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Investigation of the effects of YM-31636, a novel 5-HT_3 receptor agonist, on defecation in normal and constipated ferrets

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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 newly synthesized 5-HT_3 receptor agonist, on defecation in normal and constipated ferrets, and evaluated it as an agent against constipation. YM-31636 facilitated defecation without inducing diarrhea or emetic episodes. This effect occurred within 1 h after oral administration, mostly within 30 min, whereas sodium picosulfate, a widely used laxative, tended to increase the frequency of defecation for several hours with much lower peak incidence than that of YM-31636, and induced diarrhea. UK14304 (brimonidine), an α_2 receptor agonist, and morphine reduced the frequency of defecation and YM-31636 restored it. These effects of YM-31636 were antagonized by ramosetron, a 5-HT_3 receptor antagonist. These results suggest that YM-31636 could be promising in the treatment of constipation. Because of an early and reliable onset of action compared with sodium picosulfate, YM-31636 could make it easier to control the time of defecation.

Keywords: YM-31636; 5-HT_3 receptor agonist; Defecation; Emesis; Constipation; Diarrhea

1. Introduction

Constipation is one of the most common chronic digestive disorders. The definition of constipation, however, is not sufficiently established, probably because of its various kinds of symptoms. The principal mechanism of the development of constipation is the motility disorder of the colon. Functional constipation is classified into two main categories, that is, atonic and spastic constipation (Koch, 1995). Laxatives are widely used in the treatment of constipation, although some of them are contraindicated in spastic constipation. Their onset of action is slow and dispersed, causing difficulty in control of the time of defecation. Moreover, their main action is to increase water secretion from the colonic mucosa. They, therefore, tend to cause diarrhea, dehydration and electrolyte disturbance.

5-Hydroxytryptamine (5-HT) is a biogenic amine that mediates a variety of physiological actions. In the gastrointestinal tract, 5-HT is stored in and released from enterochromaffin cells and enteric serotonergic neurons (Erspamer, 1966; Wood, 1994). 5-HT_3 receptor has been identified on enteric neurons (Mawe et al., 1986; Ito and Tamura, 1995; Zhou and Galligan, 1999). A 5-HT_3 receptor antagonist suppressed colonic motility in healthy humans (Talley et al., 1990), indicating that the 5-HT_3 receptor is involved in the regulation of colonic function in man. The mechanism of the regulation of colonic function through the 5-HT_3 receptor is considered to be as follows: 5-HT is released in compliance with stimulation of the mucosal surface by intraluminal contents, binds to the neuronally located 5-HT_3 receptors, and accelerates colonic functions through the release of acetylcholine, tachykinins, vasoactive intestinal peptide (VIP) or nitric oxide (NO), or both. 5-HT_3 receptor-stimulating agents, therefore, would be promising in the treatment of constipation.

It is well established that chemotherapy-induced emetic response is mediated by stimulation of 5-HT_3 receptors in the afferent vagus nerves, splanchnic nerves and area postrema (Kamato et al., 1993). It is, therefore, considered that 5-HT_3 receptor-stimulating agents used to treat constipation should affect colonic function selectively, and should not act on other organs.

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Our previous report indicated that YM-31636 (2-(1*H*-imidazol-4-ylmethyl)-8*H*-indeno[1,2-*d*]thiazole monofumarate), a newly synthesized 5-HT_3 receptor agonist, showed almost full agonistic activities in inducing contraction with an intrinsic activity of 0.90 compared with 5-HT in isolated guinea pig distal colon, whereas, partial agonistic activities in increasing short-circuit current with an intrinsic activity of 0.19 in the guinea pig colonic mucosa (Ito et al., 2000). The result indicates that YM-31636 potently stimulates the motility, and mildly increases the transport of chloride ions that is considered to be followed by water secretion in distal colon. Furthermore, YM-31636 showed positive chronotropic effect with a low intrinsic activity (0.23) in isolated guinea pig right atria, suggesting that YM-31636 preferentially affects colonic function. These profile would be good for a drug which treats constipation, and further in vivo studies are necessary to clarify the pharmacological profile of YM-31636 for treating constipation.

In the present study, we investigated the effects of YM-31636 on defecation in ferrets and evaluated it as an agent against constipation. To examine the effects of this compound in constipated condition, we established the animal models by using UK14304 (brimonidine), an α_2-adrenoceptor agonist (Cambridge, 1981), and morphine in ferrets.

