Brit. J. Pharmacol. (1963), **20**, 399–417.

THE ACTION OF DRUGS ON THE CIRCULAR MUSCLE STRIP FROM THE GUINEA-PIG ISOLATED ILEUM

BY

J. HARRY

From the Department of Pharmacology, King's College, Strand, London, W.C.2

(*Received November* 15, 1962)

The circular muscle strip is a new preparation for examining the action of drugs on the circular muscle of the guinea-pig isolated intestine. The preparation differed from the longitudinal muscle in that it was insensitive to drugs which act on autonomic effector tissues but, after inhibition of cholinesterase, it responded readily to choline esters, 5-hydroxytryptamine, histamine and nicotine. This behaviour necessitated the treatment of each strip with the anticholinesterase *NN*-diisopropylphosphodiamidic fluoride (mipafox) before each experiment. The contractions of the strip by 5-hydroxytryptamine, histamine and nicotine were abolished by procaine, botulinum toxin (Type A), morphine and hemicholinium, whilst the actions of acetylcholine and methacholine were unaffected. Contractions of the strip in response to each of the drugs were abolished by atropine and hyoscine. The action of nicotine was specifically antagonized by hexamethonium, that of 5-hydroxytryptamine by desensitization of the tissue to 5-hydroxytryptamine, and that of histamine either by desensitization of the tissue to histamine or by mepyramine. It is postulated that 5-hydroxytryptamine, histamine and nicotine stimulate specific receptor sites within the intramural nerve plexuses of the guinea-pig isolated ileum. Finally, botulinum toxin (Type A), morphine or hemicholinum, acting on the neuronal elements of the intramural plexuses, depressed the contractions of the circular muscle strip due to histamine or nicotine more readily than those due to 5-hydroxytryptamine.

The activity of the circular muscle coat of the isolated intestine was first reliably recorded by Trendelenburg in 1917. He measured the changes in intraluminal pressure which accompanied the peristaltic reflex and described two phases. During the first, or preparatory phase, the longitudinal muscle contracts without fluid expulsion, whilst in the second, or emptying phase, a contraction ring of circular muscle travels in an aboral direction forcing out the fluid contents. The method has since been applied in two ways ; to discover the nervous mechanisms involved in the emptying phase (Feldberg & Lin, 1949a ; Kosterlitz, Pirie & Robinson, 1956 ; Kosterlitz & Robinson, 1957 ; Bülbring, Lin & Schofield, 1958) and to examine the effect of drugs on the nervous plexus network of the isolated intestine (Paton & Zaimis, 1949 ; Schaumann, 1955, 1957 ; Ginzel, 1957).

Evans & Schild (1953) used the method with the plexus-free circular muscle of the cat duodenum whereas Beleslin & Varagić (1958) and Bülbring & Crema (1958) examined the involvement of 5-hydroxytryptamine in the peristaltic reflex. The

technique has one disadvantage for agonist-antagonist experiments, and this is the complication introduced by the physiological reactions to a positive pressure in the lumen of the intestinal segment. The circular muscle strip preparation avoids this difficulty.

Others have used strips of cat and dog intestine (Gunn & Underhill, 1914 ; Young, 1914 ; Evans & Underhill, 1923 ; Gasser, 1926) but with the object of examining the involvement of the intramural nerve plexuses of circular muscle in spontaneous activity. More recently, Evans & Schild (1953) used strips of plexus-free circular muscle to examine the action of drugs thought to act upon the intramural plexuses of the intestine.

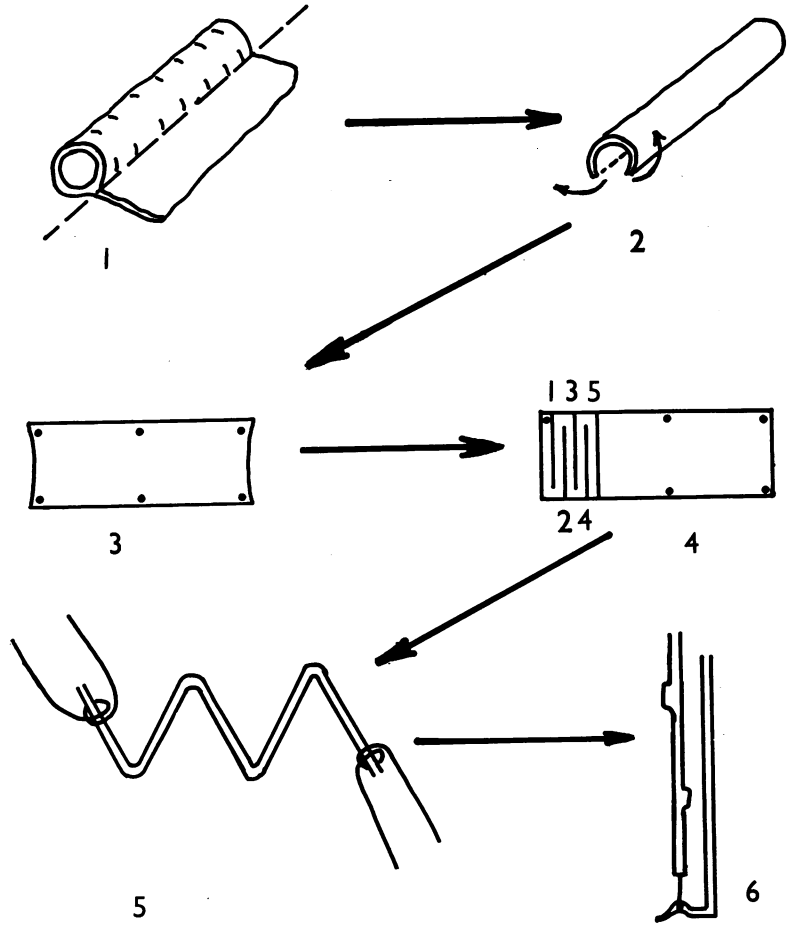

Fig. 1. The preparation of the circular muscle strip. 1: a 1·5 cm length of ileum was taken about 15 cm from the ileo-caecal junction, with its mesentery attached. 2: the mesentery was removed and the segment opened along the mesenteric border. 3: the ileum was pinned out on cork under Krebs solution with the mucosal surface uppermost. 4: the segment was cut at right-angles to its long axis as shown until five cuts were made. The thickness of the strands between the cuts was 3 to 4 mm. 5: ligatures were tied to each end of the strip. 6: one of the ligatures was tied to a glass holder, and the other to a metal hook.

The circular muscle strip from the guinea-pig intestine has been used in this study to examine the site of action of those drugs which influence it.

METHODS

Segments 1.5 cm in length were taken from the ileum 15 cm from the ileo-caecal junction. A segment was opened by a longitudinal incision through the wall along its mesenteric border, and the resultant rectangle of ileum was pinned out under Krebs solution with the mucosal surface upwards. A strip was produced by cutting the rectangle in the direction of the circular muscle fibres (Fig. 1). Usually five cuts produced a strip of circular muscle of sufficient length but, depending on the width of the ileum, four or six cuts may be made. A cotton ligature was tied around each end of the strip, one of which was tied to a glass holder and the other to a small metal hook. The holder was immersed in an organ-bath containing 23 ml. of Krebs solution at 37° C and bubbled with 95% oxygen and 5% carbon dioxide. The metal hook was attached to an isotonic lever with a frontal writing point. The load on the tissue was 290 mg; the response was magnified six times.

Two such circular muscle strips, one to serve as a control, were usually prepared under identical conditions and placed in two organ-baths, from adjacent portions of the same ileum.

Drug injection cycle. A drug was added to the bath fluid from a syringe or pipette and left in contact with the tissue for 1 min, after which the bath fluid was changed. After a further minute the bath fluid was changed once more and after another minute the drug was added to the bath fluid again, so making a 3 min drug injection cycle. When studying the effect of an antagonistic drug, the tissue was first exposed to the antagonistic compound for a given time, usually 30 min, and then the compound was added to the bath fluid each time this was changed.

