THE MODE OF NEUROMUSCULAR BLOCKING ACTION OF CHLORPROMAZINE

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The inhibitory action of chlorpromazine on skeletal muscle has been studied with isolated preparations. In the nerve-muscle preparations of the frog sartorius and the rat diaphragm, the twitch responses to indirect stimulation are much more strongly depressed by chlorpromazine than those to direct stimulation. The conductivity of the nerve trunk is unaffected. The contractures of the frog rectus abdominis muscle caused by acetylcholine are depressed by chlorpromazine, but the contractures due to KCl are not influenced. Larger doses of chlorpromazine cause contracture by themselves, and this cannot be prevented by tubocurarine. In the sartorius muscle of the toad, the depolarization due to acetylcholine is reduced by chlorpromazine. The paralysing action of chlorpromazine adds to that of tubocurarine, and is antagonized to some extent by eserine or neostigmine. Muscles treated with chlorpromazine do not completely recover on washing. High concentrations of chlorpromazine depress the release of acetylcholine by motor-nerve stimulation, although they do not affect the enzymic synthesis of acetylcholine by acetone-dried powder of guinea-pig brain. The differences between the neuromuscular block produced by chlorpromazine and that by tubocurarine are discussed.

Several papers have been published concerning the action of chlorpromazine on striated muscle. Huidobro (1954) observed that chlorpromazine did not modify the twitch of cat quadriceps muscle stimulated through its nerve. Dobkin, Gilbert, and Lamoureux (1954) reported that chlorpromazine did not block the neuromuscular junction in either frog or cat sciatic-gastrocnemius preparation.

Contrarily, many authors state that chlorpromazine inhibits striated muscle. Kopera and Armitage (1954) observed that chlorpromazine depressed the response of the gastrocnemius muscle of cats when stimulated either directly or indirectly through the sciatic nerve. They, as well as Burn (1954), considered that the action of chlorpromazine was not curare-like but was a direct action on the muscle. On the other hand, Ryall (1956) demonstrated that chlorpromazine could inhibit neuromuscular transmission in the isolated phrenic nerve-diaphragm preparation of rabbits, and Wislicki (1958) obtained the same result with the sciatic-gastrocnemius preparation of cats. Ryall (1956) suspected that chlorpromazine paralysed the muscle by decreasing acetylcholine synthesis at the motor nerve endings, but this idea has been rejected recently by Otubo

(1959). The latter author stated that chlorpromazine at first stimulated the endplate membranes and later inhibited them.

In view of these conflicting results, we decided to perform experiments to throw some light upon the mode of action of chlorpromazine on striated muscle.

METHODS

Isolated preparations of striated muscle were used throughout so as to avoid interfering factors such as the actions of chlorpromazine on the central nervous or the cardiovascular systems.

Sartorius Muscle of the Frog.—The sartorius nerve muscle preparation of *Rana tigrina* was dissected out and set up in an organ bath containing 50 ml. of frog Ringer solution kept at room temperature and constantly oxygenated. Indirect and direct single electric shocks (rectangular in shape, supramaximal and of 0.5 and 5 msec. durations respectively) were applied once every 10 sec. The stimulator was a Model 751 by Arthur H. Thomas Co. The isotonic twitches of the muscle were recorded on smoked paper.

Diaphragm of the Rat.—The phrenic nerve-diaphragm preparation of the rat (Bülbring, 1946) was suspended in 50 ml. Tyrode solution, kept at 37° to 38° and constantly oxygenated with 95% O₂ and 5%

CO₂. Contractions were caused and recorded in the manner described for the sartorius muscle of the frog.

Rectus Abdominis Muscle of the Frog.—The rectus abdominis muscle of *R. tigerina* was excised and mounted in a 5 ml. bath containing frog Ringer solution at room temperature. Dose-response curves were obtained by applying acetylcholine in concentrations of 10^{-6} , 10^{-5} , 10^{-4} , and 10^{-3} g./ml. and recording the contractions on smoked paper. The contracture produced by 10^{-3} g./ml. acetylcholine was considered as the maximum response. The dose-response curves to KCl were produced similarly.

