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BLOCKADE OF ADRENERGIC AND CHOLINERGIC TRANSMISSIONS BY EMETINE

BY

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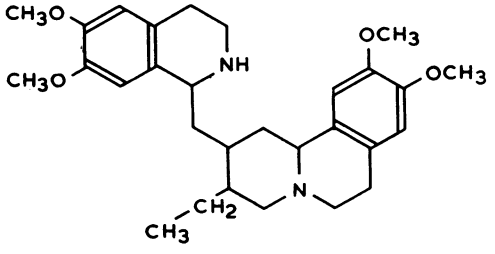
The side effects of emetine encountered in clinical practice include fall of blood pressure, diarrhoea and weakness or paralysis of skeletal muscles. These side effects resemble the effects and side effects of sympathetic blocking agents like bretylium. Emetine has the formula



which shows that it is a secondary amine. Since mecamylamine, which is also a secondary amine, has been found to act like bretylium at the sympathetic post-ganglionic terminations (Burn & Gibbons, 1964) and since mecamylamine resembles bretylium in causing block of the neuromuscular junction in skeletal muscle (Burn & Seltzer, 1965) it seemed possible that emetine might act like bretylium. In a preliminary study, Ng (1966a) showed that emetine abolished both the motor and inhibitory responses of the effector organs to stimulation of the sympathetic post-ganglionic fibres. The block was not due to an antagonism of noradrenaline. The present experiments were designed to analyse the block of adrenergic neurones by emetine, and to determine whether emetine also acts on ganglia and on the neuromuscular junction.

METHODS

The effect of emetine on the response to post-ganglionic sympathetic stimulation was studied on the Finkleman (1930) preparations of the rabbit and guinea-pig ileum and colon. The periarterial nerves were stimulated through a bipolar electrode with supra-maximal shocks (usually 20 V) of 0.5 msec at 20/sec or 50/sec for 20 to 30 sec. The transmurally stimulated Finkleman preparation (Birmingham & Wilson, 1965) of the guinea-pig ileum was taken from the small intestine at least 10 cm proximal to the ileo-caecal junction. Observations were made in 50 ml. of McEwen's solution maintained at 35° C and equilibrated with a gas mixture of 95% oxygen and 5% carbon dioxide.

In experiments on the local peristaltic reflex, the guinea-pig ileum was suspended in 50 ml. of Tyrode solution at 35° C according to the method described by Burn (1952). The solution was

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bubbled with 95% oxygen and 5% carbon dioxide. The reflex was initiated by raising the intraluminal pressure by 2 to 3 cm of Tyrode solution for 30 to 60 sec at intervals of 2 to 4 min. When only the longitudinal muscle contraction was required the guinea-pig ileum was suspended in a 15 ml. bath.

Actions at the neuromuscular junction were studied on the rat phrenic nerve-diaphragm preparations (Bülbring, 1946) mounted in a 35 ml. bath at room temperature (27–28° C). The phrenic nerve was stimulated maximally with rectangular pulses of 0.5 msec duration at the rate of 12/min. The action of emetine on the acetylcholine contraction of skeletal muscle was observed on the rectus abdominis of the local toad (*Bufo melanostictus*) in the manner described by Burn (1952).

Experiments on the nictitating membrane were performed on cats anaesthetized with ether followed by chloralose (80 mg/kg body weight). In all experiments, the vagi and the cervical sympathetic chains were sectioned. The systemic arterial pressure was measured from the carotid artery by a mercury manometer, and the drugs were given through a cannula in the femoral vein. The preganglionic fibres of the left cervical sympathetic nerve were separated from the vagus and laid across shielded electrodes for stimulation. In some animals, separation of the two nerves was continued above the superior cervical ganglion so that post-ganglionic stimulation was possible. The contraction of the nictitating membrane in response to supramaximal nerve stimulation (0.5 msec; 20/sec for 10 sec) was recorded on smoked paper with a frontal-writing lever.

