Effects of 5-Hydroxytryptamine on the Lower Esophageal Sphincter In Vivo

EVIDENCE FOR MULTIPLE SITES OF ACTION

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ABSTRACT Intravenous administration of 5-hydroxytryptamine (5-HT) caused a dose-dependent contraction in the lower esophageal sphincter in the opossum. The smallest dose of 5-HT which caused a detectable contraction of the sphincter was 0.5 μg/kg, and a maximal sphincter contraction was produced by a dose of 40 μg/kg. Methysergide converted the contractile effect of 5-HT to a dose-dependent fall in the sphincter pressure; maximal inhibition of 77.2 ±7.2% of the resting pressure occurred with a dose of 40 μg/kg. The inhibitory effect of 5-HT was antagonized by tetrodotoxin, 5 MeO-DMT, and 5-HT tachyphylaxis. 5 MeO-DMT enhanced 5-HT-induced contraction of the sphincter. In the presence of 5 MeO-DMT and methysergide, 5-HT still caused a brief contraction of the sphincter; this contraction appeared to be due to stimulation of postganglionic cholinergic neurons as it was antagonized by tetrodotoxin or atropine. Reserpinization caused enhancement of the sphincter contraction by 5-HT. In the reserpinized animals in the presence of methysergide, 5-HT caused a small initial contraction followed by prolonged inhibition; atropine antagonized the initial contraction, while inhibition was antagonized by 5 MeO-DMT. These studies are consistent with the view that 5-HT exerts several different effects on the sphincter. 5-HT causes contraction of the sphincter by its direct action on the muscle and also by stimulation of cholinergic excitatory neurons. In addition, 5-HT inhibits the sphincter by stimulation of nonadrenergic inhibitory neurons.

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INTRODUCTION

Serotonin or 5-hydroxytryptamine (5-HT)[1] has been shown to be widely distributed in various parts of the gastrointestinal tract in man and animals (1–4). 5-HT has been localized mainly to enterochromaffin cells, and it has also been demonstrated in the myenteric plexus (1, 2, 5). The physicological role of 5-HT on the motor function of the gut is not known, but several studies have suggested that 5-HT may play an important role in the modulation of motor activity of the gut (1, 6), and it has been implicated in the pathogenesis of dumping syndrome (7), in the action of morphine-like agents on the gut (8), and in the disturbed gastrointestinal motility in patients with carcinoid syndrome (9).

The effect of 5-HT on the different parts of the gut is variable. It is known to stimulate small bowel activity in man and animals, but inhibits gastric and colonic motor activity (1, 2, 9–11). The effect of 5-HT on gastrointestinal sphincters has not been systematically examined. Clark and Vane (12) reported that 5-HT caused contraction of the lower esophageal sphincter (LES) in the cat. We report here studies

[1] *Abbreviations used in this paper*: 5-HT, 5-hydroxytryptamine (serotonin); LES, lower esophageal sphincter; LESP, lower esophageal sphincter pressure; 5 MeO-DMT, 5 methoxy,N,N,dimethyltryptamine; methysergide, 1-methyl-d-lysergic acid butanolamide.

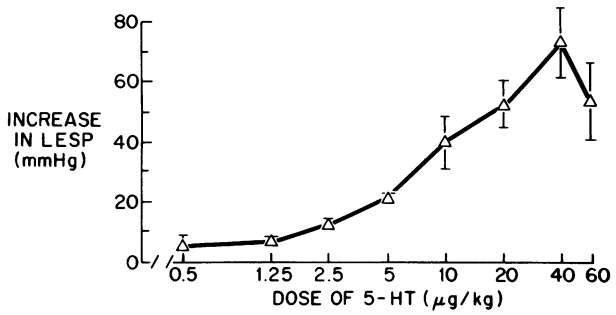

FIGURE 1 Dose-response curve of the effect of 5-HT on LESP. Each point is a mean ± 1 SE of five observations in five animals. Note that 40 μg/kg 5-HT produced maximal contraction of the LES. The values represent peak responses.

which show that 5-HT can activate receptors at at least three different sites to modify the lower esophageal sphincter pressure (LESP) in intact opossums.

METHODS

The studies were conducted in 49 opossums (*Didelphis virginiana*). In this species the lower esophageal sphincter, as in man, is composed of smooth muscle fibers (13). The animals of either sex, weighing between 2.5 and 5.1 kg, were fasted overnight and were anesthetized with pentobarbital sodium (14). The anesthesia was maintained by periodic i.v. administration of pentobarbital (3 mg/kg) so that the animals showed no conjuctival reflex, their jaw muscles were flaccid, and toe withdrawal on pinching was absent. The animals were strapped supine on an animal board, put on an artificial ventilator, and their jugular veins were cannulated for i.v. administration of drugs. The carotid artery was cannulated with a catheter filled with heparinized saline. The catheter was connected with a pressure transducer (Statham model P23Db; Statham Instruments Div., Gould Inc., Oxnard, Calif.) to record blood pressure. All the animals had bilateral cervical vagotomy to eliminate the influence of extrinsic reflexes mediated by the vagus nerves.

