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THE SITES OF ACTION OF 5-HYDROXYTRYPTAMINE IN NERVE-MUSCLE PREPARATIONS FROM THE GUINEA-PIG SMALL INTESTINE AND COLON

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1 The sites of action of 5-hydroxytryptamine (5-HT) were examined in isolated segments of guinea-pig intestine. Mechanical records were taken from the longitudinal muscle of the ileum and proximal colon and from the circular muscle of the ileum and distal colon.

2 In order to examine direct actions of 5-HT, nerve-mediated responses were blocked with tetrodotoxin (0.2 μg/ml). There was a gradient in the responsiveness of the longitudinal muscle of the ileum; in the proximal ileum it was usually unresponsive, whereas in the distal ileum about 30% of the amplitude of contraction was caused by a direct effect on the muscle. In the circular muscle from all parts of the ileum, direct effects on the muscle were weak or absent. In the distal colon, the circular muscle was almost always unresponsive to direct effects of 5-HT even when concentrations of 5-HT as great as 100 μg/ml were used. All direct actions of 5-HT on intestinal muscle were blocked by methysergide (1 μg/ml), which itself did not affect nerve-mediated responses.

3 Excitatory cholinergic nerves and excitatory and inhibitory nerves which released unidentified substances were all stimulated by 5-HT. The contractions mediated through cholinergic nerves were blocked by hyoscine (0.6 μg/ml).

4 Tachyphylaxis to the action of 5-HT occurred both for effects mediated through nerves and for direct effects on the muscle. Responses returned promptly after 5-HT was washed from the organ bath.

5 While 5-HT blocked its own action on neural receptors, it did not antagonize the stimulation of nicotinic receptors on cholinergic neurones by 1-1 dimethyl-4-phenylpiperazinium iodide (DMPP). Moreover, pentolinium markedly reduced contractions caused by DMPP without significantly affecting responses to 5-HT. In contrast, (+)-tubocurarine, another nicotinic receptor antagonist, was effective in reducing contractions caused by 5-HT.

6 Phenyldiguanide, which has been reported to antagonize the stimulant action of 5-HT on cholinergic neurones in the mouse small intestine, did not cause any significant reduction in the action of 5-HT on cholinergic neurones in the guinea-pig ileum unless a concentration of 1 mg/ml was used. However, contractions elicited by carbachol and DMPP were antagonized to a similar extent by phenyldiguanide at this concentration. Antagonism of the action of 5-HT at neural receptors by bromolysergic acid and by tryptamine was found but it was not specific, these drugs causing comparable decreases in responses to 5-HT, carbachol and DMPP.

7 The present results, which show that 5-HT has little or no direct effect on the circular muscle of the ileum and colon, imply that, if 5-HT is a transmitter in enteric reflexes, it must be released from interneurones.

Introduction

There have been several suggestions, based mainly on pharmacological studies, that 5-hydroxytryptamine (5-HT) or a similar substance might be an intestinal neurotransmitter (Bülbring & Gershon, 1967; Costa & Furness, 1976; Gershon, 1977). Consistent with these suggestions are the findings that some intestinal nerves contain tryptophan hydroxylase, aromatic amino-acid decarboxylase and monoamine oxidase, enzymes which could be involved in the synthesis and degradation of 5-HT (Furness & Costa, 1971; Costa, Furness & McLean, 1976; Dreyfus, Bornstein & Gershon, 1977; Gershon, Dreyfus, Pickel, Joh & Reis,

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1977). Some intrinsic intestinal neurones are also capable of taking up and storing indoleamines (Gershon, Robinson & Ross, 1976; Dreyfus, Sherman & Gershon, 1977; Furness & Costa, 1978) and 5-HT taken up into intestinal nerves can be released by electrical stimulation (Schulz & Cartwright, 1974).

In the present work, the sites of action of 5-HT in several preparations in which there is evidence for a non-cholinergic excitatory transmitter (the ileum, the proximal colon and the distal colon of the guinea-pig) have been examined. In the proximal colon, release of an unknown substance from electrically excited nerves leads to a contraction of the longitudinal muscle (Costa & Furness, 1972). It is known that 5-HT also contracts the muscle and that it stimulates enteric inhibitory nerves in this preparation (Furness & Costa, 1973) but whether 5-HT causes contraction by a direct action on the muscle or by releasing another substance is not known. In the distal colon and ileum of the guinea-pig a contraction of the circular muscle is elicited above a point of distension (Costa & Furness, 1976; Furness & Costa, 1976). This reflex contraction on the oral side is blocked by methysergide and is also blocked when the muscle is made tachyphylactic to the action of 5-HT. No analysis of the action of 5-HT on the circular muscle of the distal colon has been published although Pruitt, Grubb, Jaquette & Burks (1974) have shown that 5-HT contracts the circular muscle of the proximal colon. In circular strips of ileum, Harry (1963) found that the muscle was insensitive to 5-HT except when the strips were pretreated with an anticholinesterase. The strips were then contracted by 5-HT and the contraction was blocked by atropine, procaine, hemicholinium or botulinum toxin, implying that 5-HT had an indirect action through the stimulation of cholinergic nerves.

Experiments to determine the sites of action of 5-HT in longitudinal preparations of guinea-pig ileum have produced apparently contradictory results (Gaddum & Picarelli, 1957; Brownlee & Johnson, 1963; Garattini & Valzelli, 1965). The observations of Gaddum & Picarelli indicate that 5-HT contracts the ileum both by a direct action on the muscle and indirectly by releasing acetylcholine from intrinsic nerves, whereas Brownlee & Johnson found no direct effect on the muscle. Since the former authors used the terminal part of the ileum, while the latter used the mid-part, responses to 5-HT have been systematically evaluated along the ileum in the present work.