The drugs used were emetine hydrochloride, noradrenaline acid tartrate, hexamethonium bromide, dopamine hydrochloride, dexamphetamine sulphate, cocaine hydrochloride, hyoscine hydrobromide, atropine sulphate, nicotine hydrogen tartrate, acetylcholine chloride and physostigmine salicylate. All drug concentrations are given in terms of their salt.

RESULTS

Rabbit ileum with periarterial sympathetic nerves

The inhibition of the pendular movement of the isolated rabbit ileum caused by the electrical stimulation of the sympathetic nerve was abolished by emetine 2×10^{-6} g/ml. (Fig. 1). The block persisted on repeated washings. Concentrations greater than 1×10^{-5} g/ml. reduced the tone and the rhythm of the pendular movement, so that most observations were made with concentrations varying from 1 to 5×10^{-6} g/ml. When the response to nerve stimulation was abolished, the direct action of noradrenaline was unaltered or potentiated.

Hexamethonium (5×10^{-5} to 1×10^{-4} g/ml.) did not affect the inhibitory response to sympathetic nerve stimulation. Neither did it prevent emetine from causing a block of the sympathetic inhibition.

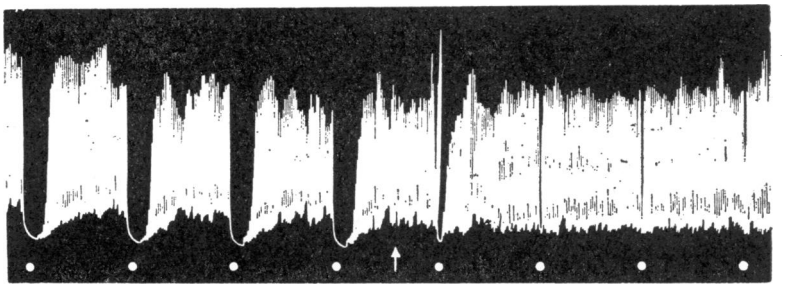

Fig. 1. Isolated rabbit ileum. At the dots, the periarterial nerves were stimulated (20 V; 0.5 msec; 50/sec for 20 sec) at intervals of 3 min. At the arrow, emetine was added in a concentration of 2×10^{-6} g/ml. The effect of stimulation was abolished at the end of 12 min.

Dopamine (1×10^{-6} to 1×10^{-5} g/ml.), dexamphetamine 1×10^{-6} to 1×10^{-5} g/ml.), and cocaine (1×10^{-6} to 5×10^{-5} g/ml.) did not restore the inhibitory response to periarterial nerve stimulation when the blockade was complete.

Rabbit colon with lumbar sympathetic nerve

Relaxation of the colon was seen in response to nerve stimulation at 20/sec or 50/sec applied for 30 sec in every 3.5 min. The effect of emetine (3×10^{-6} g/ml.) in one experiment is illustrated in Fig. 2. The inhibition produced by sympathetic nerve stimulation was gradually reduced and abolished at the end of 20 min. In this experiment, a restoration of the response was seen after washing out the drug. In most experiments, the relaxation produced by noradrenaline was greater during an emetine-induced failure of nerve stimulation than it had been initially.

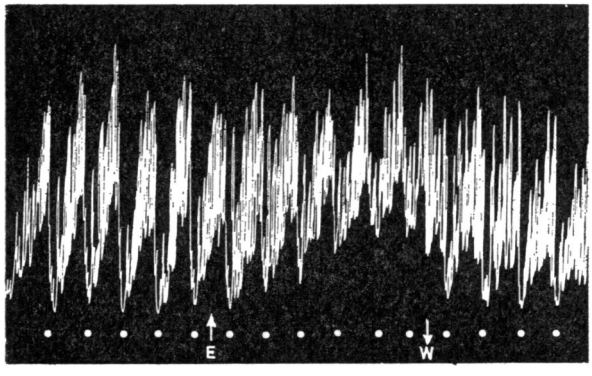

Fig. 2. Isolated rabbit colon. Periarterial nerve stimulation (20 V; 1.0 msec; 20/sec for 30 sec) was applied at the dots at intervals of 3 min. At E, emetine 3×10^{-6} g/ml. was added to the bath and, at W, the bath fluid was changed.