2. Materials and methods

2.1. Animals

Male ferrets weighing 0.8–1.5 kg ($n = 60$) were used. The animals were fed an ordinary laboratory chow and allowed free access to water under a constant 12-h light–dark cycle. The food was given once daily in the middle of the light cycle (1:00 p.m.). The animals were used repeatedly with 1-week washout periods between the different tests. All experiments were performed in compliance with the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical.

2.2. Defecation and emetic episodes in normal ferrets

YM-31636 or sodium picosulfate was administered orally at 10:00 a.m. and defecation, emetic episodes including vomiting and retching, and some other behavioral changes were observed. In the preliminary observations on the duration of the effects of these compounds on defecation, the effect of YM-31636 finished within 2 h after administration, although the effect of sodium picosulfate continued for 12 h. We observed, therefore, the effects of YM-31636 and sodium picosulfate for 2 and 12 h, respectively. No food was supplied during the observation. In the antagonist study, ramosetron 0.1 μg/kg was administered subcutaneously 0.5 h before the administration of YM-31636. The stools were collected and dried (110 °C, 24 h). The water content was estimated by measuring both wet and dry weights of stools. In the case of repeated dosing, YM-31636 was orally administered once daily at 10:00 a.m. for 5 days, and the frequency of defecation for 1 h after administration was observed each day.

2.3. Defecation in constipation models

In order to establish the constipation model, we first investigated the normal defecation pattern in untreated ferrets. The frequency of defecation spontaneously increased 3 to 5 h after food intake. Next, we examined the effects of UK14304 and morphine on the increased defecation 3 to 5 h after food intake. Subcutaneously administered UK14304 or morphine 1.5 h after feeding inhibited defecation in a dose-dependent manner with minimum effective doses of 3 μg/kg and 0.3 mg/kg, respectively. We, therefore, established the experimental protocol as follows: UK14304 (3 μg/kg) or morphine (0.3 mg/kg) were administered subcutaneously 1.5 h after feeding to suppress the increase in frequency of defecation 3 to 5 h after food intake. YM-31636 was orally administered 3 h after feeding, the frequency of defecation was observed and the stools were collected to estimate the water content for the following 2 h. In the antagonist study, ramosetron 0.1 μg/kg was administered subcutaneously 0.5 h before the administration of YM-31636.

2.4. Drugs

YM-31636, ramosetron HCl and UK14304 were prepared by Yamanouchi Pharmaceutical. Sodium picosulfate was extracted with water from tablets of Laxoberon® (Teijin, Japan) and purified by reversed phase column chromatography, and recrystallized from ethanol. Morphine HCl was purchased from Takeda Chemical Industries (Osaka, Japan). YM-31636 and sodium picosulfate were suspended in 0.5% methylcellulose solution and given to ferrets orally at a dose of 2 ml/kg. Ramosetron, UK14304 and morphine were dissolved in physiological saline and injected in ferrets subcutaneously at a dose of 1 ml/kg.

2.5. Statistical analysis

Values were expressed as the mean ± S.E.M. Statistical analysis of the data was performed with Student's *t*-test or Dunnett's test for multiple comparison. In the case of repeated measurement, the two-way repeated measures analysis of variance (ANOVA) was performed followed by Dunnett's test to compare the differences between control and treatment groups at each time point. Probability values of < 0.05 were considered significant.

3. Results

3.1. Effects on defecation in normal ferrets

YM-31636 facilitated defecation within 1 h, mostly within 30 min, after oral administration in ferrets (Fig. 1A). The between-subjects and within-subjects effects and the interaction were significantly different by two-way repeated measures ANOVA. On the other hand, sodium picosulfate tended to increase the frequency of defecation 2 to 10 h after administration with much lower peak incidence than that of YM-31636 (Fig. 1B). As shown in Fig. 2, YM-31636 significantly increased the frequency of defecation within 1 h after administration with a minimum effective dose of 0.03 mg/kg p.o. and the effect of YM-31636 was antagonized by ramosetron, a selective 5-HT_3 receptor antagonist.