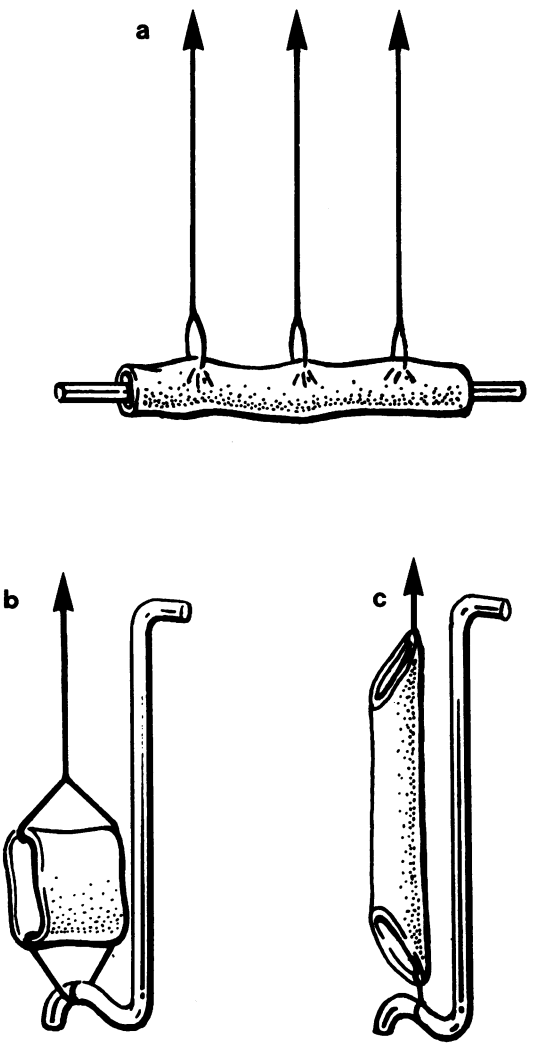

Figure 1 Arrangements used to record tension changes in isolated preparations of intestine. (a) Recording of circular muscle activity in a segment of intestine passed over a rigid rod. (b) Recording from the circular muscle of a ring of intestine. (c) Recording longitudinal muscle movements.

Methods

Adult albino guinea-pigs of either sex, weighing 200 to 600 g, were killed by a blow on the head and bled from the carotid arteries. Segments of ileum and of proximal and distal colon were dissected out and placed in an organ bath containing a modified Krebs solution with the composition (mM): Na^+ 151.0, K^+ 4.7, Ca^{2+} 2.8, Mg^{2+} 0.6, Cl^- 143.7, $H_2PO_4^-$ 1.3, HCO_3^- 16.3, SO_4^{2-} 0.6 and dextrose, 7.7 (Furness, 1969). The bath was maintained at 36 ± 1°C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2.

Changes in the mechanical activity of intestinal muscle in response to 5-HT and other agonists was

recorded isometrically with a Grass force-displacement transducer (FT03C) or isotonically with a Harvard rotary motion transducer and displayed on a Grass polygraph. The mechanical activity of the circular muscle was recorded in three ways: (1) segments of intestine 3 to 6 cm long were secured at the bottom of a 100 ml organ bath by a metal bar passed through the lumen and heart clips (Palmer) were attached to the wall and connected via cotton threads to the transducers. In this way the mechanical activity of the circular muscle was recorded with little or no interference by movements of the longitudinal muscle (Figure 1a); (2) rings of intestine, 5, 10 or 15 mm in length, were mounted in a 10 ml organ bath; cotton threads were tied to opposite sides of each ring and one end was connected to a tissue holder and the other to the transducer (Figure 1b); (3) segments of intestine were slit open along the mesenteric attachment and the external muscle layer, which consists of circular and longitudinal muscle with the myenteric plexus in between, was separated from the submucosa and mucosa. Strips of muscle, 5 mm wide, were cut in the direction of the long axis of the circular muscle and mounted in a 10 ml organ bath so that their mechanical activity could be recorded with a force-displacement transducer.

Movements of the longitudinal muscle were recorded from 2 to 4 cm segments of intestine mounted in 10 or 25 ml organ baths and connected to force-displacement transducers (Figure 1c). For some experiments, nerve-free longitudinal muscle strips were prepared by stripping off the longitudinal muscle under a dissecting microscope. The complete removal of the nerves was verified by the glyoxylic acid fluorescence histochemical method of Furness & Costa (1975).

Segments from the small intestine of the mouse, set up according to the procedure of Drakontides & Gershon (1968), were used in some experiments.

Preparations were allowed to equilibrate in Krebs solution for at least 30 min before agonists were added in small volumes (less than 1% of bath volume). Agonists were left in contact with the tissue for periods of 20 to 60 s at intervals of 4 or more minutes. Antagonists were added at least 15 min before agonists were tested.

Significance of differences in the amplitudes of responses have been evaluated with Student's *t* test, the 5% level of probability being taken to indicate that findings were significantly different.

Drugs used were D-2-bromolysergic acid diethylamide (BOL), carbamylcholine chloride (carbachol), 1-1 dimethyl-4-phenylpiperazinium iodide (DMPP), 5-hydroxytryptamine creatinine sulphate (5-HT), hyoscine hydrobromide, methysergide bimaleate, pentolinium tartrate, phenyldiguanide, tetrodotoxin, tryptamine hydrochloride, (+)-tubocurarine chloride. All concentrations given in the text refer to the concentrations of the salts in the organ bath.

Results

Longitudinal muscle of the ileum

The small intestine between the duodenal-jejunal flexure and the ileo-caecal junction was divided into five equal lengths, referred to as segments one to five, numbering from the oral end. These segments were each about 20 cm in length. Up to four preparations from each segment were used in any one experiment. In all segments, 5-HT caused a contraction which was rapid in onset, reaching a peak in about 5 s or less. When the 5-HT was left in the organ bath, the contraction soon began to fade and the basal tone was re-established in 30 to 40 s. The concentration-response curves were not significantly different in oral and anal segments. 5-HT was used in experiments to analyze its sites of action in a concentration (1 μg/ml) which gave sub-maximal or just maximal contractions. The contractions elicited by 5-HT were reduced by hyoscine (0.6 μg/ml) to different extents depending on the segment used. This concentration of hyoscine caused a 1000-fold shift to the right of the concentration-response curve for carbachol; similar shifts were found for the antagonism by hyoscine of muscarinic receptors in the other areas of intestine investigated in the present work. Figure 2 shows that the contraction elicited by 5-HT in the first segment of the ileum was almost abolished by hyoscine while the percentage of the 5-HT response remaining increased in segments towards the ileo-caecal junction reaching one third of the maximal contraction in the fifth segment. Responses still occurring despite the action of hyoscine were further reduced by tetrodotoxin (0.2 μg/ml) and the component which still remained was abolished by methysergide (10 μg/ml); in other experiments, when methysergide was added soon after hyoscine was fully effective, the response was further reduced leaving a small response that was abolished by tetrodotoxin. In Figure 3, the cumulative effects of hyoscine, methysergide and tetrodotoxin are shown for the action of 5-HT on longitudinal preparations from segment five. Nerve-free strips from segment five were also contracted by 5-HT (1 μg/ml). These contractions were unaffected by hyoscine (0.6 μg/ml), but were blocked by methysergide (10 μg/ml). In the most oral segments, methysergide (10 μg/ml) did not reduce the response to 5-HT and in fact slightly potentiated the responses caused by 5-HT (0.1 to 10 μg/ml; Figure 4). Hyoscine (0.6 μg/ml) abolished or substantially reduced the responses to 5-HT at all concentrations.

