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5-HYDROXYTRYPTAMINE RECEPTORS IN THE MOUSE DUODENUM

BY

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The pharmacological action of 5-hydroxytryptamine (5-HT) in the gastrointestinal tract of a variety of species has been extensively studied (Garattini & Valzelli, 1965; Erspamer, 1966). Actions of 5-HT have been found, mediated indirectly through nerves, and directly on smooth muscle. In the guinea-pig ileum, the presence of two types of receptor for 5-HT was suggested by Gaddum & Picarelli (1957). These were called the M receptor, blocked by morphine, and the D receptor, blocked by phenoxybenzamine (Dibenzyline). The M receptor was thought to be in nervous tissue and the D receptor on the smooth muscle.

The use of the agents, morphine and phenoxybenzamine, to describe receptor sites specific for 5-HT presents a number of difficulties. Morphine has been shown to depress the twitch height and tetanus of transmurally stimulated guinea-pig ileum by reducing the amount of acetylcholine released from cholinergic nerve endings (Paton, 1957a). Therefore morphine may block the excitatory neural action of 5-HT, not by antagonizing 5-HT at specific receptors, but by inhibiting release of acetylcholine from excitatory nerve endings. Morphine may also have other actions, such as the liberation of histamine (Paton, 1957b). Similarly, it is difficult to define a receptor by the use of phenoxybenzamine, because this drug also is non-specific. Besides blocking 5-HT, phenoxybenzamine blocks α-receptors for catecholamines (Nickerson, 1949), prevents uptake of noradrenaline into sympathetic nerve endings (Iversen, 1963), and has an atropine-like action (Boyd, Burnstock, Campbell, Jowett, O'Shea & Wood, 1963). Moreover, Day & Vane (1963) have shown that phenoxybenzamine antagonizes both the muscular and the neural actions of 5-HT. Thus, although 5-HT receptors have been found in two locations in the gut, the receptors themselves need to be characterized further.

Moreover, the analysis of 5-HT receptors is complicated by the presence within the intrinsic nervous tissue of the intestine of both excitatory and inhibitory neurones (Ambache, 1951; Burnstock, Campbell & Rand, 1966) which can both be stimulated by 5-HT (Gershon, 1967). Thus 5-HT may act at three different sites in the wall of the intestine, directly on muscle, and indirectly on the two kinds of neurones. The duodenum of the mouse has proved to be a preparation in which all three sites may be clearly demonstrated and studied. In the present study, therefore, this tissue was used to characterize 5-HT receptors further.

METHODS

Adult mice of either sex, derived originally from the strains *DBA/2* and *BALB/c* and kept in this laboratory for 7 years, were used. The animals ranged in weight from 20 to 35 g. After the cervical spinal cord had been crushed and the abdominal cavity exposed, the duodenum was cut approximately 8 mm from the pyloric sphincter and a strip 3–4 cm long was dissected free of mesentery and pancreas. The anal end was left open and mounted on a J-shaped glass tube immersed in a 10 ml. organ bath containing Krebs solution. The oral end was occluded and attached to the lever of a Phipps and Bird model ST-2 linear motion transducer. Isotonic contractions of the longitudinal muscle of the gut were displayed on a potentiometric pen recorder.

The J-shaped tube contained a platinum wire loop electrode which extended into the lumen of the duodenum. A stainless steel screen placed within the organ bath served as a second electrode. The gut was stimulated transmurally between the two electrodes. A Grass Model S4 stimulator was used to deliver rectangular pulses of 0.01 msec duration at frequencies of 5–30 pulses/sec. The intensity of the current was measured across a 1 Ω resistor in series with the electrodes and was 500 mA. Trains of pulses lasting 5 sec were applied at 1 min intervals.

Duodenal segments were allowed to equilibrate for 40–60 min before the commencement of any experimental procedures. The contents of the organ bath were washed at intervals of 15, 45, and 75 sec after the introduction of drugs, unless otherwise noted in the text.

Krebs solution, modified according to Bülbring (1953), was equilibrated with 95% oxygen and 5% carbon dioxide at 37° C. It had the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.3, $NaHCO_3$ 16, $CaCl_2$ 2.7, $MgCl_2$ 0.17, dextrose 7.07. The pH ranged between values of 7.38 and 7.42.

All drugs were dissolved in distilled water; 0.1 ml. of the solution was added to the organ bath. The drugs used were: acetylcholine chloride (ACh), dimethylphenylpiperazinium iodide (DMPP), eserine sulphate, hexamethonium chloride, 5-hydroxytryptamine creatinine sulphate (5-HT), methysergide maleate, nicotine hydrogen tartrate, phenylbiguanide, tetrodotoxin citrate (Sankyo) and tryptamine hydrochloride. All drug concentrations refer to the salts and are given in μg/ml.