Guinea-pig colon with lumbar sympathetic nerves

Stimulation of the sympathetic nerve caused the colon to relax and the same response was obtained with noradrenaline (1×10^{-7} g/ml.). Emetine (1 to 4×10^{-6} g/ml.) reduced the response to sympathetic nerve stimulation and caused complete blockade after 15 to 20 min (Fig. 3).

Transmurally stimulated Finkleman preparation of the guinea-pig ileum

Munro (1953) showed that the response of the guinea-pig ileum to stimulation through the periarterial nerves was relaxation in the proximal part and contraction in the distal terminal part. Therefore observations were made from segments taken from the proximal or mid-ileum. In these preparations, stimulation of the periarterial nerves, like noradrenaline (5×10^{-7} g/ml.), lowered the tone of the preparation and inhibited the response to transmural stimulation.

Ng (1966b) showed that the contractions to transmural stimulation were unaffected by emetine (2×10^{-6} g/ml.), but the inhibition of these responses by sympathetic stimulation was abolished and replaced by augmented twitches. Observations were made to determine

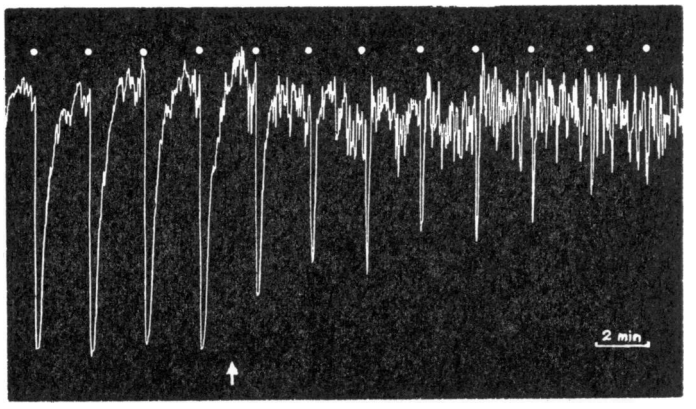

Fig. 3. Isolated guinea-pig colon. At the dots, periarterial nerve stimulation (20 V ; 0.5 msec ; 50/sec for 10 sec) was applied at intervals of 2 min. Emetine, 2×10^{-6} g/ml. added at the arrow, gradually abolished the inhibitory response.

the effect of emetine on the response to sympathetic stimulation alone. Fig. 4 shows that emetine (2×10^{-6} g/ml.) blocked the inhibitory effect of sympathetic stimulation and reversed the inhibitory response to a motor effect. The motor response was augmented by hexamethonium 1×10^{-4} g/ml., but it was abolished by hyoscine 1×10^{-7} g/ml.

Nicotine response of the guinea-pig ileum

Emetine 1 to 5×10^{-6} g/ml. did not contract the longitudinal muscle of the ileum, while 6×10^{-6} to 5×10^{-5} g/ml. reduced the tone and diminished the spontaneous movement. It depressed the response of the muscle to acetylcholine, the extent of reduction in

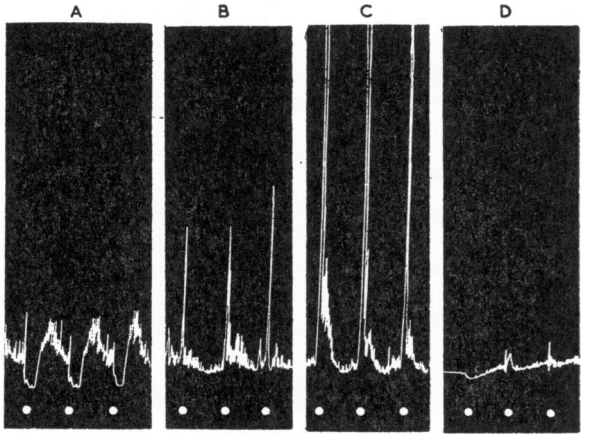

Fig. 4. Isolated guinea-pig ileum. At the dots, the periarterial nerves were stimulated (20 V ; 0.5 msec ; 50/sec for 30 sec) at intervals of 2 min. (A) shows control observations. (B) 14 min after the addition of emetine 2×10^{-6} g/ml. to the bath, nerve stimulation now gave rise to a motor response. (C) the motor response was augmented in the presence of hexamethonium 1×10^{-4} g/ml. (D) the motor response was abolished by hyoscine 1×10^{-7} g/ml.

contractility being dependent on the concentration in the bath. The minimal effective concentration was 3 μg/ml.