These results suggest that there is little or no direct

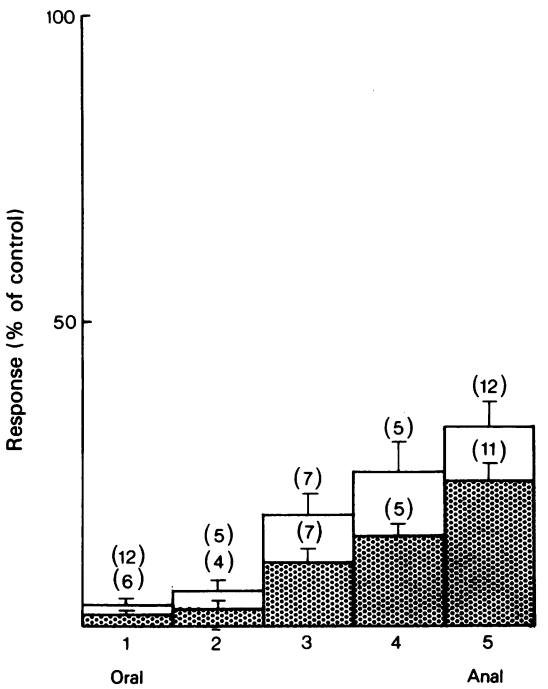

Figure 2 Differences in the effects of hyoscine and tetrodotoxin on responses of the longitudinal muscle of segments 1 to 5 of the ileum. The amplitudes of contractions caused by 5-hydroxytryptamine (5-HT, 1 μg/ml) with no other drug present are taken as 100%. The full heights of the columns (stippled plus unshaded) represent the mean amplitudes of contractions remaining (expressed as percentages of the original) in the presence of hyoscine (0.6 μg/ml) and the heights of the stippled sections represent response amplitudes in the presence of hyoscine plus tetrodotoxin (TTX; 0.2 μg/ml). These remaining responses were blocked by methysergide (10 μg/ml). The number of experiments used for each determination is given in parentheses. Bars indicate s.e. mean.

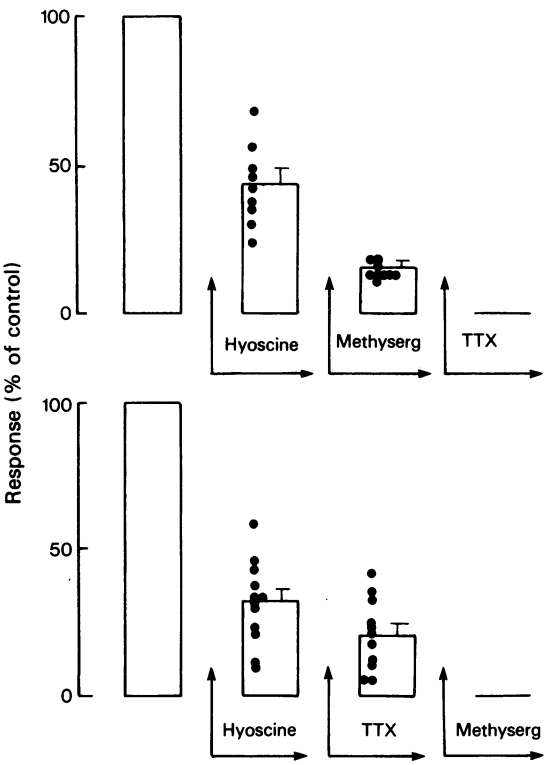

Figure 3 The combined effects of the successive addition of hyoscine, methysergide (Methyserg) and tetrodotoxin (TTX) on the contractions caused by 5-hydroxytryptamine (5-HT, 1 μg/ml) in the longitudinal muscle of segment 5 of the ileum (drug concentrations given in the text). The dots show the results obtained in individual experiments, while the heights of the columns indicate the means obtained from all experiments with the s.e. given by the vertical lines. After all three drugs (fourth column), there were no responses in any experiment.

effect of 5-HT on the muscle in the most oral (jejunal) segments of the ileum where the effect appears to be mediated almost entirely through the stimulation of cholinergic neurones and that there is a progressive increase in the responsiveness of the muscle to the direct effect of 5-HT as more distal sections are approached.

The possibility that 5-HT acts through nicotinic receptors on intrinsic cholinergic neurones was tested in preparations from the first two segments of ileum by comparing the effectiveness of pentolinium in antagonizing responses to 5-HT and to the nicotinic agonist, DMPP. The contractions elicited by DMPP (1 μg/ml) were completely blocked by tetrodotoxin (0.2 μg/ml). Hyoscine (0.6 μg/ml) reduced the response to DMPP to 20% and the remaining response was abolished by tetrodotoxin. These results indicate that DMPP contracts the ileum by stimulating excitatory nerves, that cholinergic nerves cause most of the response and that non-cholinergic nerves are probably also stimulated. Pentolinium (10 μg/ml), which reduced contractions in response to DMPP to, on average, less than 20%, had no significant effect on those caused by 5-HT. However, when tubocurarine was used as a nicotinic receptor antagonist instead of pentolinium, the response elicited by 5-HT (1 μg/ml) was significantly reduced (Figure 5).

It has been shown that 5-HT antagonizes its own action on the intestine (Gaddum, 1953; Rocha e Silva, Valle & Picarelli, 1953; Brownlee & Johnson, 1963).