RESULTS

Responses to transmural stimulation

Responses to transmural stimulation varied in different preparations. Most responses began as contractions, but in spite of continuing stimulation many preparations subsequently relaxed, some to a length greater than the resting length. Five types of responses could be defined and are illustrated in Fig. 1. In general, the magnitude of the excitatory response varied inversely with the resting tone, and the magnitude of the inhibitory response was directly related to the muscle tone. In 74% of the preparations the responses were predominantly excitatory, whereas 26% showed responses in which inhibitory components were dominant. Inhibitory responses were frequently followed by an augmentation of the amplitude of the spontaneous activity. This post-inhibitory rebound phenomenon is similar to that described previously by Bennett (1966). In all preparations in which the responses were predominantly inhibitory (Fig. 1, 5), the excitatory component of the diphasic response became dominant after repetitive stimulation.

Responses to transmural stimulation were not affected by the addition of hexamethonium (10 μg/ml.) DMPP (10 μg/ml.), or nicotine (10 μg/ml.) to the organ bath. In 85% of the preparations, contractile responses were potentiated by eserine (0.1 μg/ml.) (Fig. 2A). In the presence of hyoscine (0.1 μg/ml.), the contractile response was abolished and replaced by a relaxation (Fig. 2B).

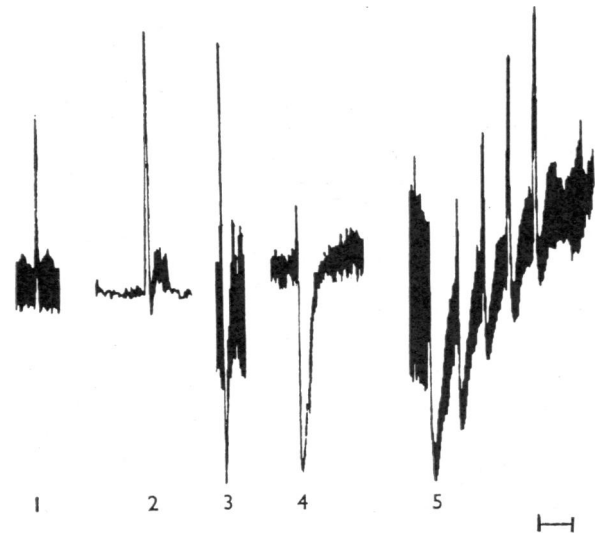

Fig. 1. Responses to transmural stimulation in five preparations of mouse duodenum. Stimulus, 500 mA, 0.01 msec duration, 5 pulses/sec for 5 sec. The traces illustrate different types of responses: 1, contractile responses only; 2, biphasic response with a dominant contractile response and a small relaxation with subsequent increase in spontaneous activity; 3, biphasic response, the contractile response having twice the amplitude of the relaxant response; 4, biphasic response with relaxation dominant and a slow return to resting level; 5, the first stimulus caused relaxation but subsequent stimuli repeated at 1 min intervals induced biphasic responses with diminution of the relaxant phase. Horizontal line, 1 min.

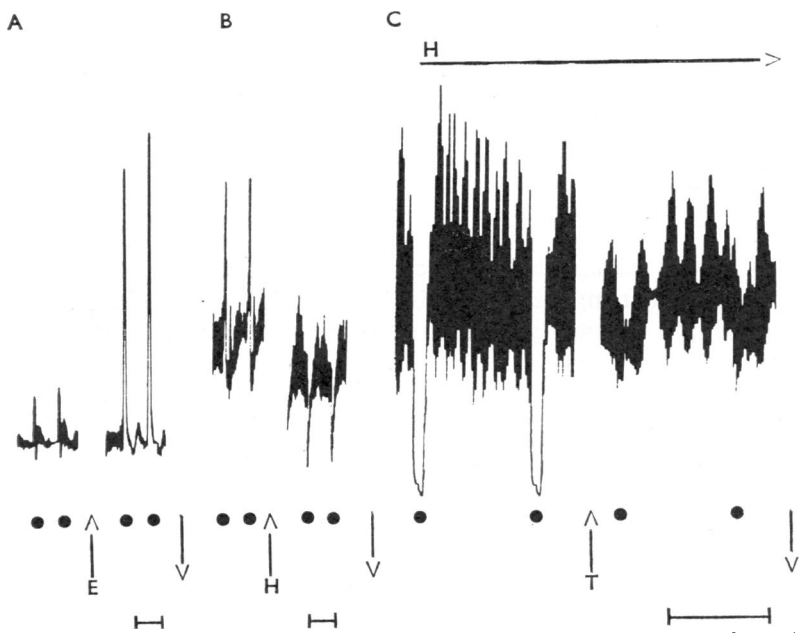

Fig. 2. Responses to transmural stimulation in three preparations, before and after administration of eserine (E), hyoscine (H) and tetrodotoxin (T). ↑ indicates addition and ↓ washing out of drug. A: Addition of eserine (0.1 μg/ml.) resulted in a potentiation of the response to transmural stimulation. B: After addition of hyoscine (0.1 μg/ml.), transmural stimulation resulted in relaxation. C: The relaxant response to transmural stimulation in the presence of hyoscine (0.1 μg/ml.) was abolished by administration of tetrodotoxin (0.1 μg/ml.). Horizontal lines represent 1 min.

