STUDIES ON CENTRAL NERVOUS SYSTEM DEPRESSANTS (IX)

The action of some tranquillizing agents on the coaxically stimulated guineapig ileum

By

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Tranquillizers, namely, benactyzine hydrochloride, pipethanate hydrochloride, trifluoperazine, rescinnamine, carisoprodol, captodiamine, and emylcamate were investigated for their mechanism of action on the intestine. All the carbamate derivatives except emylcamate failed to cause reduction or abolition of twitch due to electrical stimulation. From our findings it appears that some of the tranquillizers namely reserpine, rescinnamine, chlorpromazine, prochlorperazine, azacyclonal, and hydroxyzine act on intestine by competitive antagonism of acetylcholine at the receptor groups, whereas drugs like benactyzine, pipethanate, captodiamine and trifluoperazine appear to depress the twitch of intestine by directly acting on the muscle.

Paton (1957) has shown that morphine and morphine-like analgesics depress the twitch and tetanus of coaxically stimulated guineapig ileum by reducing acetylcholine (Ach.) released from cholinergic nerve endings. Schaumann (1957) also, though using a different technique, has shown that morphine inhibited the release of Ach. from isolated small intestine of guineapig. Experimental evidence has been presented by this worker which suggests that reduced release of Ach. could not be explained by an inhibition of the synthesis of Ach. nor by stabilization of the bound form of Ach. in the tissue. Apparently morphine reduces the excitability of postganglionic structures and thereby the liberation of Ach. from nerve endings during the process of excitation. Kosterlitz and Robinson (1955) found that morphine has an inhibiting effect on the action of 5-hydroxytryptamine (serotonin), whose action on the ileum is now believed to involve nervous pathway (Robertson, 1953 ; Rocha E Silva, et al., 1953 and Gaddum and Hameed, 1954).

In clinical practice, it has been observed that several tranquillizers also act on the gastrointestinal tract, causing thereby various disturbances of gastrointestinal tract. The effects of some of the ataractic drugs on coaxically stimulated guineapig ileum were reported earlier from this laboratory (Dandiya, 1963). The results of this study warranted similar investigation on some more ataractic drugs in an effort to elucidate their mechanism of action on intestine. The compounds studied were :

Benactyzine = 2-diethylaminoethyl ester of benzilic acid.

Pipethanate = 2-piperid-1-ylethyl benzilate.

Trifluoperazine = 10-[3-(4-methylpiperazin-1-yl) propyl]-2-trifluoromethylphenothiazine.
Rescinnamine = 3 : 4 : 5-trimethoxycinnamate ester of methylreserpate.
Carisoprodol = 2-Carbamyloxymethyl-2-isopropyl-carbamyloxymethylpentane.
Captodiamine = p-butylthiodiphenylmethyl-2-dimethylaminoethyl sulphide.
Emylcamate = Carbamate of 1-ethyl-1-methyl-1-propanol.

MATERIALS AND METHODS

The technique of Paton (1957) with modifications as reported from this laboratory earlier (Dandiya, 1963) was employed. All experiments were done on pieces of guineapig's small intestine. Guineapig was killed by a blow on the neck. The small intestine was taken out and placed in Kreb's solution. A small piece (nearly $1\frac{1}{2}''$) of the ileum was taken, emptied of its contents and tied off at both the ends to prevent the mucus in the lumen from oozing into the bath. One end of the gut piece was tied on to a glass tubing and suspended in Kreb's solution bubbled with oxygen, while the other end was clipped to a silver electrode. The capacity of bath was 50 ml. and temperature was controlled between 35°–37° C. Current pulses of 1 m. sec. duration and 4 volts to produce maximal response to a single shock were applied to the electrodes after an interval of every 15 seconds. The contractions of the gut were recorded on a smoked drum by means of a sufficiently light lever. The silver wire was attached to the lever for recording the intestinal movements.

Appropriate quantity of the drug under test was added to the bath in the form of an aqueous solution. All the drug solutions were made in distilled water excepting that of rescinnamine which was dissolved in a few drops of glacial acetic acid and the volume was made up with water. The volume of each solution added to the bath was less than 1 ml. Appropriate concentrations of Ach. were also added to the bath, so as to cause a muscle contraction almost equivalent to that induced by electrical stimulation. The sensitivity of the muscle to Ach. was always tested before and after treatment with the drug. If pretreatment with the drug did not bring any response due to control doses of Ach. then higher doses of Ach. were tried.