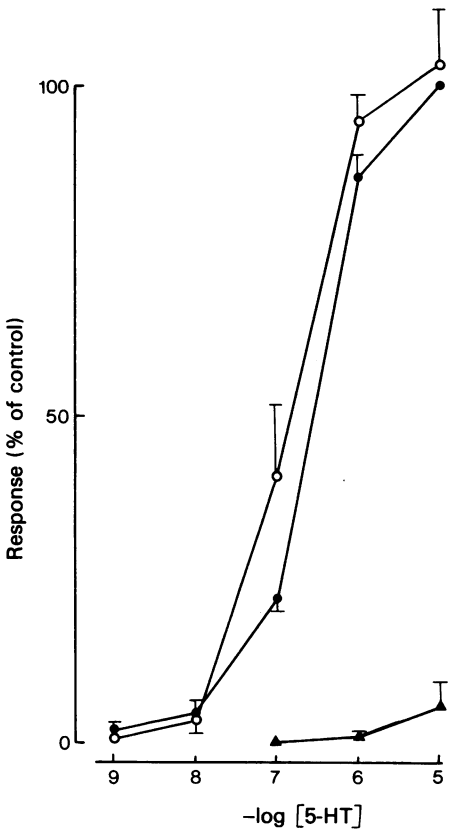

Figure 4 Concentration-response curves for the action of 5-hydroxytryptamine (5-HT) in control preparations from segments 1 and 2 (●), in the presence of methysergide (1 μg/ml, ○) and in the presence of methysergide plus hyoscine (0.6 μg/ml, ▲). Vertical bars show s.e. mean. Abscissa scale: concentration (g/ml).

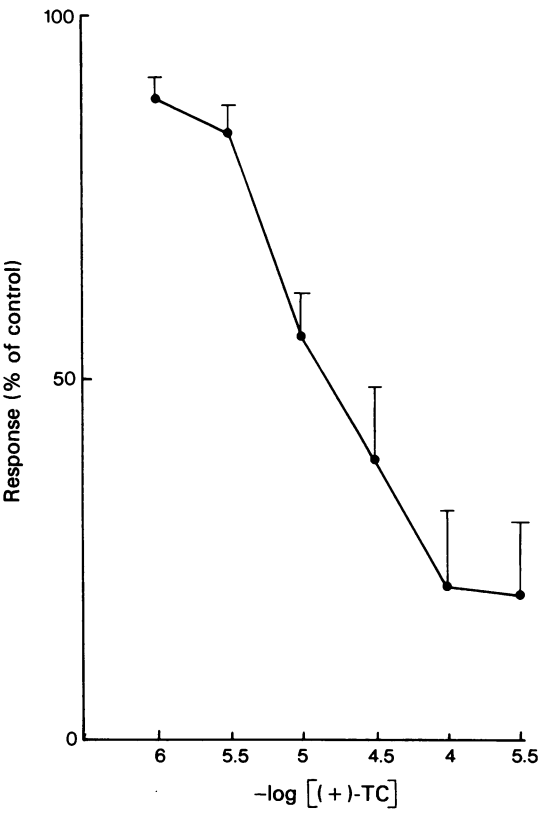

Figure 5 The reduction by (+)-tubocurarine ((+)-TC) of contractions elicited by 5-hydroxytryptamine (5-HT, 10 μg/ml) in the longitudinal muscle of the proximal ileum. Control = 100%. Vertical bars represent standard errors of the mean. Abscissa scale: concentration (g/ml).

In segments one and two, where practically all the 5-HT contraction is nerve-mediated, the contraction to 5-HT (1 μg/ml) was reduced to 10% if the same concentration of 5-HT had been applied 30 min before and left in the organ bath; a concentration of 10 μg/ml completely abolished the contraction caused by the subsequent addition of 1 μg/ml, whereas contractions elicited by DMPP (1 μg/ml) were not significantly affected (Figure 6). The contractions elicited by 5-HT (1 μg/ml) in segments four and five in the presence of hyoscine (0.6 μg/ml) were also abolished when 5-HT (5 μg/ml) was left in the organ bath. Tachyphylaxis to 5-HT was readily reversed by washing the drug from the bath.

Circular muscle of the ileum

5-HT caused contractions of the circular muscle which were brief and not sustained, similar to those elicited in the longitudinal muscle. However, the circular muscle was considerably less sensitive. Rings of circular muscle from each segment of the ileum were taken. In many cases the rings were not affected by 5-HT (1 μg/ml) and even when a contraction could be elicited, the response was not always repeatable after washing the bath free of 5-HT. A greater concentration (10 μg/ml) always contracted the muscle and, on average, this concentration produced 90% of the maximal response; an even higher concentration (100 μg/ml) gave a maximal contraction but responses to successive brief exposures to this concentration became smaller and smaller. A concentration of 10 μg/ml 5-HT was used for testing its sites of action. In 18 of 20 ring preparations from different parts of the ileum, the contraction was blocked by hyoscine (0.6 μg/ml) and in the other 2 cases it was reduced.

In segments of ileum mounted on a bar (see Methods, Figure 1) 5-HT (1 μg/ml) consistently

