Br. J. Pharmac (1983), **78**, 103–110

Indirect action of 5-hydroxytryptamine on the isolated muscularis mucosae of the guinea-pig oesophagus

Yuichiro Kamikawa & Yasuo Shimo

Department of Pharmacology, Dokkyo University School of Medicine, Mibu, Tochigi 321-02, Japan

1 The site of action of 5-hydroxytryptamine (5-HT) was examined on the isolated muscularis mucosae attached to the submucous plexus of the guinea-pig oesophagus. Isotonic responses of the longitudinal muscularis mucosae were recorded.

2 5-HT produced a transient contraction of the muscularis mucosae at concentrations higher than 3 μM. The contraction was rapid in onset, reaching a peak in about 15 s or less, and was restored to the basal level after 20 to 30 s without washing out 5-HT. When the 5-HT-induced contraction faded to the basal tone, successive applications of 5-HT no longer produced any contracture.

3 Nicotine (Nic), at concentrations higher than 10 μM, also produced a transient contraction which had a very similar pattern to that induced by 5-HT. Again, the successive application of Nic no longer produced any contracture following prior treatment with Nic itself. However, the 5-HT-induced contraction was not modified by the presence of Nic.

4 Exogenously applied acetylcholine (ACh) produced a concentration-dependent contraction of the muscularis mucosae, the 50% effective concentration (EC_{50}) was 69 ± 5.6 nM. The contraction was sustained during incubation with ACh, and was not modified by prior treatment with 5-HT or Nic.

5 The 5-HT (100 μM)-induced contraction was completely abolished by tetrodotoxin (0.2 μM) and atropine (0.2 μM). This means that the action is mediated by stimulating cholinergic nerves in the submucous plexus attached to muscularis mucosae. Moreover, the stimulating action of 5-HT does not involve nicotinic receptors, since the action was not blocked by hexamethonium (100 μM).

6 Among several 5-MT antagonists examined, methysergide (1 μM), ketanserin (1 μM) and morphine (100 μM) failed to modify the 5-HT (100 μM)-induced contraction significantly. Cinanserin (0.1–3 μM), cyproheptadine (3–100 nM) and phenoxybenzamine (0.1–3 μM) inhibited the 5-HT-induced contraction, in a concentration-dependent manner, and each highest concentration abolished the response. However, none of these antagonists was specific for 5-HT, but the Nic (100 μM) or ACh (0.1 μM)-induced contractions were also inhibited by them.

7 The present results indicate that 5-HT contracts the muscularis mucosae of the guinea-pig oesophagus indirectly by stimulating cholinergic nerves in the submucous plexus, and has no direct action on the muscularis mucosae. In addition, the type of 5-HT receptors responsible for the stimulant action may be different from those in other parts of the gastrointestinal tract, blood vessels or brain, because of the different effects of 5-HT antagonists.

Introduction

There is much evidence to suggest that 5-hydroxytryptamine (5-HT) may be a gastrointestinal neurotransmitter (Bülbring & Gershon, 1967; Costa & Furness, 1976; Gershon, Robinson & Ross, 1976; Wood & Mayer, 1978; 1979; Costa & Furness, 1979a; Gershon & Tamir, 1981; Ahlman, Demagistris, Zinner & Jaffe, 1981; Gershon, 1981; Furness & Costa, 1982). Endogenous 5-HT observed in the mammalian gastrointestinal tract is stored mainly in mucosal enterochromaffin cells (Erspamer & Asero, 1952) but partly in intramural nerves, and may regulate gut motility. Exogenously applied 5-HT contracts gastrointestinal smooth muscles. The response is mediated not only by a direct action on the smooth muscle but also by an indirect action on intramural nerve plexuses (Gaddum & Picarelli, 1957; Day & Vane, 1963; Brownlee & Johnson, 1963; Gershon, 1967; Drakontides & Gershon, 1968; Yamaguchi, 1972; Burleigh, 1977; Costa & Furness, 1979b). These two kinds of action of 5-HT seem to be

0007-1188/83/010103-08-$01.00

mediated by two types of 5-HT receptors, which had been named the M and D receptors by Gaddum & Picarelli (1957). The M receptors, which can be blocked by morphine, are present in nervous structures and the D receptors, which can be blocked by phenoxybenzamine, are in the smooth muscle. However, this nomenclature has been questioned because of the poor specificity of these blocking drugs (Paton, 1957; Drakontides & Gershon, 1968; Cook, 1971) and at present more than two subtypes of 5-HT receptor have been proposed (Wallis, 1981).