All responses to transmural stimulation were abolished by tetrodotoxin (0.1 μg/ml.) (Fig. 2C); this effect was fully reversed after removal of tetrodotoxin from the bathing solution. Because tetrodotoxin prevents the generation of action potentials in nerve and striated muscle but has no effect on smooth muscle, it can be used to differentiate direct responses of the smooth muscle from those initiated indirectly by nerve stimulation (Toida & Osa, 1965; Kuriyama, Osa & Toida, 1966; Bülbring & Tomita, 1967; Gershon, 1967). The observed responses to transmural stimulation must therefore be mediated by excitation of neural elements. The responses are also resistant to hexamethonium and nicotine, so they appear to be postganglionic. As in other species, intrinsic excitatory and inhibitory neurones both appear to be activated by transmural stimulation, and the excitatory component is cholinergic (Paton, 1955; Burnstock *et al.*, 1966).

Responses to 5-HT

Addition of 5-HT to the organ bath caused the longitudinal muscle of the mouse duodenum to contract. The minimal effective concentration ranged from 0.0001 to 0.001 μg/ml. and the maximal effect was produced with a concentration of 1 to 10 μg/ml. The response diminished at a concentration of 100 μg/ml. (Fig. 3).

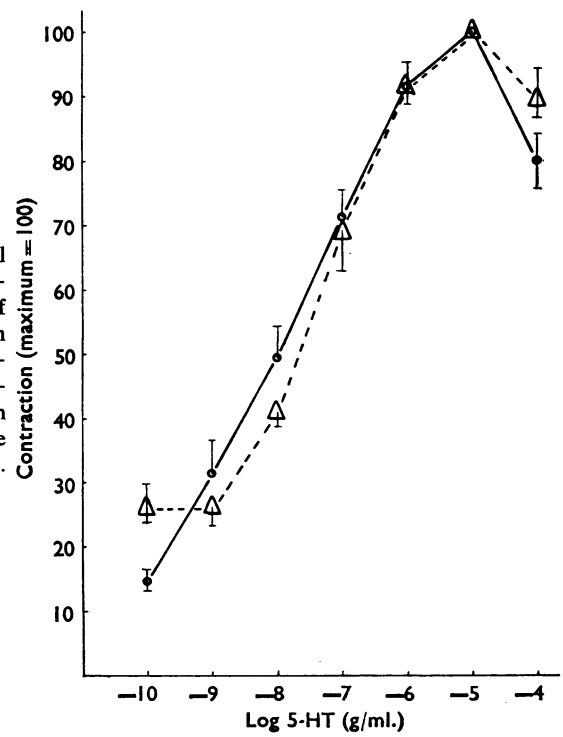

Fig. 3. Effects of 5-HT on the longitudinal muscle of the mouse duodenum. Dose-response curves obtained in the absence of (——) and presence (- - - -) of tetrodotoxin (0.1 μg/ml.). Abscissa, log of concentration of 5-HT (g/ml.). Ordinate, contraction expressed as percentage of maximum contraction. Each point represents the mean of responses from four preparations. Vertical lines indicate S.E. of mean.

Tetrodotoxin (0.1 μg/ml.) did not block responses to 5-HT. While responses to concentrations of 5-HT below 0.1 μg/ml. were never altered by tetrodotoxin, the response to higher concentrations of 5-HT appeared occasionally to be either potentiated or diminished, but tetrodotoxin never produced a consistent change.

