. 2. The results show that the effects of CB.2487 are surmountable by strychnine. Furthermore, since the dose-response lines do not deviate significantly from parallelism and the log interval space between these lines is constant, these results suggest competitive antagonism between the drugs (Ariëns, Rossum & Simonis, 1956).

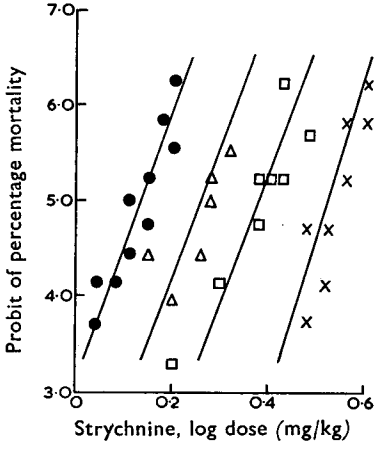

FIG. 2. Log dose/probit mortality plots for strychnine in the presence of geometrically increasing doses of CB.2487. Both drugs were injected intraperitoneally into mice. ●—● No antagonist. △—△ 25 mg/kg CB.2487. □—□ 50 mg/kg CB.2487. ×—× 100 mg/kg CB.2487.

The comparative PD50 values mg/kg (95% fiducial limits) of CB.2487, mephenesin and phenytoin, administered orally against maximal electric shock in mice, are: 190·0 (165–218), 198·0 (157·5–254) and 4·70 (3·56–6·21) respectively. Both CB.2487 and mephenesin exhibited weak activity in this test compared with the activity of phenytoin, a drug known to be potent in mice and rats in this test (Goodman, Toman & Swinyard, 1948).

In a further experiment, the durations of activities of these three compounds were investigated (Fig. 3). Again CB.2487 and mephenesin had fleeting activity even at high doses whereas the effect of phenytoin at 10 mg/kg lasted for more than 2 hr.

Antagonism of fighting behaviour in mice. The oral ED50 values mg/kg (95% fiducial limits) of CB.2487, mephenesin and meprobamate in suppressing fighting episodes in mice are 127 (95·8–169), 125 (91·3–174) and 61·7 (39·2–96·8) respectively. Only compounds which suppress

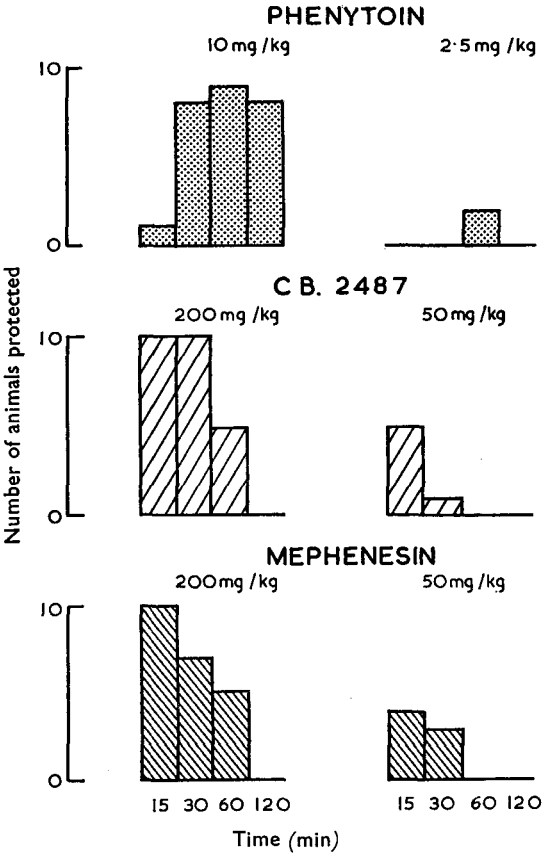

FIG. 3. Effects of CB.2487, mephenesin and phenytoin, administered orally to groups of 10 mice, in preventing seizures induced by maximal electric shock. The ordinate gives the interval between the administration of drug and the application of the shock.

fighting behaviour at doses substantially less than those causing paralysis (see Table 1) can be considered to have a selective action. The ratio, ED50 causing paralysis/ED50 in suppressing fighting episodes, is a convenient means of assessing selectivity, a high ratio indicating high selectivity. For CB.2487 the figure is 1·058, for mephenesin, 1·296 and for meprobamate $>3{\cdot}241$.

