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A novel 5-HT_3 receptor agonist, YM-31636, increases gastrointestinal motility without increasing abdominal pain

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Abstract

We examined the effects of YM-31636 (2-(1*H*-imidazol-4-ylmethyl)-8*H*-indeno[1,2-*d*]thiazole monofumarate), a novel 5-HT_3 receptor agonist, on gastrointestinal functions including visceral pain reflex in rats. Injection of YM-31636 increased the number of fecal pellets. This effect was completely inhibited by ramosetron, a 5-HT_3 receptor antagonist. YM-31636 also increased the intracolonic pressure measured in both conscious and anesthetized rats. In isolated distal colon, YM-31636 increased the short-circuit current response. This effect was abolished by ramosetron. Both the maximal response and the potency of YM-31636 were weaker than those of other 5-HT_3 receptor agonists. In two visceral pain reflex models, YM-31636 neither changed the magnitude of pressor response to colonic distension in anesthetized rats nor affected the visceromotor threshold to colorectal distension in conscious rats. In conclusion, YM-31636 facilitated defecation without increasing visceral pain. Consequently, 5-HT_3 receptor agonists like YM-31636 would be promising in the treatment of chronic constipation.

Keywords: YM-31636; 5-HT_3 receptor agonist; Visceral pain; Constipation; Defecation; Short-circuit current

1. Introduction

5-Hydroxytryptamine (5-HT) receptors are now classified into five principal subtypes: 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, and 5-HT_7 receptors. Of these subtypes, the 5-HT_3 receptor is a member of the ligand-gated ion channel family. Until recently, only one subunit of the 5-HT_3 receptor had been cloned, but distinct subunit, 5-HT_{3B}, has been discovered (Davies et al., 1999). Transcripts of this subunit are co-expressed with the established 5-HT_3 receptor subunit, 5-HT_{3A}, in the specific brain region.

The 5-HT_3 receptor has been suggested to play a role in the regulation of colonic motor function in several animal species and humans. Selective 5-HT_3 receptor antagonists suppress stress- or 5-HT-induced defecation in rats (Miyata et al., 1992) and reduce 5-HT-induced colonic motility in rats and dogs (Kamato et al., 1992; Nagakura et al., 1996). It has also been reported that 5-HT_3 receptor antagonists inhibit colonic motility in healthy humans (Talley et al., 1990) and decrease defecation frequency in women with irritable bowel syndrome (Camilleri et al., 1999).

YM-31636 (2-(1*H*-imidazol-4-ylmethyl)-8*H*-indeno [1,2-*d*]thiazole monofumarate) is a novel 5-HT_3 receptor agonist. YM-31636 causes contraction of isolated guinea pig colon with an intrinsic activity of 0.90 compared with 5-HT (Ito et al., 2000). This agonist also facilitates defecation in ferrets with a minimum effective dose of 0.03 mg/kg p.o., without inducing diarrhea or emetic episode (Ito et al., 2001). Moreover, YM-31636 restores the frequency of defecation in two constipation models that use ferrets, including morphine-induced constipation (Ito et al., 2001). These results indicate that 5-HT_3 receptor agonists, such as YM-31636, would be promising in the treatment of constipation. However, there are many reports that gastrointestinal hypermotility sometimes causes visceral pain sensation (Connell et al., 1965; Louvel et al., 1996; Sarna et al., 1991). Furthermore, involvement of 5-HT_3 or 5-HT_3-like receptors has been described in visceral pain, since some 5-HT_3 receptor antagonists reduce visceral pain responses in rats (Banner et al., 1995; Banner and Sanger, 1995; Morteau et al., 1994; Moss and Sanger, 1990) and dogs (Miura et al., 1999), and relieve abdominal discomfort in women with irritable bowel syndrome (Camilleri et

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al., 1999). Consequently, one problem preventing the potential clinical use of agents like YM-31636 concerns these possible adverse effects.