The animals were made to swallow a specially designed catheter assembly described elsewhere (15). The catheters were continuously perfused with boiled water at a rate of 0.33 ml/min using a thin polyethylene tubing (Clay Adams, Div. of Becton, Dickinson & Co., Parsippany, N.J.; model PE10; ID = 0.28 and OD = 0.61 mm). The compliance of this system was very low; sudden occlusion of the catheter tip caused a rate of pressure rise of 100 mm Hg in 0.1 s. The pressures were recorded on a Beckman Dynograph recorder (model R411; Beckman Instruments, Inc., Fullerton, Calif.) with Statham transducers (model P23Db).

The catheter assembly was anchored in the LES. The details of this technique have been described elsewhere (15). Briefly, the catheter assembly was first passed into the stomach orally, so that a few centimeters of its terminal part rested in the stomach. The animal was then prepared for laparotomy, and the abdomen was opened with a midline incision. The catheter assembly was gradually withdrawn so that one of the openings recorded the highest LESP. The catheter assembly was anchored in place by inserting two ordinary pins, 5 mm apart, through the sphincter area and the central core of the manometric catheter (15). Unless stated otherwise, drugs were administered as single 30-s boluses followed by flushing with a 2-ml sterile saline solution. The volumes of drugs varied from 0.2 to 0.8 ml.

Whenever needed, continuous infusion was carried out with a B. Braun-Melsungen continuous infusion pump (Bronwill Scientific, a division of Will Scientific, Inc., Rochester, N. Y.). Different doses of 5-HT were given at random, and an interval of at least 30 min elapsed between two doses. All the responses were measured as the peak effect. Except for the studies on the reserpinized animals, the effect of 5-HT was studied before and after the antagonists in the same animals. In this way each animal served as its own control, and the statistical significance was calculated with the paired *t* test. The LES responsiveness to unrelated agonists was tested after treatment with antagonists. For catecholamine depletion experiments, the animals were treated with intraperitoneal administration of reserpine (3 mg/kg) 48 and 24 h before the experiment as described elsewhere (16). Such a treatment abolished the effect of tyramine on the LES (16, 17). Moreover, de Carle et al. (18) have reported disappearance of catecholamine fluorescence in the esophagus after treatment of opossums with a smaller reserpine dose than that used in this study.

In experiments where tetrodotoxin was used, tetrodotoxin was administered intravenously in doses of 10 μg/kg (15). After administration of tetrodotoxin, the LES response to esophageal distention by balloon was recorded. In control, before administration of tetrodotoxin, 4 ml distention of balloon with air for 4 s produced 77.5±4.2% (SEM) fall in the sphincter pressure (n = 8, two observations each in four animals). The basal sphincter pressure in these animals at the time of esophageal distention was 46.5±2.9 mm Hg (n = 8, two observations each in four animals). The administration of tetrodotoxin was continued (10 μg/kg at a time) 10 min apart until no LES relaxation was seen on esophageal distention. The sphincter pressure at the time of esophageal distention after complete neural antagonism with tetrodotoxin was 47.3 ±1.2 mm Hg (n = 8, two observations each in four animals). Apart from the response to esophageal distention, the influence of vagal stimulation and local stimulation (15) was also tested. Local stimulation was applied with a small electrode consisting of two stainless steel wires 3 mm apart. The electrode was inserted into the region of the LES (15). Electrical stimuli were provided by a Grass stimulator (model S48, Grass Instrument Co., Quincy, Mass). The total dose of tetrodotoxin which produced complete neural antagonism (19, 20) in these animals varied from 10 to 50 μg/kg.

The following drugs were used: 5-HT (Schwarz/Mann, Div. Becton, Dickinson & Co., Orangeburg, N. Y.); 1-methyl-d-lysergic acid butanolamide (methysergide, a gift from Sandoz Pharmaceuticals, Division of Sandoz, Inc., East Hanover, N. J. 07936); 5-methoxy,N,N,dimethyltryptamine (5 MeO-DMT), a bufotenine derivative (Aldrich Chemical Co., Inc., Milwaukee, Wis.); atropine sulfate (Eli Lilly and Co., Indianapolis, Ind.); reserpine (The Vitarine Co., Inc., New York); tetrodotoxin (Calbiochem, San Diego, Calif.); nicotine sulfate (Sigma Chemical Co., St. Louis, Mo.).

RESULTS

Effect of 5-HT on the lower esophageal sphincter pressure. Intravenous administration of 5-HT in a dose of 40 μg/kg produced a contraction of the LES. This contraction began with a latency of 15.3±2.2 s (n = 5, one observation each in five animals), and the duration of contraction varied from 1 to 4 min. The LES contraction in response to 5-HT was dose related (Fig. 1). A dose of 0.5 μg/kg produced sphincter con-

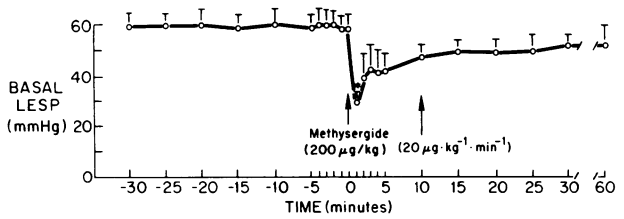

FIGURE 2 Effect of methysergide on LESP. Each point is mean±1 SE of five observations in five animals. Note that methysergide caused a transient fall in LESP. After the initial fall, the sphincter pressure stabilized near pre-infusion levels.