The doses of various drugs employed are expressed in terms of their salts.

RESULTS

Benactyzine was found to be very potent in abolishing the twitch of guineapig ileum due to electrical stimulation and in suppressing the response to Ach. In doses as low as 1 μg it was able to prevent the twitch due to electrical stimulation, response to very high doses (250 times the control dose) of Ach. was less than that due to the control dose prior to drug treatment (Fig. 1).

Pipethanate in doses as low as 1 μg was able to abolish twitch due to electrical stimulation, but response to twice the control dose of Ach. was less than that due to the control dose of Ach. prior to drug treatment (Fig. 2).

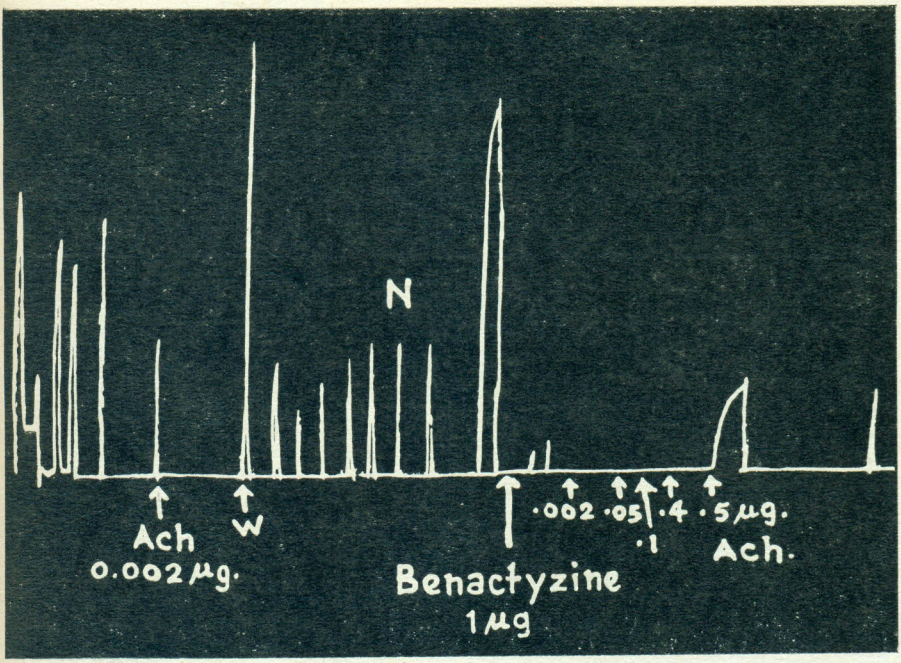

Fig. 1. The effect of benactyzine hydrochloride on twitches (4/min) and acetylcholine response of guineapig ileum. At 'N' normal twitches are shown. Acetylcholine 0.002 µg was added to the bath while electrical stimulation was temporarily stopped. At 'W' the ileum was washed. On treatment with benactyzine hydrochloride (1 µg) the twitch was reduced and then abolished. Response to acetylcholine after treatment with the drug could be elicited only with 0.5 µg.

