

Species Differences in the 5-Hydroxytryptamine-Induced Contraction in the Isolated Distal Ileum

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ABSTRACT—We investigated the mechanism of 5-hydroxytryptamine (5-HT)-induced contraction in the longitudinal muscle of isolated distal ileum from ferrets, piglets and rats. 5-HT and 5-methoxytryptamine concentration-dependently contracted the ileum of ferrets, piglets and rats. 2-Methyl-5-HT and *m*-chlorophenylbiguanide concentration-dependently contracted the ferret ileum, whereas they had no effect in piglets and rats. In ferrets, the 5-HT-induced contraction was inhibited by methysergide and by ramosetron, but not by ketanserin or GR113808. Atropine and tetrodotoxin suppressed contractions elicited by 5-HT, 2-methyl-5-HT and *m*-chlorophenylbiguanide in ferrets, but not that elicited by 5-methoxytryptamine. In piglets, 5-HT-induced contraction was inhibited by methysergide and by tetrodotoxin, but not by ketanserin, ramosetron, GR113808 or atropine. In rats, 5-HT-induced contraction was inhibited by methysergide and by ketanserin, but not by ramosetron or tetrodotoxin. In contrast, GR113808 enhanced contractions elicited by 5-HT or 5-methoxytryptamine. These results suggest that 5-HT-induced contraction in ferrets is mediated via 5-HT₁ receptors on the muscle and by release of acetylcholine via 5-HT₃ receptors. In piglets, 5-HT-induced contraction appears to be mediated by release of neurotransmitters other than acetylcholine via 5-HT₁ receptors. 5-HT-induced contraction in rats is evoked via 5-HT₁ and 5-HT₂ receptors on the muscle. Furthermore, 5-HT₄ receptors may participate in the relaxation elicited by 5-HT in rats.

Keywords: Species difference, 5-HT receptor, Distal ileum, Longitudinal muscle

5-Hydroxytryptamine (5-HT) is known to be distributed in blood platelets, the nervous system and enterochromaffin cells. In the gastrointestinal tract, 5-HT is reported to contribute to the regulation of the motility and secretion via several 5-HT-receptor subtypes. The involvement of the 5-HT-receptor subtype in 5-HT-induced intestinal contraction has been well-examined in the guinea pig small and large intestines (1–8). In contrast, the effect of 5-HT on the regulation of intestinal motility has not been established in species other than guinea pigs.

Defecation and intestinal transit in rats are frequently utilized as an *in vivo* system for the evaluation of the action of 5-HT on the motility of the small and large intestine. However, the involvement of 5-HT receptor subtypes in 5-HT-elicited responses has been less extensively investigated *in vitro* by the use of selective 5-HT receptor antagonists in rat isolated intestine. In the present study, we examined the participation of 5-HT₁-, 5-HT₂-, 5-HT₃- and 5-HT₄-receptor subtypes in 5-HT-induced contrac-

tion in rat isolated distal ileum.

Since the ferret and piglet have been shown to have similarities with respect to anatomical and physiological characteristics of the gut to that of humans (9, 10), the intestinal responses in ferret and piglet are suggested to be good models of some of the physiologic and pharmacologic processes in humans. Furthermore, 5-HT-containing neurons were observed in the submucosal and myenteric plexus of ferret and pig intestine as well as that of humans (11–16). The participation of 5-HT in the modulation of ileal motility, however, has not been evaluated in ferrets and piglets. Therefore, we also evaluated the mechanism of 5-HT-induced contraction in the isolated distal ileum of ferrets and piglets as well as in that of rats. We obtained evidence of a species difference among ferrets, piglets and rats in the mechanism of 5-HT-induced ileal contraction.

MATERIALS AND METHODS

Tissue preparation

Male ferrets (0.95–1.6 kg; Charles River Japan, Yokohama), male Wistar rats (230–350 g; SLC, Hamamatsu) and piglets of both genders (0.95–2.5 kg; Hamri Co., Sashima, Ibaraki) were killed by cervical dislocation and exsanguinated. The distal portion of the ileum (discarding the terminal 2–3 cm) was removed. The ileum was divided longitudinally into segments approximately 2 cm in length. The longitudinal muscle of isolated rat ileum with myenteric plexus was removed by gentle stroking with a cotton swab at an angle to the mesenteric attachment. In the isolated ileum of ferret and piglet, the whole segment was used. The tissues were vertically suspended in a 10-ml organ bath containing Krebs-bicarbonate solution (118.4 mM NaCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 4.7 mM KCl, 1.2 mM MgSO₄, 25.0 mM NaHCO₃ and 10.0 mM glucose) maintained at 37°C and gassed with a mixture of 95% O₂ and 5% CO₂. Tissues were attached to isometric force-displacement transducers (SB-1T; Nihon Kohden, Tokyo) connected to a recorder (MC 6621; Graphtec, Tokyo) through a carrier amplifier (AP-621G, Nihon Kohden). The tissues were allowed to equilibrate for approximately 30 min before exposure to test compounds. Isometric contractions under a loading tension of 0.5 (the tissue from rats) or 1 g (the tissues from ferrets and piglets) were recorded because stable contractions were recorded under this condition.