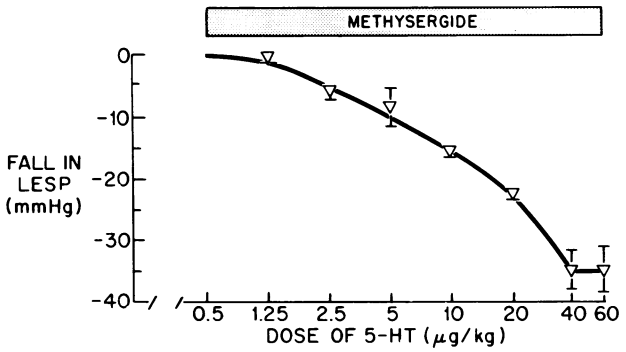

FIGURE 3 Dose-response curve of the effect of 5-HT on LESP in the presence of methysergide. In the presence of methysergide, 5-HT caused a dose-dependent fall in LESP. The maximal fall also occurred with a dose of 40 μg/kg. Each point is mean of five observations in five animals.

traction of 5.4±2.2 mm Hg (SEM). The smallest dose which produced maximal effect was 40 μg/kg; this dose caused LES contraction of 72.8±11.8 mm Hg.

Influence of tetrodotoxin on the effect of 5-HT on LESP. The effect of 40 μg/kg 5-HT was studied in animals in whom neural responses were blocked with tetrodotoxin (15). Tetrodotoxin failed to antagonize the contractile effect of 5-HT on the LES. The rise in sphincter pressure with 5-HT in control and after neural antagonism was 54.4±11.6 and 56.6±19.8 mm Hg, respectively ($P > 0.05$, $n = 7$).

Studies during methysergide infusion

Effect of methysergide on resting LESP. Methysergide was initially administered intravenously as a single bolus in a dose of 200 μg/kg. 10 min after the bolus, continuous infusion of methysergide at the dose rate of 20 μg/kg per min was started to insure a steady level of the antagonist in the circulation. The effect of methysergide on resting LESP in five animals is summarized in Fig. 2. The basal sphincter pressure of 58.8 ±5.6 mm Hg (calculated at 0 min) dropped to 30.4±5.6 mm Hg at the end of the 1st min, after administration of 200 μg/kg methysergide ($P < 0.05$, $n = 5$). The change in sphincter pressure during subsequent time intervals, as illustrated in Fig. 2, was not significant, although the pressures remained at slightly lower levels than during control period. At the end of 10 min, there was a substantial recovery of the sphincter pressure, and at that time continuous infusion of methysergide (20 μg/kg per min) was started. The continuous infusion of methysergide did not cause any further decline in the sphincter pressure.

Influence of methysergide on the effect of 5-HT dose-response curve. The effect of 5-HT on the LES was studied 20 min after the continuous infusion of methysergide was begun. In the presence of methysergide, 5-HT instead of producing contraction caused a fall in the sphincter pressure. This fall in sphincter pressure was also dose related (Fig. 3). The maximal fall of 34.6 ±3.3 mm Hg (77.2±7.2%) occurred with the dose of 40 μg/kg of 5-HT, the same dose which caused maximal contractile effect. The duration of the LES inhibition lasted from 1 to 5 min.

Influence of tetrodotoxin on the effect of 5-HT in the presence of methysergide. The effect of tetrodotoxin on the fall in LESP caused by 5-HT in the presence of methysergide was studied in four animals. The neural antagonism with tetrodotoxin antagonized this inhibitory effect of 5-HT (Fig. 4). The residual fall in sphincter pressure with 5-HT after tetrodotoxin was only 7.4±1.9 mm Hg, as compared to a fall of 34.6±3.3 mm Hg before tetrodotoxin ($P < 0.01$).

Influence of 5-HT tachyphylaxis on the effect of 5-HT in the presence of methysergide. A large dose of 5-HT (2 mg/kg) antagonized all responses to subsequently administered 5-HT. To insure tachyphylaxis specifically to the neural effects of 5-HT, 5-HT tachyphylaxis was

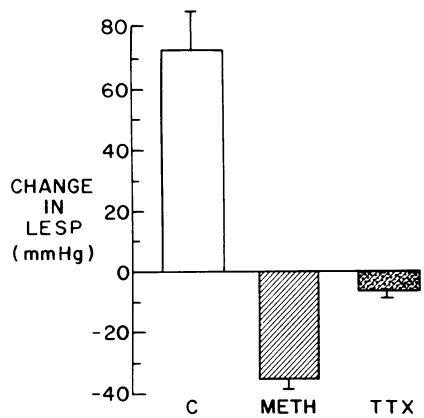

FIGURE 4 Effect of tetrodotoxin on the fall in LESP caused by 5-HT (40 μg/kg) in the presence of methysergide. Note increase in LESP with 5-HT during control period (C) and fall in the sphincter pressure in the presence of methysergide (Meth). After neural block with tetrodotoxin, the effect of 5-HT was almost abolished. These results are mean±SE of four observations in four animals.