Most physiological and pharmacological studies on the action of 5-HT in the gastrointestine have been performed on longitudinal or circular smooth muscle and on the myenteric plexus which is located between these two muscle layers. However, there are few reports based on experiments using the muscularis mucosae (Bartlet, 1968) and the submucous plexus (Hirst & Silinsky, 1975) which are located in submucosal layers. The isolated muscularis mucosae of the guinea-pig oesophagus contains a submucous plexus which includes cholinergic nerve cell bodies, independent of the myenteric plexus (Kamikawa & Shimo, 1979). This is a suitable preparation for examining the actions of drugs on the submucous plexus and muscularis mucosae of the alimentary tract (Kamikawa, Shimo & Uchida, 1982; Kamikawa & Shimo, 1982a). Previously, Bartlet (1968) showed that 5-HT produced a contraction of this tissue which is partially blocked by methysergide or hyoscine. Our previous paper (Kamikawa & Shimo, 1982a) demonstrated that low concentrations (less than 3 μM) of 5-HT facilitate cholinergic neurotransmission in this preparation via a prejunctional mechanism. This led us to re-examine the action of 5-HT and the receptor types in the submucous plexus and muscularis mucosae. The results indicate that 5-HT produces a contraction of the isolated muscularis mucosae of the guinea-pig oesophagus induced by stimulating cholinergic nerves in the submucous plexus, and that it has no direct action on muscularis mucosae. A preliminary account of some of these results has been given (Kamikawa & Shimo, 1982b).

Methods

Male guinea-pigs (300 to 600 g) were stunned, the oesophagus excised and the isolated muscularis mucosae attached to the submucous plexus was prepared (Kamikawa & Shimo, 1979). Briefly, the excised oesophagus was pinned on a cork mat immersed in Tyrode solution. The outer striated muscle coat was cut longitudinally, and gently peeled away leaving an inner tube. The tube including the longitudinal muscularis mucosae, about 15 mm long without a load, was immersed in a 15 ml organ bath filled with Tyrode solution of the following composition (mM): NaCl 136.8, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, disodium edetate (EDTA) 0.03, ascorbic acid 0.12 and glucose 5.56 (pH 7.4). The Tyrode solution always contained 20 μM choline chloride and was bubbled with 5% CO_2 and 95% O_2, and maintained at 37°C. The preparation was suspended under a 0.3 g load and 60 min was allowed to elapse before experiments were started. Responses of the longitudinal muscularis mucosae were recorded isotonically by means of an isotonic transducer (MEC-1411) and a Nihon Kohden polygraph recorder (RJG-4004).

The data obtained are expressed as mean ± s.e.mean. Each experimental group consisted of 8–15 preparations taken from different animals. Student's t tests for paired or unpaired observations were used for statistical evaluation of the data. P values smaller than 0.05 were considered to be significant.

Drugs used were 5-hydroxytryptamine creatinine sulphate (Nakarai), nicotine tartrate, hexamethonium chloride, atropine sulphate (Wako), acetylcholine chloride (Daiichi), tetrodotoxin (Sankyo), methysergide bimaleate (Sandoz), ketanserin tartrate (R41 468, Janssen-Kyowa), morphine hydrochloride (Dainippon), cinanserin hydrochloride (Squibb), cyproheptadine hydrochloride (Nippon Merck-Banyu) and phenoxybenzamine hydrochloride (Tokyo Kasei). To prepare stock solutions, all drugs were dissolved in 0.9% w/v NaCl solution (saline). Further dilutions were made with Tyrode solution each day. The molar concentrations of drugs described in this paper refer to the final bath concentrations.

Results

The isolated muscularis mucosae of the guinea-pig oesophagus usually showed neither tone nor spontaneous activity. 5-HT, at concentrations up to 1 μM, had no significant influence on the tone of the muscularis mucosae. Above 1 μM, 5-HT produced a contraction of the muscularis mucosae which had a rapid onset, reaching a peak in about 15 s or less (Figure 1a). When 5-HT was left in the organ bath, the contraction soon began to fade and the basal tone was re-established within 20 to 30 s. In a few preparations (8 out of 72), the 5-HT-induced transient contraction was followed by a gradual increase in tone, the height of which was usually smaller than that of the initial contraction. When the basal tone had been re-established without washing out 5-HT from the bath, further addition of a higher concentration of 5-HT no longer produced any contracture (Figure 1b). The response to 5-HT was also reduced when the agonist