Drugs. The drugs used were: acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate, histamine phosphate, nicotine acid tartrate, *NN*-diisopropylphosphodiamidic fluoride (mipafox), atropine sulphate, hyoscine hydrobromide, procaine hydrochloride, hexamethonium bromide, dimethylphenylpiperazinium iodide, botulinum toxin (Type A), morphine sulphate, hemicholinium hydrochloride and mepyramine maleate. All drugs were dissolved in Krebs solution, the lower concentrations made freshly each day from concentrated stock solutions in distilled water. Drug concentrations are expressed as μg/ml. referring to the final bath concentration of the bases, with the exception of mipafox, hyoscine, dimethylphenylpiperazinium, botulinum toxin, morphine and hemicholinium, which are expressed as the salts. The dried botulinum toxin was suspended in Krebs solution.

RESULTS

The effect of mipafox on the circular muscle strip and on its responses to acetylcholine, 5-hydroxytryptamine, histamine and nicotine

Mipafox (100 μg/ml.) appeared to have no direct muscarinic action on the circular muscle strip since no activity was seen until the tissue had been in contact with the drug for 30 min. Then there occurred a gradual increase in the spontaneous activity of the preparation (Fig. 2, upper record); this was reduced by atropine (0.5 μg/ml.) (Fig. 2, lower record).

Before inhibition of cholinesterase, the strip responded only to acetylcholine in high concentration and not to 5-hydroxytryptamine, histamine or nicotine (Fig. 3). Both test and control strips responded to acetylcholine (10 to 100 μg/ml.) but, after exposure of the test strip to mipafox (100 μg/ml.) for 90 min, it responded readily to acetylcholine (0.025 to 0.2 μg/ml.), 5-hydroxytryptamine (0.5 to 4.0 μg/ml.), histamine (1.0 to 4.0 μg/ml.) and nicotine (1.0 to 4.0 μg/ml.).

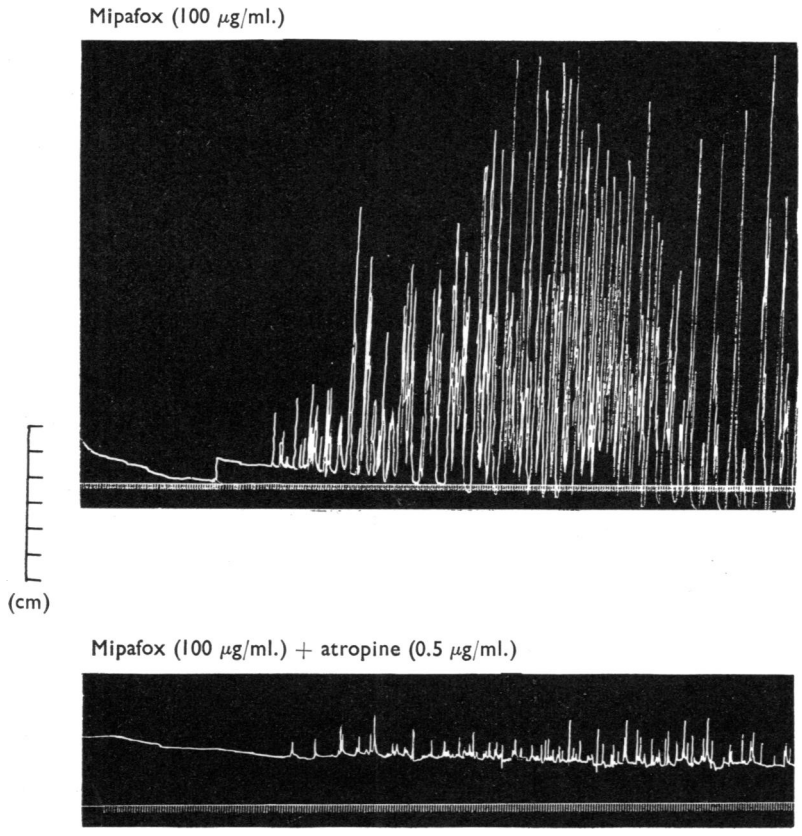

Fig. 2. The upper record shows the response of a circular muscle strip to mipafox (100 μg/ml.) added to the bath at the start of the trace and remaining in the bath throughout. The lower record shows the response of a strip taken from the adjacent portion of the same ileum, exposed to mipafox (100 μg/ml.) and atropine (0.5 μg/ml.) together. Time signal, 30 sec.

From Fig. 3 it may be seen that the response of the circular muscle strip to acetylcholine has been potentiated about 4,000 times. No evaluation of the potentiation of the responses of the strip to 5-hydroxytryptamine, histamine or nicotine could be made because the tissue did not respond to these drugs before treatment with mipafox. The control strip, to which no anticholinesterase was added, showed no change in its response to these compounds. Each preparation described in the remainder of the results was exposed to mipafox (100 μg/ml.) for 90 min before experiments were made.

The effects of atropine and hyoscine on the responses of the circular muscle strip

Exposure to atropine or hyoscine (0.1 μg/ml., Fig. 4) for 30 min abolished the responses of the test strip to acetylcholine, 5-hydroxytryptamine, histamine and nicotine. The control strip (Fig. 4), to which no antagonist was added, showed little change in its responses to these compounds.

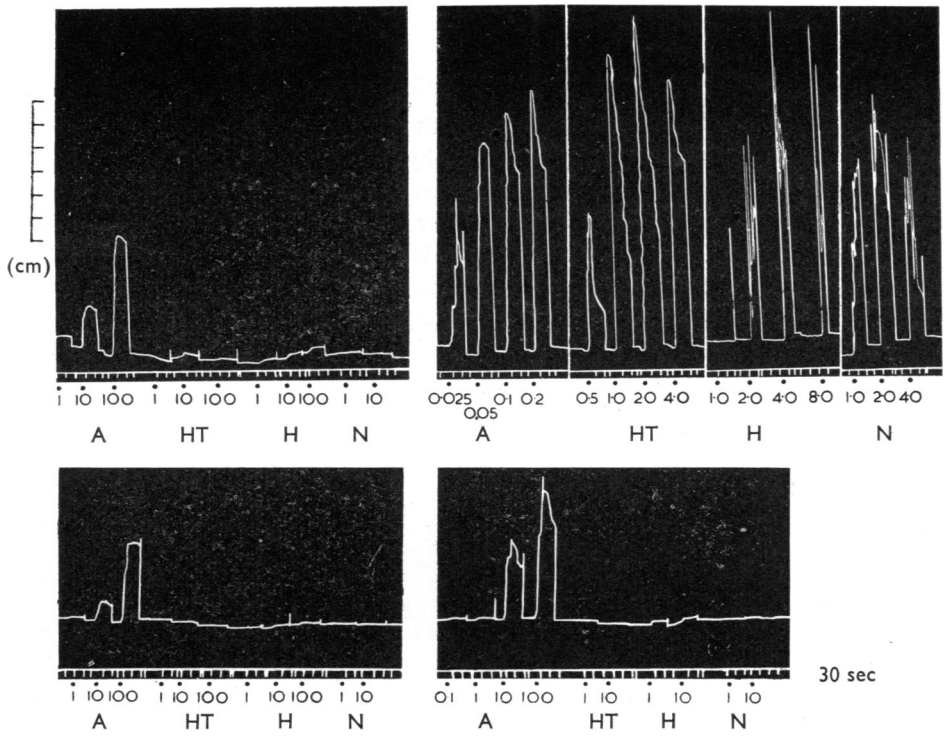

Fig. 3. The effect of mipafox on the responses of a circular muscle strip to acetylcholine(A), 5-hydroxytryptamine (HT), histamine (H) and nicotine (N). The upper record is that of the test strip, and the lower one that of the control strip. The left-hand panels show the responses of both strips. The upper right-hand panel shows the responses of the test strip to the compounds after exposure of the tissue to mipafox (100 μg/ml.) for 90 min. The lower right-hand panel shows the responses of the control strip to which no mipafox was added. Numbers, in this and subsequent Figs., refer to drug concentrations expressed as μg/ml. of bath fluid. Time signal, 30 sec.