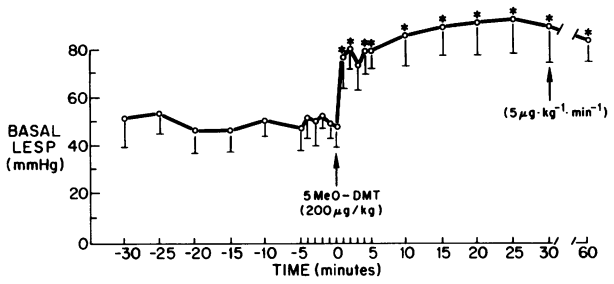

FIGURE 5 Effect of 5 MeO-DMT on LESP. Note that unlike methysergide, 5 MeO-DMT caused a marked and sustained increase in sphincter pressure. The values represent mean ±SE in three animals.

produced in the presence of methysergide. In these animals, 5-HT (40 μg/kg) caused a contraction of 52.5 ±9.9 mm Hg. After infusion of methysergide, 5-HT produced an inhibition of 37.3±4.7 mm Hg. To produce 5-HT tachyphylaxis, two massive doses of 5-HT (1 mg/kg each) were administered 10 min apart. The effect of 5-HT was studied after the stabilization of LES pressure, while methysergide was still present. After this treatment, the fall in LESP with 5-HT was significantly antagonized to 5.0±2.1 mm Hg ($P < 0.001$, $n = 6$).

Studies during 5 MeO-DMT infusion

The effect of 5 MeO-DMT on basal LESP. 5 MeO-DMT is a derivative of 5-HT and is known to antagonize certain effects of 5-HT. 5 MeO-DMT was administered as a single bolus in a dose of 200 μg/kg. At the end of the 1st min after administration, the sphincter pressure increased from 47.3±8.2 to 76.3±13.6 ($P < 0.05$, paired t test in three animals, one observation each). The sphincter pressure remained elevated during the subsequent time intervals (Fig. 5). At the end of ½ h after the bolus of 5 MeO-DMT, a continuous infusion of 5 MeO-DMT (5 μg/kg per min) was started.

Influence of 5 MeO-DMT on the dose-response curve of 5-HT. The effect of 5-HT on the LES was studied after a continuous infusion of 5 MeO-DMT was begun. The effect of different doses (5, 10, 20, and 40 μg/kg) of 5-HT was studied in five animals during control as well as during infusion of 5 MeO-DMT. 5 MeO-DMT augmented 5-HT responses (Fig. 6); the shift of the 5-HT dose-response curve in the presence of 5 MeO-DMT as compared to control was statistically significant ($P < 0.05$; sign test (21) for all the individual points on the curve).

Influence of 5 MeO-DMT on the effect of nicotine on the LES. The presence of 5 MeO-DMT did not interfere with the action of nicotine on the LES. There was a fall of 57.5±2.5% and 59.7±2.9% after nicotine (50 μg/kg) in control and during infusion of 5 MeO-DMT, respectively ($P > 0.05$, $n = 4$).

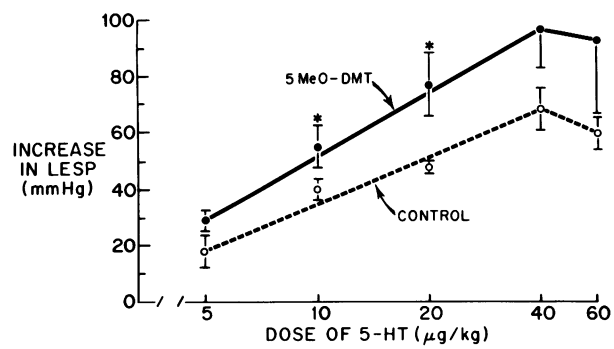

FIGURE 6 Dose-response curve of the effect of 5-HT in the presence of 5 MeO-DMT. Note the augmentation of the increase in LESP with 5-HT after 5 MeO-DMT as compared to control response. Each point represents mean±SE of five observations in five animals. (*) indicates that the difference was statistically significant ($P < 0.05$).

Studies during infusion of methysergide plus 5 MeO-DMT

Effect of methysergide plus 5 MeO-DMT on basal LESP. The mean basal sphincter pressure was 48.5 ±3.6 during control period and 58.8±5.4 mm Hg after treatment with 5 MeO-DMT and methysergide. This difference in pressure was statistically significant ($P < 0.01$, six animals).

Influence of 5 MeO-DMT on the effect of 5-HT in the presence of methysergide. The influence of a combination of the two 5-HT antagonists, methysergide and 5 MeO-DMT, on the effect of 5-HT was studied in six animals as follows: The effect of 40 μg/kg 5-HT was studied in control period, then in the presence of methysergide and finally in the presence of methysergide and 5 MeO-DMT. As shown in Fig. 7A, 5-HT alone in control experiments caused a contraction of 65.0±10.4 mm Hg. After methysergide, 5-HT caused a fall of 35.4 ±4.8 mm Hg in LESP. Addition of 5 MeO-DMT not only antagonized the inhibitory effect of 5-HT, but now 5-HT produced a sphincter contraction of 18.3±0.9 mm Hg.

