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THE ACTION OF CHLORPROMAZINE ON THE SKELETAL MUSCLE OF FROG

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ABSTRACT

The action of Chlorpromazine on the skeletal muscle of frog when tested under Gaddum superfusion technique was found to be (1) an anticholinergic effect in small doses and (2) a spasmogenic effect in large doses, this spasmogenic effect was not blocked by curare.

INTRODUCTION

CHLORPROMAZINE (CPZ) is one of the phenothiazine derivatives widely used in psychiatry and also in the treatment of spastic conditions. The untoward effects of this drug include parkinsonian like symptoms and dyskinetic symptoms which may be mistaken for tetanus, meningitis, poliomyelitis, etc. These symptoms are explained as the effect of the drug on the extrapyramydal system. This work was taken up to find the effect of graded concentration of CPZ on the skeletal muscle.

MATERIALS AND METHOD

Experiments were carried out on the skeletal muscle of frog *Rana tigrina* under Gaddum superfusion technique[4,5]. The ringer solution prepared according to Burn J. H. (1952) was used. The contractions were recorded on a slow moving smoked drum. Acetylcholine chloride (ACH) was used as an agonist in a dose of 1–2 mcg.

CPZ dissolved in distilled water was used in doses of 0·1, 1, 10, 100 ng[1] and 1, 10 and 100 mcg in 0·1 ml volume. The effect of CPZ in higher doses like 100 mcg and above were recorded on a stopped drum. In these experiments ACH was not used as an agonist, and they were repeated in potassium-free, calcium-free, sodium-free ringer solution and the solution containing twice the concentration of potassium ion. Tubocurarine 10 mg/ml was used as a blocking agent.

All drugs were dropped from a tuberculine syringe along with ringer solution. The contractions due to ACH were recorded for 20 seconds. After obtaining a set of submaximal contractions due to ACH, the CPZ was dropped followed by ACH after 20 seconds. The inhibition or potentiation of ACH induced contractions by CPZ was expressed as the height of contraction in mm.

RESULTS

The effects of CPZ on skeletal muscle can be grouped under three headings.

1. Anticholinergic action in doses of 0·1 ng to 10 mcg (Fig. 1).
2. Potentiation of ACH induced contraction in 0·1 ng and 1 ng.
3. A spasmogenic action at 100 mcg and above (Fig. 2),

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TABLE I

Showing the anticholinergic action of CPZ expressed as percentage inhibition of ACH induced contractions in 20 experiments

	0·1 ng*	1 ng	10 ng	100 ng	1 mcg	10 mcg
% inhibition ± standard error	27·8 ± 2·34	19·5 ± 0·81	24·08 ± 1·00	30·4 ± 1·2	35·9 ± 1·55	48·34 ± 2·0

*ng = nanogram.

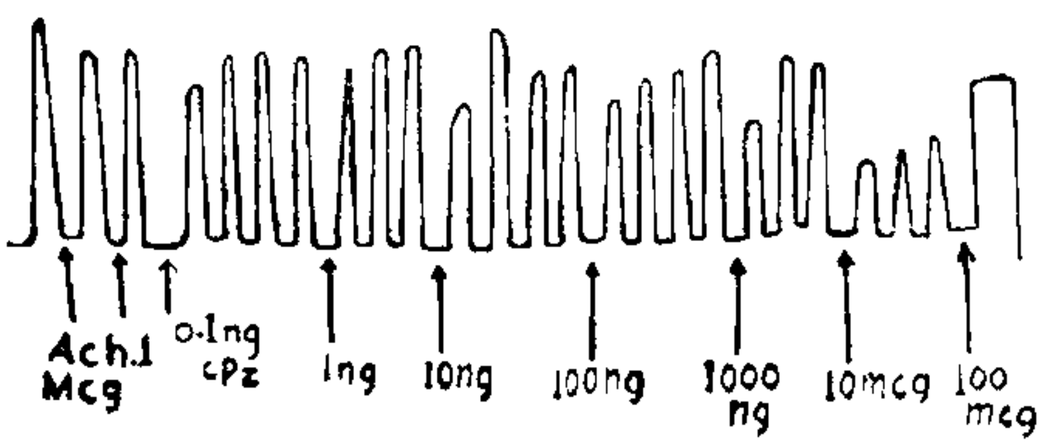

FIG. 1. Showing anticholinergic action of CPZ in doses of 0·1 ng to 10 mcg, each dose of CPZ is followed by 1 mcg of ACH except at 100 mcg.