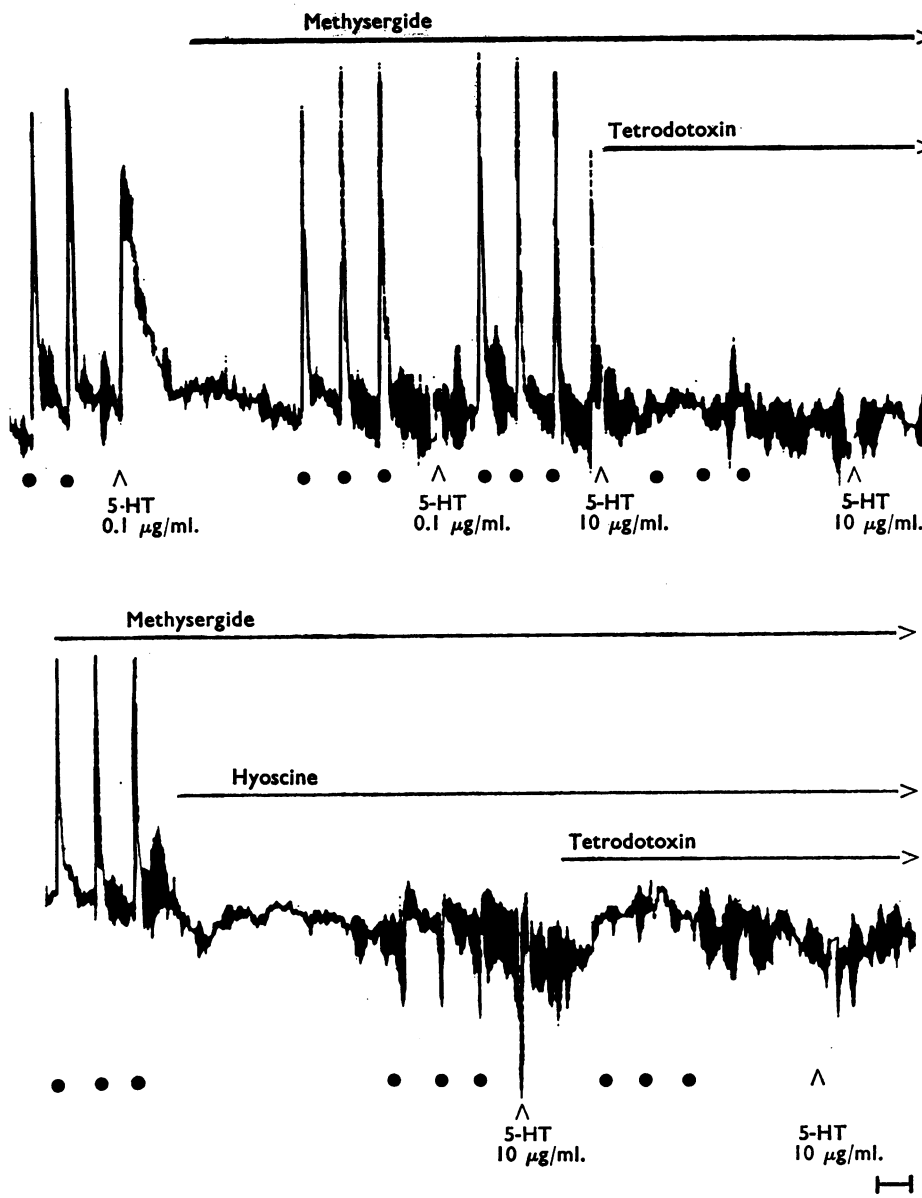

Fig. 4. Effects of methysergide and tetrodotoxin on the responses of the mouse duodenum to 5-HT and transmural stimulation. The upper and lower tracings are a continuous record from one preparation. In the upper trace, two responses to transmural stimulation (●) were followed by administration of 5-HT (0.1 μg/ml.). Note the prolonged recovery phase of the 5-HT response. Methysergide (0.1 μg/ml.) was added and maintained in the bath for the remainder of the experiment. Addition of 5-HT (0.1 μg/ml.) was now without effect, but the responses to transmural stimulation were not affected. 5-HT (10 μg/ml.) caused a transient contraction. Tetrodotoxin (0.1 μg/ml.) abolished responses to transmural stimulation and to 5-HT (10 μg/ml.). After tetrodotoxin was washed out, responses to transmural stimulation returned. After addition of hyoscine (0.1 μg/ml.), transmural stimulation and 5-HT caused relaxant responses which were abolished by tetrodotoxin (0.1 μg/ml.). Horizontal line, 1 min.

The addition of methysergide (0.1 μg/ml.) was followed occasionally by a fall in muscle tone, but usually it had no effect on either tone or spontaneous activity ; this concentration of methysergide did not antagonize responses to acetylcholine, DMPP, nicotine, adrenaline or transmural stimulation. In the presence of methysergide, however, the responses to low concentrations of 5-HT (<0.1 μg/ml.) were blocked (Fig. 4). In 70% of preparations, the responses to 5-HT in a concentration of 0.1 μg/ml. were also blocked and in the remaining 30% the responses were much reduced ; concentrations of 5-HT greater than 0.1 μg/ml. still resulted in a contractile response. These responses to 5-HT of the methysergide-treated gut were abolished by tetrodotoxin (0.1 μg/ml.). Thus, in the presence of methysergide, contractile responses to 5-HT seemed to be mediated by neural elements (Fig. 4, upper trace).