Influence of methysergide on the effect of 5-HT in the presence of 5 MeO-DMT. In a different set of experiments (Fig. 7B) the order of the addition of the two 5-HT antagonists was reversed; 5 Meo-DMT was added first, and then methysergide was added. 5-HT in control produced a contraction of 67.0±6.2 mm Hg. In the presence of 5 MeO-DMT, this contraction increased to 89.7±14.0 mm Hg. In the presence of 5 MeO-DMT, methysergide reduced this contraction to 17.8 ±1.3 mm Hg ($P < 0.05$, paired t test).

Influence of tetrodotoxin on the effect of 5-HT in the presenct of 5 MeO-DMT and methysergide. Tetrodotoxin completely antagonized the residual 5-HT-pro-

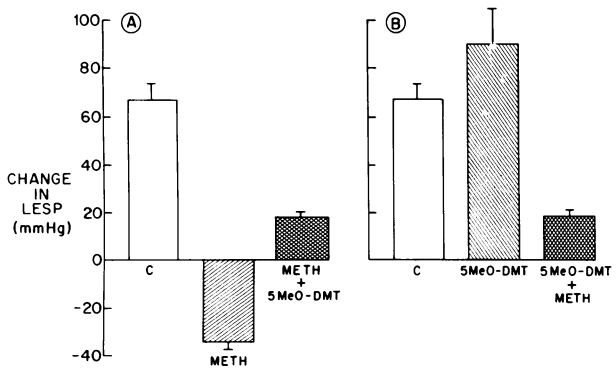

FIGURE 7 Influences of combination of methysergide and 5 MeO-DMT on the effect of 5-HT. In the experiment shown in panel (A), effect of 5-HT (40 μg/kg) was studied during control period, in the presence of methysergide and finally after addition of 5 MeO-DMT. This experiment again shows fall in LESP with 5-HT in the presence of methysergide. Addition of 5 MeO-DMT not only antagonized the inhibitory effect of 5-HT but it converted it to a contraction (mean±SE = 18.3 ±0.9 mm Hg, n = 3). In the experiments shown in panel (B), the order of addition of the two 5-HT antagonists was reversed. Note that 5 MeO-DMT enhanced the contraction by 5-HT (40 μg/kg). Addition of methysergide significantly reduced but did not abolish the LES contraction by 5-HT (n = 6).

duced contraction in the presence of 5 MeO-DMT and methysergide (Fig. 8A).

Influence of atropine on the effect of 5-HT in the presence of 5 MeO-DMT and methysergide. The contraction of LES with 5-HT in the presence of 5 MeO-DMT and methysergide was significantly antagonized by atropine (30 μg/kg). Atropine reduced contraction from 17.8±1.3 mm Hg to 3.3±1.7 mm Hg ($P < 0.05$) (Fig. 8B). However, 5 MeO-DMT, methysergide and atropine treatment did not reduce the effect of pentagastrin (1 μg/kg) on LESP.

Influence of 5 MeO-DMT, methysergide, and tetrodotoxin on the effect of bethanechol and isoproterenol on LESP. To assess the status of the responsiveness of the LES after treatment with various 5-HT antagonists and tetrodotoxin, the effect of an excitatory

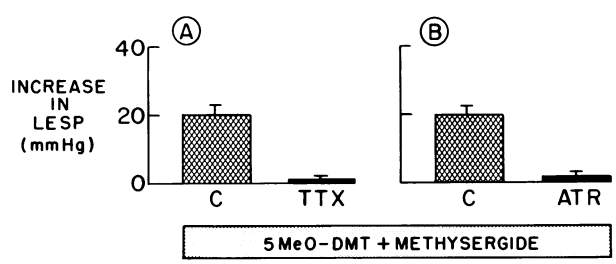

FIGURE 8 Influence of tetrodotoxin or atropine (30 μg/kg) on the LES contraction caused by 5-HT (40 μg/kg) in the presence of 5 MeO-DMT and methysergide. Note that 5-HT produced LES contraction which was antagonized by tetrodotoxin or atropine (n = three animals for each set of experiments).

TABLE I

Effect of Bethanechol and Isoproterenol on the LES Pressure during Control Period and during Antagonism with 5 MeO-DMT, Methysergide, and Tetrodotoxin

	Increase in LESP (mm Hg) with 20 μg/kg bethanechol	Fall in LESP (mm Hg) with 2.5 μg/kg isoproterenol
	Mean±SE	*Mean±SE*
Control (n = 3)	32.0±2.0	36.0±2.1
After 5 MeO-DMT plus methysergide (n = 3)	37.3±2.7 ($P > 0.05$)	29.3±4.4 ($P > 0.05$)
After 5 MeO-DMT plus methysergide plus tetrodotoxin (n = 3)	32.0±1.5 ($P > 0.05$)	31.0±2.6 ($P > 0.05$)

agent (bethanechol) and an inhibitory agent (isoproterenol) was examined. These results are summarized in Table I. Note that these antagonists did not impair the responsiveness of the LES to bethanechol or to isoproterenol.

Studies in reserpinized animals

Effect of reserpine on LESP. The mean LESP in reserpine-treated animals was 65.0±2.7 mm Hg, and it was 58.5±1.6 mm Hg in the control animals. This difference was not significant ($P > 0.05$). These findings are similar to our previous studies (16) in which we also did not find any influence of reserpinization on the LESP.