Both acetylcholine and nicotine stimulated the ileum to contract but, with nicotine, the onset of contraction was usually delayed for a few seconds. Low concentrations of emetine abolished the nicotine response with minimal effect on the contractions produced by acetylcholine. In the experiment illustrated in Fig. 5, 5.3×10^{-8} g/ml. of acetylcholine produced the same height of contraction as 4×10^{-6} g/ml. of nicotine. However, in the presence of 3×10^{-6} g/ml. of emetine, the nicotine contraction was almost abolished

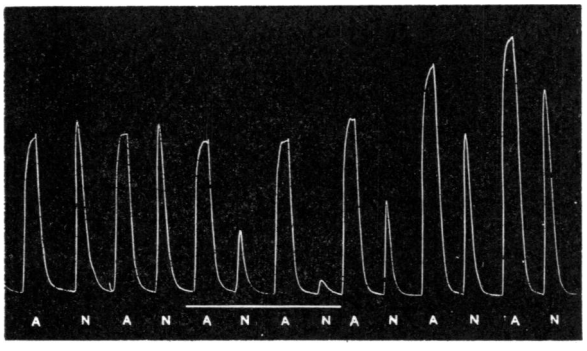

Fig. 5. Isolated guinea-pig ileum showing contractions of the longitudinal muscle to acetylcholine (A) 5.3×10^{-8} g/ml. and nicotine (N) 4×10^{-6} g/ml. for 1 min duration at 1.5 min intervals. The white line indicates observations made in the presence of emetine 3×10^{-6} g/ml. Note that emetine abolished the nicotine response without affecting the action of acetylcholine.

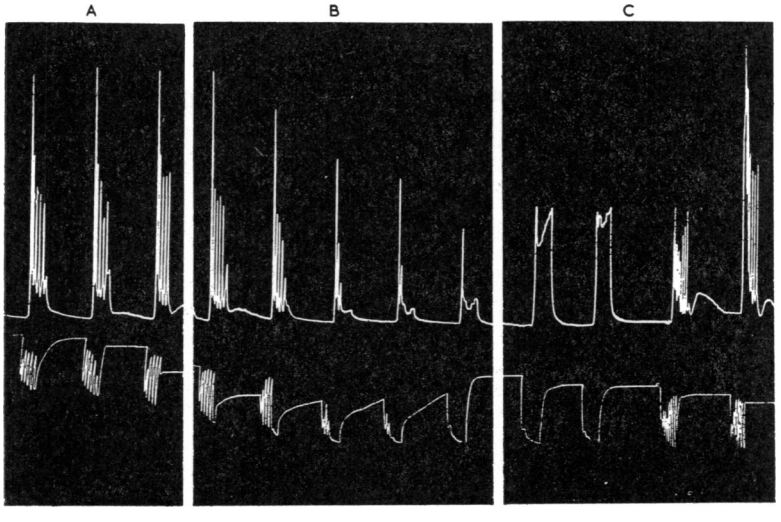

Fig. 6. Isolated guinea-pig ileum. The upper tracing records contractions of the longitudinal muscle, the lower tracing the intestinal volume. Peristalsis was initiated by raising the intraluminal pressure to 2 cm Tyrode solution for 30 sec every 2.5 min. (A) shows control observations; (B) in the presence of 5×10^{-6} g/ml. of emetine; and (C) after the drug was washed out.

while the direct action of acetylcholine remained unchanged. When emetine was washed out from the bath, the sensitivity of the tissue to acetylcholine was increased, while the response to nicotine gradually recovered.