It is clear that only meprobamate has a selective action. Suppression of fighting episodes by CB.2487 and mephenesin occurred at, or near to, doses which induced much depression of motor activity. Again it is seen that CB.2487 resembles mephenesin and both these compounds may be easily differentiated from meprobamate.

Antagonism of drug effects in mice by bemegride. The effects of bemegride on the duration of paralysis induced by CB.2487, mephenesin or pentobarbitone are illustrated in Fig. 4. Bemegride had little or no

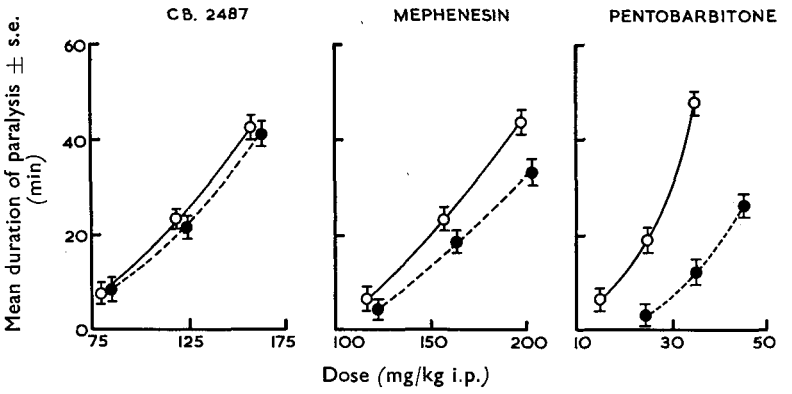

FIG. 4. Effects of bemegride on the durations of paralysis induced by CB.2487, mephenesin and pentobarbitone in the mouse. ○—○ Drug alone. ●···● Drug + bemegride, 10 mg/kg i.p.

effect on the paralysis induced by CB.2487 or mephenesin but had the expected antagonism to the barbiturate. Since bemegride is believed to be a selective barbiturate antagonist (Shaw, Simon, Cass, Schulman, Anstee & Nelson, 1954) it is probable that neither CB.2487 nor mephenesin possesses barbiturate-like activity.

Action on spinal reflexes. In cats anaesthetised with chloralose CB.2487, 5 to 10 mg/kg intravenously, selectively depressed contractions of the quadriceps femoris muscle elicited through a multisynaptic pathway (crossed extensor reflex). The degree of depression was dependent upon the dose injected. At these dose levels CB.2487 did not depress contractions of the quadriceps muscle elicited through a monosynaptic pathway (patellar reflex). In agreement with published reports (Kaada, 1950; Taverner, 1952) mephenesin also selectively blocked the multisynaptic

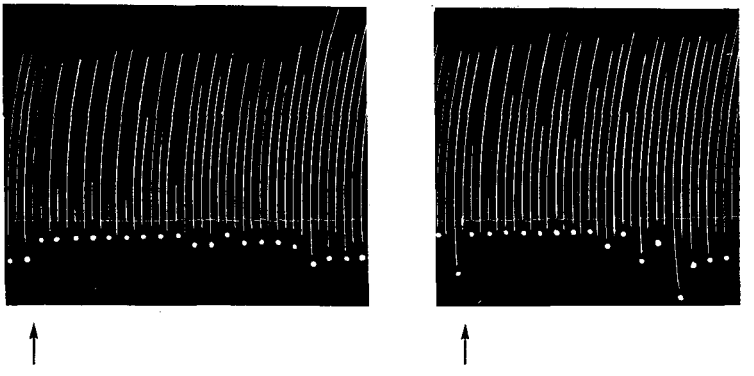

FIG. 5. Cat, light chloralose anaesthesia. Alternate crossed extensor and patellar reflex contractions of the quadriceps femoris muscle every 30 sec (i.e. 1 min between reflex contractions of the same type). Crossed extensor reflexes indicated by white dots. Left-hand panel, 10 mg/kg, CB.2487; right-hand panel, 10 mg/kg, mephenesin. Both drugs injected intravenously.

reflex, being about 0·6 times as active as CB.2487; equipotent doses of these compounds had similar durations of action. The results are summarised in Table 2 and one experiment is illustrated in Fig. 5. At the dose levels examined neither drug had any significant effect on arterial blood pressure.