Experimental protocol

Cumulative concentration-response curves for the 5-HT-receptor agonists were constructed by increasing the bath concentrations of each agonist by approximately threefold. The preliminary study showed the concentration-response curve for 5-HT to be not reproducible in the same preparation. Because of this problem all subsequent experiments were controlled by use of paired preparations taken from adjacent portions of distal ileum in the same animal. The concentration-response curve for 5-HT in the first tissue was not significantly different from that in the paired partner. Thus one tissue received agonist alone and the paired partner received agonist plus antagonist for antagonist studies. Antagonists were allowed to pre-equilibrate for 30 min prior to the construction of concentration-response curves for agonists.

Data analyses

All values are expressed as the mean \pm S.E.M. or as the mean with 95% confidence limits. Responses were measured as an increase in isometric tension and expressed as a percentage of the maximal contraction obtained with 5-HT. EC₅₀ values were obtained by linear regression with

the least square method. Significance between groups was assessed by one-way ANOVA. Probabilities of <5% ($P < 0.05$) were considered significant.

Drugs

Ramosetron hydrochloride (YM060), GR113808 {[1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidyl]methyl 1-methyl-1*H*-indole-3-carboxylate}], 2-methyl-5-HT and 1-(*m*-chlorophenyl)biguanide hydrochloride (mCPB) were prepared by Yamanouchi Pharmaceutical Co. (Tsukuba). Methysergide hydrogen maleate was kindly donated by Sandoz, Ltd. (Basle, Switzerland). 5-HT creatinine sulfate and 5-methoxytryptamine hydrochloride (5-MOT) were purchased from E. Merck (Darmstadt, Germany) and Fluka AG (Buchs, Switzerland), respectively. Ketanserin, atropine sulfate and tetrodotoxin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ramosetron, GR113808 and methysergide were dissolved in a minimal amount of a 0.1 N HCl solution and diluted with Krebs-bicarbonate solution. Ketanserin was dissolved in a minimal amount of a tartaric acid solution and diluted with Krebs-bicarbonate solution. The reported concentrations are the final bath concentrations.

RESULTS

The 5-HT-receptor agonist-induced contraction

5-HT (0.1–100 μ M), the 5-HT-receptor agonist 5-MOT (0.01–30 μ M), and selective 5-HT₃-receptor agonists 2-methyl-5-HT (0.1–100 μ M) and mCPB (0.01–10 μ M) concentration-dependently induced contraction in the isolated distal ileum of ferrets (Fig. 1A). The maximal contractions of 5-MOT (3 μ M), 2-methyl-5-HT (10 μ M) and mCPB (3 μ M) were 117%, 38% and 38% of the maximal response of 5-HT (10 μ M) in the ferret ileum, respectively. 5-HT and 5-MOT also produced concentration-dependent contraction in piglets and rats, whereas 2-methyl-5-HT and mCPB had no effect up to 10 μ M (Fig. 1: B and C). The EC₅₀ values for 5-HT, 5-MOT, 2-methyl-5-HT and mCPB are given in Table 1. On the basis of EC₅₀ values, the agonistic activity of 5-HT in rats was approximately 10 times weaker than that in ferrets and piglets.