Local peristaltic reflex of the guinea-pig ileum

Emetine 5×10^{-6} to 2×10^{-5} g/ml. had an inhibitory action on both the preparatory and emptying phases of the peristaltic reflex (Fig. 6). The effect was observed 2 to 8 min after the drug was introduced into the bath, recovery taking place within 4 to 6 min when the bath fluid was changed. In a few preparations, the contractions of the circular muscle during the emptying phase persisted until the drug was removed from the bath.

Rat isolated diaphragm with phrenic nerve

Emetine 1 to 3×10^{-4} g/ml. reduced or abolished the twitch response of the rat diaphragm to electrical stimulation of the phrenic nerve. The onset of the blockade was slow and the effect persisted even 2 hr after the drug was removed from the bath. When the response to nerve stimulation was completely abolished, direct stimulation of the muscle was still effective, though the response was not sustained and much less than before the addition of emetine to the bath (Fig. 7).

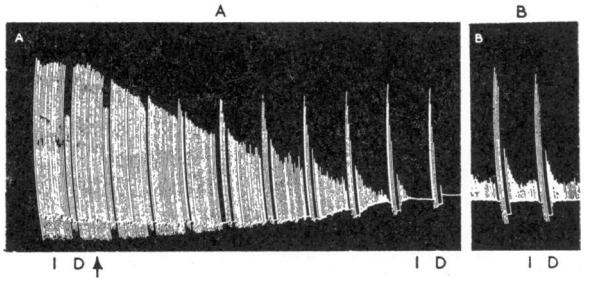

Fig. 7. Isolated rat diaphragm. Phrenic nerve stimulation is indicated by (I) and muscle stimulation by (D). In panel A, emetine 1.4×10^{-4} g/ml. was added at the arrow. Between A and B, the bath was washed repeatedly, and panel B shows observations 70 min after the drug was removed from the bath.

The blockade was not antagonized by physostigmine 1×10^{-6} to 1×10^{-5} g/ml., which normally reversed the curare-blockade completely.

Toad rectus

Low concentration (1×10^{-6} to 1×10^{-5} g/ml.) of emetine did not affect the response of the rectus to acetylcholine, while concentration greater than 3×10^{-5} g/ml. reduced the height of contraction in proportion to the dosage (Fig. 8).

Nictitating membrane of the cat

Ten experiments were done on the cat's nictitating membrane and the results were variable. In two experiments, the response of the nictitating membrane to post-

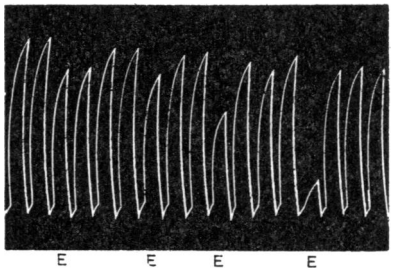

Fig. 8. Contractions of the toad rectus in response to 1.5×10^{-6} g/ml. of acetylcholine for 60 sec at 3.5 min intervals. The third contraction was made in the presence of 1×10^{-6} g/ml. of emetine, seventh contraction in 1×10^{-5} g/ml., tenth contraction in 3×10^{-5} g/ml. and fourteenth contraction in 1×10^{-4} g/ml. of emetine.

ganglionic stimulation was reduced, while the response to pre-ganglionic stimulation was abolished after 10 mg/kg of emetine was given intravenously. One of the results is illustrated in Fig. 9. In three other experiments, the response to pre-ganglionic stimulation was reduced by 20–30% at the end of 90 min and further administration of emetine caused cardiac arrest of the animals. In the remaining five experiments, no result was obtained with emetine 5 mg/kg and the cats did not survive after a further dose of emetine.