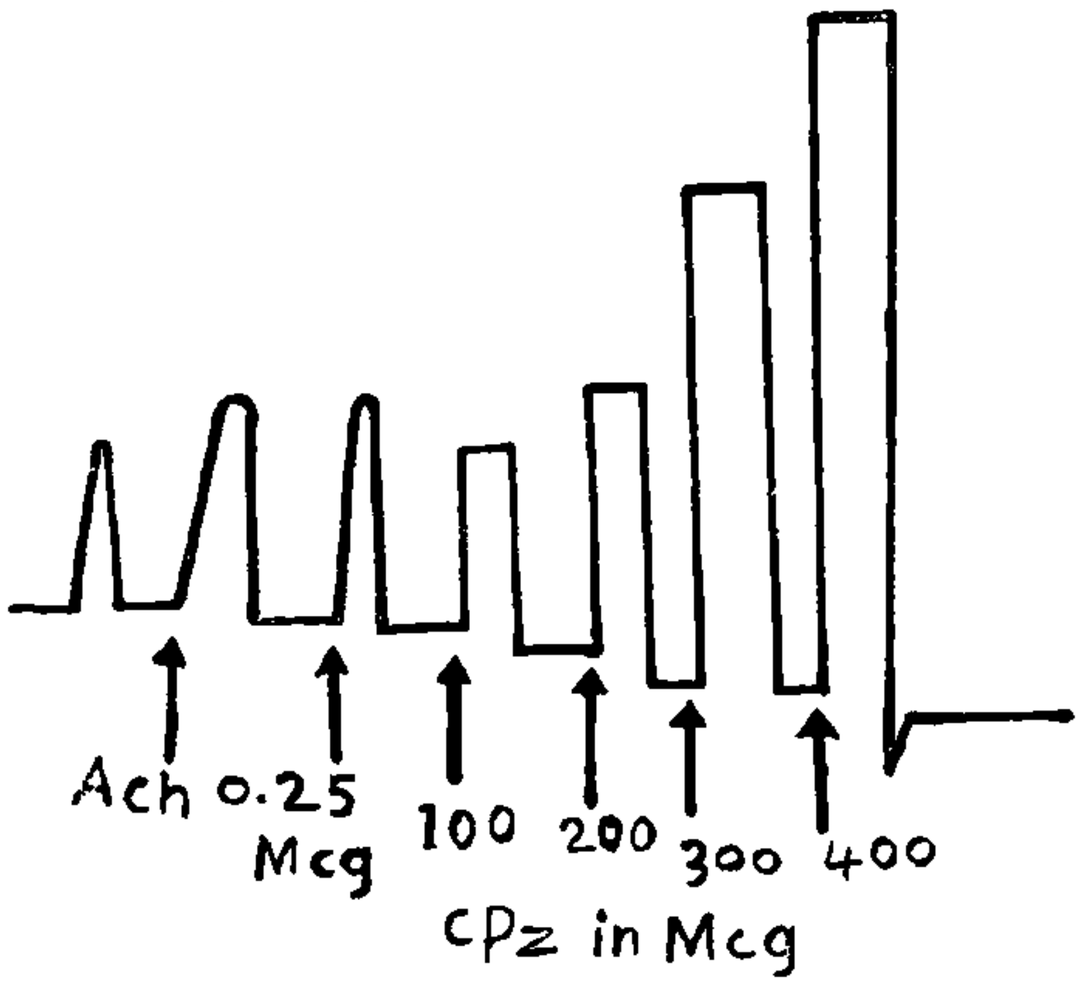

FIG. 2. Showing the spasmogenic action of CPZ in 100 200, 300 and 400 mcg, ACH is not used after CPZ.

1. CPZ in doses from 0·1 ng to 10 mcg produced a dose-dependent block of ACH induced contractions on skeletal muscle. The block is directly proportional to the dose at 100 ng to 10 mcg.

2. In 7 out of 20 experiments CPZ in doses of 0·1 ng and 1 ng produced either an immediate or a delayed potentiation of 17·2 ± 2·47% of ACH induced contraction of the skeletal muscle. In one out of these 7, the potentiation lasted during the entire course of the experiment, whereas in others it lasted for a short time.

3. *Spasmogenic action*: CPZ when used at 100 mcg and above produced a classic phasic contracture of the skeletal muscle which develops very slowly in over 20–30 minutes. During the first few minutes the tissue does not exhibit any effect, then slowly, an upward deflection reaching a maximum height of 30–70 mm depending upon the dose. The tissue fails to recover completely even after 6 hours, during which, it remains insensitive to ACH also. This action of CPZ was not blocked even by 200 mcg of curare.