The mechanism of 5-HT-induced contraction in the ferret ileum

In the ferret ileum, the 5-HT-induced contraction was significantly inhibited by methysergide (10 μ M), a 5-HT₁- and 5-HT₂-receptor antagonist, and by ramosetron (0.3 μ M), a selective 5-HT₃-receptor antagonist (Fig. 2: A and C). However, it was not affected by ketanserin (0.1 μ M), a selective 5-HT₂-receptor antagonist, or GR113808 (0.3 μ M), a selective 5-HT₄-receptor antagonist (Fig. 2: B and

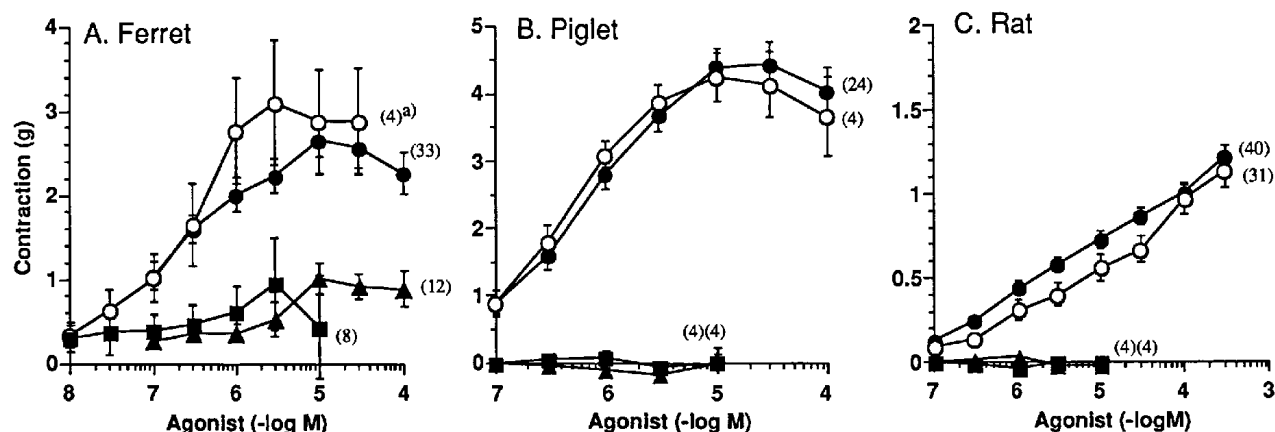


Fig. 1. Concentration-response curves for the 5-HT (●), 5-MOT (○), 2-methyl-5-HT (▲) and mCPB (■)-induced contraction in the isolated distal ileum of ferrets (A), piglets (B) and rats (C). Each point represents the mean \pm S.E.M. of 4 to 40 preparations. ^aFigures in parentheses represent the number of preparations.

Table 1. Estimate of EC₅₀ values and maximum (Max) responses of 5-HT-receptor agonists-induced contraction in the longitudinal muscle of isolated distal ileum from ferrets, piglets and rats

Species	5-HT-receptor agonists-induced contraction EC ₅₀ (M) [Max response (g)]			
	5-HT	5-MOT	2-Methyl-5-HT	mCPB
Ferret	1.8 (0.63–3.1) $\times 10^{-7}$ [2.7 \pm 0.2] (33) ^a	1.8 (0.70–5.2) $\times 10^{-7}$ [3.1 \pm 0.7] (4) ^a	9.0 (3.2–23.8) $\times 10^{-7}$ [1.0 \pm 0.2] (12) ^a	7.9 (0.37–71.0) $\times 10^{-8}$ [0.95 \pm 0.6] (8) ^a
Piglet	5.3 (4.0–6.8) $\times 10^{-7}$ [4.4 \pm 0.3] (24) ^a	3.8 (2.8–5.3) $\times 10^{-7}$ [4.2 \pm 0.4] (4) ^a	N.E. (4) ^a	N.E. (4) ^a
Rat	4.0 (3.0–5.3) $\times 10^{-6}$ [1.2 \pm 0.1] (40) ^a	9.2 (5.4–15.6) $\times 10^{-6}$ [1.1 \pm 0.1] (31) ^a	N.E. (4) ^a	N.E. (4) ^a

EC₅₀ values are the mean with 95% confidence limits. Max responses are expressed as the mean \pm S.E.M. N.E. = no effect. ^aNumber of preparations.

D). Tetrodotoxin (1 μ M) and atropine (1 μ M) significantly suppressed the contraction elicited by 5-HT (Fig. 3). The inhibitory effects of ramosetron and tetrodotoxin, however, were significant only on the contractions elicited by the higher concentrations of 5-HT ($\geq 10 \mu$ M). Combinations of methysergide plus ramosetron and of methysergide plus atropine abolished the 5-HT-induced contraction (data not shown, $n=3$). Although the 5-MOT-induced contraction was abolished by methysergide, it was not inhibited by ketanserin, ramosetron, GR113808 or tetrodotoxin (data not shown, $n=3-5$). The contractions elicited by 2-methyl-5-HT and by mCPB were abolished by ramosetron, tetrodotoxin or atropine, but they were not affected by methysergide (data not shown, $n=4-11$).