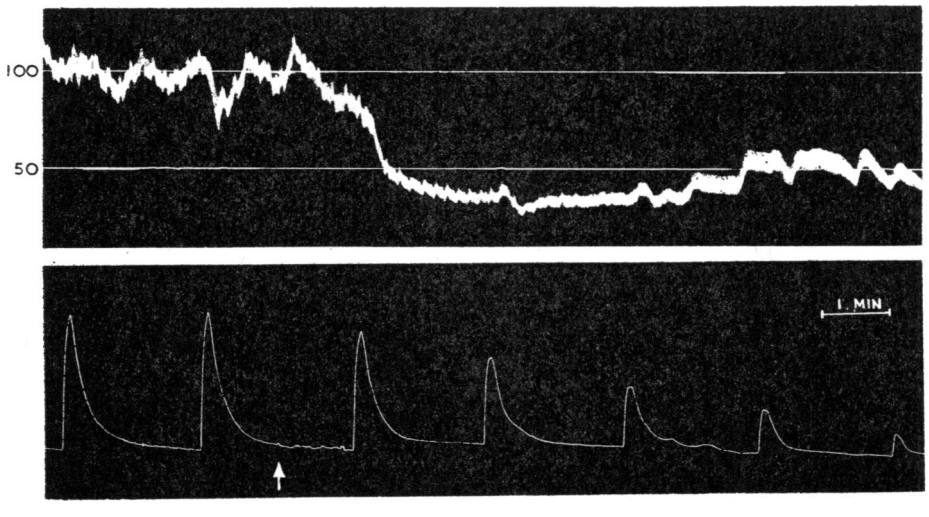

Fig. 9. Female cat 2.1 kg. Upper tracing: arterial blood pressure in mm Hg. Lower tracing: contractions of the left nictitating membrane in response to supramaximal stimulation of pre-ganglionic fibres with 0.5 msec pulses (20 V) at 20/sec for 10 sec at 2 min intervals. The cat had previously recovered from emetine 5 mg/kg. At the arrow, emetine 10 mg/kg was administered. Note the fall in blood pressure and the reduction in response of the nictitating membrane to sympathetic nerve stimulation.

DISCUSSION

The present experimental findings confirm the early observations by Ng (1966a, b) that emetine has an adrenergic neurone blocking activity. In addition to this, emetine has also been shown to have ganglion and neuromuscular blocking actions.

Like xylocholine, bretylium, and guanethidine, for example, the inhibitory response to sympathetic nerve stimulation was abolished by emetine while the inhibitory action of noradrenaline was unaltered or potentiated. However, emetine differs from these adrenergic neurone blocking agents in that the sympathetic blockade was not reversed by dexamphetamine, dopamine or cocaine (Day, 1962).

It is reasonable to suspect that emetine may cause sympathetic blockade by a local anaesthetic action. However, previous workers have shown repeatedly the irritant property of emetine. Thus Duckworth (1871) and Lowin (1903) found that emetine produced a violent inflammation on the conjunctiva. Chopra, Gupta & Pillai (1927-28) showed that emetine caused irritation of the tongue with a marked flow of saliva. Pellini & Wallace (1916) observed a local irritation following a subcutaneous or intramuscular injection of emetine into patients.

The observation of the effect of emetine on the response of the guinea-pig ileum to transmural and periarterial nerve stimulation which was previously made (Ng, 1966b), is of interest in relation to the mechanism of adrenergic neurone blockade by emetine. In this preparation, emetine blocks the response to sympathetic adrenergic stimulation without affecting the cholinergic response mediated by transmural stimulation. This suggests that emetine has a predilection for sympathetic fibres and that its action on these neurones is not due to a conventional local anaesthetic action. When the inhibitory response to sympathetic stimulation was abolished, a motor effect was revealed which was not blocked by hexamethonium but was abolished by hyoscine or atropine. The evidence suggests its origin from the post-ganglionic sympathetic fibre. On the hypothesis of Burn & Rand (1959, 1965) that the release of noradrenaline at the sympathetic nerve terminations is mediated by acetylcholine, it is possible that emetine unmasks the cholinergic link while preventing the release of noradrenaline by acetylcholine.