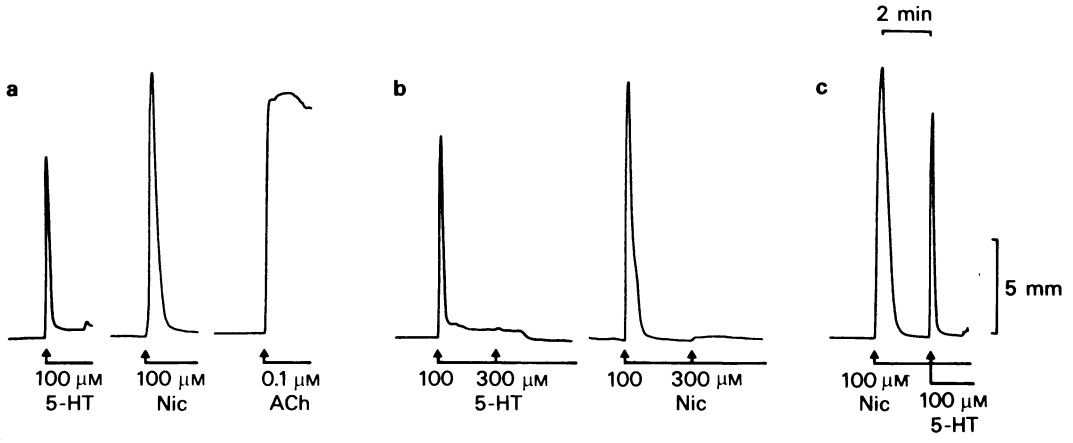

Figure 1 The contractile responses to 5-hydroxytryptamine (5-HT, 100 μM), nicotine (Nic, 100 μM), and acetylcholine (ACh, 0.1 μM) of the isolated muscularis mucosae of the guinea-pig oesophagus. Both 5-HT and Nic produced a transient contraction, while ACh produced a sustained one (a). When the tone induced by 5-HT and Nic had declined to the basal level without washing out either drug, further additions of a higher concentration of each agonist no longer produced any contracture (b). However, prior treatment with Nic did not modify the 5-HT-induced contraction (c). Vertical calibration shows 5 mm shortening of the tissue; horizontal calibration is 2 min.

was re-applied to the bath within 10 min of washing out the previous dose of 5-HT. These results indicate a rapid development of tachyphylaxis or desensitization to the action of 5-HT in this tissue. To obtain reproducible responses, the interval between doses required was 20–30 min. A very similar pattern of contractile responses of the muscularis mucosae was also observed with nicotine (Nic) at concentrations higher than 10 μM. The contraction reached a peak in about 30 s or less, and soon began to fade, the basal tone being re-established within about 60 s (Figure 1a). When the contraction had faded completely, the successive addition of Nic produced no contracture (Figure 1b). However, as shown in Figure 1c, 5-HT (100 μM) produced a contraction even in the presence of Nic (100 μM). A similar result was obtained when the order of application of these agonists was reversed. The results suggest that the contractile responses to 5-HT and Nic are mediated by separate types of receptors. On the other hand, acetylcholine (ACh, 0.003–3 μM) produced a contraction of the muscularis mucosae which had a rapid onset and which was sustained throughout the drug application (Figure 1a). The contraction was not significantly modified by pretreatment with 5-HT (100 μM or Nic (100 μM). The contractile response to ACh was dependent on the concentration and the maximum was obtained at 3 μM (Figure 2). As shown in Figure 2, the concentration-response curves for 5-HT and Nic were located to the right of that for ACh and the maximum responses to 5-HT and Nic were approximately 60% and 70% of the maximum induced by ACh (3 μM), respectively, As suggested from the standard errors of the mean, the magnitudes of the responses to 5-HT and Nic varied from preparation to preparation, while that to ACh was quite constant in all preparations. In subsequent experiments to analyse its site of action, 5-HT was used in a concentration of 100 μM, and was compared with responses to Nic (100 μM) and ACh (0.1 μM). To avoid the

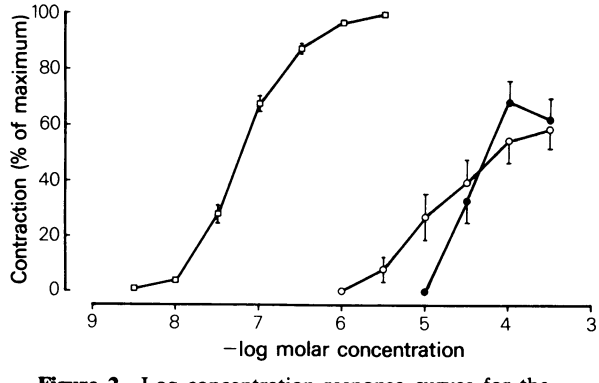

Figure 2 Log concentration-response curves for the contractile responses to acetylcholine (ACh, □, $n = 15$), 5-hydroxytryptamine (5-HT, ○, $n = 12$) and nicotine (Nic, ●, $n = 11$) of the isolated muscularis mucosae of the guinea-pig oesophagus. ACh was added cumulatively. 5-HT and Nic were applied to the tissue in single doses; each concentration was applied at random with a 30 min interval between doses. The concentrations (μM) of ACh, 5-HT and Nic which produced a 50% contraction of the maximum (induced by 3 μM of ACh) were 0.069 ± 0.006, 158.4 ± 50.7 and 107.9 ± 31.6, respectively. Each point represents the mean response; vertical lines show s.e.mean.