The mechanism of 5-HT-induced contraction in the piglet ileum

In the piglet ileum, the 5-HT-induced contraction was significantly inhibited by methysergide (10 μ M, Fig. 4A)

and by tetrodotoxin (1 μ M, Fig. 5A), but it was not inhibited by ramosetron (0.3 μ M, Fig. 4C), GR113808 (0.3 μ M, data not shown, $n=2$) or atropine (1 μ M, Fig. 5B). Although ketanserin (0.1 μ M) failed to influence the contraction elicited by lower concentrations of 5-HT (0.1–10 μ M), it significantly enhanced the contraction elicited by higher concentrations of 5-HT (30–100 μ M) (Fig. 4B).

The mechanism of 5-HT-induced contraction in the rat ileum

In the rat ileum, the 5-HT (0.1–30 μ M)-induced contraction was significantly inhibited by methysergide (10 μ M) and by ketanserin (0.1 μ M) (Fig. 6: A and B). In contrast, higher concentrations of 5-HT (100–300 μ M)-induced contractions were significantly inhibited by methysergide but not by ketanserin (Fig. 6: A and B). Ramosetron (0.3 μ M, Fig. 6C) and tetrodotoxin (1 μ M, data not shown, $n=3$) failed to influence the response to 5-HT. Although methysergide inhibited the 5-MOT-induced contraction, ketanserin and tetrodotoxin had no

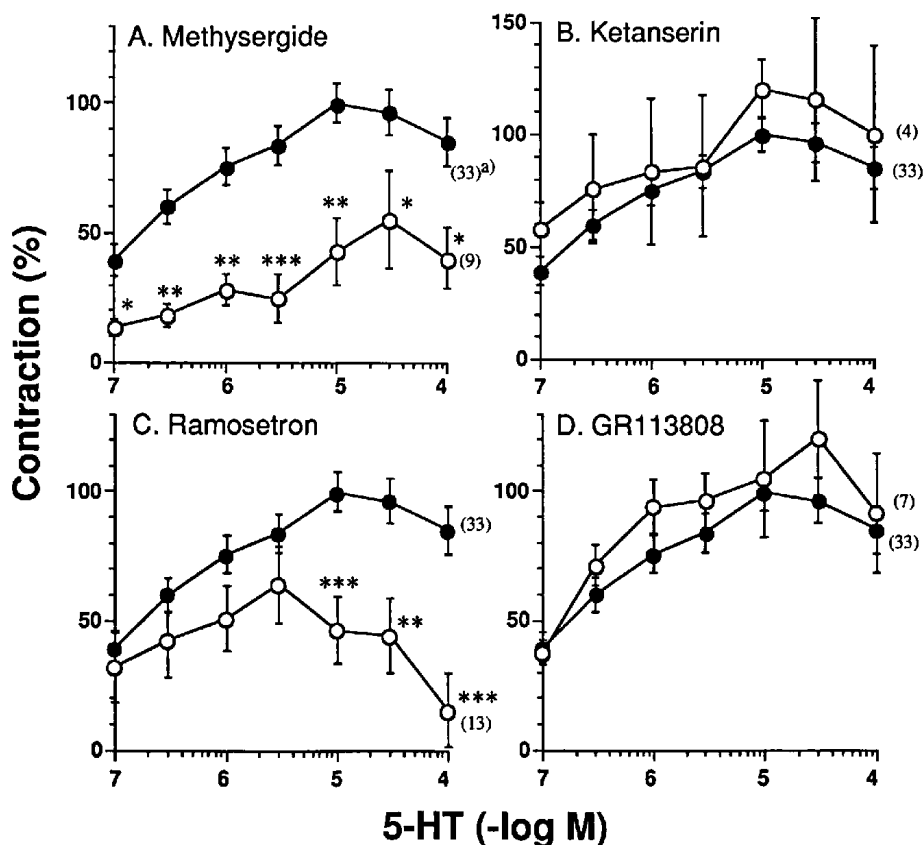


Fig. 2. Inhibitory effects of methysergide (10 μ M, A), ketanserin (0.1 μ M, B), ramosetron (0.3 μ M, C) and GR113808 (0.3 μ M, D) on the 5-HT-induced contraction in the isolated distal ileum of ferrets. Antagonists were preincubated 30 min before 5-HT application. Absence (●) or presence (○) of antagonists. Each point represents the mean \pm S.E.M. of 4 to 33 preparations. ^aFigures in parentheses represent the number of preparations. * P < 0.05, ** P < 0.01, *** P < 0.001, compared with the control group without antagonists.