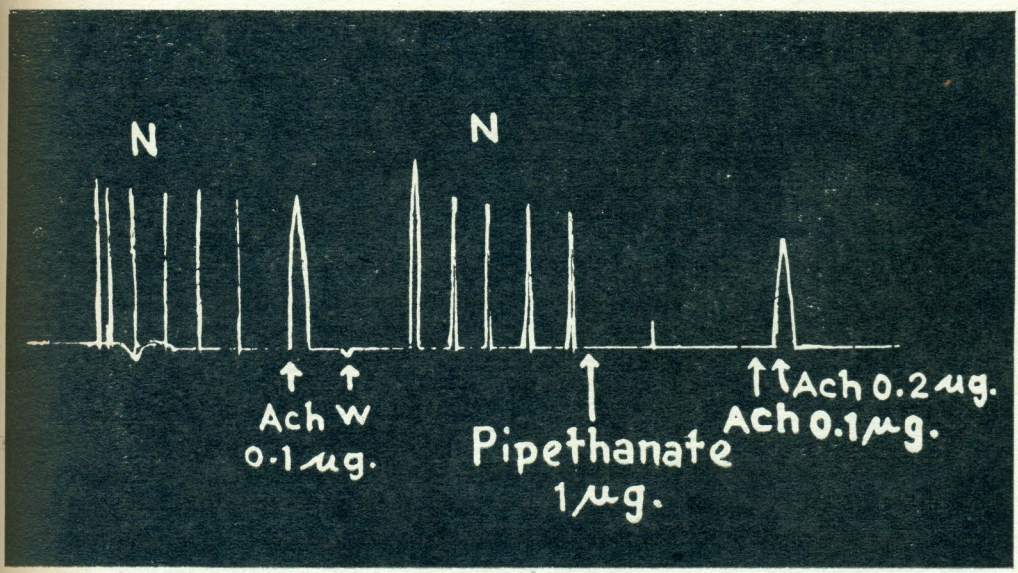

Fig. 2. The effect of pipethanate hydrochloride on twitches (4/min) and acetylcholine response of guineapig ileum. At 'N' normal twitches are shown. Acetylcholine 0.1 µg was added to the bath while electrical stimulation was temporarily stopped. At 'W' the ileum was washed. On treatment with pipethanate hydrochloride (1 µg) the twitch was first reduced and then abolished. Response to acetylcholine after treatment with the drug could be elicited only with 0.2 µg.

Captodiamine in moderate doses (30 to 100 μg) gradually abolished the twitch due to electrical stimulation, and high doses of Ach. failed to elicit response (Fig. 3).

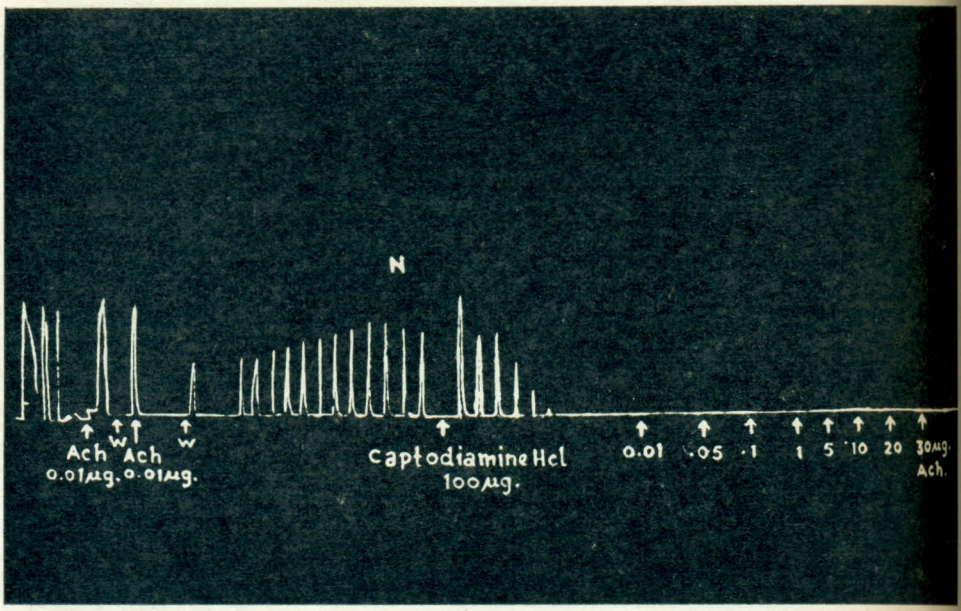

Fig. 3. The effect of captodiamine hydrochloride on twitches (4/min) and acetylcholine response of guineapig ileum. At 'N' normal twitches are shown. Acetylcholine 0.01 μg was added to the bath while electrical stimulation was temporarily stopped. At 'W' the ileum was washed. On treatment with captodiamine hydrochloride (100 μg) the twitch was reduced and gradually abolished. Response to acetylcholine after treatment with the drug could not be elicited even with 30 μg.