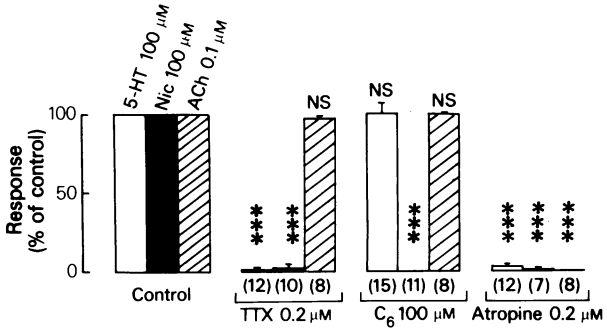

Figure 3 The effects of tetrodotoxin (TTX, 0.2 μM), hexamethonium (C_6, 100 μM) and atropine (0.2 μM) on the contractile responses to 5-hydroxytryptamine (5-HT, 100 μM; open columns), nicotine (Nic, 100 μM; solid columns) and acetylcholine (ACh, 0.1 μM; hatched columns) of the isolated muscularis mucosae of the guinea-pig oesophagus. The antagonists were applied to the tissues 5 min before the addition of agonists. 5-HT-induced contractions were abolished by TTX and atropine, but not by C_6. Numbers in parentheses under each column are the numbers of observations. *** $P < 0.001$; NS, not significant. The differences were examined by the paired t test.

development of tachyphylaxis, the intervals between doses of these agonists were 30 min.

The 5-HT (100 μM)-induced transient contraction was abolished by tetrodotoxin (0.2 μM) and atropine (0.2 μM), but not by hexamethonium (100 μM) (Figure 3). The biphasic response to 5-HT observed in a few preparations ($n = 8$) was also abolished by tetrodotoxin (0.2 μM) and atropine (0.2 μM). The Nic (100 μM)-induced contraction was also abolished by tetrodotoxin (0.2 μM), hexamethonium (100 μM) and atropine (0.2 μM), whereas that to ACh (0.1 μM) was abolished only by atropine (0.2 μM) (Figure 3). These results indicate that 5-HT contracts the isolated muscularis mucosae of the guinea-pig oesophagus by an indirect action by stimulation of cholinergic nerves via non-nicotinic receptors in the attached submucous plexus. Both methysergide and ketanserin, antagonists of 5-HT, at concentrations less than 1 μM, failed to modify the 5-HT-induced transient contraction. At 1 μM, the antagonists appeared to augment the response to 5-HT but not to Nic and ACh, but the effect was not statistically significant (Figure 4). Above 10 μM, both antagonists produced a weak contraction of muscularis mucosae. Morphine, in concentrations up to 100 μM, also failed to modify the

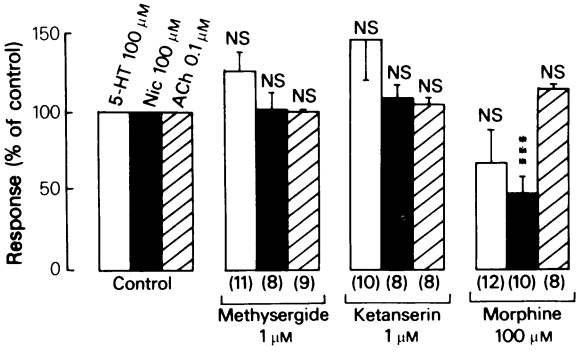

Figure 4 The effects of methysergide (1 μM), ketanserin (1 μM) and morphine (100 μM) on the contractile responses to 5-hydroxytryptamine (5-HT, 100 μM; open columns), nicotine (Nic, 100 μM; solid columns) and acetylcholine (ACh, 0.1 μM; hatched columns) of the isolated muscularis mucosae of the guinea-pig oesophagus. The antagonists were applied to the tissues 5 min before the addition of agonists. 5-HT-induced contractions were not significantly modified by methysergide, ketanserin and morphine, while Nic-induced contractions were significantly depressed by morphine. Numbers in parentheses under each column show numbers of observations. *** $P < 0.001$; NS, not significant. The differences were examined by the paired t test.

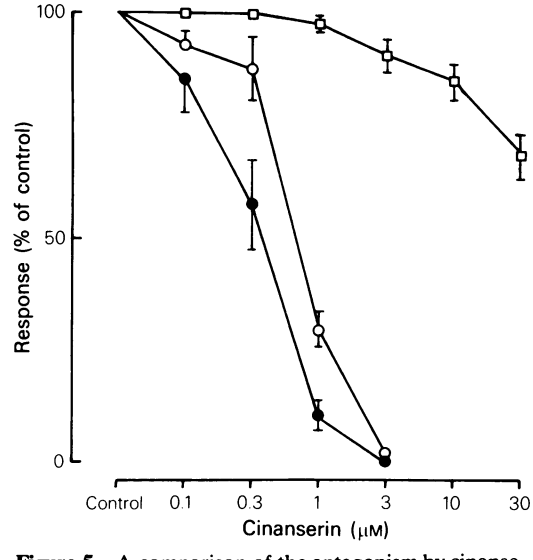

Figure 5 A comparison of the antagonism by cinanserin of the contractions induced by 5-hydroxytryptamine (5-HT, 100 μM; ○), nicotine, (100 μM; ●) and acetylcholine (ACh, 0.1 μM; □) in the isolated muscularis mucosae of the guinea-pig oesophagus. Cinanserin was applied to the tissues 5 min before the addition of agonists. The ordinate scale shows the amplitude of the response as a percentage of the control. Each point represents the mean response; vertical lines show s.e. mean. Numbers of observations are shown in Table 1.