In the present study, we investigated the effect of YM-31636 on the visceral pain reflex in rat models, which were most commonly used. However, since the effective doses of YM-31636 in rats were not known, we also examined the effects of YM-31636 on defecation, colonic motility, and water secretion.

2. Methods

2.1. Animals

Male Wistar rats weighing 200–400 g were used. The animals were maintained on ordinary laboratory chow and tap water ad libitum under a constant 13:11-h light–dark cycle. All experiments were performed in compliance with the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical.

2.2. Effect of YM-31636 on defecation

The effect of subcutaneously administered YM-31636 on defecation was evaluated using fed rats. Because the effect of YM-31636 on defecation lasts for approximately 1 h, the number of fecal pellets expelled by each animal was measured 1 h after YM-31636 injection. Ramosetron was given s.c. 30 min before YM-31636 administration.

2.3. Recording of intracolonic pressure

The experiments were performed in both conscious and anesthetized rats. Under light ether anesthesia for conscious animals, or under urethane (1.25 g/kg i.p.) anesthesia for anesthetized animals, a 1 cm latex balloon was inserted into the colon through the anus and positioned such that the end of the balloon was 4 cm from the anus. The balloon catheter was fixed in place by taping to the base of the tail and connected to a pressure transducer (MPU-0.5, Nihon Kohden; Tokyo, Japan). The pressure signal was amplified with a carrier amplifier (AP-621G, Nihon Kohden) and stored in a gut motility measuring system (ESC-820A, Star Medical; Tokyo, Japan). Intracolonic pressure was quantified by determining the motility index, which was calculated by the computer system (ESC-820C, Star Medical). The motility index was defined as the integrated area between the pressure wave and the base line during a certain fixed time period. For conscious animals, the rats were kept in Ballman cages and allowed to recover from ether anesthesia. For anesthetized animals, a jugular vein catheter (PE-50) was inserted for intravenous drug administration. The measurement of intracolonic pressure was started and at least 2 h later, after the intracolonic pressure became stable, the drug was administered.

2.4. Recording of short-circuit current (I_{sc}) response in colonic mucosa

Experiments were performed as previously described (Kiso et al., 1997). Briefly, the distal colon was removed and two adjacent preparations of the mucosa were prepared by dissection of the muscle layers. These preparations were then mounted in Ussing chambers, bathed on both sides with Krebs-bicarbonate solution warmed to 37 °C and equilibrated with 95% O_2: 5% CO_2. Preparations were short-circuited by use of a short-circuit current amplifier (CEZ-9100, Nihon Kohden) and the I_{sc} was continuously recorded. Concentration–response curves of agonists were constructed in a cumulative manner. For agonist studies, one preparation received 5-HT and the paired preparation received one of several 5-HT_3 receptor agonists. For antagonist studies, one preparation received YM-31636 alone and the paired preparation was exposed to ramosetron for 30 min before the addition of YM-31636.

2.5. Pressor response

The rats were anesthetized with urethane and a polyethylene tube was inserted into the trachea to provide artificial ventilation. A polyethylene catheter (PE-50) was inserted to the left carotid artery and connected to a pressure transducer. The blood pressure signal was amplified and continuously recorded (WI-641G, Nihon Kohden). Some rats were cannulated with a polyethylene catheter into the left femoral vein for drug administration. To achieve colonic distension, a 1 cm latex balloon was inserted into the colon through the anus and positioned such that the end of the balloon was 5–6 cm from the anus. The balloon catheter was fixed to the base of the tail and connected to a pressure transducer. The catheter was also connected to a water bottle via a three-way tap. After performing preliminary distension, a stepwise distension pressure series (10, 20, 40, 60, and 80 mm Hg) was produced. Each step lasted until the blood pressure response became stable, and one series completed within 5 min. The stepwise distension was performed again 30 min after the first distension. Drugs were administered 5–30 min before the second distension.