Depolarization of the Toad Sartorius Muscle.—A method similar to that of Fatt (1950) was used. The sartorius muscle of the toad (*Bufo melanostictus*) was mounted in a glass chamber containing 50 ml. of frog Ringer solution. An electrode was placed at the pelvic end and another near the bottom of the chamber in contact with the solution. In order to determine the position of maximum density of endplates, a preliminary record was obtained of the potential differences along the surface of each muscle preparation in the following manner: the level of the Ringer solution in the bath was lowered stepwise by draining 1 ml. away each time, and the potentials were measured by means of a potentiometer. With part of the muscle immersed in solution, the potential difference recorded was that between the upper end and a point on the muscle at the surface of the bathing solution. This procedure was repeated after substituting a solution of acetylcholine (8×10^{-6} g./ml.) for the Ringer. The point on the muscle surface which gave the maximum potential difference before and after using acetylcholine represented the focus of maximum endplate density. The depolarization by acetylcholine at the region of maximum endplate density was measured again, and the value was taken as 100%. The same procedure was repeated at 15 min. intervals after the application of chlorpromazine.

Estimation of Acetylcholine Release from the Rat Diaphragm.—A sector of the diaphragm, with phrenic nerve attached but without ribs, was suspended in a 3 ml. bath in Krebs bicarbonate solution containing 5×10^{-6} g./ml. neostigmine at 37°. After tetanizing at 25 shocks/sec. for 20 min. the fluid was withdrawn and assayed for acetylcholine by its depressor effect on the blood pressure of the anaesthetized rat (Straughan, 1958). Since the resting output of acetylcholine was negligible, no correction for this was made.

Acetylcholine Synthesis by Guinea-pig Brain.—The acetone-dried powder from guinea-pig brain was prepared and incubated at 37° with the reaction mixture of Feldberg and Hebb (1947). The acetylcholine produced was assayed on the frog's rectus abdominis muscle according to the method of Feldberg and Mann (1945).

The drugs used were chlorpromazine hydrochloride (Wintermin, Shionogi), tubocurarine chloride (Intocostrin-T, Squibb), acetylcholine chloride (Ovisot,

Daiichi), neostigmine methylsulphate (Vagostigmine, Shionogi), and eserine salicylate (Merck). Concentrations of drugs are expressed as weights of salts, or as μ M. or mM.

RESULTS

Sartorius Muscle of the Frog

The muscle contractions elicited by indirect stimulation were depressed markedly by chlorpromazine in concentrations of 30 to 50 μ M. (11 to 18 μ g./ml. of chlorpromazine hydrochloride) after an initial slight augmentation. The responses to direct stimulation also suffered inhibition which was, however, much smaller (Fig. 1). When a

FIG. 1.—Frog, motor nerve-sartorius muscle. Amplitudes of single twitches in response to supramaximal stimulation. Solid line, direct stimulation; broken line, indirect stimulation. a, 10 μ M.; b, 30 μ M.; and c, 50 μ M. of chlorpromazine given at zero time.

segment of the nerve trunk between the muscle and the electrodes was soaked in 10 to 100 μ M. solutions of chlorpromazine, no change in the amplitude of contraction occurred for more than 1 hr. Similar results were obtained in a few experiments with sciatic nerve-gastrocnemius preparations of the frog.

These results indicate that chlorpromazine exerts primarily a neuromuscular blocking action, plus a smaller action on the muscle fibres.

The slight augmentation of the contractions by 10 μ M. of chlorpromazine and the initial transient augmentation after 30 to 50 μ M. might have been due to the mild anticholinesterase activity of this drug (Erdös, Baart, Shanor, and Foldes, 1958).

The diminished contractions of this preparation could usually be restored by repeated washing with

Ringer's solution, even after a complete block had occurred. When the blocking action was fully developed the tetanic contraction caused by repetitive stimulation was no longer sustained, and no post-tetanic potentiation was found (Fig. 2).