Trifluoperazine also had similar effect on the twitch height. In doses ranging between 30 μg to 100 μg it was able to gradually abolish the twitch due to electrical stimulation, and Ach. in high doses (20 times the control dose) could elicit response (Fig. 4).

Rescinnamine when tried in moderate doses (100 μg) abolished the twitch due to electrical stimulation. Soon after the treatment with the drug, control dose of Ach. was able to produce subnormal response but after a lapse of 1-2 minutes only exceptionally high doses of Ach. (2500 times the control dose) could elicit response and that too was less than due to control dose prior to treatment with the drug. But when tried in low doses (10-30 μg) rescinnamine exhibited depressant effect very gradually. After treatment with the drug for 10 minutes, twitch in response to electrical stimulation was gradually suppressed, and control dose of Ach. could elicit only subnormal response (Fig. 5).

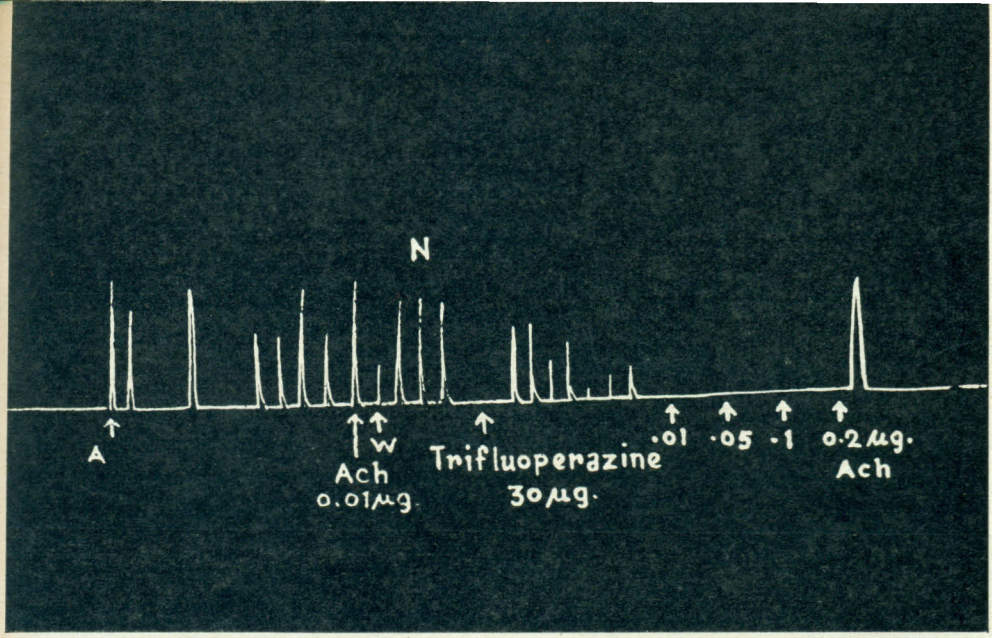

Fig. 4. The effect of trifluoperazine on the twitches (4/min) and acetylcholine response of guineapig ileum. At 'N' normal twitches are shown. Acetylcholine 0.01 µg was added to the bath while electrical stimulation was temporarily stopped. At 'W' the ileum was washed. On treatment with trifluoperazine (30 µg) the twitch was gradually reduced and then abolished. Response to acetylcholine after treatment with the drug could be elicited only with 0.2 µg.