2.6. Visceromotor reflex

Under sodium pentobarbital (50 mg/kg i.p.) anesthesia, an electrode (M-1.5I, Star Medical) for electromyographic (EMG) recordings was stitched into the external oblique muscle. For some experiments, a jugular vein catheter was inserted for intravenous drug administration. The ends of

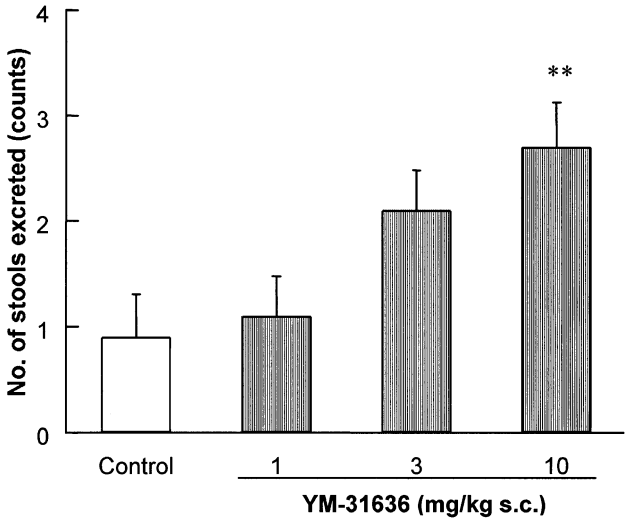

Fig. 1. Effect of YM-31636 on stool excretion in fed rats. The number of fecal pellets excreted by each animal was measured 1 h after s.c. injection. Each bar represents the mean ± S.E.M. for 10 animals. $^{**}P < 0.01$, statistically significant compared with the control group (Dunnett's multiple-range test).

both the electrode and the catheter were brought subcutaneously to a skin incision made at the back of the neck and protected by a protective Velcro jacket. The experiments started at least 5 days after surgery. On the day of the experiments, a 6-cm latex balloon was inserted into the colon through the anus while the animals were under light ether anesthesia and positioned such that the end of the balloon was 2 cm from the anus. The balloon catheter was fixed in place by taping to the base of the tail and connected to a pressure transducer. The catheter was also connected to a water bottle, which provides a uniform pressure increase in the balloon, via a three-way tap. The pressure signal within the balloon was amplified with a carrier amplifier. After recovery from the ether anesthesia in a cage ($23.5 \times 19 \times 19$ cm), repeated incremental colorectal distensions were started. Visceromotor threshold to colorectal distension, defined as the pressure within the balloon when the EMG activity in the abdominal muscle increased abruptly, was determined and at this threshold point the stimulus was immediately removed. A cutoff distension pressure of 80 mm Hg was employed to avoid tissue damage (Traub et al., 1992). The EMG activity was amplified with an amplifier (AB-621G, Nihon Kohden) and quantified with data recording interface (1401 plus, Cambridge Electronic Design; Cambridge, England). Colorectal distensions were performed every 4 min. To ensure the responses were stable (Kolhekar and Gebhart, 1994), more than five colorectal distensions were performed to determine a control value. All data were reported as the mean of results from three consecutive colorectal distensions. A test drug was administered 1–2 min after the last (third) control colorectal distension. The effects of drugs were determined at 15, 19, and 23 min after i.v. administration; at 39, 43, and 47 min after s.c. administration; or at 62, 66, and 70 min after p.o. administration.

2.7. Statistical analysis

Data are expressed as means ± S.E.M. The statistical significance of values for fecal pellet output was determined by Dunnett's multiple range test. The statistical analysis of the data for the motility index or pressor response was performed by two-way repeated measures analysis of variance (ANOVA). EC_{50} values for 5-HT_3 receptor agonists in rat colonic mucosa were calculated by curve fitting on the statistical computer program (SAS ver. 6.11). The statistical analysis of values for visceromotor threshold was performed by paired Student's *t*-test.