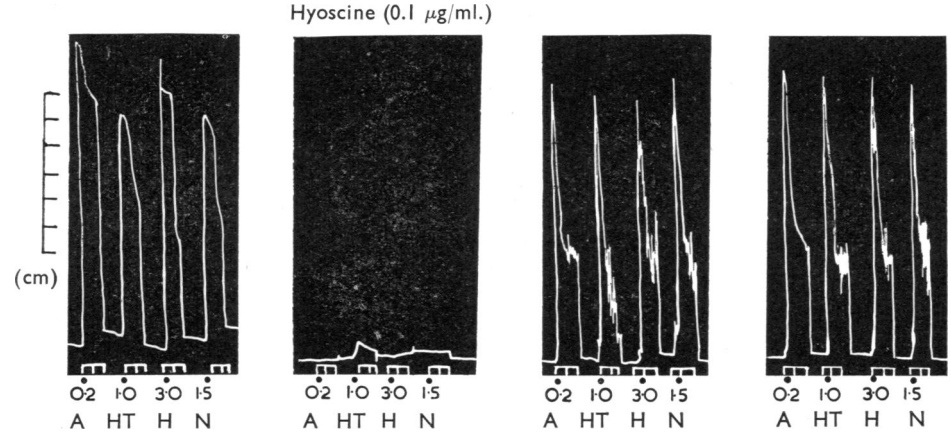

Fig. 4. The effect of hyoscine hydrobromide on the responses of a circular muscle strip to acetylcholine (A), 5-hydroxytryptamine (HT), histamine (H) and nicotine (N). The two left-hand panels show the responses of the test strip, before and in the presence of hyoscine hydrobromide (0.1 μg/ml.). The two right-hand panels show the responses of the control strip, to which no hyoscine was added, over the same time period. Time signal, 30 sec.

The effect of procaine on the responses of the circular muscle strip

Procaine (100 μg/ml.) was left in contact with the circular muscle strip for 30 min. This reduced the responses to acetylcholine, 5-hydroxytryptamine and methacholine, but after increasing the concentration of agonist five times the responses were again produced by acetylcholine and methacholine but not by 5-hydroxytryptamine (Fig. 5). The effects of nicotine also were abolished by treatment with procaine. The control strip showed no change in its responses to these compounds. In other experiments, the response of the strip to histamine was abolished by procaine (50 μg/ml.), and increasing the concentration of histamine did not overcome the blockade.

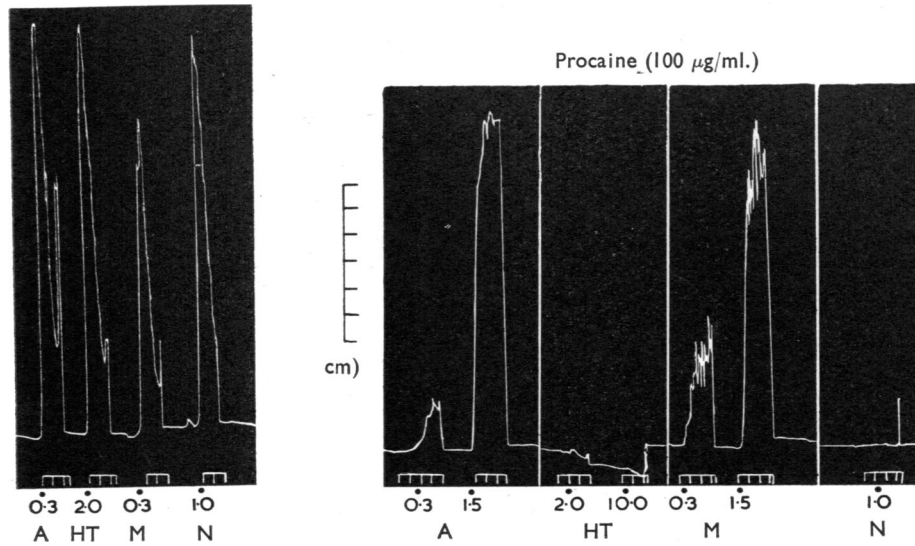

Fig. 5. The effect of procaine on the responses of a circular muscle strip to acetylcholine (A), 5-hydroxytryptamine (HT), methacholine (M) and nicotine (N). The left-hand panel shows the responses of the strip, and the right-hand panel the responses in the presence of procaine (100 μg/ml.). The record of the control strip has been omitted. Time signal, 30 sec.

The effects of botulinum toxin (Type A), morphine and hemicholinium on the responses of the circular muscle strip

Botulinum toxin can reduce the amount of acetylcholine released from the cholinergic nerve endings in the walls of the guinea-pig ileum (Harry, 1962). After exposure of the circular muscle strip for 3 hr to 1×10^6 mean lethal doses/mouse/ml. of botulinum toxin (Type A) and then removing the excess toxin from the bath fluid, the tissue responded to acetylcholine and methacholine but not to 5-hydroxytryptamine, histamine or nicotine (Fig. 6). The responses of the control strip showed little change during this time. On another strip from a different guinea-pig, exposure of the tissue to 6×10^5 mean lethal doses/mouse/ml. of botulinum toxin (Type A) for 3 hr abolished the responses produced by histamine and nicotine, whilst the response to 5-hydroxytryptamine was reduced and that to acetylcholine unaffected (Fig. 7).

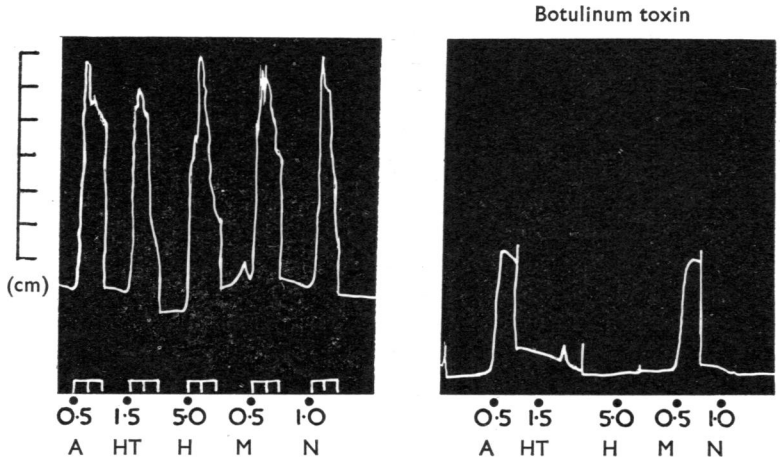

Fig. 6. The effect of botulinum toxin (Type A) on the responses of a circular muscle strip to acetylcholine (A), 5-hydroxytryptamine (HT), histamine (H), methacholine (M) and nicotine (N). The left-hand panel shows the responses of the strip, and the right-hand panel the responses after exposure of the tissue to 1×10^6 mouse mean lethal dose of botulinum toxin/ml. The record of the control strip has been omitted. Time signal, 30 sec.

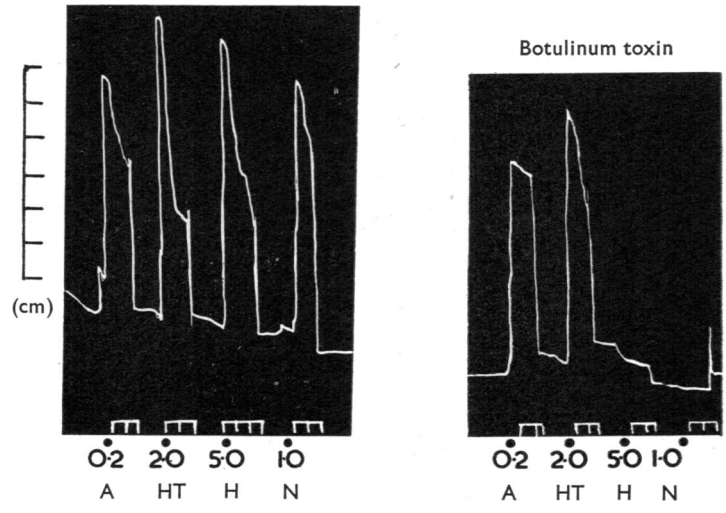

Fig. 7. The effect of botulinum toxin (Type A) on the responses of a circular muscle strip to acetylcholine (A), 5-hydroxytryptamine (HT), histamine (H) and nicotine (N). The left-hand panel shows the responses of the strip, and the right-hand panel the responses after exposure of the tissue to 6×10^5 mouse mean lethal doses of botulinum toxin/ml. The record of the control strip has been omitted. Time signal, 30 sec.