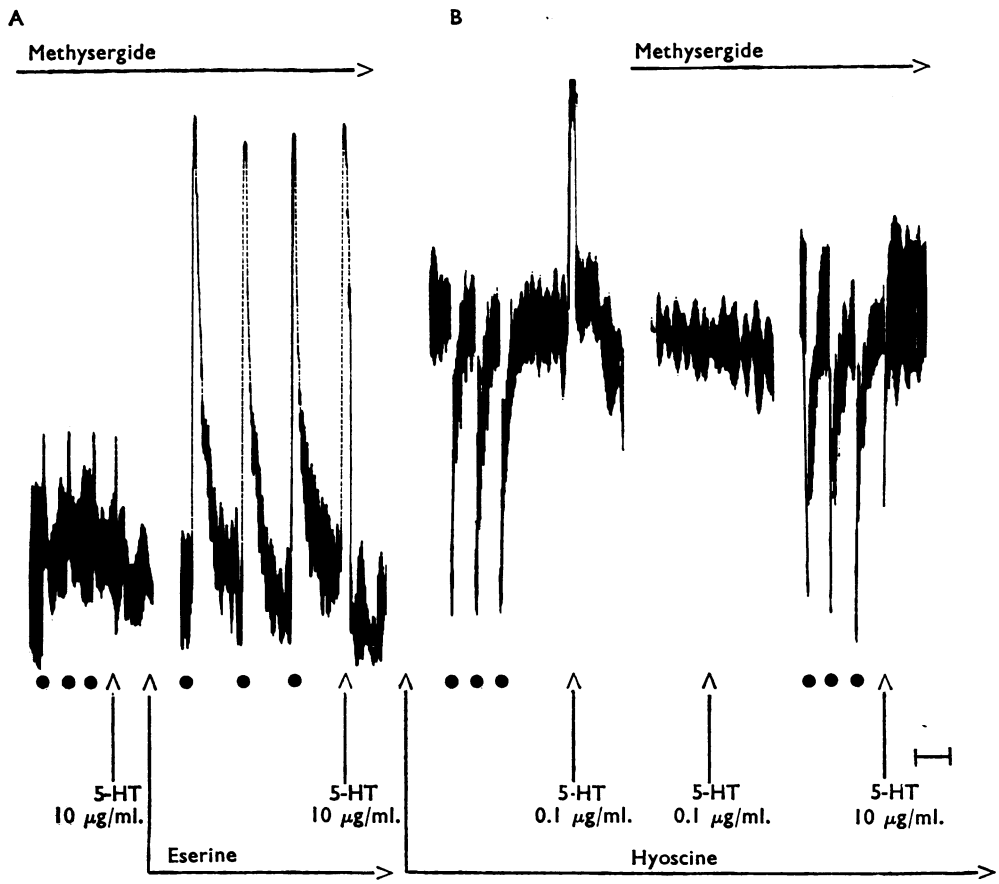

Fig. 5. Effects of methysergide, eserine and hyoscine on the responses of two preparations of mouse duodenum to 5-HT and transmural stimulation. A: Responses to transmural stimulation (●) and 5-HT (10 μg/ml.) in the presence of methysergide (0.1 μg/ml.) are both potentiated by eserine (0.1 μg/ml.). B: Hyoscine (0.1 μg/ml.) was present throughout. Although transmural stimulation (●) relaxed the gut, 5-HT (0.1 μg/ml.) produced a contraction. Addition of methysergide (0.1 μg/ml.) blocked the response to 5-HT (0.1 μg/ml.), while the response to transmural stimulation was unaffected ; 5-HT (10 μg/ml.) caused relaxation. Horizontal line, 1 min.

In preparations not treated with methysergide, neither eserine (0.1 μg/ml.) nor hyoscine (0.1 μg/ml.) affected responses to 5-HT. The contractions produced by high concentrations of 5-HT (>1 μg/ml.) in the presence of methysergide, however, were potentiated by eserine (0.1 μg/ml.) (Fig. 5A). Although transmural stimulation in the presence of hyoscine resulted in relaxation, the addition of 5-HT (0.1 μg/ml.) caused a contraction of the duodenum (Fig. 5B). When methysergide was added to the hyoscine-treated preparation, 5-HT in a concentration of 0.1 μg/ml. had no effect but a concentration of 10 μg/ml. caused relaxation (Fig. 5B). The lower trace of Fig. 4 also illustrates that, in the presence of methysergide, hyoscine converts the contractions caused by transmural stimulation and 5-HT (10 μg/ml.) to relaxations. In the methysergide-treated gut, tetrodotoxin abolished both the contractile 5-HT responses, which had been potentiated by eserine, and the relaxant 5-HT response obtained in the presence of hyoscine. Neither of these responses, however, was affected by hexamethonium ; this indicates that, although mediated by excitation of neural elements, these responses did not involve a nicotinic synapse.

Responses to nicotinic ganglion stimulants

Nicotine (10 μg/ml.) and DMPP (10 μg/ml.) produced transient contractions of the duodenum. In the presence of eserine, these contractile responses were potentiated. In the presence of hyoscine and methysergide, however, both nicotine and DMPP relaxed the gut (Fig. 6). Tetrodotoxin abolished all responses to nicotine and to DMPP, whether or not hyoscine or eserine was present.

Responses to nicotine and DMPP therefore seem to be similar to responses of the methysergide-treated gut to 5-HT as far as their modification by eserine, hyoscine and

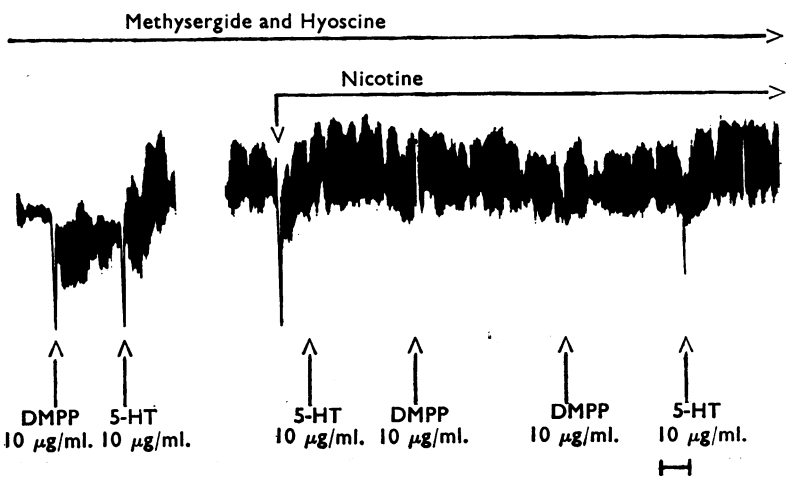

Fig. 6. Effects of methysergide and hyoscine, and of methysergide, hyoscine and nicotine, on the responses of the mouse duodenum to 5-HT and DMPP. Methysergide (0.1 μg/ml.) and hyoscine (0.1 μg/ml.) were present throughout the experiment. Nicotine (100 μg/ml.) was present in the bath as indicated and caused a transient relaxation at the time of addition ; 1–4 min after addition of nicotine, responses to both 5-HT and DMPP were blocked ; 12–16 min later, in the continued presence of nicotine, the responses to 5-HT, but not those to DMPP, returned. Horizontal line, 1 min.

tetrodotoxin is concerned. In contrast to the response of the methysergide-treated gut to 5-HT, however, all responses to nicotine and DMPP were blocked by hexamethonium. These results suggest that the effect of 5-HT may be caused by the activation on ganglion cells of receptors distinct from the nicotinic receptors for acetylcholine. In order to determine whether this was so, the effect of nicotine on the responses of the methysergide-treated gut to 5-HT was studied.