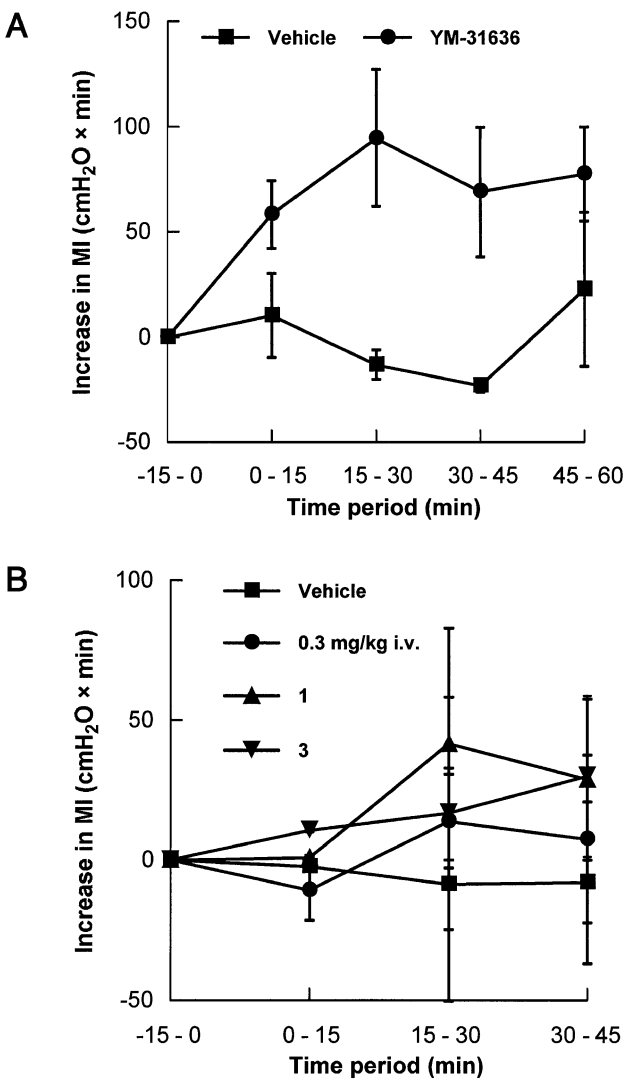

Fig. 2. (A) Effect of YM-31636 (10 mg/kg s.c.) on the intracolonic pressure in conscious rats. (B) Effect of YM-31636 (0.3–3 mg/kg i.v.) on the intracolonic pressure in anesthetized rats. Intracolonic pressure was measured with a balloon inserted into the colon through the anus and the increase in motility index (MI) for 15-min periods was determined. The MI is equivalent to the integrated area between the pressure wave and base line. Each point represents the mean ± S.E.M. for 3–7 animals.

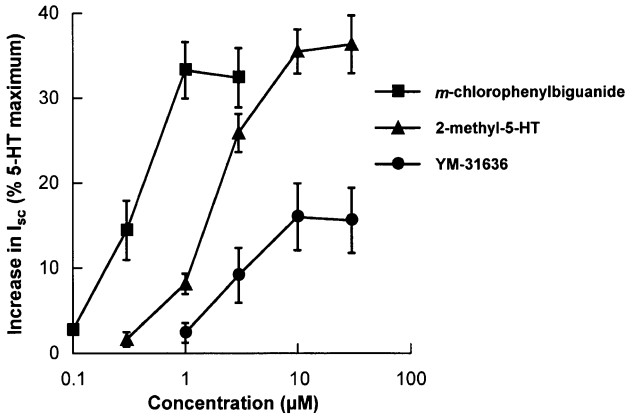

Fig. 3. Effect of 5-HT_3 receptor agonists on the short-circuit current (I_{sc}) response in rat colonic mucosa. The preparations of the mucosa were mounted in Ussing chambers and the I_{sc} was recorded. Each point represents the mean ± S.E.M. for 8–9 preparations.