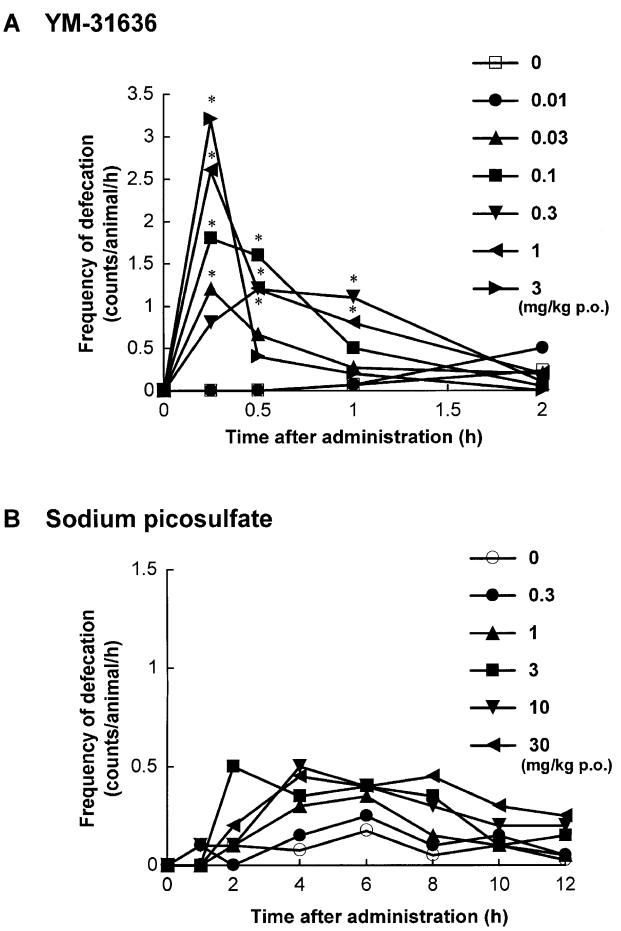

Fig. 1. The time course of the cathartic effects of YM-31636 (A) and sodium picosulfate (B) in normal ferrets. YM-31636 (0.01–3 mg/kg, $n = 10$–30) or sodium picosulfate (0.3–30 mg/kg, $n = 20$) was administered orally and the frequency of defecation was observed for 2 and 12 h after administration of YM-31636 and sodium picosulfate, respectively. Each point represents the mean. $^{*}P < 0.05$ compared with the control group at each time point with Dunnett's test.

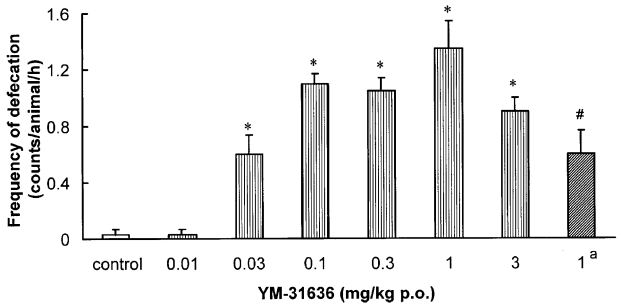

Fig. 2. The effect of YM-31636 on defecation in normal ferrets. YM-31636 (0.01–3 mg/kg, $n = 10$–30) was administered orally and the frequency of defecation was observed. Each value represents the mean ± S.E.M. for 1 h after administration. a—Pretreatment with ramosetron, 1 μg/kg s.c., 0.5 h before administration of YM-31636. $^{*}P < 0.05$ compared with the control group with Dunnett's test. $^{\#}P < 0.05$ compared with the YM-31636 (1 mg/kg) treatment group with Student's *t*-test.

3.2. Effects on water content of stools in normal ferrets

YM-31636 slightly, but not significantly, increased the water contents of stools (Fig. 3A). No fluid stools were