In the presence of methysergide and hyoscine, the relaxant responses to 5-HT were antagonized by nicotine (100 μg/ml.) provided that the addition of 5-HT followed that of nicotine in less than 15 min. If a longer time was allowed to elapse between additions of nicotine and 5-HT, nicotine no longer antagonized the action of 5-HT. When, in spite of the continuing presence of nicotine, the responses to 5-HT returned, the effect of DMPP (10 μg/ml.) was still abolished (Fig. 6); thus the responses to 5-HT returned although nicotinic receptors remained blocked. Because nicotine does prevent responses to 5-HT during the early, probably depolarizing, phase of its action (Paton & Perry, 1953; Trendelenburg, 1957; Brownlee & Johnson, 1963), however, nicotine and 5-HT seem to act on the same cells.

Responses to phenylbiguanide

The results so far indicate that in the mouse duodenum 5-HT is able to activate receptors in both nerve and muscle. Only the receptors in muscle are blocked by methy-

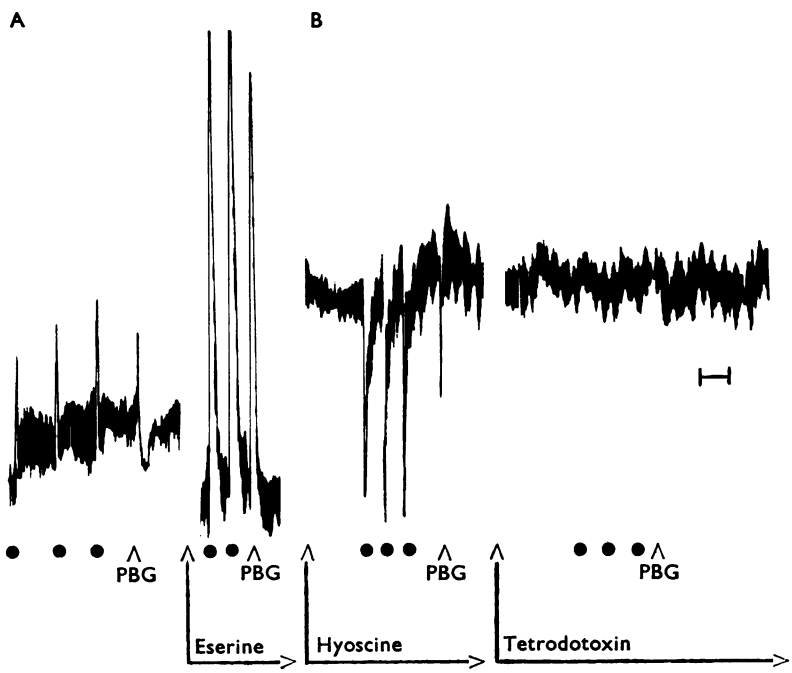

Fig. 7. Effects of eserine, hyoscine and tetrodotoxin on the responses of the mouse duodenum to phenylbiguanide and transmural stimulation. A: Responses to transmural stimulation (●) and to phenylbiguanide (PBG, 10 μg/ml.) were potentiated. B: In the presence of hyoscine (0.1 μg/ml.), transmural stimulation and PBG relaxed the gut. Tetrodotoxin (0.1 μg/ml.) abolished the responses to transmural stimulation and PBG. Horizontal line, 1 min.

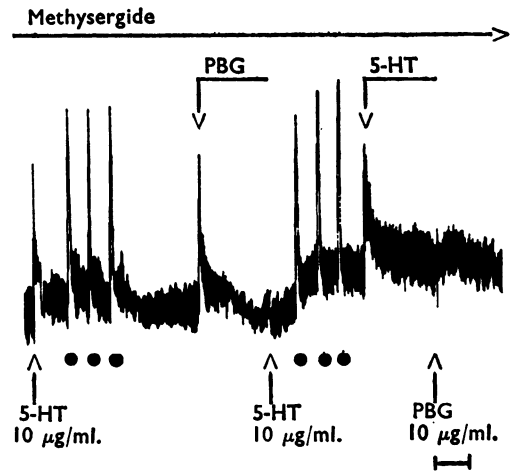

Fig. 8. Mouse duodenum pretreated with methysergide; the effects of phenylbiguanide on the contractile responses to 5-HT and transmural stimulation and the effects of 5-HT on the responses to phenylbiguanide. Methysergide (0.1 μg/ml.) was present throughout the experiment. The first half of the tracing illustrates responses to 5-HT (10 μg/ml.) and transmural stimulation (●). Phenylbiguanide (PBG, 10 μg/ml.) caused a transient contractile response. In the continued presence of phenylbiguanide, 5-HT (10 μg/ml.) had no effect. At the end of the horizontal line marked PBG, phenylbiguanide was washed out. In the presence of 5-HT (10 μg/ml.), phenylbiguanide (10 μg/ml.) had no effect. Horizontal line, 1 min.