TABLE 2. THE DEPRESSANT EFFECTS OF CB.2487 AND MEPHENESIN ON THE CROSSED EXTENSOR REFLEX (QUADRICEPS FEMORIS MUSCLE) IN ANAESTHETISED CATS

Compound	Dose mg/kg i.v.	Mean maximal depression (%) of quadriceps twitch ± s.e.	Mean duration of effect (min) ± s.e.
CB.2487	2·5	0 (2)	0
	5·0	31·6 ± 13·5(3)	3·7 ± 2·7
	7·5	53·0	4·0
	10·0	90·6 ± 4·2 (4)	12·5 ± 1·2
	20·0	100 (2)	24·0 ± 2·0
Mephenesin	2·5	0 (2)	0
	5·0	7·9 ± 3·1 (2)	0
	10·0	55·5 ± 16·4 (5)	4·6 ± 1·6
	20·0	85·9 ± 12·4 (3)	13·7 ± 2·3

Figures in parentheses indicate number of determinations. Duration was taken as the time between dosage and recovery of the reflex response. The point of recovery was arbitrarily chosen as the first of three consecutive responses having 75% or more of the mean value of three consecutive responses immediately before dosage.

It has already been shown that neither CB.2487 nor mephenesin affected the monosynaptic patellar reflex at doses which abolished or substantially reduced the crossed extensor reflex. In fact, the patellar reflex was found to be very resistant to both drugs, no effect being seen when the dose was increased to 8 times that required to reduce the crossed extensor reflex.

In spinalised animals CB.2487 or mephenesin, 10 to 20 mg/kg intravenously, still suppressed the crossed extensor reflex. However, the effect of these drugs on the patellar reflex was variable; usually there was no effect but enhanced contractions were produced in some preparations.

In a further series of experiments using cats anaesthetised with chloralose, the effects of CB.2487 and mephenesin on a multisynaptic flexor reflex were compared. The reflex chosen involved contraction of the tibialis anterior muscle. Both drugs were found to suppress this reflex at slightly higher dosage than that needed to suppress the crossed extensor reflex. Again CB.2487 was more active than mephenesin but at equipotent doses had about the same duration of action. The results are summarised in Table 3.

In experiments to investigate the effect of CB.2487 on spinal reflexes augmented by strychnine, it was found that intravenous doses of strychnine, 10 to 30 μg/kg, had no effect on the patellar reflex but caused marked potentiation of the crossed extensor reflex. This preferential effect of strychnine has been previously observed by Kaada (1950), Naess (1950) and Bernhard, Taverner & Widen (1951). At higher dosage, 40 μg/kg, strychnine also enhanced the patellar reflex contractions. When this did occur, generalised muscular activity was also evident. In cats given strychnine, 20 μg/kg intravenously, to augment the crossed extensor

TABLE 3. THE DEPRESSANT EFFECTS OF CB.2487 AND MEPHENESIN ON A FLEXOR REFLEX (TIBIALIS ANTERIOR MUSCLE) IN ANAESTHETISED CATS

Compound	Dose mg/kg i.v.	Mean maximal depression (%) of tibialis twitch ± s.e.	Mean duration of effect (min) ± s.e.
CB.2487	5	25·7	2·0
	10	57·3 ± 11·9 (2)	11·0 ± 1·0
	15	91·6 ± 8·4 (2)	11·5 ± 5·5
	20	94·4 ± 4·6 (3)	19·7 ± 3·8
Mephenesin	5	16·8 ± 11·3 (2)	0
	10	29·3 ± 1·5 (3)	3·3 ± 0·9
	15	69·6 ± 3·6 (3)	7·0 ± 1·5
	20	79·8 ± 20·2 (2)	9·5 ± 2·5

Figures in parentheses indicate number of determinations. Duration was taken as the time between dosage and recovery of the reflex response. The point of recovery was arbitrarily chosen as the first of three consecutive responses having 75% or more of the mean value of three consecutive responses immediately before dosage.

reflex, CB.2487 (6·25 mg/kg intravenously) antagonised the augmentation caused by strychnine and, in higher doses, reduced or abolished the reflex itself. Mephenesin was found to have a similar action in this preparation but was only about one half as active as CB.2487.

Action on the myoneural junction. In the anaesthetised cat, CB.2487 in intravenous doses up to 40 mg/kg neither potentiated nor depressed the maximal twitches of the tibialis anterior muscle stimulated through its motor nerve. At these dose levels mephenesin was also without effect on this preparation. Clearly CB.2487 and mephenesin do not exhibit neuromuscular blocking activity in intravenous doses approximately 4 times those required to depress reflex contractions of the tibialis anterior muscle elicited through a multisynaptic pathway.