Neostigmine and eserine (2×10^{-7} to 5×10^{-6} g./ml.) exerted a slight antagonistic effect. Adrenaline (5×10^{-7} to 5×10^{-6} g./ml.) was totally ineffective. The paralytic potencies of a dose of chlorpromazine, of an equivalent dose of tubocurarine and of the mixture of half-doses of the two agents were approximately equal, judging from the time required for complete paralysis.

Phrenic Nerve-Diaphragm Preparation of the Rat

Chlorpromazine in concentrations of 10 to 30 μ M. reduced the muscle responses to indirect stimulation, after a brief augmentation. With a 40 μ M. solution, a complete neuromuscular block was observed within 30 min., while the muscle still responded well to direct stimulation. Repetitive stimulation usually caused a well-sustained tetanic contraction (Fig. 3).

Washing sometimes retarded the blocking process, but the amplitude of twitches never recovered appreciably. Larger doses of chlorpromazine tended to provoke contracture of the diaphragm. Our results are in agreement with those of Ryall (1956). Eserine and neostigmine were only feebly effective unless applied early, when they generally enhanced the twitches (Fig. 4).

FIG. 2.—Frog, motor nerve-sartorius muscle. Tetanic stimulations 15 min. (left) and 20 min. (right) after application of 50 μ M. chlorpromazine. Pulse frequency: 60/sec. (T1 and T3) and 120/sec. (T2 and T4).

FIG. 3.—Rat, phrenic nerve-diaphragm. Supramaximal indirect stimulation. CPZ, 40 μ M. chlorpromazine, tetanic stimulation at T. Direct stimulation at D.S. Time in min.

FIG. 4.—Rat, phrenic nerve-diaphragm. CPZ, chlorpromazine 30 μ M. (above) and 50 μ M. (below). Eserine 10^{-6} g./ml. at E. Time in min.

Rectus Abdominis Muscle of the Frog

The amplitudes of contracture caused by acetylcholine decreased after treatment of the muscle with solutions of chlorpromazine in concentrations higher than 0.1 μ M. This effect was slower and weaker, on the molar basis, than that of tubocurarine. The action proceeded gradually and approached completion in 60 min.

The higher the concentration of acetylcholine, the more the response to it was depressed, and repeated washing did not lessen the depression appreciably (Fig. 5). Thus the action of chlorpromazine is at least partly irreversible.

On the other hand, chlorpromazine showed an approximately additive effect when used in combination with tubocurarine (Fig. 6). The addition of eserine or neostigmine completely antagonized chlorpromazine as far as the responses to low concentrations of acetylcholine were concerned, whereas the responses to higher concentrations of acetylcholine were depressed (Fig. 5). This suggests a mode of action different from that of tubocurarine.

Responses to KCl were not depressed by chlorpromazine (Fig. 7), since Ringer's solution alone after 1 hr. flattened the dose-response curve as much as chlorpromazine did. This was confirmed

by the observation that, at the end of the tests with acetylcholine, the addition of an excess of KCl (300 mm.) still produced a maximum contracture.

FIG. 5.—Frog, rectus abdominis. Responses to acetylcholine. a, Control. b, 2 μ M. chlorpromazine for 60 min.. c, 2 μ M. chlorpromazine for 60 min. followed by washing for 60 min. d, 2 μ M. chlorpromazine and 10^{-6} g./ml. neostigmine for 60 min.

FIG. 6.—Frog, rectus abdominis. Responses to acetylcholine. a, Control. b, 2 μ M. chlorpromazine for 60 min. c, 0.5 μ M. tubocurarine for 60 min. d, 1 μ M. chlorpromazine and 0.25 μ M. tubocurarine for 60 min.

High concentrations of chlorpromazine caused contracture of the rectus muscle, which relaxed only very slowly on washing (Fig. 8). Previous treatment of the muscle with tubocurarine (4×10^{-5} g./ml. for 30 min.) had no influence on it, as Ryall (1956) observed for the diaphragm of the rabbit. The contracture produced by chlorpromazine, therefore, does not demonstrate an endplate depolarizing action of this agent, but seems to require some other explanation.