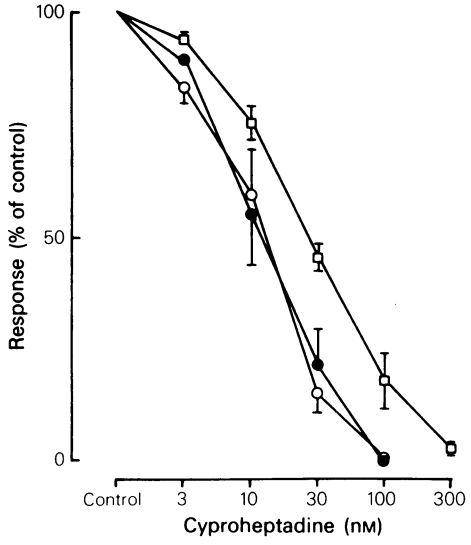

Figure 6 A comparison of the antagonism by cyproheptadine of the contractions induced by 5-hydroxytryptamine (5-HT, 100 μM; ○), nicotine (Nic, 100 μM; ●) and acetylcholine (ACh, 0.1 μM; □) on the isolated muscularis mucosae of the guinea-pig oesophagus. Cyproheptadine was applied to the tissues 5 min before the addition of agonists. Each point represents the mean response; vertical lines show s.e.mean. Numbers of observations are shown in Table 1.

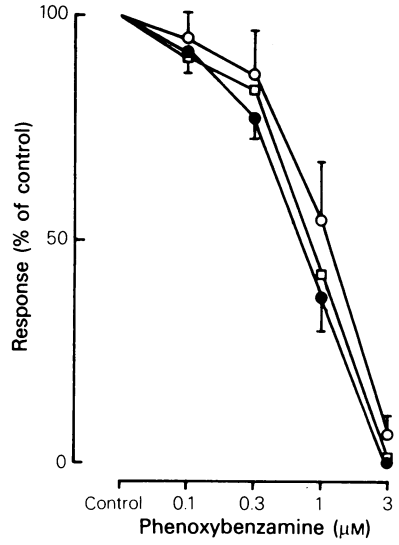

Figure 7 A comparison of the antagonism by phenoxybenzamine of the contractions induced by 5-hydroxytryptamine (5-HT, 100 μM; ○), nicotine (Nic, 100 μM; ●) and acetylcholine (ACh, 0.1 μM; □) in the isolated muscularis mucosae of the guinea-pig oesophagus. Phenoxybenzamine was applied to the tissues 5 min before the addition of agonists. Each point represents the mean response; vertical lines show s.e. mean. Numbers of observations are shown in Table 1.

response to 5-HT, but reduced that to Nic by some 50% whereas that to ACh was unaffected (Figure 4). Cinanserin, at concentrations above 0.1 μM, inhibited the 5-HT-induced contractions, in a concentration-dependent manner, and at 3 μM abolished the response (Figure 5). However, cinanserin more effectively inhibited Nic-induced contractions whereas that to ACh was hardly affected. On the other hand, cyproheptadine at concentrations higher than 3 nM, inhibited the responses to all three agonists, being most effective against 5-HT and Nic, and slightly less effective against ACh; at 100 nM cyproheptadine abolished the responses to 5-HT and Nic (Figure 6). Phenoxybenzamine also inhibited the responses to all three agonists to similar degrees, at concentrations higher than 0.1 μM, and at 3 μM completely abolished all responses (Figure 7). Table 1 summarizes the 50% inhibitory concentrations (IC_{50}) of cinanserin, cyproheptadine and phenoxybenzamine on the contractile responses to the three agonists. The IC_{50} value for cinanserin against 5-HT was significantly higher than that against Nic, but clearly lower than that against ACh. The value for cyproheptadine against 5-HT was not significantly different from that against Nic, but was significantly lower than that against ACh. The values for phenoxybenzamine against the three agonists were not significantly different. The inhibitory ac-

Table 1 Concentrations causing 50% inhibition (IC_{50}) for cinanserin, cyproheptadine and phenoxybenzamine on the contractile responses to 5-hydroxytryptamine (5-HT), nicotine (Nic) and acetylcholine (ACh) of the isolated muscularis mucosae of the guinea-pig oesophagus

	IC_{50} (μM)		
	5-HT (100 μM)	*Nic (100 μM)*	*ACh (0.1 μM)*
Cinanserin	0.79 ±0.07 (10)	0.47 ±0.08 (10)*	>30 (10)
Cyproheptadine	0.016±0.002 (11)	0.020±0.005 (10)^{NS}	0.027±0.002 (8)**
Phenoxybenzamine	1.24 ±0.24 (12)	0.89 ±0.16 (10)^{NS}	0.99 ±0.16 (8)^{NS}

Numbers in parentheses show numbers of observations. Values are mean ± s.e.mean. *$P<0.05$; **$P<0.01$; NS, not significant. These were obtained by comparison with the value for 5-HT using an unpaired *t* test. (See also Figures 5–7.)

tions of cinanserin and cyproheptadine on the contractile responses to 5-HT or Nic were fully reversible and 60 min after washing out these antagonists from the bath almost the same size of contraction as with the control was obtained. However, the inhibitory action of phenoxybenzamine was entirely irreversible and even 3 h after washing out the antagonist the responses to three agonists were not restored.