2.8. Drugs

YM-31636, ramosetron HCl, 2-methyl-5-HT and *m*-chlorophenylbiguanide were prepared by Yamanouchi Pharmaceutical (Tsukuba, Japan). 5-HT creatinine sulfate was purchased from Merck (Darmstadt, Germany). 5-hydroxy-L-tryptophan (5-HTP) was obtained from Sigma-RBI (Natick, MA, USA). Morphine HCl was from Takeda Chemical Industries (Osaka, Japan). Capsaicin was purchased from Wako (Osaka, Japan). All drugs were dissolved in saline or Krebs solution except capsaicin, which was dissolved in 50% ethanol. In the case of oral administration, YM-31636 was suspended in 0.5% methylcellulose solution. All drug doses are stated in terms of the free base.

3. Results

3.1. Effect of YM-31636 on defecation

The administration of YM-31636 (1, 3, or 10 mg/kg s.c.) dose-dependently increased the number of stools excreted in fed rats (Fig. 1). A subcutaneous injection of 10 mg/kg YM-31636 resulted in a significant increase in defecation. A selective 5-HT_3 receptor antagonist ramosetron (1 mg/kg s.c.) inhibited YM-31636-induced increase in fecal output by 85%.

3.2. Intracolonic pressure

In conscious rats, s.c. injection of YM-31636 at 10 mg/kg, which significantly increased the number of fecal pellets, also increased the intracolonic pressure (Fig. 2A). The maximal response of YM-31636 was obtained at the 15- to 30-min period after administration. Similarly, intravenous administration of YM-31636 (1 or 3 mg/kg) produced an increase in the motility index in anesthetized rats (Fig. 2B). The maximal response was obtained at the 15- to 30-min or the 30- to 45-min period.

3.3. I_{sc} response in colonic mucosa

The 5-HT_3 receptor agonists *m*-chlorophenylbiguanide (0.1–3 μM), 2-methyl-5-HT (0.3–30 μM), and YM-31636 (1–30 μM) concentration-dependently increased I_{sc} in rat colonic mucosa (Fig. 3). The EC_{50} values for *m*-chlorophenylbiguanide, 2-methyl-5-HT, and YM-31636 were 0.3