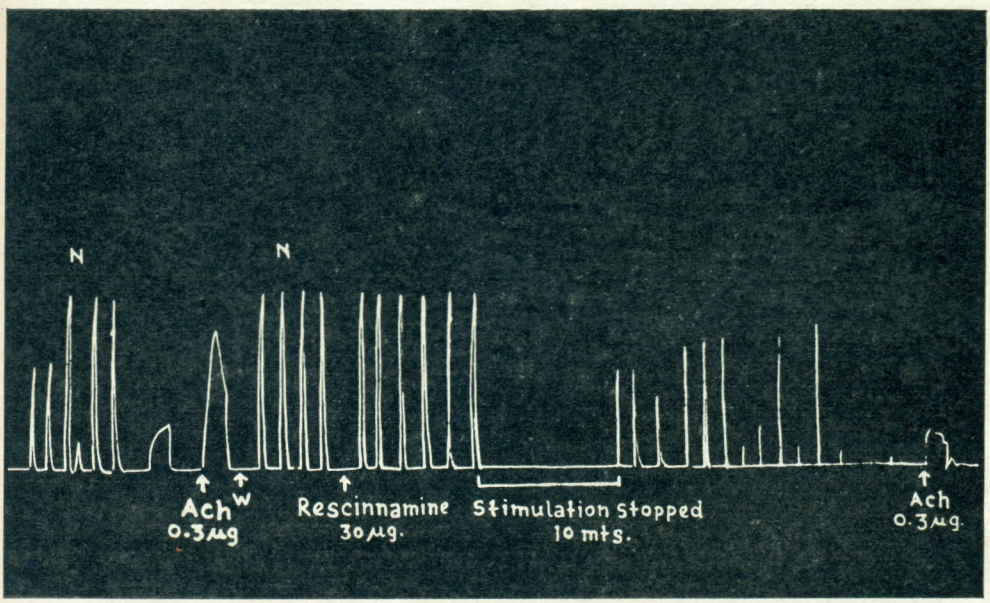

Fig. 5. The effect of rescinnamine on twitches (4/min) and acetylcholine response of guineapig ileum. At 'N' normal twitches are shown. Acetylcholine 0.3 µg was added to the bath while electrical stimulation was temporarily stopped. At 'W' the ileum was washed. On treatment with rescinnamine (30 µg) no effect on twitches was observed but after lapse of 10 minutes the twitches first became variable and reduced, and then were abolished. When electrical stimulation failed to produce response, it was stopped and acetylcholine 0.3 µg was added to the bath. The response to acetylcholine obtained was considerably shorter as compared to pretreatment response.

Carisoprodol in moderate doses (100 μg) had no effect on the twitch height due to electrical stimulation. In this property it resembles with another dicarbamate derivative meprobamate as reported earlier.

Emylcamate in doses ranging between 100 μg to 300 μg had no effect on the twitch height due to electrical stimulation and on the response due to Ach. But in doses as high as 1 mg., it gradually abolished the twitch due to electrical stimulation and control doses of Ach. could elicit subnormal response. (Fig. 6).

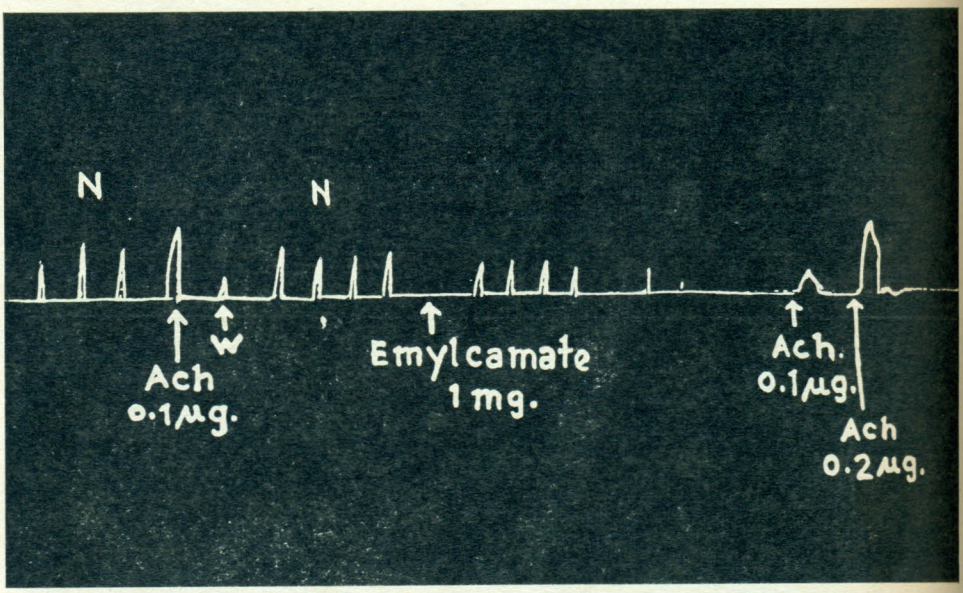

Fig. 6. The effect of emylcamate on twitches (4/min) and acetylcholine response of guineapig ileum. At 'N' normal twitches are shown. Acetylcholine 0.1 μg was added to the bath while electrical stimulation was temporarily stopped. At 'W' the ileum was washed. On treatment with emylcamate 1 mg. the twitches were gradually abolished. The twitch produced by Acetylcholine 0.1 μg was considerably shorter as compared to the contraction in untreated muscle.