Discussion

These experiments demonstrate that, in the isolated muscularis mucosae of the guinea-pig oesophagus, 5-HT produces a contraction induced by stimulation of cholinergic nerves in the attached submucous plexus, and not by any direct action on the muscularis mucosae itself. Thus 5-HT-induced contractions were abolished by both tetrodotoxin and atropine. A very similar indirect action in this tissue was also produced by Nic. However, the contractile responses to both 5-HT and Nic are probably mediated by separate receptors, since cross tachyphylaxis between 5-HT and Nic was not observed and hexamethonium, an antagonist of nicotinic receptors in autonomic ganglia, abolished the response to Nic, but not that to 5-HT. The lack of a direct action of 5-HT on the muscularis mucosae contrasts with previous results obtained on the same tissue (Bartlet, 1968), in which hyoscine-sensitive, hyoscine-resistant and methysergide-sensitive contractions had been produced by 5-HT. The pattern of 5-HT contracture observed was also different from those obtained in the present investigation. The cause of these differences is not clear, but may be due to differences in the preparation of the isolated muscularis mucosae. We have noticed that the 5-HT-induced contraction varied from preparation to preparation, the reason for this may be the amount of intact submucous plexus attached to the isolated muscularis mucosae. To obtain the maximum contraction to 5-HT, it is necessary to isolate very carefully the muscularis mucosae from the excised oesophagus.

To characterize the type of 5-HT receptors responsible for the stimulant action on the intramural cholinergic nerves, the effects of several 5-HT antagonists on the 5-HT-induced contraction were examined. Methysergide never inhibited, but rather slightly augmented the 5-HT-induced contraction, in spite of evidence to the contrary (Bartlet, 1968). Methysergide does antagonize the direct action of 5-HT on some smooth muscles (Day & Vane, 1963; Gyermek, 1966; Drakontides & Gershon, 1968; Frankhuijzen & Bonta, 1974; Carrol & Nasveld, 1978). Therefore, the lack of antagonism by methysergide might be comparable with the lack of a direct action of 5-HT on the muscularis mucosae. Ketanserin, which was recently developed as a specific 5-HT_2 receptor antagonist in the brain or blood vessels (Leysen, Awouters, Kennis, Laduron, Vandenberk & Janssen, 1981; Van Nueten, Janssen, Van Beek, Xhonneux, Verbeuren & Vanhoutte, 1981; Van Nueten, Janssen, De Ridder & Vanhoutte, 1982) also did not inhibit the response to 5-HT. This may mean that the types of 5-HT receptor in this tissue differ from those in the brain or blood vessels. Morphine also failed to modify the 5-HT-induced contractions even at a high concentration (100 μM). Previously, it had been demonstrated that morphine effectively inhibits the neural action of 5-HT in the guinea-pig gastrointestine (Gaddum & Picarelli, 1957; Day & Vane, 1963; Yamaguchi, 1972) and heart (Kamikawa, 1978). However, the effect of morphine is not specific for 5-HT, and electrical stimulation, barium ions, angiotensin and other agonist-induced contractions of the intestine were also inhibited by the drug (Paton, 1957; Weinstock, 1971). Our previous paper (Kamikawa & Shimo, 1982a) showed that morphine was less effective in inhibiting the electrically-induced twitch contractions of this muscularis mucosae, while in the present results, the Nic-induced contraction was reduced to half by the drug. These findings confirmed that morphine is not a specific antagonist of the neural action of 5-HT. All other three antagonists examined, cinanserin, cyproheptadine and phenoxybenzamine, inhibited 5-HT-induced contractions, in a concentration-dependent manner; cyproheptadine was the most effective. However, none of these antagonists is specific for 5-HT and both Nic and ACh-induced contractions were also inhibited. Although the nonspecificity of these antagonists has also been reported in other tissues (Gaddum & Picarelli, 1957; Stone, Wenger, Ludden, Stavorski & Ross, 1961; Day & Vane, 1963; Rubin, Piala, Burke & Craver, 1964; Gyermek, 1966; Cook, 1971; Leysen *et al.*, 1981; Williams & Martin, 1982), it has been considered that the antagonistic activity of these drugs on the action of 5-HT is due to their direct interactions on the 5-HT receptors. This seems to be true in the present preparation, since cinanserin and cyproheptadine, but not phenoxybenzamine, more effectively inhibited the response to 5-HT than that to ACh. Wallis (1981) has proposed that 5-HT receptors in the peripheral nervous system are not uniform in their sensitivity to agonists and antagonists. According to his criteria for classification, the excitatory 5-HT receptors present in intramural cholinergic nerves of the present tissue can be characterized as follows; the receptors readily display tachyphylaxis, can be blocked by 5-HT itself, cyproheptadine, cinanserin and phenoxybenzamine, but are insensitive to blockade by methysergide, ketanserin and

morphine. These characteristics are different from those in other peripheral nervous systems such as sympathetic and parasympathetic ganglia or in the myenteric plexus (Wallis, 1981) and therefore may suggest the presence of different type of 5-HT receptors in the muscularis mucosae of the guinea-pig oesophagus. To determine the exact type of receptor in this tissue, a specific 5-HT antagonist will be required and the sensitivity to analogues of 5-HT examined.