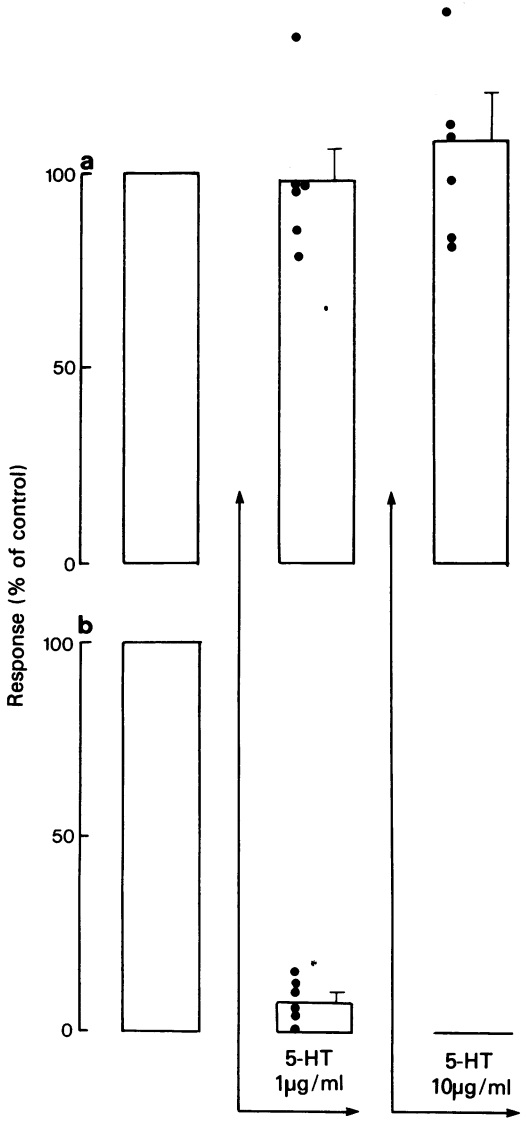

Figure 6 Comparison of the effects of sustained exposures to 5-hydroxytryptamine (5-HT, 1 and 10 μg/ml) on contractions elicited by 1-1 dimethyl-4-phenylpiperazinium (DMPP, 1 μg/ml) in (a), and 5-HT itself (1 μg/ml) in (b). Tachyphylaxis to 5-HT developed but did not change the contraction caused by DMPP. The dots indicate results obtained in individual experiments and the vertical bars give s.e. mean.

elicited contractions which were reduced by hyoscine (0.6 μg/ml) to 9.9% ± 1.7% ($n = 31$) of the control. Tetrodotoxin (0.2 μg/ml) caused similar reductions in the responses. Unlike the longitudinal muscle preparations, no significant trend in results from different parts of the ileum was detected. The small response remaining after treatment with hyoscine was blocked by methysergide (10 μg/ml) but was not altered by tetrodotoxin (0.2 μg/ml). In the absence of hyoscine, methysergide (10 μg/ml) did not significantly affect contractions elicited by 5-HT (10 μg/ml). When 5-HT (10 μg/ml) was left in the bath the responses to subsequent applications of 5-HT were abolished, while the responses to carbachol (20 ng/ml) were not significantly affected. Washing 5-HT from the bath restored the responses to brief exposures to 5-HT.

Longitudinal muscle of the proximal colon

The longitudinal muscle of the proximal colon has sufficient resting tone to allow relaxations to be easily recorded. 5-HT (1 μg/ml) elicited either contraction or relaxation but more frequently a biphasic response, i.e. an initial relaxation followed by a contraction.

Hyoscine (0.6 μg/ml) enhanced or revealed the relaxation which was then abolished by tetrodotoxin (0.2 μg/ml) indicating that 5-HT stimulates inhibitory neurones. 5-HT (1 or 5 μg/ml) left in the bath quickly abolished the relaxation elicited by subsequent applications of 5-HT. The 5-HT antagonism of its own responses was easily reversed by washing 5-HT out of the bath. Methysergide (10 μg/ml) did not affect the relaxation caused by 5-HT. These 5-HT relaxations were always brief, lasting only 5 to 20 s even when 5-HT was applied for longer periods.

The contractions elicited by 5-HT were difficult to study because of their variability and tendency to fade following only a few repeated applications. Whether 5-HT elicited contractions only or contractions after an initial relaxation, the response was abolished by exposure of the preparation to 5-HT (5 μg/ml). Tetrodotoxin (0.2 μg/ml) reduced but did not abolish the contractions. The residual response was slightly reduced by hyoscine (0.6 μg/ml) and abolished by methysergide (10 μg/ml).

Circular muscle of the distal colon

Strips of external musculature 5-HT (1 to 100 μg/ml) had a variable effect on these preparations. In most cases, the response was a rapidly developing, brief contraction of variable amplitude for the same concentration of 5-HT. The responsiveness of the muscle appeared to increase after the initial applications of 5-HT. In 22 out of 30 strips tested, hyoscine (0.6 μg/ml) abolished the contractions produced by 5-HT, in five reduced it and in three had no effect. Methysergide (10 μg/ml) tested on five strips had no clear effect in four and reduced the response in one. The hyoscine-resistant contraction caused by 5-HT was not consistently affected by methysergide which sometimes enhanced and sometimes antagonized the responses. Tetrodotoxin (0.2 μg/ml) abolished the small

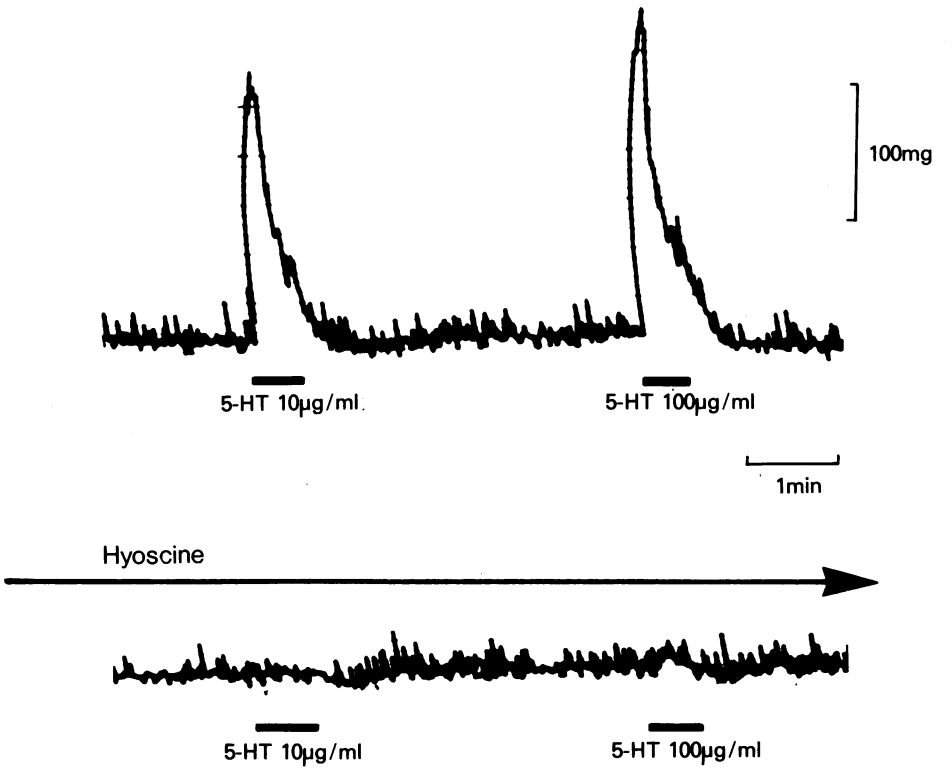

Figure 7 Mechanical records taken from the circular muscle of a segment of distal colon mounted on a rod. With no drug present, 5-hydroxytryptamine (5-HT, 10 and 100 μg/ml) contracted the muscle, whereas with hyoscine in the organ bath, there was no contraction caused by 5-HT. Bars represent 20 s exposures to 5-HT. Note that 5-HT causes a rapid contraction of the muscle which is not sustained.

contractions elicited by 5-HT remaining after methysergide or methysergide plus hyoscine, indicating that 5-HT not only stimulates cholinergic neurones but also non-cholinergic excitatory neurones.