Morphine also reduces the release of acetylcholine from cholinergic nerve endings in the smooth muscle of the guinea-pig ileum (Paton, 1957). After exposure of the circular muscle strip to morphine (1.0 μg/ml.) for 30 min and in its presence, the responses of the tissue to histamine and nicotine were reduced and the responses to 5-hydroxytryptamine and acetylcholine were unaffected (Fig. 8). Higher concentrations of morphine (5.0 μg/ml.) abolished the responses of the tissue to histamine and nicotine, reduced that to 5-hydroxytryptamine and did not affect that to acetylcholine. By increasing the concentration of morphine still further to 50 μg/ml., the response of the strip to 5-hydroxytryptamine was further reduced but that to acetylcholine remained unchanged (Fig. 8). The control strip, to which no morphine had been added, showed little change in its responses to these compounds during the same time period.

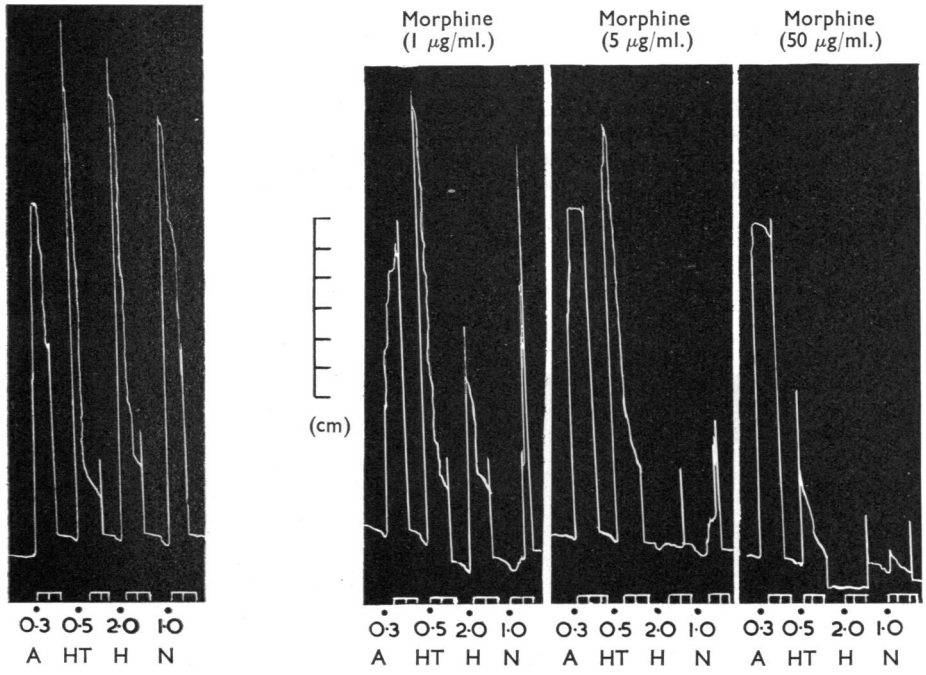

Fig. 8. The effect of increasing concentrations of morphine on the responses of a circular muscle strip to acetylcholine (A), 5-hydroxytryptamine (HT), histamine (H) and nicotine (N). The left-hand panel shows the responses of the circular strip and the three right-hand panels show the responses in the presence of morphine (1.0 μg/ml., 5.0 μg/ml. and 50.0 μg/ml.). The record of the control strip has been omitted. Time signals, 30 sec.

Dose/response curves (Fig. 9) were constructed from the contractions of the circular muscle strip to acetylcholine, 5-hydroxytryptamine, histamine and nicotine, in the presence of several concentrations of morphine (0.1 to 10.0 μg/ml.). The dose/response curves for acetylcholine were least affected ; those for 5-hydroxytryptamine were less affected than those for histamine and nicotine.

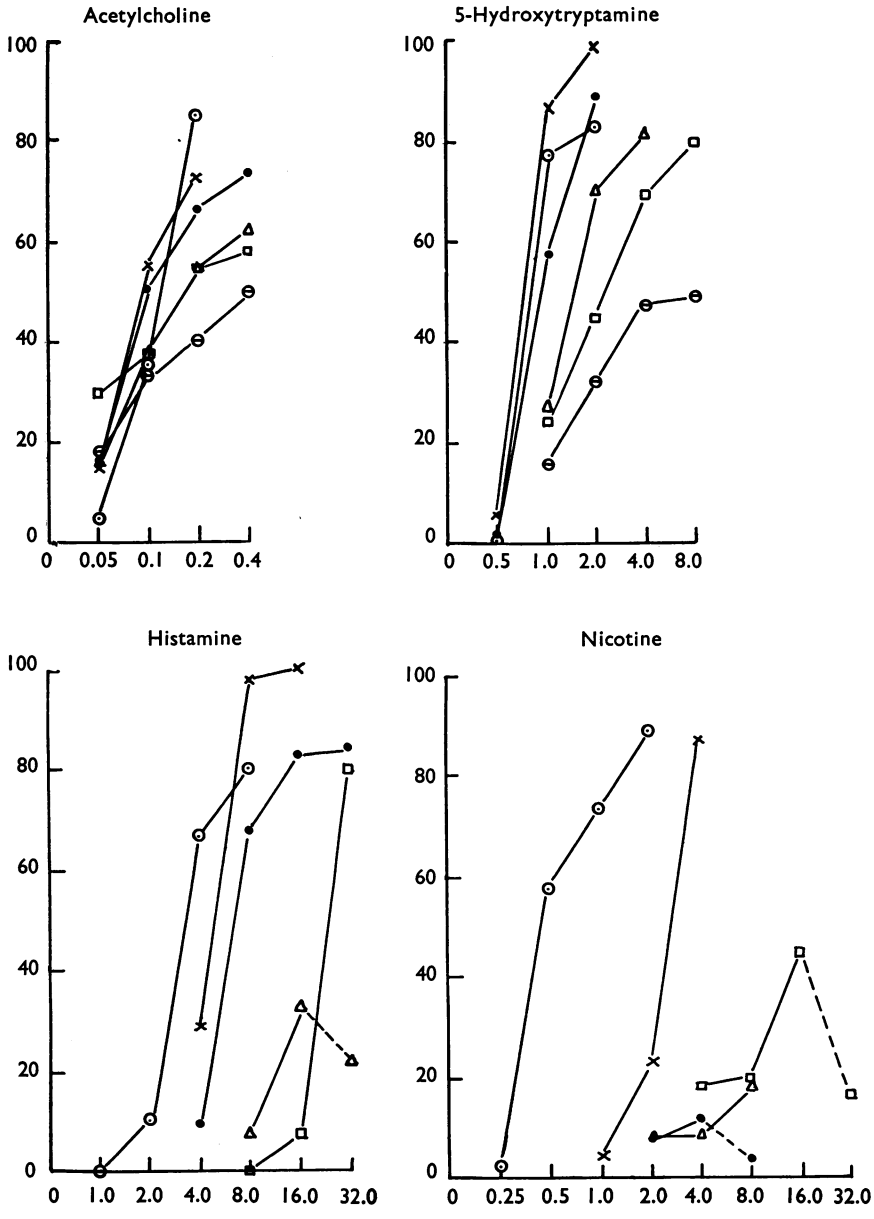

Fig. 9. The effect of increasing concentrations of morphine on dose/response curves to acetylcholine, 5-hydroxytryptamine, histamine and nicotine, obtained from the circular muscle strip. Abscissae=$\log_2$ concentration of the agonists. Ordinates=contractions expressed as percentages of maxima. ⊙——⊙, no morphine in the bath fluid; x——x, 0.1 μg/ml.; ●——●, 0.5 μg/ml.; Δ——Δ, 1.0 μg/ml.; □——□, 5.0 μg/ml.; ⊖——⊖, 10.0 μg/ml. of morphine in the bath fluid.