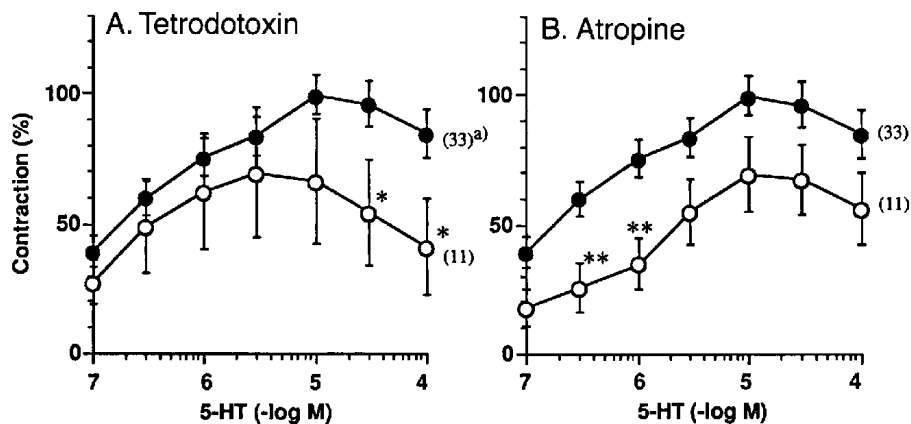


Fig. 3. Inhibitory effects of tetrodotoxin (1 μ M, A) and atropine (1 μ M, B) on the 5-HT-induced contraction in the isolated distal ileum of ferrets. Antagonists were preincubated 30 min before 5-HT application. Absence (●) or presence (○) of antagonists. Each point represents the mean \pm S.E.M. of 11 or 33 preparations. ^aFigures in parentheses represent the number of preparations. * P < 0.05, ** P < 0.01, compared with the control group without antagonists.

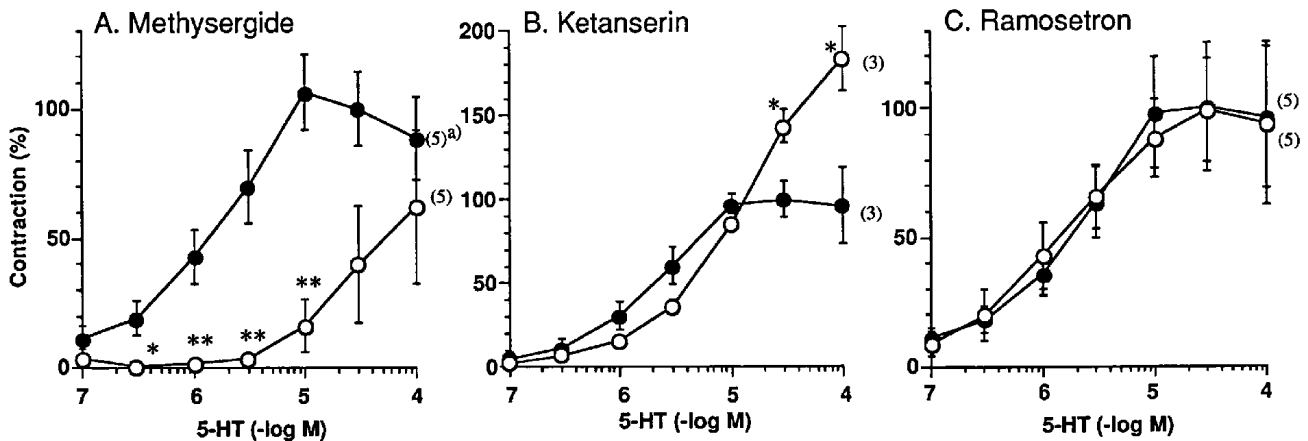


Fig. 4. Inhibitory effects of methysergide (10 μ M, A), ketanserin (0.1 μ M, B) and ramosetron (0.3 μ M, C) on the 5-HT-induced contraction in the isolated distal ileum of piglets. Antagonists were preincubated 30 min before 5-HT application. Absence (●) or presence (○) of antagonists. Each point represents the mean \pm S.E.M. of 3 or 5 preparations. ^a) Figures in parentheses represent the number of preparations. * $P < 0.05$, ** $P < 0.01$, compared with the control group without antagonists.