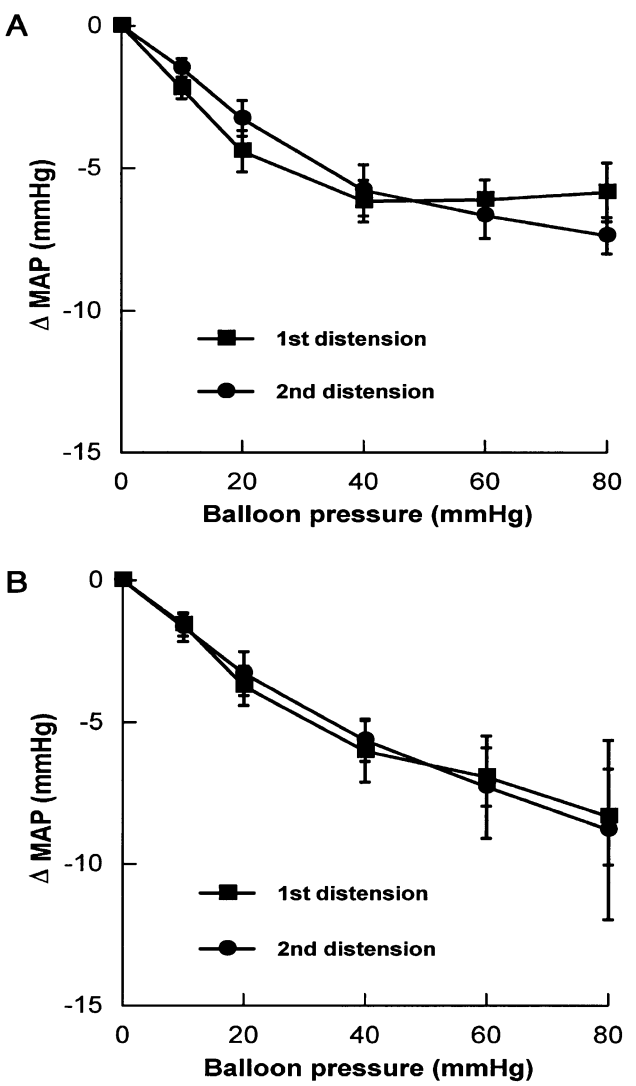

Fig. 4. Effect of YM-31636 on the blood pressure decrease induced by colonic distension in anesthetized rats. Responses are expressed as ΔMAP in mm Hg. A stepwise colonic distension series was performed and the 2nd distension was repeated 30 min after the first distension. YM-31636 was intravenously injected 15 min before, or subcutaneously administered 30 min before, the second distension. (A) YM-31636 3 mg/kg i.v. (B) YM-31636 10 mg/kg s.c. Each point represents the mean ± S.E.M. for 6–8 animals.

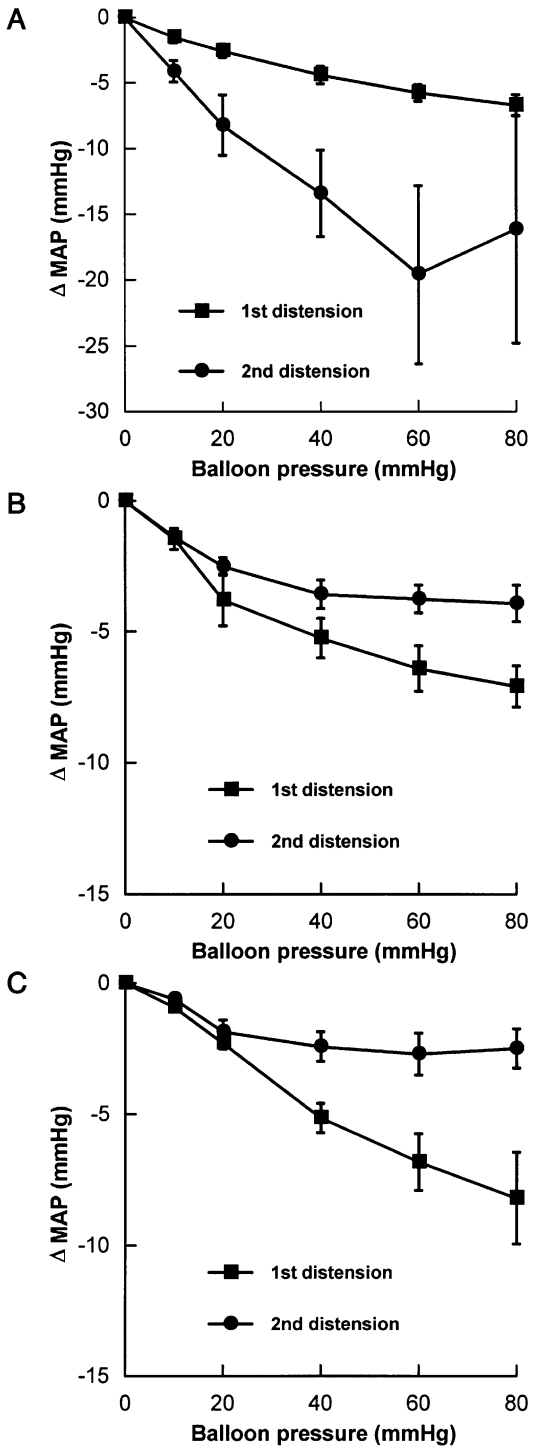

Fig. 5. Effect of drug treatments on the pressor response induced by colonic distension in anesthetized rats. Responses were expressed as ΔMAP in mm Hg. A stepwise colonic distension series was performed and the second distension was repeated 30 min after the first distension. Capsaicin (A) was injected intracolonically 5 min before the second colonic distension. Alternatively, intravenous injection of morphine at 5 mg/kg (B), or co-administration of morphine and YM-31636 (C), was performed 30 min before the second distension. Each point represents the mean ± S.E.M. for 6–8 animals.