The previous observation in the guinea-pig small intestine that iontophoretically applied 5-HT depolarizes some submucous neurones (Hirst & Silinsky, 1975) and that some myenteric nerve cell bodies showing 5-HT-like immuno-reactivity send axons in an anal direction to supply submucous ganglia (Furness & Costa, 1982) agree well with the present results. Since 5-HT has no direct action on the muscularis mucosae (present result), these findings suggest that 5-hydroxytryptaminergic neurones in the enteric nervous system play a role as interneurones and that their activation could depolarize cholinergic nerves in the submucous plexus, and lead to contracture of the muscularis mucosae.

We are grateful to Janssen-Kyowa Pharmaceuticals for a gift of ketanserin, to E.R. Squibb & Sons, Inc. for cinanserin and to Nippon Merck-Banyu Co. for cyproheptadine.

References

AHLMAN, H., DEMAGISTRIS, L., ZINNER, M. & JAFFE, B.M. (1981). Release of immunoreactive serotonin into the lumen of the feline gut in response to vagal nerve stimulation. *Science*, **213**, 1254–1255.

BARTLET, A.L. (1968). Actions of 5-hydroxytryptamine and histamine on the neural structures and muscularis mucosae of the guinea-pig oesophagus. *Br. J. Pharmac. Chemother.* **33**, 184–192.

BROWNLEE, G. & JOHNSON, E.S. (1963). The site of the 5-hydroxytryptamine receptor on the intramural nervous plexus of the guinea-pig isolated ileum. *Br. J. Pharmac. Chemother.*, **21**, 306–322.

BÜLBRING, E. & GERSHON, M.D. (1967). 5-Hydroxytryptamine participation in the vagal inhibitory innervation of the stomach. *J. Physiol.*, **192**, 823–846.

BURLEIGH, D.E. (1977). Evidence for more than one type of 5-hydroxytryptamine receptor in the human colon. *J. Pharm. Pharmac.*, **29**, 538–541.

CARROLL P.R. & NASVELD, P.E. (1978). The effects of methysergide on the constrictor response to serotonin on the isolated, perfused rabbit ear artery. *Aust. J. exp. Biol. Med. Sci.*, **56**, 493–497.

COOK, D.A. (1971). Blockade by phenoxybenzamine of the contractor response produced by agonists in the isolated ileum of the guinea-pig. *Br. J. Pharmac.*, **43**, 197–209.

COSTA, M. & FURNESS, J.B. (1976). The peristaltic reflex: an analysis of the nerve pathways and their pharmacology. *Naunyn-Schmiedebergs Arch. Pharmac.*, **294**, 47–60.

COSTA, M. & FURNESS, J.B. (1979a). On the possibility that an indoleamine is a neurotransmitter in the gastrointestinal tract. *Biochem. Pharmac.*, **28**, 565–571.

COSTA, M. & FURNESS, J.B. (1979b). The site of action of 5-hydroxytryptamine in nerve-muscle preparations from the guinea-pig small intestine and colon. *Br. J. Pharmac.*, **65**, 237–248.

DAY, M. & VANE, J.R. (1963). An analysis of the direct and indirect actions of drugs on the isolated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **20**, 150–170.

DRAKONTIDES, A.B. & GERSHON, M.D. (1968). 5-Hydroxytryptamine receptors in the mouse duodenum. *Br. J. Pharmac. Chemother.*, **33**, 480–492.

ERSPAMER, V. & ASERO, B. (1952). Indentification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature*, **169**, 800–801.

FRANKHUIJZEN, A.L. & BONTA, I.L. (1974). Receptors involved in the action of 5-HT and tryptamine on the isolated rat stomach fundus preparation. *Eur. J. Pharmac.*, **26**, 220–230.

FURNESS, J.B. & COSTA, M. (1982). Neurons with 5-hydroxytryptamine-like immunoreactivity in the enteric nervous system: their projections in the guinea-pig small intestine. *Neuroscience*, **7**, 341–349.

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

GERSHON, M.D. (1967). Effects of tetrodotoxin on innervated smooth muscle preparations. *Br. J. Pharmac. Chemother.*, **29**, 257–279.

GERSHON, M.D. (1981). The enteric nervous system: multiplicity of neurotransmitters outside of the brain. In *Smooth Muscle: An Assessment of Current Knowledge.* ed. Bülbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 263–284. London: Edward Arnold.

GERSHON, M.D., ROBINSON, R.G. & ROSS, L.L. (1976). Serotonin accumulation in the guinea-pig myenteric plexus: Ion dependence, structure activity relationship and the effect of drugs. *J. Pharmac. exp. Ther.*, **198**, 548–561.