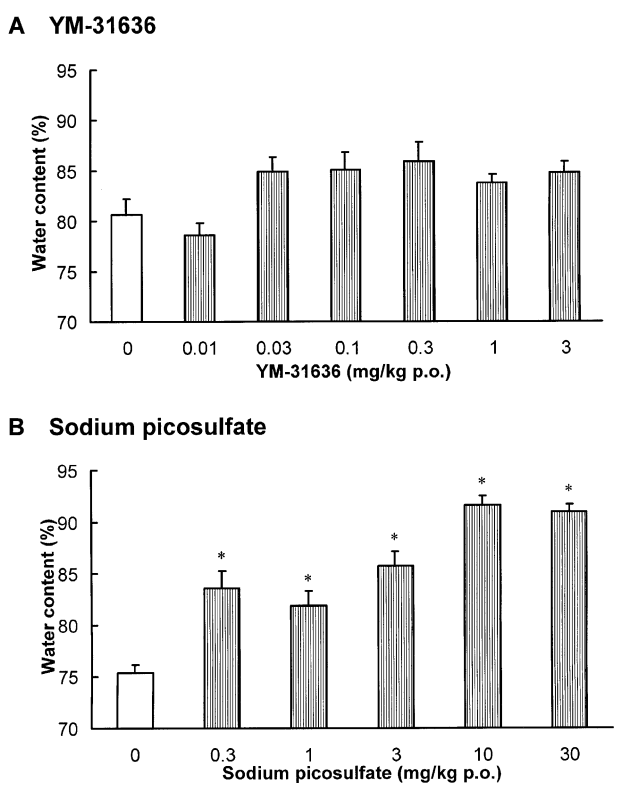

Fig. 3. The effects of YM-31636 (A) and sodium picosulfate (B) on the water contents of stools in normal ferrets. The stools were collected for 2 and 12 h after the administration of YM-31636 (0.01–3 mg/kg, $n = 10$–30) and sodium picosulfate (0.3–30 mg/kg, $n = 20$), respectively. The water content was estimated by measuring both wet and dry weights of stools. Each value represents the mean ± S.E.M. The number of stools was 5 to 11 (A) and 13 to 31 (B), respectively. $^{*}P < 0.05$ compared with the control group with Dunnett's test.

observed after the administration of YM-31636. On the other hand, sodium picosulfate significantly increased the water content of stools (Fig. 3B). At high doses, the increased contents exceeded 90% and fluid stools were observed.

3.3. Emetic effect of YM-31636 in normal ferrets

YM-31636 did not induced vomiting, retching, or any other behaviours suggestive of nausea-like symptoms up to 30 mg/kg p.o., whereas it facilitated defecation at doses of more than 0.03 mg/kg p.o. leading to a safety margin of 1000 (data not shown).

3.4. Effect of repeated dosing of YM-31636 in normal ferrets

The effect of YM-31636 on defecation did not change after repeated dosing for 5 days (Fig. 4). The between-subjects effects were significantly different, whereas the within-subjects effects and the interaction were not significant by two-way repeated measures ANOVA.

3.5. Constipation models

UK14304, when administered 1.5 h after the food intake, inhibited defecation 3 to 5 h after feeding, in a dose-dependent manner, whereas the water content of stools did not significantly change. In the same way, morphine dose-dependently reduced the frequency of defecation and also the water content of stools (data not shown). Both UK14304 and morphine did not induce emetic episodes at the dosage up to 10 μg/kg and 1 mg/kg s.c., respectively.

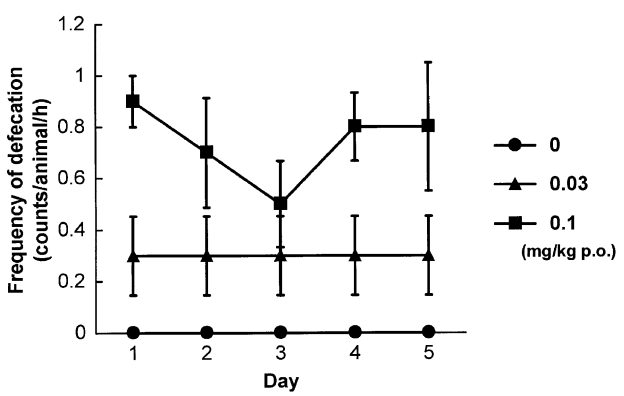

Fig. 4. The effect of repeated dosing of YM-31636 on defecation in ferrets. YM-31636 (0.03, 0.1 mg/kg, $n = 10$) was administered orally once daily for 5 days, and the frequency of defecation for 1 h after administration was observed each day. Each point represents the mean ± S.E.M.