(0.2–0.4) μM, 2.3 (1.5–3.1) μM, and 2.9 (2.1–3.6) μM, respectively. The maximal responses to *m*-chlorophenylbiguanide, 2-methyl-5-HT and YM-31636 were 74.5 ± 6.7, 66.6 ± 4.3 and 29.9 ± 6.6 μA/cm^2, respectively. The I_{sc} response to YM-31636 was completely abolished by ramosetron at 0.3 μM.

3.4. Pressor response

Colonic distension caused a decrease in blood pressure, which correlated with the intensity of distension, in anesthetized rats. Neither intravenous (0, 1, 3, or 10 mg/kg) nor subcutaneous (0, 3, 10, or 30 mg/kg) administration of YM-31636 affected this response induced by colonic distension (Fig. 4). A nociceptive agent, capsaicin (0.3%, 1 ml; administered intracolonically) significantly enhanced this blood pressure decrease (Fig. 5A). In contrast, intravenous injection of the antinociceptive drug morphine (5 mg/kg) significantly inhibited the response to distension (Fig. 5B). YM-31636 (10 mg/kg s.c.) did not change the effect of morphine (Fig. 5C).

3.5. Visceromotor reflex

Injection of morphine (3 mg/kg i.v.) significantly increased the visceromotor threshold in response to colorectal distension in conscious rats (Table 1). However, administration of the 5-HT precursor, 5-HTP (30 mg/kg s.c.) significantly decreased this threshold. YM-31636 (10 mg/kg i.v. or 30 mg/kg p.o.) did not affect the visceromotor reflex threshold during the test period.

Table 1
The effect of drug treatments on the visceromotor threshold to colorectal distension

Treatment	Dose	Threshold (mm Hg)	
		Before treatment	After treatment
Saline	1 ml/kg i.v.	30.6 ± 4.8	30.4 ± 5.6
	1 ml/kg s.c.	37.2 ± 1.9	40.6 ± 2.8
0.5% methylcellulose	2 ml/kg p.o.	34.1 ± 2.2	34.2 ± 3.0
Morphine	3 mg/kg i.v.	29.3 ± 2.6	45.8 ± 7.3[a]
5-HTP	30 mg/kg s.c.	37.8 ± 1.9	25.6 ± 2.1[a]
YM-31636	10 mg/kg i.v.	27.9 ± 3.0	27.1 ± 4.6
	30 mg/kg p.o.	32.8 ± 4.0	33.9 ± 4.4

Each rat was given repeated incrementing colorectal distensions (colorectal distensions). The visceromotor threshold to colorectal distension is defined as the pressure within the balloon at the time when the EMG activity in the abdominal muscle increased abruptly. The effects of drugs were determined at 15, 19, and 23 min after i.v. administration; at 39, 43, and 47 min after s.c. administration; or at 62, 66, and 70 min after p.o. administration. Values represent the mean ± S.E.M. for four animals.

[a] $P < 0.05$, statistically significant compared with the pre-treatment value (paired *t*-test).