Influence of reserpine pretreatment on the dose-response curve of the effect of 5-HT on LESP. As shown in Fig. 9, reserpinization augmented the contractile response of the LES to 5-HT at all of the doses tested. There was a significant shift of the dose-

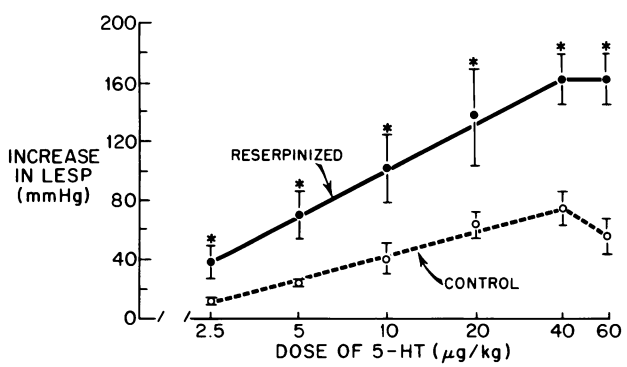

FIGURE 9 Effect of reserpine pretreatment on the dose-response curve of the effect of 5-HT on LESP. The response curve in reserpinized animals (n = four animals) was significantly different from that in another group (four animals) of nonreserpinized (control) animals. (*) indicates that values were significantly different ($P < 0.05$).

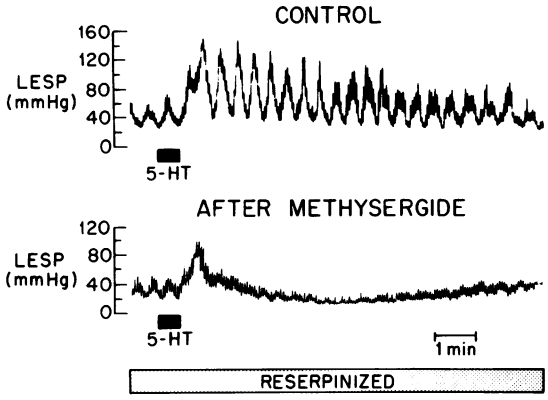

FIGURE 10 Influence of methysergide on the effect of 5-HT on LESP in reserpinized animals. Note a marked rhythmic contraction of the LES after 5-HT (40 μg/kg) in the control period. After methysergide, 5-HT produced a biphasic response; there was an initial contraction which was followed by a more prolonged inhibition. The initial contraction with 5-HT in the presence of methysergide was not observed in the nonreserpinized (control) animals.

response curve after reserpine treatment as compared to the curve in another group of nonreserpinized (control) animals.

Influence of methysergide on the effect of 5-HT in reserpinized animals. To examine the possibility that the inhibitory component of the action of 5-HT was due to participation of catecholamines, the effect of 5-HT on LESP was examined in the reserpinized animals in the presence of methysergide. In these experiments, 40 μg/kg 5-HT produced a biphasic response. There was an initial contraction followed by a prolonged inhibition (Fig. 10). Such studies in four animals showed an initial contraction of 30.0±3.9 mm Hg followed by peak inhibition of 27.5±3.2 mm Hg (Fig. 11). These results in reserpinized animals were very different from those obtained in nonreserpinized animals, as described earlier.

Influence of atropine and 5 MeO-DMT on the response to 5-HT (Fig. 11). The initial contraction caused by 5-HT in the presence of methysergide in the reserpinized animals was antagonized by atropine (30 μg/kg). The sphincter contraction was 30.0±3.9 mm Hg before atropine and 2.5±1.5 mm Hg after atropine ($P < 0.01$). Atropine did not influence the inhibitory response which followed the initial contraction.

As shown in Fig. 11, addition of 5 MeO-DMT antagonized the inhibitory component of the effect of 5-HT in these experiments ($P < 0.01$).

DISCUSSION

The lower esophageal sphincter provides a good model for the study of excitatory and inhibitory effects of drugs. The sphincter muscle maintains a basal tone at rest; against this tone, both inhibition and excitation can be easily demonstrated and quantitated.

These studies show that contraction of the LES caused by 5-HT is a net effect of stimulation of at least three different receptor sites. A model of the possible location of the 5-HT receptors is summarized in Fig. 12. According to this model, 5-HT causes contraction by: (*a*) a direct excitatory effect on the sphincter muscle; (*b*) an indirect excitatory effect due to stimulation of cholinergic excitatory neurons; and (*c*) an indirect inhibitory effect due to stimulation of nonadrenergic inhibitory neurons in the LES.

Gaddum and Picarelli (22) suggested that stimulation of the guinea pig ileum by 5-HT was due to activation of two kinds of receptors for 5-HT, namely a muscle receptor and a neural receptor. The muscle receptor was designated as D type because the effect of 5-HT on this receptor was antagonized by Dibenzyline (phenoxybenzamine) (Smith Kline & French Laboratories, Philadelphia, Pa.). On the other hand, neural receptor was designated as M type because the effect of 5-HT on this receptor was antagonized by morphine. However, it is now known that both Dibenzyline and morphine exert several nonspecific effects. Therefore, the classification of the 5-HT receptors into D or M types is not currently favored (23), but it is generally agreed that 5-HT may exert both direct and indirect excitatory effects on the gut muscle. In certain parts of the gut such as duodenum (mouse), the direct effect of 5-HT is more prominent (23), while in others such as guinea pig ileum, the indirect neural effect of 5-HT is predominant (24, 25).