There have been controversial views on the action of emetine on the gastrointestinal tract. Pick & Wasicky (1916) thought that emetine being chemically related to papaverine would have a paralysing action. Epstein (1932) observed that emetine relaxed the tone of the isolated cat and rabbit ileum. On the other hand, Chopra & Ghosh (1922) found that low concentrations of emetine increased the tone of the rabbit and guinea-pig intestine while high concentrations caused an initial stimulation followed by marked depression. Chopra, Gupta & Pillai (1927-28) confirmed the stimulant action of emetine in the intestine of the cat both *in vitro* and *in vivo*. In this work, emetine was found to diminish the tone and spontaneous movement of the isolated rabbit or guinea-pig ileum. In the latter, it reduced the action of acetylcholine, abolished the nicotine response and blocked both the emptying and preparatory phases of the local peristaltic reflex. Boura & Green (1959) and Kosterlitz & Lees (1961) showed a similar action with bretylium on the peristaltic reflex of the guinea-pig ileum.

Though the effect of emetine on the response of the cat's nictitating membrane to pre- and post-ganglionic stimulations was variable, the evidence derived from the

inhibitory action of emetine on the nicotine response and on the peristalsis of the guinea-pig ileum is strongly suggestive of its ganglion-blocking activity.

High concentrations of emetine antagonized the action of acetylcholine on the toad rectus and caused a curare-like paralysis of the rat diaphragm to nerve stimulation. But, unlike curare, the paralysis was not antagonized by physostigmine. This observation is consistent with that of Dixit, Gulati & Gokhale (1961) who showed that the neuromuscular blockade by bretylium was not reversed by physostigmine.

There are reports of muscular weakness or paralysis following the use of emetine in clinical practice. Brown (1935) suggested that the neuromuscular manifestations represented a form of " neuritis." In animal experiments, however, Young & Tudhope (1926) failed to find evidence of inflammation along the course of the nerve or sheath, but they observed swelling and cellular degeneration of the skeletal muscle. Klatskin & Friedman (1948) attributed the syndrome to a primary disturbance of the muscle rather than to a disturbance of the nervous system. On the present experimental findings this clinical condition is probably due to the action of emetine at or near the neuromuscular junction.

SUMMARY

1. Emetine blocked the response of the isolated rabbit and guinea-pig intestines to stimulation of the sympathetic adrenergic nerves but did not alter the inhibitory effects of added noradrenaline.

2. In the presence of hexamethonium, which did not reduce the inhibitory response to sympathetic nerve stimulation, emetine still caused sympathetic blockade.

3. The blockade was not reversed by dopamine, dexamphetamine or cocaine.

4. Emetine abolished the inhibitory effect of sympathetic stimulation on the guinea-pig ileum, revealing a motor effect which was not blocked by hexamethonium but was blocked by hyoscine or atropine.

5. Emetine reduced the response of the cat's nictitating membrane to post-ganglionic stimulation and abolished the response to pre-ganglionic stimulation.

6. The action of acetylcholine on the guinea-pig ileum was not affected by a low concentration of emetine which abolished the nicotine response, but it was antagonized by a high concentration of emetine.

7. Emetine inhibited both the preparatory and emptying phases of the local peristaltic reflex of the guinea-pig ileum.

8. Emetine blocked the response of the isolated rat diaphragm to stimulation of the phrenic nerve and depressed the response to direct stimulation.

9. Emetine antagonized the action of acetylcholine on the toad rectus.

10. The results indicate a blocking action of emetine on the adrenergic neurones, the ganglia and the neuromuscular junction.

11. It is suggested that emetine blocks the release of noradrenaline by acetylcholine at the adrenergic nerve endings.

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REFERENCES

BIRMINGHAM, A. T. & WILSON, A. B. (1965). An analysis of the blocking action of Dimethylphenylpiperazinium iodide on the inhibition of isolated small intestine produced by stimulation of the sympathetic nerves. *Br. J. Pharmac. Chemother.*, **24**, 375–386.

BOURA, A. L. A. & GREEN, A. F. (1959). The actions of bretylium: adrenergic neurone blocking and other effects. *Br. J. Pharmac. Chemother.*, **14**, 536–548.