4. Discussion

YM-31636 is a potent 5-HT_3 receptor agonist in ferrets. This compound facilitates defecation in normal ferrets (Ito et al., 2001) or restores the frequency of defecation in constipated ferrets (Ito et al., 2001). It exhibits a minimum effective dose of 0.03 mg/kg p.o. and does not induce emesis up to 30 mg/kg p.o. Results from the present study show that this compound also increases the fecal output and colonic motility in rats. However, the effective dose of YM-31636 in rats is 10 mg/kg s.c., which is much higher than that in ferrets. One possible cause of the difference of effective dose of YM-31636 between rats and ferrets is species difference of 5-HT_3 receptor. There are several reports about inter-species differences of 5-HT_3 receptors (Kilpatrick and Tyers, 1992; Miyake et al., 1995), although no marked species differences of 5-HT_3 receptors between rats and ferrets in the von Bezold–Jarisch reflex system have been reported (Yamano et al., 1995). In addition to inter-species differences, intra-species differences, especially between 5-HT_3 receptors involved in colonic motility and those involved in the von Bezold–Jarisch reflex system, have also been demonstrated (Ito et al., 1997). The pharmacological response of the 5-HT_3 receptor from ferret colon closely resembles that of human 5-HT_3 receptor, and not that of rat 5-HT_3 receptor (Mochizuki et al., 2000). Taken together, these results suggest the possibility that different types of 5-HT_3 receptor exist in the same species, and 5-HT_3 receptors those involved in the regulation of colonic motility are different between rats and ferrets.

Other possible cause of the difference of effective dose of YM-31636 between rats and ferrets are difference of pharmacokinetics or that of contribution of 5-HT_3 receptors to gastrointestinal motility. In isolated distal ileum, 5-HT induces contraction in ferrets via 5-HT_1 and 5-HT_3 receptors, whereas in rats, via 5-HT_1 and 5-HT_2 receptors (Yamano et al., 1997). Further studies are necessary to confirm the cause of the difference of effective dose of YM-31636 between rats and ferrets.

The increase in the secretion of Cl^- from rat colonic mucosa results in an increase in I_{sc}. In this study, YM-31636 increases I_{sc} in rat colonic mucosa, although the maximal response is less than 50% that of other 5-HT_3 receptor agonists. Similarly, this agonist shows partial agonistic activities in increasing I_{sc} response in guinea-pig distal colon, while showing almost full agonistic activities in inducing contraction in isolated guinea-pig distal colon (Ito et al., 2000). YM-31636 also facilitated defecation without inducing diarrhea in ferrets (Ito et al., 2001). These results show that YM-31636 increases colonic motility and weakly stimulates colonic secretion to the extent of not causing diarrhea in different species. These properties would be very useful to expel feces. The maximal I_{sc} responses, the potencies of *m*-chlorophenylbiguanide and YM-31636, and the rank order of potency in rats are very different from those in guinea pigs. This finding indicates the inter-species differences of 5-HT_3 receptors between rats and guinea pigs.

The visceral pain reflex models most commonly used are pressor response or visceromotor reflex in response to colonic or colorectal distension in rats. YM-31636, at a dose more than that which increases defecation and colonic motility, neither increases the intensity of pressor response to colonic distension nor decreases the visceromotor threshold in response to colorectal distension. These results indicate that YM-31636 facilitates defecation without reducing the visceral pain threshold or increasing the intensity of visceral pain. In addition to not affecting visceral pain reflex, this compound does not inhibit or enhance the antinociceptive effect of morphine. Opioids like morphine are frequently given to patients with cancer or chronic pain (McQuay, 1999). However, opioids produce many adverse effects, for example, nausea, dizziness, and constipation, among others. Of these adverse effects, constipation is the most common symptom. Moulin et al. (1996) reported that 41% of morphine-treated patients became constipated. YM-31636 relieves constipation induced by morphine in ferrets (Ito et al., 2001). Taken together, these results suggest that 5-HT_3 receptor agonists such as YM-31636 would be useful compounds to relieve constipation, including opioid-induced constipation.

In conclusion, YM-31636 facilitates defecation and increases colonic motility in rats, without affecting the visceral pain reflexes. Consequently, 5-HT_3 receptor agonists like YM-31636 would be promising in the treatment of chronic constipation.

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