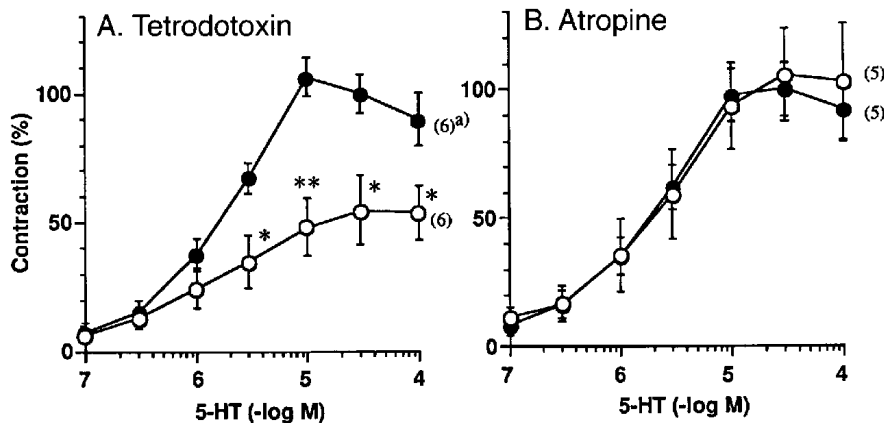


Fig. 5. Inhibitory effects of tetrodotoxin (1 μ M, A) and atropine (1 μ M, B) on the 5-HT-induced contraction in the isolated distal ileum of piglets. Antagonists were preincubated 30 min before 5-HT application. Absence (●) or presence (○) of antagonists. Each point represents the mean \pm S.E.M. of 5 or 6 preparations. ^a) Figures in parentheses represent the number of preparations. * $P < 0.05$, ** $P < 0.01$, compared with the control group without antagonists.

effect on it (data not shown, $n = 3-5$). GR113808 (0.3 μ M) enhanced the contractions elicited by 5-HT (Fig. 6D) or 5-MOT (data not shown, $n = 5$).

DISCUSSION

Species differences in the effect of 5-HT in the mammalian cardiovascular system have been elucidated. The tachycardia elicited by 5-HT is species-dependent and is mediated by 5-HT₁ (cat), 5-HT₂ (rat, dog), 5-HT₃ (rabbit, dog, guinea pig) or 5-HT₄ (piglet, human) receptors, or by a tryptamine-like (guinea pig) mechanism (17-20). We also reported that there was a species difference between guinea pigs and other species in the participation of 5-HT-receptor subtypes in 5-HT-induced bradycardia (21).

Endogenous and exogenous 5-HT affects not only the cardiovascular system but also the gastrointestinal system in most species. Therefore, it may be hypothesized that a species difference exists in the involvement of 5-HT-receptor subtypes in the effect of 5-HT on intestinal motility. Although the effect of 5-HT on intestinal motility has been well-examined in guinea pigs, its effect in other species has not been clear. The aim of the present study was to investigate the action of 5-HT on the motility of the longitudinal muscle of the distal ileum of ferrets, piglets and rats in vitro. Species differences among these animals in the mechanism of 5-HT-induced contraction in the isolated distal ileum were also examined.

We found that 5-HT induced concentration-dependent contractions in the isolated distal ileum of ferrets, piglets