BROWN, P. W. (1935). Results and dangers in the treatment of amebiasis. A summary of fifteen years' clinical experience at the Mayo Clinic. *J. Am. med. Ass.*, **105**, 1319–1325.

BÜLBRING, E. (1946). Observations on the isolated phrenic nerve diaphragm preparation of the rat. *Br. J. Pharmac. Chemother.*, **1**, 38–61.

BURN, J. H. (1952). *Practical Pharmacology*. Blackwell: Oxford.

BURN, J. H. & GIBBONS, W. R. (1964). The sympathetic postganglionic fibre and the block by bretylium; the block prevented by hexamethonium and imitated by mecamylamine. *Br. J. Pharmac. Chemother.*, **22**, 549–557.

BURN, J. H. & RAND, M. J. (1959). Sympathetic post-ganglionic mechanism. *Nature, Lond.*, **184**, 163–165.

BURN, J. H. & RAND, M. J. (1965). Acetylcholine in adrenergic transmission. *A. Rev. Pharmac.*, **5**, 163–182.

BURN, J. H. & SELTZER, J. (1965). Substances blocking sympathetic post-ganglionic fibres and the neuromuscular junction. *J. Physiol., Lond.*, **179**, 569–576.

CHOPRA, R. N. & GHOSH, B. N. (1922). The therapeutics of emetine. *Indian med. Gaz.*, **57**, 248–253.

CHOPRA, R. N., GUPTA, J. C. & PILLAI, K. V. (1927–28). Observations on the action of emetine on the gastro-intestinal tract. *Indian J. med. Res.*, **15**, 883–888.

DAY, M. D. (1962). Effect of sympathomimetic amines on the blocking action of guanethidine, bretylium and xylocholine. *Br. J. Pharmac. Chemother.*, **18**, 421–439.

DIXIT, B. N., GULATI, O. D. & GOKHALE, S. D. (1961). Action of bretylium and guanethidine at the neuromuscular junction. *Br. J. Pharmac. Chemother.*, **17**, 372–379.

DUCKWORTH, D. (1871). Observations upon the action of ipecacuanha and its alkaloid emetia. *St. Bart's Hosp. Rep.*, **7**, 91–125.

EPSTEIN, D. (1932). The actions of emetine and cephaeline on the circulation, uterus and intestine. *Q. Jl. Pharm. Pharmac.*, **5**, 21–32.

FINKLEMAN, B. (1930). On the nature of inhibition in the intestine. *J. Physiol., Lond.*, **70**, 145–157.

KLATSKIN, G. & FRIEDMAN, H. (1948). Emetine toxicity in man: studies on the nature of early toxic manifestations, their relation to the dose level, and their significance in determining safe dosage. *Ann. intern. Med.*, **28**, 892–915.

KOSTERLITZ, H. W. & LEES, G. M. (1961). Action of bretylium on the isolated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **17**, 82–86.

LOWIN, C. (1903). Beiträge zur kenntnis der ipecacuanha; ueber die ipecacuanha-alkaloide. *Archs. int. Pharmacodyn. Ther.*, **11**, 9–55.

MUNRO, A. F. (1953). Effect of autonomic drugs on the responses of isolated preparations from the guinea-pig intestine to electrical stimulation. *J. Physiol., Lond.*, **120**, 41–52.

NG, K. K. F. (1966a). A new pharmacological action of emetine. *Br. med. J.*, **i**, 1,278–1,279.

NG, K. K. F. (1966b). An adrenergic neurone blocking action of emetine. *J. Pharm. Pharmac.*, **18**, 255–256.

PELLINI, E. J. & WALLACE, G. B. (1916). The pharmacology of emetin. *Am. J. med. Sci.*, **152**, 325–336.

PICK, E. P. & WASICKY, R. (1916). Zur pharmakologischen analyse des emetins. *Arch. exp. Path. Pharmak.*, **80**, 147–160.

YOUNG, W. A. & TUDHOPE, G. R. (1926). The pathology of prolonged emetin administration. *Trans. R. Soc. trop. Med. Hyg.*, **20**, 93–99.