DISCUSSION

Present observations when considered along with the results reported earlier from this laboratory (Dandiya, 1963), throw light on the mechanism of action of these drugs.

The combined results are classified in the Table I.

Benactyzine and pipethanate have been found to be more potent as compared to hydroxyzine, azacyclonal, and captodiamine. These latter compounds have prevented the response due to electrical stimulation only in high concentrations.

TABLE I

Class	Name	Inhibition of response to electrical stimulation	Inhibition of response to Ach.
Diphenyl methane Derivatives ...	Azacyclonal	++	++
	Benactyzine	+++++	+++++
	Pipethanate	+++++	+++
	Captodiamine	+++	++++
	Hydroxyzine	++	++
Phenothiazines ...	Chlorpromazine	++	++
	Prochlorperazine	++	++
	Trifluoperazine	++++	++++
Rauwolfia Alkaloids ...	Reserpine	++	+
	Rescinnamine	++	+
Carbamates ...	Meprobamate	0	0
	Carisoprodol	0	0
	Emylcamate	+	+
	Phenaglycodal	0	0

Key —Very Strong +++++ Strong ++++
Moderate +++ Mild ++
Very Mild + No effect 0

These results are not surprising keeping in view the fact that benactyzine and pipethanate possess potent anticholinergic action.

Among phenothiazines, trifluoperazine has been found to be depressant of intestine in doses of 30-100 μg and in this respect it has been found to be almost three times more potent than chlorpromazine and prochlorperazine. On comparing the structure and activity of the above mentioned phenothiazines, it appears that the potent action of trifluoperazine on intestine may be due to 2-trifluoromethyl substitution in the phenothiazine molecule in place of the 2-chloro group. It is well known that trifluoperazine is many times more potent in its tranquillising action than chlorpromazine, although unlike the latter compound it has not been shown to possess potent anticholinergic activity (Bradley, 1963). This anomaly therefore needs further investigation.

The pattern of action on intestine shown by reserpine and rescinnamine is more or less similar. However in doses of 100 μg the onset of inhibition demonstrated by rescinnamine as compared to that due to reserpine, is slower but more complete.

Carbamate derivatives have hardly exhibited any action worth mentioning except that of emylcamate which has shown mild depressant action. These observations on carbamates confirm the present knowledge that these drugs act centrally and there is very little direct effect of these drugs on the intestine.

In conclusion it can be stated that although like morphine and codeine, the tranquillising agents reserpine, rescinnamine, chlorpromazine, prochlorperazine, azacyclonal, and hydroxyzine were able to abolish twitch due to electrical stimulation when employed in appropriate concentrations, unlike the two analgesic agents these also appreciably prevented the response to Ach. Although these drugs do not possess any structural similarity with Ach. but the possibility of a competitive antagonism cannot be altogether ruled out.

On the other hand, some of the drugs namely benactyzine, pipethanate, captodiamine, and trifluoperazine when used in appropriate concentrations, not only abolished the twitch due to electrical stimulation but also the response due to Ach. even when tried in considerably high doses. It appears that there is likelihood that these drugs act directly on the muscle.

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Trifluoperazine Hcl. (Stelazine)	—	Smith, Kline & French Labs.
Captodiamine Hcl. (Suvren)	—	Ayerst, Mckenna & Harrison Ltd., Montreal.
Carisoprodol (Soma)	—	Wallace Labs., New Brunswick.

Pipethanate Hcl. (Sycotrol) — Reed & Carnrick, New Jersey.

Benactyzine Hcl (Suavitil) and Emylcamate — Merck Sharp & Dohme Research Lab, West Point, Pa.

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