In seven strips, hyoscine reversed the contractions caused by 5-HT to relaxations, which were sometimes followed by contractions. The relaxations were not affected by guanethidine (1 μg/ml) or methysergide (10 μg/ml) but were abolished by tetrodotoxin (0.2 μg/ml). Thus, 5-HT appears to stimulate inhibitory neurones in this preparation.

Rings of distal colon 5 to 15 mm long In 13 out of 14 rings tested the contraction elicited by 5-HT (1 to 100 μg/ml) was abolished by hyoscine (0.6 μg/ml) which in 4 strips revealed a relaxation.

Segments of distal colon

When lengths of colon mounted on a rod were used, 5-HT (1 to 100 μg/ml) elicited contractions of the circular muscle which lasted 30 to 120 s (Figure 7); in only one out of eight experiments which were performed with no other drug present the response was biphasic with an initial relaxation followed by contraction. The responses to 5-HT often became smaller with successive applications of 5-HT and thus antagonists were added at or near the beginning of the experiments. Hyoscine (0.6 μg/ml) caused a rapid and complete block of the contractions caused by 5-HT (Figure 7). In the presence of hyoscine, 5-HT elicited either a relaxation or a biphasic response in which relaxation was followed by contraction. In 4 out of 16 experiments the initial relaxation was not apparent but the contraction appeared with a delay of several seconds. Tetrodotoxin (0.2 μg/ml) abolished both the relaxation and the delayed contraction. When both hyoscine and tetrodotoxin were in the bath there was usually no response to 5-HT in the concentration range tested (up to 100 μg/ml). In a very few experiments small slow contractions, which appeared without significant delay, were recorded. These contractions were abolished by methysergide (10 μg/ml). All

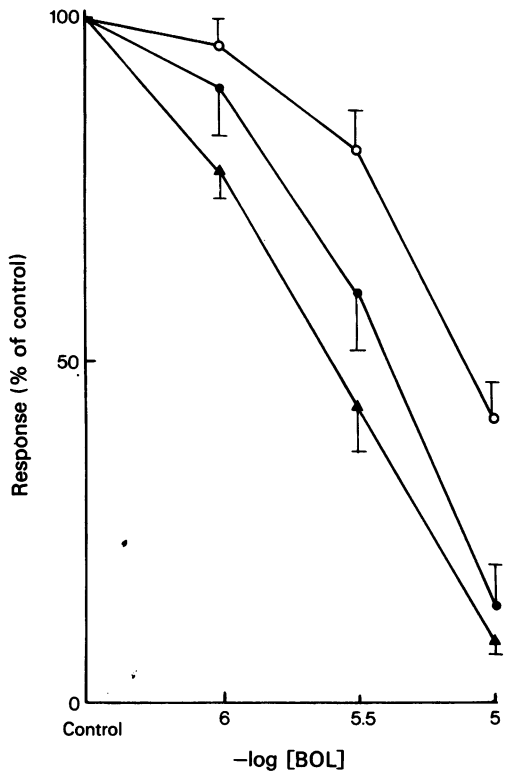

Figure 8 Comparison of the antagonism by bromolysergic acid diethylamide (BOL) of contractions elicited by 5-hydroxytryptamine (5-HT, ●), carbachol (○) and 1-1 dimethyl-4-phenylpiperazinium (DMPP, ▲) in longitudinal preparations from the proximal part of the guinea-pig ileum. Agonist concentrations used were: 5-HT, 1 μg/ml; carbachol, 2 ng/ml; DMPP, 1 μg/ml. The ordinate scale shows the amplitude of the response as a percentage of the control. The vertical bars indicate s.e. mean. Abscissa scale: concentration (g/ml).

responses to 5-HT were abolished by leaving 5-HT (5 μg/ml) in the bath.

These results are consistent with the results on circular muscle strips and rings of distal colon which together suggest that 5-HT is capable of stimulating excitatory cholinergic neurones and inhibitory neurones and that it has a weak excitatory action by stimulating non-cholinergic excitatory neurones. A weak, direct, excitatory action on the muscle which was mediated through methysergide-sensitive receptors was demonstrable in less than 5% of all circular muscle preparations of the distal colon.

Effects of bromolysergic acid, phenyldiguanide and tryptamine

One of the problems in analyzing the possible role of 5-HT in the intestine is the lack of availability of a relatively specific antagonist of its action on neurones. The results described above show that methysergide can be used as a selective antagonist at receptors on muscle and that 5-HT itself, through the tachyphylaxis it produces, can be used as an antagonist acting on both nerve and muscle. BOL, phenyldiguanide and tryptamine were tested as antagonists of the stimulant actions of 5-HT, DMPP and carbachol in the proximal ileum, where the effect of 5-HT is mediated through nerves. The particular concentrations of 5-HT (1 μg/ml), DMPP (10 μg/ml) and carbachol (20 ng/ml) which were used in this series of experiments were chosen because they gave less than maximal responses on the steep parts of their respective concentration-response curves.

BOL reduced the responses to all three agonists, being most effective against DMPP, slightly less effective as an antagonist of 5-HT and least effective against carbachol, although in a concentration of 10 μg/ml it reduced responses to all three drugs by more than 50% (Figure 8).