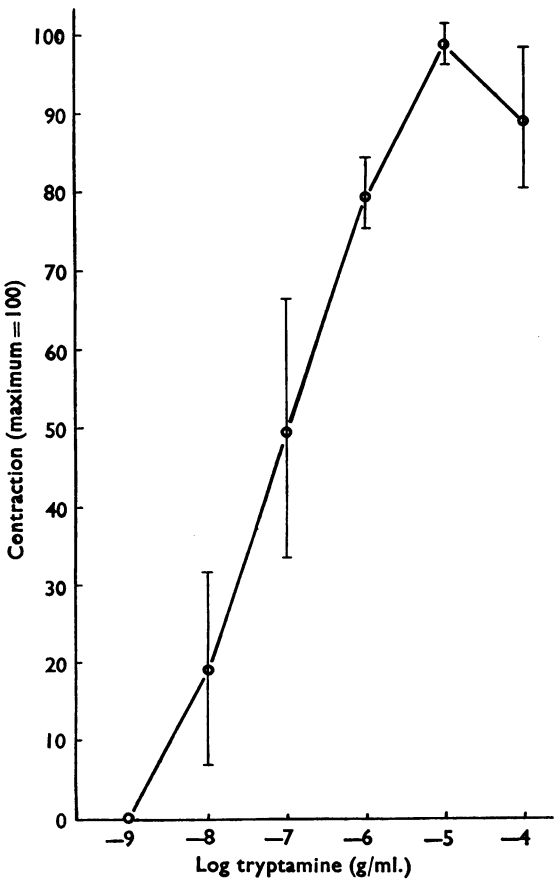

Fig. 9. Effects of tryptamine on the longitudinal muscle of the mouse duodenum. Abscissa, log of concentration of tryptamine (g/ml.). Ordinate, contraction expressed as percentage of maximum contraction. Each point represents the mean of responses from four preparations. Vertical lines indicate S.E. of mean.

sergide, so the neural receptors for 5-HT appear to be different from the muscle receptors. Phenylbiguanide was used to characterize further the neural receptors because it has many pharmacological properties similar to those of 5-HT (Fastier, McDowall & Waal, 1959); in the inferior mesenteric ganglion phenylbiguanide first stimulates and then blocks 5-HT receptors (Gyermek, 1966).

Phenylbiguanide (10 μg/ml.) caused a transient contraction of the longitudinal muscle of the mouse duodenum. This contractile effect was potentiated by eserine (0.1 μg/ml.), whereas, in the presence of hyoscine (0.1 μg/ml.), phenylbiguanide produced a relaxation. Because these responses were all abolished by tetrodotoxin (0.1 μg/ml.) they appeared to be mediated by neural elements (Fig. 7). The response to phenylbiguanide was unaltered by hexamethonium (10 μg/ml.) or methysergide (0.1 μg/ml.) but was blocked by exposure of the gut to 5-HT (10 μg/ml.) for 2 min. This treatment of the gut with 5-HT did not affect responses to DMPP (10 μg/ml.), nicotine (10 μg/ml.) or transmural stimulation. Conversely, 5-HT produced no response in the methysergide-treated gut in the presence of phenylbiguanide (Fig. 8). Phenylbiguanide did not antagonize responses to DMPP or transmural stimulation. In the absence of methysergide, the responses to 5-HT were not antagonized by phenylbiguanide. Thus phenylbiguanide seems to activate and then block the same neural receptor sites as 5-HT, yet has no action on the 5-HT receptors in muscle.

Responses to tryptamine

Addition of tryptamine to the organ bath caused the longitudinal muscle of the mouse duodenum to contract. The minimal effective concentration was 0.01 μg/ml. (Fig. 9). These responses to tryptamine were unaltered by tetrodotoxin (0.1 μg/ml.), hyoscine (0.1 μg/ml.), or phenylbiguanide (10 μg/ml.). Treatment of the gut with methysergide (0.1 μg/ml.), however, abolished responses to all concentrations of tryptamine. Thus tryptamine appears to activate muscle receptors only. This is in contrast to 5-HT, which, in the methysergide-treated preparation, stimulates neural receptors.

DISCUSSION

The present investigation indicates that, in the mouse duodenum, 5-HT can activate receptors at three different sites, namely smooth muscle, excitatory neurones and inhibitory neurones. The receptors in neural tissue seem to differ from those in muscle. The neural receptors are insensitive to tryptamine and are not blocked by methysergide; they are, however, first stimulated and then blocked by phenylbiguanide. On the other hand, the receptors in muscle are activated by tryptamine and blocked by methysergide, but are insensitive to phenylbiguanide. The smooth muscle is very much more sensitive to 5-HT than are either the excitatory or inhibitory neurones.