Hemicholinium inhibits the synthesis of acetylcholine in nervous tissue (MacIntosh, Birks & Sastry, 1956). The circular muscle strip was exposed to hemicholinium (40 μg/ml.) for 90 min and the bath was then washed out. Only the response of the tissue to nicotine was abolished (Fig. 10). After exposure to twice the dose of

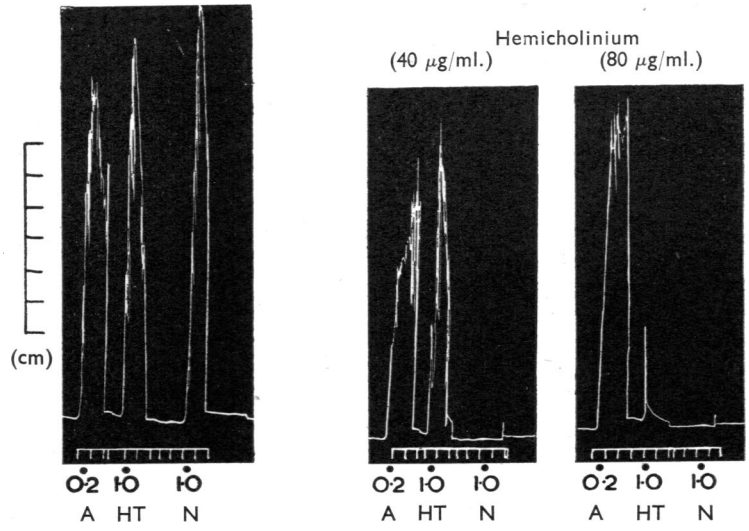

Fig. 10. The effect of hemicholinium on the responses of a circular muscle strip to acetylcholine (A), 5-hydroxytryptamine (HT) and nicotine (N). The left-hand panel shows the responses of the strip, and the two right-hand panels show the responses in the presence of hemicholinium (40 μg/ml. and 80 μg/ml.). The control strip record has been omitted. Time signal, 30 sec.

hemicholinium (80 μg/ml.) for a further 30 min, the response of the strip to 5-hydroxytryptamine was also abolished but that to acetylcholine was unaffected (Fig. 10). The control strip showed little change in its responses to these compounds over the same time. On another preparation, the response to histamine also was abolished by hemicholinium.

The effect of ganglionic blocking agents on the responses of the circular muscle strip

Paton & Zaimis (1949) showed that hexamethonium could block ganglionic transmission. Hexamethonium (50 μg/ml. for 30 min) antagonized the response of the strip to nicotine whilst not affecting the responses to acetylcholine, 5-hydroxytryptamine or histamine (Fig. 11). The control strip, to which no hexamethonium had been added, showed no change in its response to nicotine. On another strip dimethylphenylpiperazinium (5.0 μg/ml.) antagonized the stimulatory actions only of nicotine and of dimethylphenylpiperazinium (Fig. 12).

The effects of high (blocking) concentrations of 5-hydroxytryptamine and of histamine on the responses of the circular muscle strip

Gaddum (1953) showed that high concentrations of 5-hydroxytryptamine blocked responses to 5-hydroxytryptamine on the guinea-pig ileum. 5-Hydroxytryptamine (10 μg/ml.) produced a strong stimulation of the circular muscle strip with a

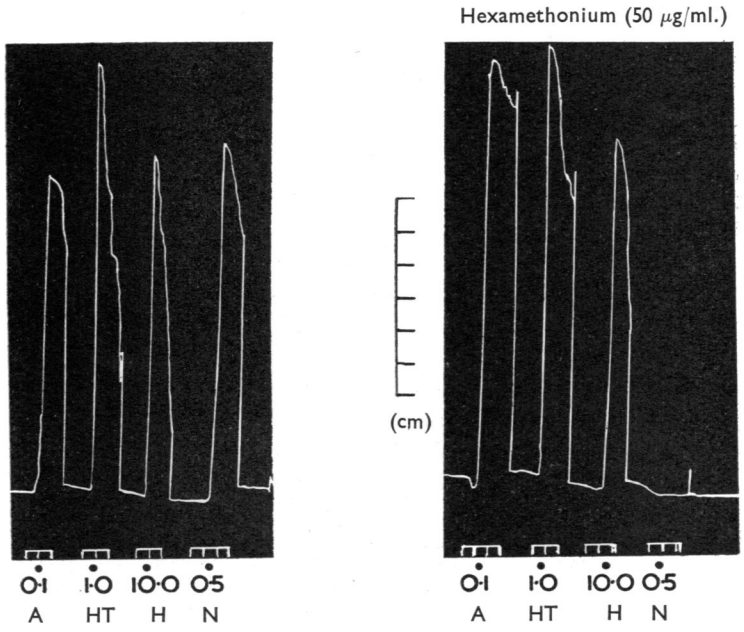

Fig. 11. The effect of hexamethonium on the contractions of a circular muscle strip produced by acetylcholine (A), 5-hydroxytryptamine (HT), histamine (H) and nicotine (N). The left-hand panel shows the responses of the strip, and the right-hand panel the responses in the presence of hexamethonium (50 μg/ml.). The record of the control strip has been omitted. Time signal, 30 sec.

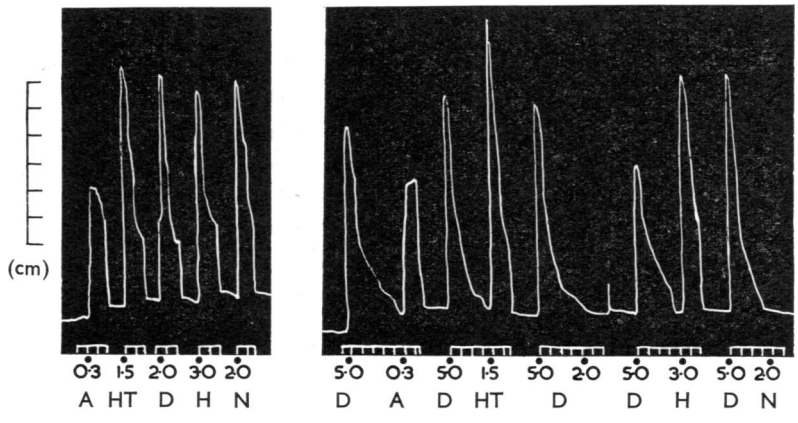

Fig. 12. The effect of a blocking concentration of dimethylphenylpiperazinium (D) on the responses of a circular muscle strip to acetylcholine (A), 5-hydroxytryptamine (HT), histamine (H) and nicotine (N). The left-hand panel shows the responses of the circular strip, and the right-hand panel the responses after each drug addition has been preceded (by 2 min) by dimethylphenylpiperazinium (5.0 μg/ml.). The record of the control strip has been omitted. Time signal, 30 sec.

subsequent relaxation to the base-line within 2 min. This desensitized preparation, from which the 5-hydroxytryptamine was not removed by washing, remained sensitive to acetylcholine and to nicotine but was insensitive to further doses of 5-hydroxytryptamine (Fig. 13). Responses of the control strip remained unchanged throughout. In other experiments the sensitivity to histamine was unchanged in the presence of concentrations of 5-hydroxytryptamine which desensitized.

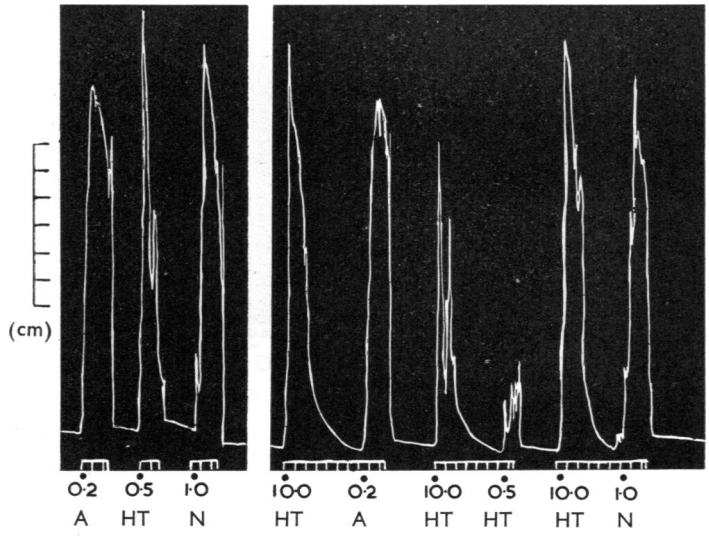

Fig. 13. The effect of a blocking concentration of 5-hydroxytryptamine on the responses of a circular muscle strip to acetylcholine (A), 5-hydroxytryptamine (HT) and nicotine (N). The left-hand panel shows the responses of the strip, and the right-hand panel the responses after each drug addition has been preceded (by 2 min) by 5-hydroxytryptamine (10 μg/ml.). The record of the control strip has been omitted. Time signal, 30 sec.

A similar experiment was made with histamine. In the presence of a desensitizing concentration of histamine (20 μg/ml.) the circular preparation of the ileum responded as usual to acetylcholine, 5-hydroxytryptamine or nicotine but the stimulant action of 4.0 μg/ml. of histamine was antagonized (Fig. 14). Further evidence to support the presence of histamine receptor sites in the circular muscle strip was obtained from the use of the antihistamine agent mepyramine (0.05 μg/ml.), which antagonized only the stimulatory response of the circular strip to histamine without modifying the responses to acetylcholine, 5-hydroxytryptamine or nicotine (Fig. 15).