Phenyldiguanide has been shown by Drakontides & Gershon (1968) to be an effective antagonist of 5-HT at neural receptors in the mouse small intestine. However, in the present work it was found to be a weak and non-selective antagonist of neuronal receptors in the guinea-pig (Figure 9); even in a concentration of 100 μg/ml, responses to 5-HT were reduced by only 20% and the reduction in the response to carbachol was almost identical. In additional experiments on the mouse duodenum, on the other hand, phenyldiguanide was found to be effective in antagonizing nerve-mediated responses to 5-HT, thus confirming Drakontides & Gershon's observations.

It has been claimed that the ileum of the guinea-pig can be specifically desensitized to 5-HT by exposure to high concentrations of tryptamine (Gaddum & Hameed, 1954). In the present work, tryptamine was found to be an effective antagonist not only of 5-HT but also of carbachol and DMPP on the proximal ileum of the guinea-pig (Figure 10).

Discussion

Pharmacological assumptions

Tetrodotoxin has been used to distinguish between effects of 5-HT resulting from conduction of action potentials in nerves, and effects which are either direct on the muscle or are due to displacement of excitatory substances from endogenous sources onto the muscle. Tetrodotoxin does not suppress the excitability of smooth muscle or block receptors for drugs acting on smooth muscle but it does block sodium-dependent action potentials in autonomic (and other)

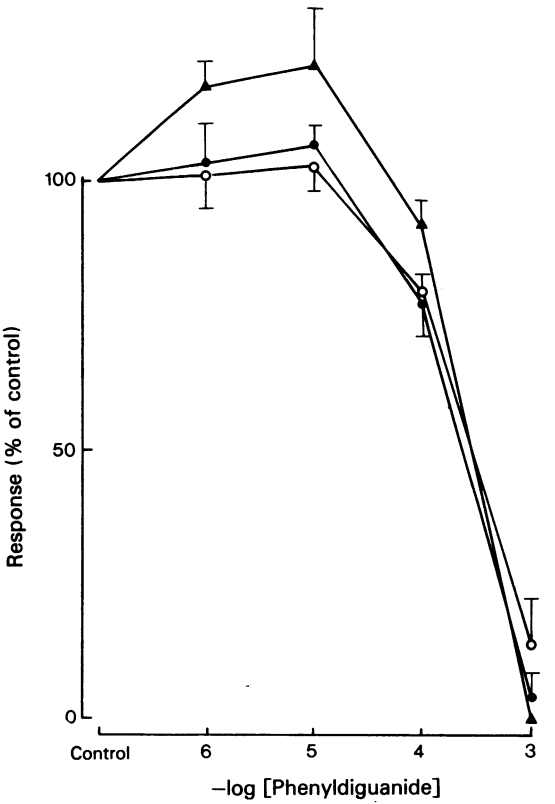

Figure 9 Comparison of the antagonism by phenyldiguanide of contractions elicited by 5-hydroxytryptamine (5-HT, ●), carbachol (○), and 1-1 dimethyl-4-phenylpiperazinium (DMPP, ▲), in longitudinal preparations from the proximal part of the guinea-pig ileum. Agonist concentrations were the same as for Figure 8. Abscissa scale: concentration (g/ml).

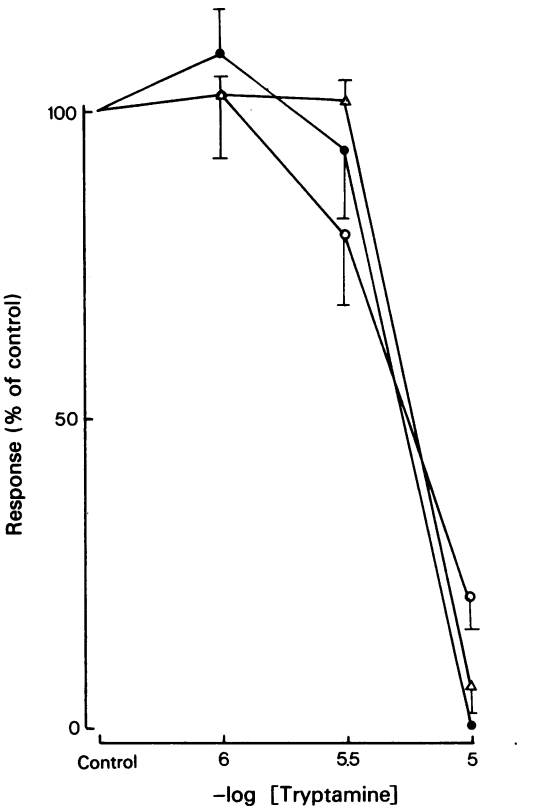

Figure 10 Comparison of the antagonism by tryptamine of contractions elicited by 5-hydroxytryptamine (5-HT, ●), carbachol (○), and 1-1 dimethyl-4-phenylpiperazinium (DMPP, ▲) in longitudinal preparations from the proximal part of the guinea-pig ileum. Agonist concentrations were the same as for Figure 8. Abscissa scale: concentration (g/ml).

nerves (Kuriyama, Osa & Toida, 1966; Gershon, 1967; Evans, 1972). Hyoscine, which is an effective antagonist of the action of acetylcholine on muscarinic receptors in smooth muscle, has been used to determine whether nerve-mediated contractions involved the release of acetylcholine. In the concentration used in the present work (0.6 μg/ml) hyoscine blocked the stimulant effect of a supramaximal concentration (10 μg/ml) of the acetylcholine analogue, carbachol, on the ileum, confirming the observations of Ambache & Freeman (1968). Hyoscine did not affect the action of 5-HT on nerve-free preparations of ileum in the present work, confirming the observation of Vane (1957) that 5-HT receptors on gastrointestinal smooth muscle are not affected by hyoscine. Responses to 5-HT which are blocked by both tetrodotoxin and hyoscine can, therefore, be concluded to be due to the stimulation of cholinergic nerves by 5-HT. When there is a tetrodotoxin-sensitive contraction remaining in the presence of hyoscine, it is assumed that a non-cholinergic excitatory nerve is being stimulated (see also Ambache & Freeman, 1968, who also interpret their results in this way). Where a contraction is partly blocked by tetrodotoxin and hyoscine has a further antagonist effect, it is postulated that acetylcholine is also being displaced without an action potential being initiated, although tetrodotoxin-resistant action potentials can be evoked in some neurones in the intestine (Hirst & Spence, 1973; North, 1973). In some experiments, 5-HT caused a relaxation that was blocked by tetrodotoxin but not affected by guanethidine, indicating that non-adrenergic intrinsic inhibitory nerves were excited; these nerves have been previously shown to have receptors for 5-HT (Bülbring & Gershon, 1967; Gershon, 1967; Bianchi, Beani, Frigo & Crema 1969; Rikimaru &

Suzuki, 1971; Drakontides & Gershon, 1972; Furness & Costa, 1973). In a number of preparations, contractions were obtained which were not blocked by hyoscine or tetrodotoxin but which were blocked by methysergide. It is presumed that these responses were due to a direct action of 5-HT on intestinal smooth muscle, although they could conceivably have been caused by displacement of an excitatory substance from cells within the preparation.