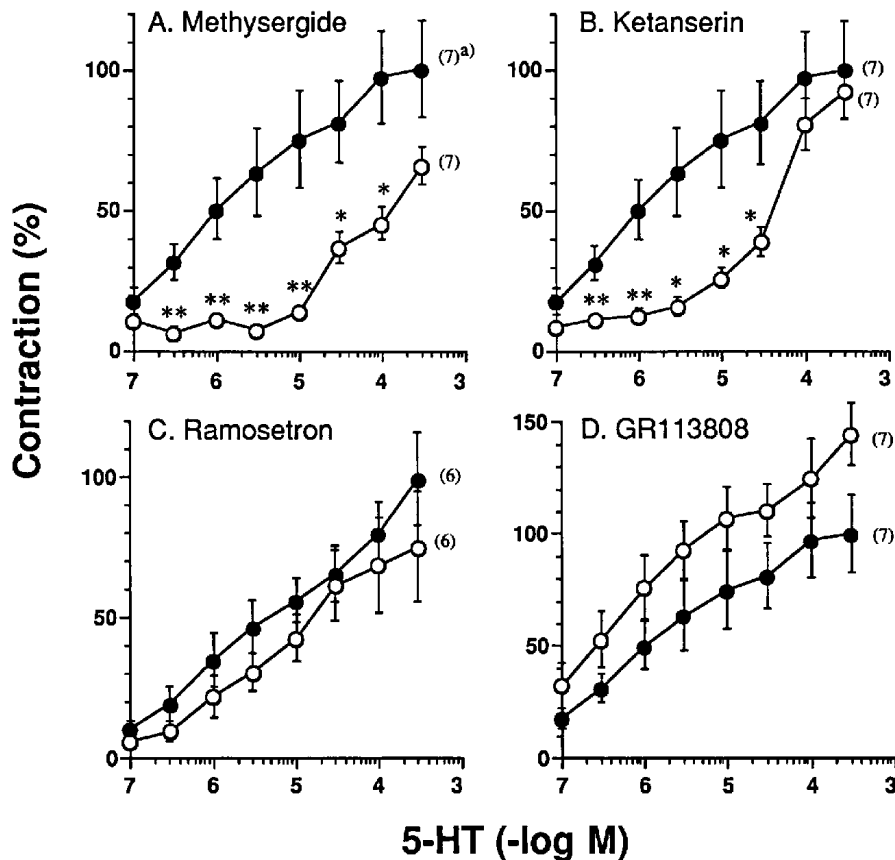


Fig. 6. Inhibitory effects of methysergide (10 μ M, A), ketanserin (0.1 μ M, B), ramosetron (0.3 μ M, C) and GR113808 (0.3 μ M, D) on the 5-HT-induced contraction in the isolated distal ileum of rats. The preparation was preincubated with the antagonists 30 min before 5-HT application. Absence (●) or presence (○) of antagonists. Each point represents the mean \pm S.E.M. of 6 or 7 preparations. ^aFigures in parentheses represent the number of preparations. * $P < 0.05$, ** $P < 0.01$, compared with the control group without antagonists.

and rats. In the longitudinal muscle of isolated ferret distal ileum, not only 5-HT but also 5-MOT, 2-methyl-5-HT and mCPB concentration-dependently induced contractions. The 5-HT-induced contraction in ferret ileum was inhibited by methysergide and by ramosetron. However, it was not affected by ketanserin or GR113808. Tetrodotoxin and atropine also suppressed the contraction elicited by 5-HT. The inhibitory effects of ramosetron and tetrodotoxin, however, were significant only on the contractions elicited by higher concentrations of 5-HT ($\geq 10 \mu$ M). Furthermore, the 5-HT₃ receptor (2-methyl-5-HT and mCPB)-mediated contraction was abolished by tetrodotoxin and by atropine, whereas the 5-HT₁ receptor-mediated contractile response to 5-MOT was insensitive to tetrodotoxin. Taken together, these results suggest that 5-HT-induced contraction in the ferret ileum is mediated by the direct activation of 5-HT₁ receptors on the muscle and the release of acetylcholine through an activation of 5-HT₃ receptors and that the lower and higher concentrations of 5-HT may act on the 5-HT₁ and

5-HT₃ receptors, respectively. Kameda et al. (22) also reported that ondansetron, a selective 5-HT₃-receptor antagonist, non-competitively inhibited 5-HT-induced contraction in the ferret ileum. Our results are consistent with their finding.

In the longitudinal muscle of isolated piglet distal ileum, 5-HT and 5-MOT produced concentration-dependent contractions. 2-Methyl-5-HT and mCPB had no effect in the piglet ileum, unlike their effect in ferret ileum. The 5-HT-induced contraction in the piglet ileum was inhibited by methysergide, but it was not suppressed by ketanserin, ramosetron or GR113808. Furthermore, the 5-HT-induced contraction in the piglet ileum was inhibited by tetrodotoxin but not by atropine, indicating that it was mediated through the stimulation of a release of neurotransmitters other than acetylcholine via 5-HT₁ receptors. Kirchgessner et al. (23) reported that the 5-HT_{1A} receptor was found on the neurons of the submucosal ganglia in the pig ileum. It was also reported that 5-HT enhanced pig jejunum motility in vivo and that this

response was not inhibited by atropine (24). In the present study, although ketanserin failed to influence the contraction elicited by lower concentrations of 5-HT, it enhanced the contraction elicited by higher concentrations of 5-HT. This result suggests that higher concentrations of 5-HT may elicit relaxation via 5-HT₂ receptors in the piglet ileum. Further studies are necessary to test this hypothesis.