Experiments on the longitudinal muscle strip

A longitudinal muscle strip may be prepared in the same way as the circular muscle strip, except that the cuts are made in the direction of the longitudinal fibres. This preparation responded to acetylcholine, methacholine, 5-hydroxytryptamine, histamine and nicotine in the concentrations usually effective on the Magnus preparation of the guinea-pig ileum.

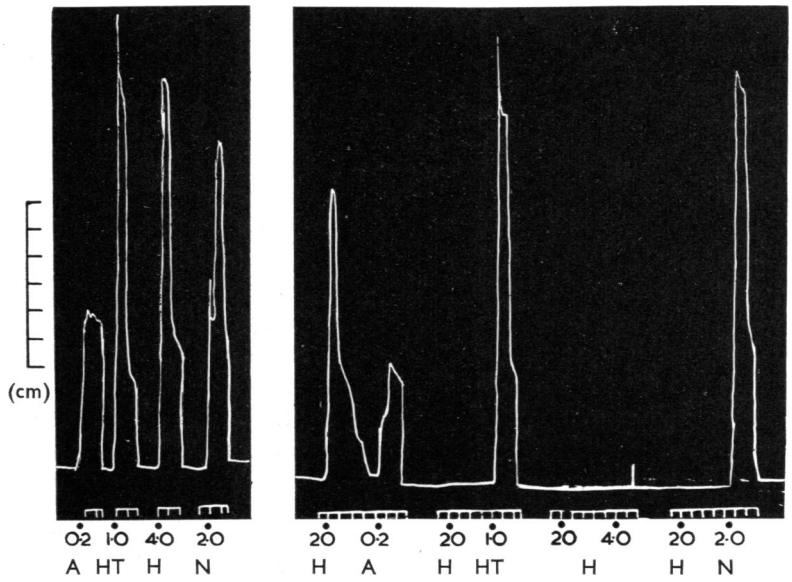

Fig. 14. The effect of a blocking concentration of histamine on the responses of a circular muscle strip to acetylcholine (A), 5-hydroxytryptamine (HT), histamine (H) and nicotine (N). The left-hand panel shows the responses of the circular strip, and the right-hand panel the responses after each drug addition has been preceded (by 3 min) by histamine (20 μg/ml.). The record of the control strip has been omitted. Time signal, 30 sec.

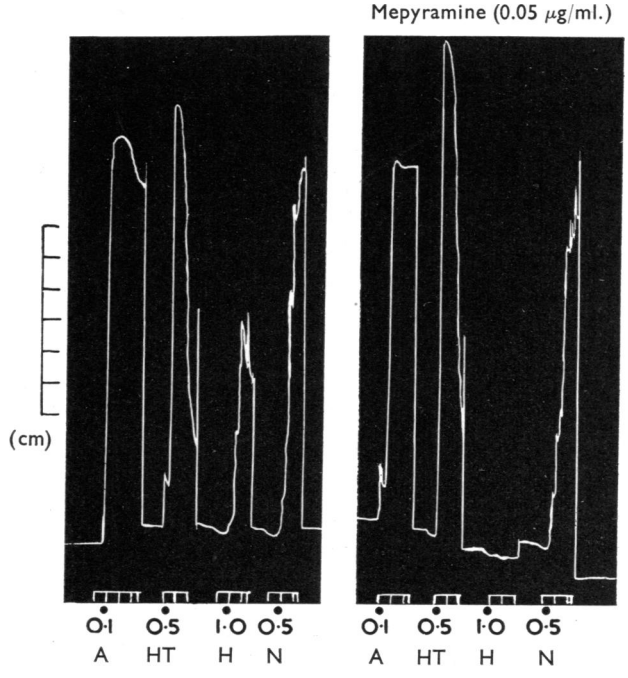

Fig. 15. The effect of mepyramine on the responses of a circular muscle strip to acetylcholine (A), 5-hydroxytryptamine (HT), histamine (H) and nicotine (N). The left-hand panel shows the responses of the circular strip, and the right-hand panel the responses in the presence of mepyramine (0.05 μg/ml.). The record of the control strip has been omitted. Time signal, 30 sec.

DISCUSSION

A circular muscle strip, prepared as described, has in its length some four small junctions of longitudinal muscle which connect the main bands of circular muscle (Fig. 1). When a strip of longitudinal muscle was prepared in a similar manner, it differed from the circular muscle strip by responding readily to directly and indirectly acting drugs without prior treatment with an anticholinesterase agent. If the small areas of longitudinal muscle present in the circular muscle strip preparation contributed to the contraction, some response to drugs should have been seen in the absence of inhibition of cholinesterase. Since this was not seen it may therefore be accepted that the contribution of these longitudinal areas to the total contraction of the circular strip was insignificant. These same tests also eliminate the possibility of an interfering effect from the longitudinal muscle which is present throughout the length of the circular strip and which contracts at right angles to the circular muscle contraction.

If the trauma of preparation contributed to the insensitivity of the circular muscle strip preparation, the longitudinal muscle strip would be similarly affected, but this was not so. Thus it seems that the circular muscle strip gives an acceptable record of the movements of the circular muscle coat of the guinea-pig ileum.

The way is now clear to consider the hypothesis that the circular muscle of the intact ileum is not as responsive to the drugs used as is the longitudinal muscle. In the Trendelenburg preparation distension insufficient to produce peristalsis may increase either the activity of the intramural nerve plexuses or the myogenic activity. These changes in underlying tone, when added to the activity induced by a stimulating compound such as 5-hydroxytryptamine, lead to an emptying response of the circular muscle. Such a property of increasing background tone has been ascribed by Feldberg & Lin (1949b, 1950), Feldberg (1950) and Chujyo (1953) to acetylcholine which they showed was released from the isolated intestine, apparently from non-nervous sources. In the circular muscle strip preparation, the background tone cannot be increased by raising the intraluminal pressure, but it is possible that the addition of mipafox brings about the same result, thus making the preparation sensitive to stimulating drugs. It is of interest to speculate about the insensitivity of the circular muscle strip to drugs which act on autonomic effector tissues. The response of the strip to acetylcholine was potentiated about 4,000 times (Fig. 3), compared to about 16 times for the longitudinal muscle. The inference is that a higher concentration of cholinesterase is associated with the cholinergic receptors in the circular muscle than with those in the longitudinal muscle.

The unmasking, by mipafox, of the stimulatory actions of 5-hydroxytryptamine, histamine and nicotine on the circular muscle strip, when considered together with the antagonism of these actions by atropine and hyoscine, suggests that the drugs stimulate the intramural nerve plexuses. This hypothesis has been challenged by employing those classical pharmacological techniques which are the accepted methods for differentiating the sites of action of drugs on the isolated intestine. Procaine can depress the neuronal activity of the isolated gut (Feldberg & Lin, 1949a) but, at the concentration used in the experiments here presented (100 μg/ml.), depression of myogenic contractile activity was evident. This depression could be

overcome by increasing the concentrations of acetylcholine or methacholine, but not by increasing the concentrations of 5-hydroxytryptamine or histamine. Botulinum toxin can prevent the release of acetylcholine selectively from cholinergic nerve endings in various isolated tissues (Ambache, 1949, 1951a, 1951b; Burgen, Dickens & Zatman, 1949; Harry, 1962). Ambache & Lessin (1955) showed that neuronal (indirect) actions of nicotine and dimethylphenylpiperazinium on the longitudinal muscle of the guinea-pig ileum were abolished by Type D botulinum toxin, whereas the myogenic (direct) actions of acetylcholine and histamine were but little affected. On the circular muscle strip preparation Type A toxin was used. This is weaker in its anticholinergic action on isolated tissues than Type D (Ambache & Lessin, 1955) and a higher concentration of Type A was needed; this may have led to a non-specific depression of the acetylcholine and methacholine responses of the circular muscle strip. The contractions of the longitudinal muscle of the guinea-pig ileum and also the emptying phase of the peristaltic reflex produced by nicotine are antagonized by morphine, which does not affect those contractions induced by acetylcholine or histamine (Trendelenburg, 1917; Schaumann, 1955; Gaddum & Picarelli, 1957; Kosterlitz & Robinson, 1958).