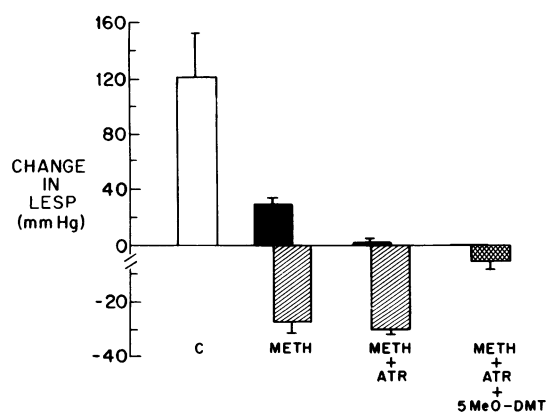

FIGURE 11 Influence of atropine and 5 MeO-DMT on the biphasic response of LES to 5-HT. Note that in these four reserpinized animals 5-HT caused contraction during control (C) period. In the presence of methysergide, 5-HT caused an initial contraction followed by inhibition of LES. Addition of 30 μg/kg of atropine antagonized the initial contraction. Both contraction and inhibition were antagonized by a combination of atropine and 5 MeO-DMT.

In the LES, 5-HT appears to exert a prominent direct stimulatory effect. LES contraction caused by 5-HT was not antagonized after antagonism of neural activity by tetrodotoxin. It is now generally agreed that methysergide selectivity antagonizes the direct effect of 5-HT on the muscle (26, 27). Interestingly, methysergide converted the effect of 5-HT from an increase in LESP to a fall in LESP. The evidence for an indirect excitatory effect of 5-HT came from studies in the reserpinized animals. After catecholamine depletion with reserpine, 5-HT caused an initial brief contraction followed by a more sustained contraction of the LES. Treatment with methysergide made the initial contraction obvious, as methysergide converted the sustained contraction to inhibition (Fig. 10). The residual brief contraction was antagonized by neural block with tetrodotoxin and also by muscarinic antagonism with atropine. These observations suggest that this contraction may be due to stimulation of cholinergic neurons. The excitatory effect of 5-HT on the cholinergic neurons could also be demonstrated when 5-HT was administered in the presence of methysergide and 5 MeO-DMT (27). In the presence of these two 5-HT receptor antagonists, 5-HT caused LES contraction which was also opposed by tetrodotoxin or atropine. These studies also revealed the inability of both methysergide and 5 MeO-DMT in antagonizing the action of 5-HT mediated by the cholinergic excitatory neuron. These studies suggest that the 5-HT receptor causing cholinergic neuron stimulation may be somewhat different from the receptor on the muscle which is blocked by methysergide and the receptor on the inhibitory neurons which is blocked by 5 MeO-DMT. The 5-HT receptor causing cholinergic neuron stimulation is, however, antagonized by 5-HT tachyphylaxis, which antagonizes the effect of 5-HT on all its receptors. Studies in other parts of the gut have shown that 5-HT can stimulate intramural cholinergic neurons (2, 8, 23).

Our findings are consistent with the view that 5-HT receptor may be present on the cholinergic neuron, but we cannot exclude the possibility that this effect may be due to stimulation of the sensory receptor organs which may cause a reflex, cholinergically mediated contraction of the LES. 5-HT has been shown to cause stimulation of sensory organs (28, 29). However, since our studies were done in bilaterally vagotomized animals, a role of vagally mediated reflex contraction by 5-HT can be ruled out. Further studies are needed to define the role of sensory receptor organ stimulation in mediating the action of 5-HT on the LES.

5-HT also exerted a marked inhibitory influence on the LES; this effect was demonstrated only when the predominant direct excitatory effect of 5-HT on the sphincter muscle was antagonized by methysergide. The inhibitory effects of 5-HT on the LES appeared to

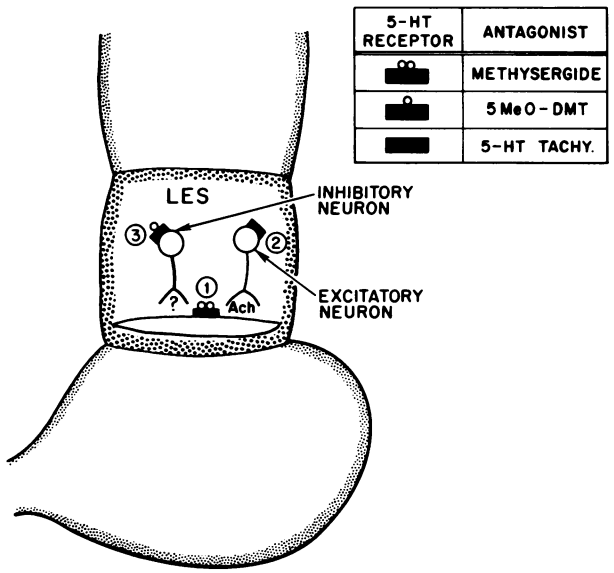

FIGURE 12 A model summarizing the possible receptor sites for 5-HT on the LES: (*1*) Shows 5-HT receptor on sphincter muscle itself. The effect of 5-HT on this receptor is antagonized by methysergide or by 5-HT tachyphylaxis. (*2*) Shows a 5-HT receptor on cholinergic excitatory neuron; this effect is antagonized by 5-HT tachyphylaxis, atropine or tetrodotoxin. Neural excitatory effect of 5-HT is not antagonized by either methysergide or 5 MeO-DMT. (3) Shows a 5-HT receptor on the nonadrenergic inhibitory neuron. The effect of 5-HT on this neural receptor is antagonized by 5 MeO-DMT and by 5-HT tachyphylaxis. This effect is also antagonized by tetrodotoxin.