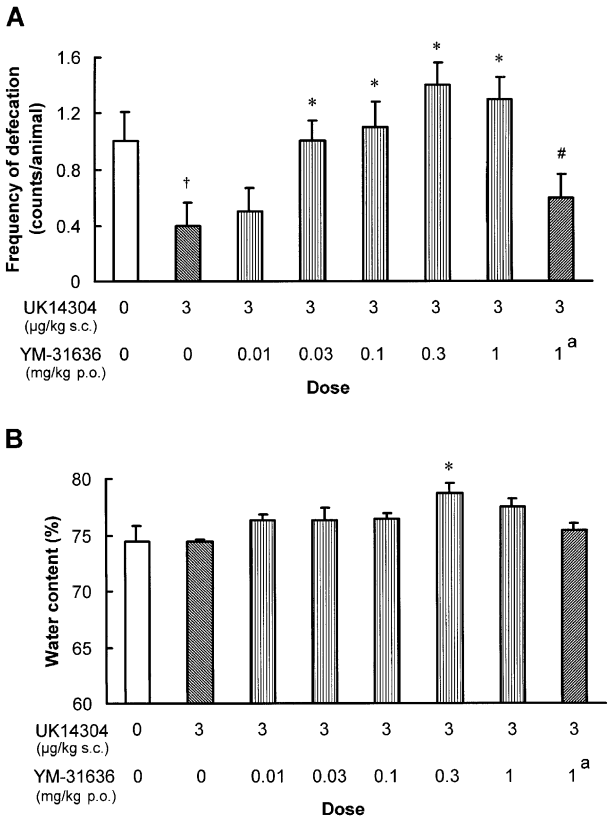

Fig. 5. The effect of YM-31636 on defecation (A) and the water content of stools (B) in UK14304-induced constipated ferrets. UK14304 (3 μg/kg, $n = 10$) was administered subcutaneously 1.5 h after feeding and YM-31636 (0.01–1 mg/kg) was administered orally 3 h after feeding, and the frequency of defecation was observed for the following 2 h. The stools were also collected during the observation time to estimate the water content. a—Pretreatment with ramosetron 1 μg/kg s.c., 0.5 h before the administration of YM-31636. Each value represents the mean ± S.E.M. The number of stools was 4 to 10. $^{\dagger}P < 0.05$ compared with the vehicle treatment group with Student's *t*-test. $^{*}P < 0.05$ compared with the UK14304 (3 μg/kg) treatment group with Dunnett's test. $^{\#}P < 0.05$ compared with the YM-31636 (1 mg/kg) treatment group with Student's *t*-test.

3.6. Effect of YM-31636 on defecation in constipated ferrets

YM-31636 restored the decreased frequency of defecation with a minimum effective dose of 0.03 mg/kg p.o., without inducing emetic episodes in either constipation model (Figs. 5A, 6A). The effects of YM-31636 were antagonized by ramosetron, a 5-HT_3 receptor antagonist.

3.7. Effect of YM-31636 on water content of stools in constipated ferrets

In UK14304-treated ferrets, the water content of stools was significantly increased by 0.3 mg/kg of YM-31636 (Fig. 5B). The increased content was, however, still under 80%, and no fluid stools were observed. In morphine-