Discussion

2-Amino-4-methyl-6-phenylamino-1,3,5-triazine (CB.2487) closely resembles mephenesin in its effects in the mouse. Both drugs cause obvious reduction in motor activity followed by paralysis without respiratory arrest. The impression that CB.2487 was mephenesin-like was further substantiated when it apparently antagonised competitively strychnine-induced toxicity, indicating that its prime locus of action was on the spinal cord. A more detailed comparison of CB.2487 and mephenesin confirmed their similarity; furthermore, it was also shown that CB.2487 could be distinguished from drugs such as meprobamate and pentobarbitone. Since CB.2487 closely resembles mephenesin in its action and since the pharmacology of mephenesin has been well documented (Berger, 1947; Kaada, 1950; Taverner, 1952) it seems probable that the effects of CB.2487 also depended on an action on the spinal cord and the drug would not be of value as a sedative or tranquilliser.

The most pronounced action of CB.2487 is its ability, in relatively small doses, to depress the crossed extensor and flexor reflexes in the anaesthetised cat. Similar results were obtained in the spinal animal as well as in the intact anaesthetised animal, showing that the effect of CB.2487 is not a consequence of an action on the brain. An action on the neuromuscular

junction, on skeletal muscle, on the muscle spindles, and on conduction in peripheral nerves (including the lower motoneurones, the γ-efferents and the Group Ia afferents) was ruled out when it was observed that CB.2487 did not depress contractions of the tibialis anterior muscle elicited by stimulation of the sciatic nerve or contractions of the quadriceps femoris muscle elicited by tapping the patellar tendon. These results therefore confirmed the location of action of CB.2487 in the spinal cord, typing the drug as a centrally acting muscle relaxant.

The selective depression of multisynaptic reflexes and not a monosynaptic reflex by CB.2487 in the cat, together with the lack of action of this compound in antagonising convulsions in mice induced by leptazol and nicotine strongly suggests that its site of action is not on the anterior horn cell of the spinal cord, since these cells and the lower motoneurones constitute the final common path in all these cases. In fact these results indicate the site of action of CB.2487 to be at some point in the interneurone chains that form part of the pathways involved in the crossed extensor and flexor reflexes. Only a speculative view can be presented on the possible mechanism by which CB.2487 exerts its effects; however, the interaction of CB.2487 with strychnine suggests a close relationship to the effects of inhibitory transmitter. Inhibition in the spinal cord might be brought about by a drug which mimicked the action of, increased the release of, or prevented the destruction of inhibitory transmitter, which stimulated inhibitory neurones or which lowered their threshold to normal physiological stimuli. According to Eccles (1964), strychnine acts by competing with the inhibitory transmitter for the same receptors on the post-synaptic cell. If CB.2487 mimicked the action or prevented the destruction of inhibitory transmitter, the apparently competitive relationship between it and strychnine could be explained on this basis. There is evidence (Curtis, 1959), as yet not fully substantiated (Anderson, Eccles, Løyning & Voorhoeve, 1963; Crawford, Curtis, Voorhoeve & Wilson, 1963), that the same transmitter is responsible for inhibition at all inhibitory synapses. If this were so then the anterior horn cells would presumably be sensitive to any substance which resembled inhibitory transmitter, yet the monosynaptic pathway was not blocked by CB.2487. The same considerations apply to the possibility that CB.2487 activates inhibitory neurones; all post-synaptic cells would be depressed, including the anterior horn cells, unless the drug selectively stimulates some inhibitory neurones but not others. Clearly without further knowledge about the identity of central transmitters and the mechanism of action of strychnine it is impossible to determine the site and mechanism of action of CB.2487 more precisely.

Possible clinical uses of centrally acting muscle relaxants include the relief of chronic spastic conditions of voluntary muscles, involving for example, hemiplegia, disseminated sclerosis, the many spastic states following brain injuries in childbirth and tetanus. To be of value in such conditions a centrally acting muscle relaxant drug would have to be long acting, selectively affect the spastic muscle and preferably be effective by mouth. Noticeable features of the pharmacological properties of

CB.2487 are the brief duration of activity and the large difference between the oral and parenteral activities, the former being much lower. The poor oral activity and brief duration of action make CB.2487 a poor prospect for use in any of these clinical conditions.

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