be neurally mediated effects, as these effects were antagonized by tetrodotoxin (20). The inhibitory effects were selectively antagonized by 5 MeO-DMT. 5 MeO-DMT is an alkylindol derivative and is known to selectively antagonize the effecct of 5-HT on certain neural 5-HT receptors (27, 30). The shift of the 5-HT dose-response curve to the left with 5 MeO-DMT was also consistent with the view that 5 MeO-DMT selectively antagonized the inhibitory effects of 5-HT and consequently enhanced the excitatory effect of 5-HT on LESP. The inhibitory effect of 5-HT on certain other parts of the gut, such as the stomach and colon in man and animals, may be due to a neurally mediated effect (26).

The inhibitory effect of 5-HT on the LES appeared to be due to stimulation of nonadrenergic inhibitory neurons, as catecholamine depletion with reserpine did not abolish the inhibitory effect. Nonadrenergic inhibitory neurons have been shown to be present in the LES (16, 31) and, moreover, these neurons have been shown to lie in the vagal pathway to the LES (16). Bülbring and Gershon have shown that 5-HT exerts inhibitory influence on the guinea pig stomach and mouse duodenum by stimulating noncholinergic, nonadrenergic inhibitory neurons (32). Our studies do not

exclude the possibility that 5-HT may stimulate mechanoreceptors (28, 29) to cause reflex fall in LESP. The afferents from the mechanoreceptors have been shown to be present mainly in the vagus (28). We can exclude the participation of the vagus in any such reflex, as these studies were done in vagotomized animals, but involvement of locally mediated reflex cannot be excluded. Moreover, our studies cannot exclude a possible effect of 5-HT on preganglionic nerve terminals synapsing with the postganglionic intramural neurons.

Reserpine treatment produced interesting modifications in the effects of 5-HT. It enhanced the excitatory effect of 5-HT, and it also unmasked the neurally mediated (by cholinergic neurons) excitatory effect of 5-HT. These effects can be explained in several ways. First, these effects could be due to depletion of catecholamines by reserpine. According to this view, 5-HT may cause release of catecholamines (33, 34), which may exert an inhibitory influence on the LES. With catecholamine depletion this inhibitory component of the effect of 5-HT is abolished which leads to apparent enhancement of excitatory effects of 5-HT. However, LES possesses alpha adrenergic excitatory and beta adrenergic inhibitory receptors (17, 35), and catecholamine release from adrenergic neurons with tyramine leads to contraction, rather than inhibition of the LES (16, 17). Second, the increased sensitivity to 5-HT may be due to interference of exogenous 5-HT uptake (36) by the putative tryptaminergic structures in the LES. Third, 5-HT supersensitivity may be due to increased cholinergic activity associated with reserpine treatment. Green et al. (37) have shown that reserpine-induced 5-HT supersensitivity in the guinea pig ileum was due to increased cholinergic activity, which was different from nonspecific supersensitivity after reserpinization seen in other structures (36, 38). Phenomenon of increased cholinergic activity associated with reserpinization can explain both increased sensitivity of the LES to excitatory effects of 5-HT at all dose levels as well as the unmasking of cholinergically mediated early contraction of the LES.

Further studies are needed to further define the mode of 5-HT supersensitivity after reserpinization. However, these experiments did help to unmask excitatory cholinergic neurons in the LES.

These studies have considerable potential physiological and clinical implications. It has been reported that a component of the inhibitory effect of vagal stimulation of the LES may be noncholinergic, as antagonism of both nicotinic and muscarinic receptors does not completely abolish the vagal response (16). These studies show that 5-HT can inhibit the LES by acting indirectly by stimulating nonadrenergic neurons. 5-HT, therefore, becomes a potential candidate involved in noncholinergic transmission of vagal inhibitory pathway to the LES. Bülbring and Gershon (32) have shown that 5-HT may participate in the vagal inhibitory responses to the guinea pig stomach. The excitatory and the inhibitory effects of different 5-HT antagonists on the LESP, particularly that of 5 MeO-DMT, also suggest a modulatory role for 5-HT on the LES.

Clinically increased circulating levels of 5-HT occur in patients with carcinoid syndrome (1). LES function in carcinoid syndrome has not been investigated. However, it is of some interest to note that methysergide therapy in these patients is associated with frequent occurrence of heartburn (4). If these studies in opossum are applicable in man, one can provide an explanation for increased frequency of heartburn in patients treated with methysergide. Methysergide treatment may cause considerable fall in sphincter pressure due to largely unopposed action of 5-HT on the inhibitory neurons.

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