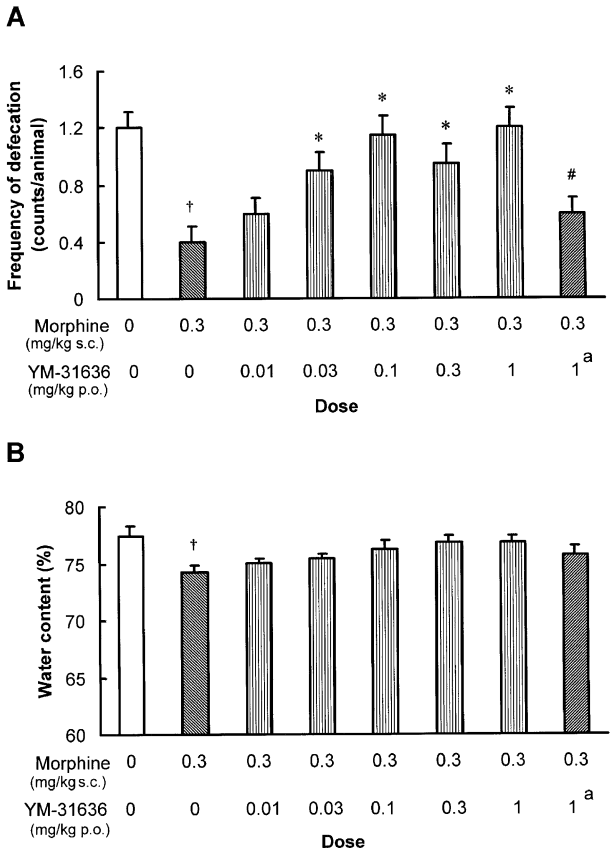

Fig. 6. The effect of YM-31636 on defecation (A) and the water content of stools (B) in morphine-induced constipated ferrets. Morphine (0.3 mg/kg, $n = 20$) was administered subcutaneously 1.5 h after feeding, YM-31636 (0.01–1 mg/kg) was administered orally 3 h after feeding, and the frequency of defecation was observed for the following 2 h. The stools were also collected during the observation time to estimate the water content. a—Pretreatment with ramosetron 1 μg/kg s.c., 0.5 h before the administration of YM-31636. Each value represents the mean ± S.E.M. The number of stools was 6 to 24. $^{\dagger}P < 0.05$ compared with the vehicle treatment group with Student's *t*-test. $^{*}P < 0.05$ compared with the morphine (0.3 mg/kg) treatment group with Dunnett's test. $^{\#}P < 0.05$ compared with the YM-31636 (1 mg/kg) treatment group with Student's *t*-test.

treated ferrets, the decreased water content seemed to be reversed, although the effect was not statistically significant (Fig. 6B). The effects of YM-31636 on the water content were also antagonized by ramosetron.

4. Discussion

In the present study, we examined the effects of YM-31636, a novel 5-HT_3 receptor agonist (Ito et al., 2000), on defecation in ferrets, and evaluated it as an agent against constipation. In normal ferrets, YM-31636 facilitated defecation with high potency and efficacy. The effect occurred within 1 h, mostly within 30 min after oral administration, whereas sodium picosulfate, a widely used laxative, tended to increase the frequency of defecation 2 to 10 h after administration with much lower peak incidence than that of YM-31636. These results suggest that YM-31636 could make it easier to control the time of defecation because of its early and reliable onset of action compared to the existing laxatives including sodium picosulfate. The effect of YM-31636 did not change after once-daily administration for 5 days, confirming further the reliable and constant effect of YM-31636 as an agent against constipation.

In addition, YM-31636 slightly, but not significantly, increased the water content of stools in normal ferrets and no fluid stools were observed after the administration. In contrast, sodium picosulfate significantly increased the water content of stools. These results are consistent with those of in vitro studies. YM-31636 increased short-circuit current in guinea pig distal colon, and the maximal response was approximately 13% of that to rhein, an active metabolite of sennoside (Ito et al, 2000). Widely used laxatives including sennoside, bisacodyl, sodium picosulfate, and so on, tend to cause diarrhea, dehydration and electrolyte disturbance, because their main action is to increase water secretion from the colonic mucosa (Corazziari et al., 1987; Duncan et al., 1992). In contrast, the increasing effect of YM-31636 on the colonic water secretion is considered to be moderate and, therefore, YM-31636 would not induce diarrhea even at doses exceeding a sufficiently effective dose.

In the present study, the effects of YM-31636 were not fully blocked by ramosetron (Figs. 2, 5, 6). It might suggest that YM-31636 facilitated defecation through not only 5-HT_3 receptor but also other receptors. In the previous report, however, we indicated that YM-31636 induced contraction of the guinea pig distal colon through the stimulation of 5-HT_3 receptor selectively, and did not affect 5-HT_1, 5-HT_2, 5-HT_4 and some other receptors (Ito et al., 2000). It is, therefore, speculated that ramosetron could not completely block the effect of YM-31636 because of considerably high affinity of YM-31636 for 5-HT_3 receptors ($pK_i = 9.67$, see Ito et al., 2000). Further investigations on the effect of YM-31636 on ferret tissues and receptors would be desirable to clarify the properties of this compound in ferrets.

It is well known that the 5-HT_3 receptor is related to the chemotherapy- and radiotherapy-induced emetic response (Kamato et al., 1993; Cunningham, 1997). It has been reported that selective 5-HT_3 receptor agonists including 2-methyl-5-HT, phenylbiguanide and *m*-chlorophenylbiguanide evoked emesis in ferrets (King, 1990; Sancilio et al., 1991; Kamato et al., 1993). We, therefore, examined the emetic effect of YM-31636 in normal ferrets. YM-31636 had no emetic effect when administered at a dosage 1000 times greater than the minimum effective dose in inducing defecation. Our previous reports suggest that 5-HT_3 receptor agonist-induced defecation and emesis may be mainly mediated, through the depolarization of colonic myenteric neurons and excitation of abdominal vagal affer-