Hemicholinium can reduce the release of acetylcholine from cholinergic nerve endings by interfering with the synthesis of acetylcholine in the rat phrenic nerve-diaphragm preparation (Reitzel & Long, 1959), in parasympathetic nerves in the rabbit atria (Chang & Rand, 1960) and in vagal nerve endings in the rabbit colon (Wong & Long, 1961). On the circular muscle strip preparation, hemicholinium abolished the effects of nicotine, 5-hydroxytryptamine and histamine, presumably through a similar mechanism of action. One disadvantage of the use of hemicholinium was the direct stimulation of the circular muscle which it produced.

The results obtained with procaine, botulinum toxin, morphine and hemicholinium on the circular muscle strip, when discussed in the light of the above evidence from the literature, allow the conclusion that 5-hydroxytryptamine, histamine and nicotine stimulate the intramural nerve plexuses, leading to a release of acetylcholine from nerve endings in the circular muscle strip (Harry, 1962), whilst the actions of acetylcholine and methacholine are peripheral to the plexuses and occur at the smooth muscle cells.

Tachyphylaxis to 5-hydroxytryptamine and histamine is characteristic of the longitudinal muscle of the guinea-pig ileum, but it was observed only to a small extent on the circular muscle strip preparation with successive increasing doses of 5-hydroxytryptamine or histamine (Fig. 3). It was not seen on any of the control strips used in experiments with procaine, botulinum toxin, morphine or hemicholinium.

Turning now to the precise location of the sites at which stimulation occurs, the experimental results provide no direct answer, but they do show the existence of separate and specific receptor sites within the plexuses for 5-hydroxytryptamine, for histamine and for nicotine.

On the longitudinal muscle of the guinea-pig ileum, hexamethonium antagonized the action of nicotine at cholinergic receptor sites on the postganglionic membranes of synapses (Feldberg, 1951a, 1951b). Because hexamethonium antagonized

specifically the action of nicotine on the circular muscle strip, it follows that nicotine was stimulating the post-synaptic membranes in the intramural nerve plexuses at specific nicotinic receptor sites. This inference is supported by the result obtained with dimethylphenylpiperazinium, a compound which first stimulates and then blocks the nicotinic receptor sites on the intramural ganglionic synapses of the guinea-pig ileum (Leach, 1957 ; Ling, 1959).

The desensitization of the circular muscle strip to 5-hydroxytryptamine but not to acetylcholine, histamine or nicotine, by exposing the tissue to a large concentration of 5-hydroxytryptamine, provided direct evidence for specific receptor sites for 5-hydroxytryptamine within the intramural plexuses.

Histamine also specifically desensitized the circular muscle strip preparation to its own stimulating action, establishing the presence of specific receptor sites for histamine within the nerve plexuses. Further, mepyramine, a competitive antagonist to the action of histamine on the longitudinal muscle coat of the guinea-pig ileum (Cambridge & Holgate, 1955), antagonized the action of histamine on the circular strip, but not that of 5-hydroxytryptamine or nicotine.

Since hexamethonium did not antagonize the stimulatory actions of 5-hydroxytryptamine or histamine, they are unlikely to be on the preganglionic fibres. The evidence presented in this paper can be interpreted to mean that 5-hydroxytryptamine and histamine have actions on receptors on the ganglion cells of the intramural autonomic plexuses which differ from the cholinergic ganglionic receptors. Such an action for 5-hydroxytryptamine has already been suggested by Gaddum (1953) and Gaddum & Hameed (1954) in work on the longitudinal muscle of the guinea-pig ileum, whilst Roche e Silva, Vallee & Picarelli (1953) showed that the 5-hydroxytryptamine receptors in the intramural plexuses of the guinea-pig isolated ileum seemed to be different from ganglionic cholinergic receptors. The evidence for an action of histamine on the intramural ganglia of the ileum is not so convincing as that for 5-hydroxytryptamine, but Ambache (1946) and Ambache & Lessin (1955) did indicate that histamine might stimulate the intramural ganglionic synapses of the rabbit isolated intestine. The suggestion that 5-hydroxytryptamine and histamine stimulate ganglia at sites other than the ganglionic cholinergic site is supported by studies on other autonomic ganglia. Thus on the perfused superior cervical ganglion of the cat, Robertson (1954) and Trendelenburg (1954, 1955, 1956a and b, 1957) showed that 5-hydroxytryptamine and histamine could stimulate the postganglionic membrane, but this stimulation was unaffected by hexamethonium and could be blocked by morphine and by mepyramine respectively. On the perfused inferior mesenteric ganglion, close intra-arterial injection of 5-hydroxytryptamine caused impulses to pass down the postganglionic fibres. This action was not antagonized by hexamethonium, although that of dimethylphenylpiperazinium was (Bindler & Gyermek, 1961). Furthermore, the action of 5-hydroxytryptamine on this ganglion could be antagonized preferentially by 5-hydroxy-3-indoleacetamide (Gyermek, 1961). Histamine can stimulate the paravertebral chain of sympathetic ganglia to increase the blood pressure of the spinal cat (Trendelenburg, 1961), whilst the ability of histamine to stimulate the adrenal medulla has been established for many years (Burn & Dale, 1926).

Perry & Talesnik (1953) showed that ganglionic transmission in both sympathetic and parasympathetic ganglia had similar features and further that the two ganglia shared common pharmacological properties. Thus it seems reasonable to infer that pharmacological results obtained from the parasympathetic autonomic ganglia of the intrinsic plexuses of the gut can be interpreted in the light of results from the sympathetic ganglia. Therefore an abundance of evidence supports the suggestion that 5-hydroxytryptamine and histamine, in producing their stimulation of the circular muscle strip preparation, are acting on postsynaptic receptor sites which are different from the cholinergic receptor sites and from each other.

In his experiments on the cat superior cervical ganglion, Trendelenburg (1956a) showed that stimulation produced by histamine could be antagonized more readily by an intravenous injection of cocaine than could the stimulation by 5-hydroxytryptamine. This is a phenomenon similar to that seen in the experiments on the circular muscle strip preparation where the stimulation caused by nicotine and histamine was more readily antagonized by botulinum toxin (Type A), morphine and hemicholinium than stimulation by 5-hydroxytryptamine. If the tissue were exposed to excessive amounts of 5-hydroxytryptamine, similar results might have been obtained, but the dose/response curves for histamine, 5-hydroxytryptamine and nicotine in the presence of increasing concentrations of morphine do not support this explanation.

I am greatly indebted to Professor G. Brownlee for his help and advice throughout this work, and in the preparation of this manuscript.

REFERENCES

AMBACHE, N. (1946). Interaction of drugs and the effect of cooling on the isolated mammalian intestine. *J. Physiol. (Lond.)*, **104**, 266–287.

AMBACHE, N. (1949). The peripheral action of *Cl. botulinum* toxin. *J. Physiol. (Lond.)*, **108**, 127–141.

AMBACHE, N. (1951a). A further survey of the actions of *Cl. botulinum* toxin in four different types of autonomic nerve fibres. *J. Physiol. (Lond.)*, **113**, 1–17.

AMBACHE, N. (1951b). Unmasking after cholinergic paralysis by botulinum toxin, of a reversed action of nicotine on the mammalian intestine, revealing the probable presence of local inhibitory ganglion cells in the enteric plexus. *Brit. J. Pharmacol.*, **6**, 51–67.

AMBACHE, N. & LESSIN, A. W. (1955). Classification of intestino-motor drugs by means of type D botulinum toxin. *J. Physiol. (Lond.)*, **124**, 449–478.

BELESLIN, D. & VARAGIĆ, V. (1958). The effect of cooling and 5-hydroxytryptamine on the peristaltic reflex of the isolated guinea-pig ileum. *Brit. J. Pharmacol.*, **13**, 266–270.

BINDLER, E. D. & GYERMEK, L. (1961). Influence of 5-HT antagonists on the ganglionic stimulant action of 5-HT and DMPP. *Fed. Proc.*, **20**, 319.

BÜLBRING, E. & CREMA, A. (1958). Observations concerning the action of 5-hydroxytryptamine on the peristaltic reflex. *Brit. J. Pharmacol.*, **13**, 444–457.