Depolarization in the Toad Sartorius Muscle by Acetylcholine

Chlorpromazine in 5 to 50 μ M. solutions did not itself cause depolarization, but greatly diminished the magnitude of the depolarization caused by acetylcholine. The effect could be eliminated very slowly by washing several times unless large doses were used. Neostigmine reversed the inhibition effectively (Fig. 9). This experiment provided further evidence that chlorpromazine exerted an important inhibitory action on the motor endplates.

Acetylcholine Release by Motor-nerve Stimulation

As shown in Table I, the release of acetylcholine by motor-nerve stimulation was greatly reduced

FIG. 7.—Frog, rectus abdominis. Response to KCl. a, Control. b, 2 μ M. chlorpromazine for 60 min. c, Frog-Ringer for 60 min.

by 50 μ M. of chlorpromazine. In one experiment (Expt. No. 3), it was completely abolished 1 hr. after the application of chlorpromazine. When 10 μ M. of chlorpromazine was applied, however, no constant results were obtained; in one experiment

FIG. 8.—Frog, rectus abdominis. Contracture caused by chlorpromazine (CPZ), a, 10 μ M., b, 100 μ M., c, 1 mM. Washing at W. Time in min.

TABLE I

ACETYLCHOLINE OUTPUT OF THE RAT EXCISED DIAPHRAGM PREPARATION BEFORE AND AFTER THE ACTION OF CHLORPROMAZINE

There was 20 min. between the first and second periods of each control experiment.

Expt. No.	Acetylcholine Output (ng./20 min.)				Concentration of Chlorpromazine (μ M.)
	Before Chlorpromazine		After Chlorpromazine		
	1st Period	2nd Period	30 Min.	60 Min.	
1	39	36	27	10	50
2	41	45	23	10	50
3	37	41	13	0	50
4	49	45	45	49	10
5	27	30	23	18	10

no reduction of acetylcholine released was found whereas in another experiment the amount of acetylcholine released was reduced by about 40% 60 min. after the application of chlorpromazine.

Synthesis of Acetylcholine by the Guinea-pig Brain

The results of a typical test are shown in Table II. It will be seen that chlorpromazine, even in concentrations as high as 100 μ M., did not affect appreciably the synthesis of acetylcholine.

FIG. 9.—Toad, sartorius muscle. Magnitudes of depolarization caused by 8×10^{-6} g./ml. acetylcholine. a, 5 μ M., b, 10 μ M., c, 50 μ M. chlorpromazine (●—●) given at zero time. Recovery in Ringer solution (Δ — Δ), and in chlorpromazine + neostigmine 10^{-6} g./ml. (X—X).

TABLE II

SYNTHESIS OF ACETYLCHOLINE BY THE GUINEA-PIG BRAIN

Figures refer to μ g. of acetylcholine produced per mg. of acetone-dried powder.

Chlorpromazine Concentration (μ M.)	Acetylcholine after Incubation Time of	
	1 hr.	2 hr.
0 (control)	0.18	0.34
1	0.18	0.32
10	0.19	0.33
100	0.18	0.32

DISCUSSION

Although Kopera and Armitage (1954), as well as Burn (1954), reported that the paralytic action of chlorpromazine in cats and rats was due to its direct action on muscle fibres, Ryall (1956) demonstrated that chlorpromazine inhibited neuromuscular transmission, with little effect on direct muscle stimulation, in the isolated phrenic nerve-diaphragm of rabbits. In our experiments using the nerve-muscle preparations of the frog sartorius and the rat diaphragm, chlorpromazine depressed the twitch responses to indirect stimulation much more markedly than those to direct stimulation. In addition, chlorpromazine decreased the size of the contractures of the frog rectus muscle due to acetylcholine while leaving those due to KCl unaffected. These facts indicate that the direct action of low concentrations of chlorpromazine on the muscle fibres is of minor importance. Since the conductivity of the nerve trunk was not affected by chlorpromazine, the main site of paralysis is probably the neuromuscular junction.

The blocking action is not of depolarizing type because the drug did not depolarize the endplates, did not cause fasciculation and did not antagonize the action of tubocurarine.