GERSHON, M.D. & TAMIR, H. (1981). Release of endogenous 5-hydroxytryptamine from resting and stimulated enteric neurons. *Neuroscience*, **6**, 2277–2286.

GYERMEK, L. (1966). Drugs which antagonize 5-hydroxytryptamine and related indolealkylamines. In *5-Hydroxytryptamine and Related Indolealkylamines, Handb. Exp. Pharmac.*, Vol. 19, ed. Erspamer, V. pp. 471–528. Berlin, Heidelberg & New York: Springer-Verlag.

HIRST, G.D.S. & SILINSKY, E.M. (1975). Some effects of 5-hydroxytryptamine, dopamine and noradrenaline on neurones in the submucous plexus of guinea-pig small intestine. *J. Physiol.*, **251**, 817–832.

KAMIKAWA, Y. (1978). Augmentation of the vagally-mediated response to 5-hydroxytryptamine in the

anaphylactically-desensitized atria of guinea-pigs 'in vitro'. *J. Pharm. Dyn.*, **1**, 238–245.

KAMIKAWA, Y. & SHIMO, Y. (1979). Cholinergic and adrenergic innervations of the muscularis mucosae in guinea-pig esophagus. *Archs int. Pharmacodyn. Thér.*, **238**, 220–232.

KAMIKAWA, Y. & SHIMO, Y. (1982a). Modulating effects of opioids, purine compounds, 5-hydroxytryptamine and prostaglandin E_2 on cholinergic neurotransmission in a guinea-pig oesophagus preparation. *J. Pharm. Pharmac.*, **34**, (in press).

KAMIKAWA, Y. & SHIMO, Y. (1982b). Pharmacological studies on the esophageal muscularis mucosae (7th report): Effect of 5-hydroxytryptamine and influences of its antagonists. *Jap. J. Pharmac.*, **32**, Suppl. (in press).

KAMIKAWA, Y., SHIMO, Y. & UCHIDA, K. (1982). Inhibitory actions of catecholamines on electrically induced contractions of the submucous plexus-longitudinal muscularis mucosae preparation of the guinea-pig oesophagus. *Br. J. Pharmac.*, **76**, 271–277.

LEYSEN, J.E., AWOUTERS, F., KENNIS, L., LADURON, P.M., VANDENBERK, J. & JANSSEN, P.A.J. (1981). Receptor binding profile of R41 468, a novel antagonist at 5-HT_2 receptors. *Life Sci.*, **28**, 1015–1022.

PATON, W.D.M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **12**, 119–127.

RUBIN, B., PIALA, J.J., BURKE, J.C. & CRAVER, B.N. (1964). A new, potent and specific serotonin inhibitor, (SQ10,643) 2'-(3-dimethylaminopropylthio) cinnamanilide hydrochloride: antiserotonin activity on uterus and on gastrointestinal, vascular, and respiratory systems of animals. *Archs int. Pharmacodyn. Thér.*, **152**, 132–143.

STONE, C.A., WENGER, H.C., LUDDEN, C.T., STAVORSKI, J.M. & ROSS, C.A. (1961). Antiserotonin-antihistaminic properties of cyproheptadine. *J. Pharmac. exp. Ther.*, **131**, 73–84.

VAN NUETEN, J.M., JANSSEN, P.A.J., VAN BEEK, J., XHONNEUX, R., VERBEUREN, T.J. & VANHOUTTE, P.M. (1981). Vascular effects of ketanserin (R41 468), a novel antagonist of 5-HT_2 serotonergic receptors. *J. Pharmac. exp. Ther.*, **218**, 217–230.

VAN NUETEN, J.M., JANSSEN, P.A.J., DE RIDDER, W. & VANHOUTTE, P.M. (1982). Interaction between 5-hydroxytryptamine and other vasoconstrictor substances in the isolated femoral artery of the rabbit; effect of ketanserin (R41 468). *Eur. J. Pharmac.*, **77**, 281–287.

WALLIS, D. (1981). Neuronal 5-hydroxytryptamine receptors outside the central nervous system. *Life Sci.*, **29**, 2345–2355.

WEINSTOCK, M. (1971). Sites of action of narcotic analgesic drug – peripheral tissues. In *Narcotic Drugs – Biochemical Pharmacology*. ed. Clouet, D.H. pp. 394–407. New York: Plenum Press.

WILLIAMS, M. & MARTIN, G.E. (1982). Selectivity of cyproheptadine as assessed by radioligand binding. *J. Pharm. Pharmac.*, **34**, 58–59.

WOOD, J.D. & MAYER, C.J. (1978). Slow synaptic excitation mediated by serotonin in Auerbach's plexus. *Nature*, **276**, 836–837.

WOOD, J.D. & MAYER, C.J. (1979). Serotonergic activation of tonic-type enteric neurons in guinea-pig small bowel. *J. Neurophysiol.*, **42**, 582–593.

YAMAGUCHI, T. (1972). Effects of 5-hydroxytryptamine on isolated strips of the guinea-pig stomach. *Br. J. Pharmac.*, **44**, 100–108.

(Received June 2, 1982.
Revised July 20, 1982.)