BÜLBRING, E., LIN, R. C. Y. & SCHOFIELD, G. (1958). An investigation of the peristaltic reflex in relation to anatomical observations. *Quart. J. exp. Physiol.*, **43**, 26–37.

BURGEN, A. S. V., DICKENS, F. & ZATMAN, T. (1949). The action of botulinum toxin on the neuromuscular junction. *J. Physiol. (Lond.)*, **109**, 10–24.

BURN, J. H. & DALE, H. H. (1926). The vasodilator action of histamine and its physiological significance. *J. Physiol. (Lond.)*, **61**, 185–214.

CAMBRIDGE, G. W. & HOLGATE, A. J. (1955). Superfusion as a method for the study of drug antagonism. *Brit. J. Pharmacol.*, **10**, 326–335.

CHANG, V. & RAND, M. J. (1960). Transmission failure in sympathetic nerves produced by hemicholinium. *Brit. J. Pharmacol.*, **10**, 326–335.

CHUJYO, N. (1953). Site of acetylcholine production in the wall of the intestine. *Amer. J. Physiol.*, **174**, 196–198.

EVANS, C. L. & UNDERHILL, S. W. F. (1923). Studies on the physiology of plain muscle. *J. Physiol. (Lond.)*, **58**, 1–14.

EVANS, D. H. L. & SCHILD, H. O. (1953). Reactions of plexus free circular muscle of cat jejunum to drugs. *J. Physiol. (Lond.)*, **119**, 376–399.

FELDBERG, W. (1950). On the origin and function of the acetylcholine in the intestinal wall. *Proc. roy. Soc., B*, **137**, 285–292.

FELDBERG, W. (1951a). Inhibitors of autonomic ganglia on the isolated guinea-pig's ileum preparation. *J. Physiol. (Lond.)*, **112**, 19*P*.

FELDBERG, W. (1951b). Effects of ganglion blocking substances on the small intestine. *J. Physiol. (Lond.)*, **113**, 483–505.

FELDBERG, W. & LIN, R. C. Y. (1949a). The action of local anaesthetics and d-tubocurarine on the isolated intestine of rabbit and guinea-pig. *Brit. J. Pharmacol.*, **4**, 33–44.

FELDBERG, W. & LIN, R. C. Y. (1949b). The effect of cocaine on the acetylcholine output of the intestinal wall. *J. Physiol. (Lond.)*, **109**, 475–487.

FELDBERG, W. & LIN, R. C. Y. (1950). Synthesis of acetylcholine in the wall of the digestive tract. *J. Physiol. (Lond.)*, **111**, 96–118.

GADDUM, J. H. (1953). Tryptamine receptors. *J. Physiol. (Lond.)*, **119**, 363–368.

GADDUM, J. H. & HAMEED, K. A. (1954). Drugs which antagonize 5-hydroxytryptamine. *Brit. J. Pharmacol.*, **9**, 240–248.

GADDUM, J. H. & PICARELLI, Z. P. (1957). Two kinds of tryptamine receptor. *Brit. J. Pharmacol.*, **12**, 323–328.

GASSER, H. S. (1926). Plexus free preparations of the small intestine. Study of their rhythmicity and their response to drugs. *J. Pharmacol. exp. Ther.*, **27**, 395–410.

GINZEL, K. H. (1957). The action of lysergic acid diethylamide (LSD25); its 2-brom derivative (BOL148) and of 5-hydroxytryptamine (5HT) on the peristaltic reflex of the guinea-pig ileum. *J. Physiol. (Lond.)*, **137**, 62*P*.

GUNN, J. A. & UNDERHILL, S. W. F. (1914). Experiments on the surviving mammalian tissue. *Quart. J. exp. Physiol.*, **8**, 275–296.

GYERMEK, L. (1961). 5-Hydroxy-3-indoleacetamide: a new type of blocking agent against 5-HT. *Nature (Lond.)*, **192**, 465.

HARRY, J. (1962). Effect of cooling, local anaesthetic compounds and botulinum toxin on the responses of and the acetylcholine output from the electrically transmurally stimulated isolated guinea-pig ileum. *Brit. J. Pharmacol.*, **19**, 42–55.

KOSTERLITZ, H. W., PIRIE, V. & ROBINSON, S. A. (1956). The mechanism of the peristaltic reflex in the isolated guinea-pig ileum. *J. Physiol. (Lond.)*, **133**, 681–694.

KOSTERLITZ, H. W. & ROBINSON, J. A. (1957). Inhibition of the peristaltic reflex of the isolated guinea-pig ileum. *J. Physiol. (Lond.)*, **136**, 249–262.

KOSTERLITZ, H. W. & ROBINSON, J. A. (1958). The inhibitory action of morphine on the contraction of the longitudinal muscle coat of the isolated guinea-pig ileum. *Brit. J. Pharmacol.*, **13**, 296–303.

LEACH, G. D. H. (1957). Ganglionic blocking action of dimethyphenylpiperazinium (DMPP). *J. Pharm. Pharmacol.*, **9**, 747–751.

LING, H. W. (1959). Actions of dimethylphenylpiperazinium. *Brit. J. Pharmacol.*, **14**, 505–511.

MACINTOSH, J. C., BIRKS, R. I. & SASTRY, P. B. (1956). Pharmacological inhibition of acetylcholine synthesis. *Nature (Lond.)*, **178**, 1181.

PATON, W. D. M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of co-axially stimulated guinea-pig ileum. *Brit. J. Pharmacol.*, **12**, 119–127.

PATON, W. D. M. & ZAIMIS, E. J. (1949). The pharmacological actions of polymethylene bistrimethylammonium and salts. *Brit. J. Pharmacol.*, **4**, 381–400.

PERRY, W. L. M. & TALESNIK, J. (1953). The role of acetylcholine in synaptic transmission at parasympathetic ganglia. *J. Physiol. (Lond.)*, **119**, 455–469.

REITZEL, N. L. & LONG, J. P. (1959). Hemicholinium antagonism by choline analogues. *J. Pharmacol. exp. Ther.*, **127**, 15–21.

ROBERTSON, P. A. (1954). *The Role of Chemical Substances in the Transmission of Effects from Nerve Endings.* Ph.D. Thesis, University of London.

ROCHE E SILVA, M., VALLEE, J. R. & PICARELLI, Z. P. (1953). A pharmacological analysis of the mode of action of serotonin (5-hydroxytryptamine) upon the guinea-pig ileum. *Brit. J. Pharmacol.*, **8**, 378–388.

SCHAUMANN, W. (1955). The paralysing action of morphine on the guinea-pig ileum. *Brit. J. Pharmacol.*, **10**, 456–461.

SCHAUMANN, W. (1957). Inhibition by morphine of the release of acetylcholine from the intestine of the guinea-pig. *Brit. J. Pharmacol.*, **12**, 115–118.

TRENDELENBURG, P. (1917). Physiologische und pharmakologische versuch über Dinndarmperistaltic. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmakol.*, **81**, 55–129.

TRENDELENBURG, U. (1954). The action of histamine and pilocarpine on the superior cervical ganglion and adrenal glands of cat. *Brit. J. Pharmacol.*, **9**, 481–487.

TRENDELENBURG, U. (1955). The potentiation of ganglionic transmission by histamine and pilocarpine. *J. Physiol. (Lond.)*, **129**, 337–351.

TRENDELENBURG, U. (1956a). The action of 5-hydroxytryptamine on the nictitating membrane and on the superior cervical ganglion of the cat. *Brit. J. Pharmacol.*, **11**, 74–80.

TRENDELENBURG, U. (1956b). Modification of transmission through the superior cervical ganglion of the cat. *J. Physiol. (Lond.)*, **132**, 529–541.

TRENDELENBURG, U. (1957). The action of morphine on the superior cervical ganglion and on the nictitating membrane of the cat. *Brit. J. Pharmacol.*, **12**, 79–85.

TRENDELENBURG, U. (1961). The pressor response of the cat to histamine and pilocarpine. *J. Pharmacol. exp. Ther.*, **131**, 65–72.

WONG, K. G. & LONG, J. P. (1961). Autonomic blocking properties of hemicholinium (HC3). *J. Pharmacol. exp. Ther.*, **133**, 211–215.

YOUNG, A. W. (1914). On movements of the isolated small intestine and the action of various drugs and extracts upon them. *Quart. J. exp. Physiol.*, **8**, 347–375.