On the contrary, chlorpromazine reduced the endplate depolarization as well as the contracture caused by acetylcholine, and it was partly antagonized by anticholinesterases. In addition, it has been reported by many authors that chlorpromazine enhances the paralytic action of tubocurarine and its analogues (Wislicki, 1958). Our results show that the combined effect of chlorpromazine and tubocurarine should be classified as summation rather than as potentiation ("supra-additive synergism") in the sense defined by Barstad (1956). It is therefore concluded

that the neuromuscular blocking action of chlorpromazine resembles that of tubocurarine in many respects.

There is evidence, however, that the action of chlorpromazine is not exactly like that of tubocurarine. First, it inhibits the muscle fibres directly and causes contracture when somewhat larger doses are applied: the mechanism of the latter action was not studied in the present work. Secondly, the anticurare agents exert only a slight antagonistic effect when the muscle is stimulated indirectly. Thirdly, post-tetanic potentiation of the muscle twitches is absent, and tetanus is well-sustained in the rat's diaphragm preparation. Fourthly, the dose response curves to acetylcholine on the frog's rectus abdominis muscle before and after treatment with chlorpromazine are not parallel, and they become bell-shaped after addition of anticurare agents.

Besides, whereas the enzymic synthesis of acetylcholine is unaffected, the release of acetylcholine by motor-nerve stimulation is inhibited by high concentrations of chlorpromazine. This inhibition may also constitute a part of the cause of neuromuscular block, indicating the multiplicity of the mode of action of chlorpromazine.

Moreover, the paralysis due to chlorpromazine could not be removed by repeated washing of the muscle preparations, except for the sartorius muscles of frogs and toads which recovered slowly. The irreversible nature of the paralysis has also been encountered in muscles of cats (Kopera and Armitage, 1954; Wislicki, 1958). Thus, the motor endplates are blocked by chlorpromazine with an affinity much stronger than that of tubocurarine, although the degree of irreversibility may vary largely among different kinds of muscle and different animal species.

Finally, although our results indicate that chlorpromazine has a direct influence on neuromuscular transmission, they do not exclude the

possible participation of a central action of the drug on muscle tone in the intact animal. On the contrary, the neuromuscular junction seems to be less sensitive than the central nervous system to the drug, judging from the relatively high concentration required for neuromuscular block *in vitro*. The same conclusion may be drawn from the observations of several authors (Ryall, 1956; Wislicki, 1958; Otubo, 1959) that inhibition of striated muscle was consistently obtained only after intra-arterial injections; either negative or inconsistent results were obtained after intravenous injections of doses sufficient to act on the central nervous system.

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REFERENCES

- Barstad, J. A. B. (1956). *Arch. int. Pharmacodyn.*, **107**, 275.
 Burn, J. H. (1954). *Proc. roy. Soc. Med.*, **47**, 617.
 Bülbring, E. (1946). *Brit. J. Pharmacol.*, **1**, 38.
 Dobkin, A. B., Gilbert, R. G. B., and Lamoureux, L. (1954). *Anaesthesia*, **9**, 157.
 Erdös, E. G., Baart, N., Shanor, S. P., and Foldes, F. F. (1958). *Arch. int. Pharmacodyn.*, **117**, 163.
 Fatt, P. (1950). *J. Physiol. (Lond.)*, **111**, 408.
 Feldberg, W., and Hebb, C. (1947). *Ibid.*, **106**, 8.
 — and Mann, T. (1945). *Ibid.*, **104**, 8.
 Huidobro, F. (1954). *Arch. int. Pharmacodyn.*, **98**, 308.
 Kopera, J., and Armitage, A. K. (1954). *Brit. J. Pharmacol.*, **9**, 392.
 Otubo, Y. (1959). *Japanese J. Anesthesiol.*, **8**, 235.
 Ryall, R. W. (1956). *Brit. J. Pharmacol.*, **11**, 339.
 Straughan, D. W. (1958). *J. Pharm., Lond.*, **10**, 783.
 Wislicki, L. (1958). *Arch. int. Pharmacodyn.*, **115**, 52.