Interpretation of results

Interpretation of the results on the basis of the pharmacological assumptions made above would indicate that 5-HT contracts the longitudinal muscle of the ileum indirectly by stimulating cholinergic and non-cholinergic excitatory nerves and by a direct action on the smooth muscle. The results show that action through cholinergic nerves is dominant and, in the upper parts of the ileum, accounts for practically all of the response. At all levels, non-cholinergic excitatory nerves contribute only a small fraction of the responses. Direct actions of 5-HT on the muscle are almost insignificant in the more oral parts of the ileum but are quite prominent in the 40 cm immediately proximal to the ileo-caecal junction. This gradient in the prominence of muscle receptors explains the different conclusions reached by Gaddum & Picarelli (1957) and Brownlee & Johnson (1963) (see Introduction).

Experiments with longitudinal muscle preparations of proximal colon and circular muscle preparations of distal colon indicate that 5-HT can also stimulate enteric inhibitory nerves (see above). The lack of evidence for the stimulation of enteric inhibitory neurones in longitudinal preparations of ileum could be due to the inherent low tone of the muscle and to the fact that the enteric inhibitory nerves do not appear to have direct inputs to the muscle (Hirst & McKirdy, 1974). In the proximal colon but not in the other preparations, in addition to a tetrodotoxin-dependent release, 5-HT appeared to displace acetylcholine from neurones without action potentials being initiated since hyoscine still reduced the contractions elicited by 5-HT after sodium-dependent nerve action potentials were abolished by tetrodotoxin. A similar observation has been made on the longitudinal muscle of the distal colon by Bianchi *et al.* (1968). A complicating factor in the analysis of the 5-HT responses is that stimulation of enteric inhibitory neurones is usually followed by a rebound contraction which is myogenic (Campbell, 1966; Furness, 1971) and this can also occur after chemical stimulation of neurones with nicotinic agonists (Hobbiger, Mitchelson & Rand, 1969). It follows that wherever 5-HT stimulates enteric inhibitory neurones, the contraction which sometimes follows could be a rebound contraction and not a direct excitatory effect of 5-HT.

Receptors on both nerve and muscle exhibited tachyphylaxis towards the stimulant action of 5-HT. This tachyphylaxis appears to be specific in that responses to DMPP, which involve the stimulation of nicotinic receptors, the conduction of a nerve impulse and the secretion and action of acetylcholine on muscarinic receptors, were not affected by exposure of the tissue to 5-HT. Methysergide was a selective antagonist of 5-HT at receptors on muscle but none of the drugs which were tested as possible specific antagonists of neural receptors (BOL, phenyldiguanide and tryptamine) were significantly more effective antagonists of 5-HT than they were of carbachol or DMPP. Phenyldiguanide was a particularly poor antagonist of the actions of all three drugs and did not show selectivity for 5-HT in the guinea-pig, which contrasts with its ability to block neural 5-HT receptors in mouse small intestine (Drakontides & Gershon, 1968). Effective antagonism of the action of 5-HT at neural receptors in the mouse was also found in the present work, thus confirming the observation of Drakontides & Gershon (1968) and pointing to a species difference in the action of phenyldiguanide.

The contraction caused by stimulation of cholinergic nerves in the ileum by 5-HT was antagonized by (+)-tubocurarine. This action was not analyzed further: it could have been due to an atropine-like action of (+)-tubocurarine (Feldberg, 1951) or to an interaction of (+)-tubocurarine with receptors for 5-HT. Gerschenfeld & Paupardin-Trisch (1974) have shown that (+)-tubocurarine is an antagonist of A receptors for 5-HT in molluscs and Hirst & Silinsky (1975) have shown that the excitatory affect of 5-HT applied iontophoretically to neurones in the submucous plexus of the ileum is blocked by (+)-tubocurarine.

The possible involvement of 5-hydroxytryptamine in enteric reflexes

The present results allow some tentative conclusions to be reached on whether 5-HT, or a 5-HT-like substance, is involved as a transmitter in the reflex pathway which leads to contraction of the circular muscle when the intestine is distended. In the distal colon, distension elicits a contraction which is only partly blocked by hyoscine, the residual response being blocked by methysergide or when the colon is continually exposed to 5-HT (Costa & Furness, 1976). This implies that a stimulant of the circular smooth muscle, other than acetylcholine, is released by nerve activity and probably acts through the same receptors as 5-HT. The present experiments indicate that 5-HT contracts the circular muscle primarily by exciting

cholinergic nerves. Direct excitatory actions on the muscle were observed in a minority of experiments and when they did occur, the contractions were usually weak and could often not be repeated. Thus, the experiments do not support the possibility of a 5-HT-like neurotransmitter being released from enteric nerves and contracting the circular muscle of the colon.

In the ileum, the reflex contraction of the circular muscle is completely abolished by hyoscine, indicating that the only excitatory agent which reaches the muscle in sufficient concentration to contract it is acetylcholine. (Furness & Costa, 1976 and unpublished results). Therefore, if 5-HT is a transmitter in the pathway of the excitatory reflex, it must be released at a neuro-neuronal junction. The reflex contraction is antagonized by methysergide or by exposure to 5-HT and, although in the present work receptors for 5-HT were found on enteric neurones, these receptors were not blocked by methysergide. The transmitter involved could be a substance similar to but not identical with 5-HT whose receptors are occupied by 5-HT as well as being blocked by methysergide, but which are different from the receptors excited when 5-HT is added to the organ bath.

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