In ion-free solutions: In calcium-free solution CPZ failed to produce the spasmogenic effect. In potassium-free solution CPZ produced the contracture of a lesser height and intensity and the tissue recovered on gently pressing down the lever in 40–60 minutes, and responded in a graded fashion to the increasing dose of CPZ.

In sodium-free ringer and ringer solution containing double the concentration of potassium ion, the tissue developed a contracture.

DISCUSSION

The substituted phenothiazines have multiple action on peripheral autonomic nervous system. These include the blockade of nicotinic and muscarinic action of ACH[8]. CPZ is said to produce the relaxation of skeletal muscle in some types of spastic conditions by selectively acting on the gamma efferent system and does not produce a blockade of the neuromuscular junction[2,9]. But a perusal of Table I shows that it blocks the action of ACH on skeletal muscle. The rectus abdominis muscle of frog is one of the preparations used to demonstrate the action of drugs at neuromuscular junction[4]. CPZ produces a curare-like action on the skeletal muscle. In these experiments the anticholinergic action is 27·77 ± 2·34 at first exposure of the tissue to the drug, whereas in next two exposures it is 19·5 ± 0·81 and 24·08 ± 0·999 though the dose of the drug is increased in a graded fashion. This may be because the tissue may show a high initial sensitivity when exposed to the drug for the first time.

The CPZ has also shown a potentiation on the action of ACH on skeletal muscle in dose of 0·1 ng and 1 ng. CPZ is reported to enhance the seizures after its depressant action of a large dose wears off[8]. Also CPZ is said to be an anicholinesterase agent[6]. Therefore the potentiation of ACH action on the skeletal muscle is probably mediated by the anticholinesterase action of the drug.

It may be seen from Fig. 2 that CPZ produces a classic phasic contracture of the skeletal muscle. The fact that it is not blocked by curare, a competitive blocker of the neuromuscular junction and CPZ itself produce a neuromuscular blocking action, shows that the drug produces contracture by acting at a receptor site other than the neuromuscular junction. The next alternative site of action would be the cell membrane or the contractile machinery. The addition of high concentration of potassium to the isolated muscle initiates the classic phase of potassium contracture, but it is not necessarily correlated with the contractility[10]. In potassium-free ringer CPZ produces contracture but relaxes quickly compared to the effect in normal ringer. Its failure to produce this effect in calcium-free ringer suggests that this action is mediated through potassium and calcium ions. CPZ is reported to be having a membrane stabilising action[6], this demonstrates that the contracture is produced by stabilising the cell membrane, so as to rise the tissue concentration of potassium and calcium ions beyond physiological limits, and more so in a rise of potassium resulting in contracture.

Thus, CPZ is shown to have an anticholinergic action in low doses and a membrane stabilising action resulting in contracture of the skeletal muscle of frog in high dose.

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RADIOCARBON DATES OF SOME LATE QUATERNARY SAMPLES

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PRESENTED below are the ^{14}C dates of Late Quaternary samples from the coastal and riverine sediments. The eustatic samples are derived from the western coast. Quite a few samples of miliolite formations of Gujarat have also been dated. The river sediments have been ^{14}C dated for their Stone Age associations.

The samples have been counted in the form of methane in gas proportional counters. The techniques have been described in detail elsewhere (Agrawal and Kusumgar, 1965; Agrawal *et al.*, 1971; Kusumgar *et al.*, 1963). Ninetyfive per cent activity of N.B.S. oxalic acid has been used as a modern standard. For all samples two dates are given in B.P. The first is based on 5568 yr. half-life value and the second, in parenthesis, on 5730 yr. None of the dates has been calibrated for any $^{14}C/^{12}C$ variations. The dates can be converted to AD/BC scale by using 1950 AD as reference year.

Though $CaCO_3$ measurements too have been expressed in terms of ^{14}C dates, yet it has to be noted that $CaCO_3$ is an inorganic chemical and the ^{14}C method, strictly speaking, does not apply to it.

These measurements were made at the Tata Institute, Bombay, but now the C^{14} lab has been shifted to Physical Research Laboratory, Ahmedabad.

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Asla, Maharashtra India

TF-1178, Late Quaternary, 9750 ± 120
(10035 ± 125)

Shells from pebbly conglomerate 2·5 m above Krishna river bed at Asla (Lat. 17° 53′ N., Long. 73° 59½′ E.), District Satara.