A number of candidates can be proposed as neurotransmitters released in the enteric nervous system involved in the 5-HT-induced contraction in the distal ileum of piglets. Neurotransmitters such as galanin, calcitonin gene-related peptide, enkephalin, neuromedin U, neuropeptide Y, somatostatin, substance P and neurokinin A are found in the enteric nervous system of pig small intestine (13, 14, 25). Although these neurotransmitters may participate in the atropine-resistant contraction elicited by 5-HT, further experiments are required to confirm which transmitter is involved.

In the myenteric plexus of rat small and large intestine, 5-HT-containing neurons have been observed (26, 27). It is known that the contractile response to 5-HT is sensitive to methysergide but insensitive to atropine and tetrodotoxin in the longitudinal muscle of isolated rat ileum and colon (28–30). In the present study, contraction of the longitudinal muscle of rat distal ileum induced by the lower 5-HT concentration was inhibited by methysergide and by ketanserin, but the contractions induced by higher concentrations of 5-HT were suppressed by methysergide but not by ketanserin. Ramosetron, GR113808 and tetrodotoxin failed to influence the 5-HT-induced contraction. In addition, methysergide inhibited the 5-MOT-induced contractions, but ketanserin, GR113808 and tetrodotoxin did not. These results indicate that the 5-HT-induced contraction in the rat ileum is evoked by the activation of both 5-HT₁ and 5-HT₂ receptors on the muscle and that the lower and higher concentrations of 5-HT act on the 5-HT₂ and 5-HT₁ receptors, respectively. Benouali-Pellissier et al. (31) reported the involvement of 5-HT₂ receptors in postprandial contraction in the rat ileum and distal colon. In the present study, GR113808 enhanced the contractile responses elicited by 5-HT or 5-MOT in the rat ileum. It was also reported that 5-HT-induced relaxation in the isolated rat ileum was antagonized by GR113808 (32, 33). Therefore, an activation of 5-HT₄ receptors may participate in the relaxation elicited by 5-HT in the rat ileum.

The involvement of the 5-HT-receptor subtype in 5-HT-induced intestinal contraction has been well-examined in guinea pig small and large intestines as mentioned in the Introduction. It was suggested that both neuronal and non-neuronal 5-HT receptors in the longitudinal muscle of guinea pig ileum were involved in the

5-HT-induced contraction (1–8). Although the nature of the non-neuronal 5-HT receptor remains unclear, the neuronal 5-HT receptor was defined as the 5-HT₃ and 5-HT₄ receptor subtypes which mediated acetylcholine release (7). The mechanism of 5-HT-elicited contraction in the isolated human ileum has not been identified. Bennett (34) reported that 5-HT-induced contraction was inhibited by methysergide but not by hyoscine in the longitudinal muscle of isolated human ileum. Borman and Burleigh (35) also reported that 5-HT-induced contraction in the human ileum was inhibited by methysergide and was unaffected by ketanserin, ondansetron, a selective 5-HT₃-receptor antagonist, and DAU6285, a 5-HT₄-receptor antagonist. Taken together, these findings indicate that an activation of the 5-HT₁ receptor was involved in the 5-HT-induced contraction in the longitudinal muscle of human ileum, but the location of these 5-HT₁ receptors has not been determined. On the basis of experimental evidence, the mechanism of the 5-HT-induced contraction in the piglet ileum may be similar to that in the human ileum. Furthermore, the mechanism of 5-HT-evoked contraction in the isolated ileum of ferrets, piglets and rats was found to be inconsistent with that in guinea pigs.

In conclusion, these results suggest that the 5-HT-induced contraction in the ferret ileum is mediated by the activation of 5-HT₁ receptors on the muscle and the release of acetylcholine through the activation of 5-HT₃ receptors. In the piglet ileum, the 5-HT-induced contraction is mediated by the release of neurotransmitters other than acetylcholine via 5-HT₁ receptors. Higher concentrations of 5-HT may elicit relaxation via 5-HT₂ receptors in the piglet ileum. In contrast, the 5-HT-induced contraction in the rat ileum is evoked by the activation of both 5-HT₁ and 5-HT₂ receptors on the muscle. In addition, the activation of 5-HT₄ receptors may participate in the relaxation elicited by 5-HT in the rat ileum. Taken together, our results demonstrate species differences in the mechanism of 5-HT-induced contraction in the longitudinal muscle of isolated distal ileum among ferrets, piglets and rats.

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