The smooth muscle of the mouse duodenum is so sensitive to 5-HT that responses seem to result from its direct action on the smooth muscle only. This finding contrasts with the results obtained on the guinea-pig ileum (Day & Vane, 1963) and the dog bladder (Gyermek, 1966), where neural elements are more sensitive to 5-HT than the smooth muscle cells. Thus the response of the mouse duodenum to 5-HT is unaltered by blockade of muscarinic receptors by hyoscine, inhibition of cholinesterase by eserine, or

paralysis of the nerves by tetrodotoxin. In contrast, neural effects elicited by transmural stimulation are potentiated by eserine, converted from a contraction to a relaxation by hyoscine and abolished by tetrodotoxin.

In the mouse duodenum the neural effects of 5-HT can be demonstrated only after the muscle receptors for 5-HT have been blocked by methysergide. Responses of the methysergide-treated gut to 5-HT are, like those to transmural stimulation, potentiated by eserine, converted from a contraction to a relaxation by hyoscine and abolished by tetrodotoxin. Methysergide itself has no effect on the responses to transmural stimulation and may therefore be used to study the neural receptors for 5-HT. Hyoscine may be used, in addition, to block the effects of cholinergic excitatory neurones, thus unmasking the action of 5-HT on inhibitory neurones.

The neural receptors for 5-HT are distinct from the nicotinic receptors for acetylcholine and the neural effects of 5-HT do not involve the participation of a nicotinic synapse. Thus the responses of the methysergide-treated gut to 5-HT are not affected by hexamethonium, or by nicotine during the late, non-depolarizing, phase of the action of nicotine (Paton & Perry, 1953 ; Trendelenburg, 1957 ; Brownlee & Johnson, 1963). The effects of nicotinic ganglion stimulants, such as DMPP, are abolished by similar treatment with hexamethonium or nicotine. This treatment with hexamethonium or nicotine has no effect on responses to transmural stimulation. On the other hand, neural 5-HT receptors seem to be located on the same cells as receptors for nicotine, since nicotine does block neural responses to 5-HT during the early, probably depolarizing, phase of its action.

Because the action of phenylbiguanide is blocked by tetrodotoxin, it is mediated by neural elements. Phenylbiguanide stimulates inhibitory as well as excitatory neurones and its contractile effect is converted to a relaxation by the presence of hyoscine. Since the neural action of phenylbiguanide is not inhibited by hexamethonium, a nicotinic site is not involved. On the other hand, phenylbiguanide desensitizes the gut to the neural action of 5-HT and 5-HT desensitizes the gut to phenylbiguanide, observations which indicate that both drugs act on the same receptor. This action of phenylbiguanide is similar to that of other biguanides noted by Bülbring & Gershon (1968) for ganglia in the wall of the guinea-pig stomach.

In contrast to phenylbiguanide, the action of tryptamine seems to be on the 5-HT receptors of smooth muscle, for it is completely blocked by methysergide and unaffected by eserine, hyoscine and tetrodotoxin.

The 5-HT receptors in the mouse duodenum seem analogous in many ways to the receptors for acetylcholine. Receptors exist for both compounds on muscle and on neurones. There is a relatively specific blocking agent for each receptor. The muscle or muscarinic receptor for acetylcholine is blocked by hyoscine but hyoscine does not antagonize the neural or nicotinic receptors for acetylcholine. The muscle receptor for 5-HT is blocked by methysergide, but methysergide does not block the neural receptor for 5-HT. Nicotine first stimulates and then blocks the neural receptor for acetylcholine, while phenylbiguanide first stimulates and then blocks the neural receptors for 5-HT. Nicotine does not stimulate the acetylcholine receptor in the muscle and phenylbiguanide does not stimulate the 5-HT receptor in muscle. Finally, muscarine activates the muscarinic muscle receptors, but not the nicotinic neural receptors for acetylcholine, and tryptamine activates the muscle receptors, but not the neural receptors, for 5-HT.

These results are therefore in agreement with the conclusions of Gaddum & Picarelli (1957), in that two kinds of receptor can be defined for 5-HT. The receptor in neural elements would correspond to their M receptor and the receptor in muscle to their D receptor. Because morphine and dibenzyline are probably not specific in their antagonist action against 5-HT (Paton, 1957a ; Paton, 1957b ; Day & Vane, 1963 ; Iverson, 1963) it is proposed that the terms M and D receptors should not be used to describe these 5-HT receptors.

SUMMARY

1. The action of 5-HT in the mouse duodenum was analysed pharmacologically ; it appears to act at three sites, muscle, excitatory neurones and inhibitory neurones.

2. All neural actions were potentiated by eserine, converted from a contraction to a relaxation by hyoscine and blocked by tetrodotoxin.

3. The neural receptor for 5-HT was distinct from the nicotinic receptor for acetylcholine, and was not blocked during the competitive phase of the action of nicotine, or by hexamethonium.

4. Neural receptors for 5-HT differed from muscle receptors. The neural receptor for 5-HT was first stimulated and then blocked by phenylbiguanide but was insensitive to tryptamine and was not blocked by methysergide. The muscle receptor for 5-HT was stimulated by tryptamine, was insensitive to phenylbiguanide and was blocked by methysergide.

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