ent nerves, respectively (Miyata et al., 1991, 1992; Ito and Tamura, 1995; Kamato et al., 1993). Since YM-31636 is considered to stimulate 5-HT_3 receptor selectively as we described above, one of the possible cause of the selective effect of YM-31636 on defecation is the differences in the number of 5-HT_3 receptors or some other conditions between the colonic neuron and vagus nerve. It has been shown that the difference in receptor number and efficiency of receptor coupling would affect the potency and intrinsic activity of agonist (Kenakin, 1984). Actually, agonist efficacy is reported to be variable among expression and assay system (Niemeyer and Lummis, 1998; Werner et al., 1994; Downie et al., 1994). In addition, the results of the present study might suggest the possibility that the different types of 5-HT_3 receptor exist in the colonic neuron and vagus nerve, and the fact that YM-31636 preferably stimulates the colonic neurons and evokes defecation. Ito et al. (1997) have reported the possibility that the 5-HT_3 receptors which mediate the von Bezold–Jarisch reflex and defecation are different in rat. Several other studies demonstrated the difference in intrinsic activities of agonists in some tissues or cells (Mochizuki et al., 2000). YM-31636 also showed the different intrinsic activities in the different tissues (Ito et al., 2000). Recently, a new class of human 5-HT_3 receptor subunit, 5-HT_{3B}, has been described (Davies et al., 1999). As compared with the homomeric receptor, the heteromeric assemblies of 5-HT_{3A} and 5-HT_{3B} subunits display similar properties to the neuronal 5-HT_3 receptor channels. This finding suggests the possibility that different types of 5-HT_3 receptor exist in the same species similarly to the nicotinic receptors, which are thought to resemble 5-HT_3 receptors, although the existence has not been confirmed by the identification of the corresponding molecule. Further studies are necessary to confirm the cause of the preferable effect of YM-31636 on defecation.

In this study, we established the animal models of constipation in ferrets by using UK14304, an α_2-adrenoceptor agonist, and morphine, a μ opioid receptor agonist. As is described above, it has been reported that the α_2-adrenoceptor agonists and μ opioid agonists showed inhibition and stimulation of colonic motility, respectively, in animals and humans (Gillis et al., 1987; Livington and Passaro, 1990; Schang et al., 1986; Corazziari, 1999), whereas they both inhibited gastrointestinal transit (Schiller et al., 1985; Rubinoff et al., 1989; Schang et al., 1986). In addition, it is known that α_2-adrenoceptor agonists and morphine evoke constipation in humans (Keenan et al., 1986; Borody et al., 1985). In this study, UK14304 and morphine both decreased the frequency of defecation in ferrets. No emesis was observed with the doses of both compounds used in this protocol, although some reports have shown that these compounds produced emesis (Lang et al., 1999; Ariumi et al., 2000). Taken together, it might be considered that UK14304 and morphine induced constipation that reflects the pathophysiology of atonic and spastic types (Koch, 1995), respectively. Further studies, however, are desirable on the mechanisms of constipation induced by UK14304 and morphine in ferrets.

YM-31636 restored the decreased frequency of defecation in both constipation models without inducing emetic episodes. In UK14304-treated ferrets, the water content of stools was increased by YM-31636. The increased content was, however, still under 80%, and no fluid stools were observed. The results suggest that YM-31636 would not induce diarrhea or emesis in constipated conditions, and would be expected to be promising as an agent against constipation, such as chronic idiopathic constipation, constipation-predominant irritable bowel syndrome, drug-induced constipation, and so on.

Our previous reports suggest that 5-HT_3 receptor agonist-induced defecation might be mainly mediated through the depolarization of colonic myenteric neurons (Miyata et al., 1991, 1992; Ito and Tamura, 1995). In this study, the effects of YM-31636 on defecation were antagonized by ramosetron, a selective 5-HT_3 receptor antagonist, suggesting that YM-31636 facilitated defecation through the stimulation of 5-HT_3 receptors on the colonic myenteric neurons, increasing the neuronal activity, and enhancing the colonic motility in ferrets.

In conclusion, YM-31636 could be promising as an agent against constipation. YM-31636 showed early and reliable onset of action as compared with sodium picosulfate, a widely used laxative, indicating that YM-31636 could make it easier to control the time of defecation. It may improve some kind of constipated conditions, such as chronic idiopathic constipation, constipation-predominant irritable bowel syndrome, drug-induced constipation, and so on, without inducing diarrhea or emetic episodes. Bowel clearance before surgery, labor or radiological examination could also be therapeutic indications. Further investigations on the mechanisms of the effects of YM-31636 on defecation, such as its effects on gastrointestinal motility, transit time, etc. are desirable.

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