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Pharmacological profile of YM-31636, a novel 5-HT_3 receptor agonist, in vitro

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Abstract

We investigated the in vitro pharmacological profile of YM-31636 (2-(1*H*-imidazol-4-ylmethyl)-8*H*-indeno[1,2-*d*]thiazole monofumarate). In cloned human 5-HT_{3A} receptors, YM-31636 had a pK_i value of 9.67 vs. ramosetron and pK_i values for other 5-HT_3 receptor agonists were less than 7. YM-31636 showed very low affinities for other receptors. YM-31636 induced contraction of isolated guinea pig distal colon. The intrinsic activity was approximately 0.90 compared with 5-hydroxytryptamine's (5-HT) 1.0, and the potency was 26 times greater than that of 5-HT. YM-31636 increased short-circuit current (I_{sc}) in the isolated guinea pig distal colon. In this case, the relative intrinsic activity was approximately 0.19. In isolated guinea pig right atrium, YM-31636 induced tachycardia with the relative intrinsic activity of approximately 0.23. All these effects of YM-31636 were antagonized by ramosetron, a selective 5-HT_3 receptor antagonist. These results suggest that YM-31636 is a potent and selective 5-HT_3 receptor agonist, preferentially acting on the contraction of the colon. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: YM-31636; 5-HT_3 receptor agonist; (Guinea pig); Colon, distal; Atrium, right

1. Introduction

5-Hydroxytryptamine (5-HT) is a biogenic amine that mediates a variety of physiological actions. 5-HT receptors are now classified into seven major groups (Hoyer and Martin, 1997). Of these subtypes, the 5-HT_3 receptor has been shown to be a ligand-gated ion channel that causes fast, depolarizing responses in neuronal cells (Yakel and Jackson, 1988; Ito and Tamura, 1995). The 5-HT_3 receptor has been investigated in neurons of various central and peripheral regions including the cerebral cortex (Kilpatrick et al., 1987), vagus nerve (Ireland and Tyers, 1987) and intestine (Derkach et al., 1989; Zhou and Galligan, 1999).

Many actions attributable to the 5-HT_3 receptor have been described in both the peripheral and central nervous systems. Several selective 5-HT_3 receptor antagonists, including ondansetron, granisetron and ramosetron, have been developed (Butler et al., 1988; Sanger and Nelson, 1989; Miyata et al., 1991), and have already been evaluated as anti-emetic agents for cancer chemotherapy and radiotherapy (Kamato et al., 1993; Cunningham, 1997). In addition, these drugs have undergone clinical trials to assess their efficacy in treating gastrointestinal tract and central nervous system disorders (Greenshaw and Silverstone, 1997; Farthing, 1998), and among them, alosetron has recently been approved as an agent against irritable bowel syndrome (Camilleri et al., 2000).

We recently synthesized YM-31636 (2-(1*H*-imidazol-4-ylmethyl)-8*H*-indeno[1,2-*d*]thiazole monofumarate as a novel 5-HT_3 receptor agonist (Fig. 1). The purpose of this study was to examine the 5-HT_3 receptor agonistic activity of YM-31636 on the distal colon and right atrium tissue sections from guinea pigs. To confirm the selectivity of this compound for the 5-HT_3 receptor, the affinity of

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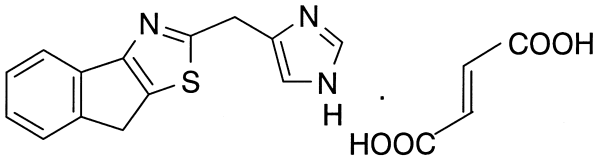

Fig. 1. Structure of YM-31636, 2-(1*H*-imidazol-4-ylmethyl)-8*H*-indeno-[1,2-*d*]thiazole monofumarate.

YM-31636 for several other neurotransmitter receptors was examined.

2. Materials and methods

2.1. Radioligand binding assay for cloned human 5-HT$_3$ receptors

The isolation of the human 5-HT type 3A (5-HT$_{3A}$) receptor cDNA and its nucleotide sequence were previously reported (Miyake et al., 1995). The cDNA fragment was subcloned into the mammalian expression vector pEF-BOS. CV-1 origine, SV40 (COS)-1 cells were transfected with plasmids using the DEAE-dextran/chloroquine method. COS-1 cells ($1–2 \times 10^6$ cells) were incubated overnight with plasmid DNA (15 μg) carried by DEAE-dextran (0.25 mg/ml) for 14 h, and further exposed to 0.1 mM chloroquine for 2.5 h. After 3 days, the transfected cells were homogenized in 50 mM HEPES, pH 7.4, and centrifuged at $48{,}000 \times g$ for 10 min. The membrane homogenates were stored at −80°C until required for the radioligand binding assay.

5-HT$_3$ receptor binding studies were performed as described below using [^{3}H]ramosetron, a potent and selective 5-HT$_3$ receptor ligand (Akuzawa et al., 1995). Assay tubes containing 50 μl of [^{3}H]ramosetron, 50 μl of buffer or competing drug and 400 μl of the membrane homogenate were incubated at 25°C for 30 min. The incubation was terminated by rapid vacuum filtration through GF/B glass-fiber filters, presoaked with 0.1% polyethyleneimine, using a Brandel Cell Harvester. The filters were washed three times with 3 ml of ice-cold 50 mM HEPES buffer. Radioactivity bound to the filter was measured by liquid scintillation spectroscopy. Non-specific binding was defined in the presence of tropisetron (1 μM).

5-HT$_3$ receptor binding studies with rabbit ileum were also performed using [^{3}H]GR65630, another selective 5-HT$_3$ receptor ligand (Champaneria et al., 1992).

2.2. Binding studies for other receptors

Other radioligand binding assays were performed as described by the following investigators with minor changes: [^{3}H]5-HT binding to 5-HT$_1$ receptors (Peroutka and Snyder, 1981), [^{3}H]ketanserin binding to 5-HT$_2$ receptors (Leysen et al., 1982), [^{3}H]prazosin binding to α_1-adrenoceptors (Terai et al., 1983), [^{3}H]rauwolsine binding to α_2-adrenoceptors (Zhang et al., 1992), [^{3}H]dihydroalprenolol binding to β-adrenoceptors (Galant et al., 1979), [^{3}H]SCH23390 binding to dopamine D$_1$ receptors (Brière et al., 1987), [^{3}H]raclopride binding to dopamine D$_2$ receptors (Hall and Wedel, 1986), [^{3}H]pyrilamine binding to histamine H$_1$ receptors (Billah et al., 1990), [^{3}H]quinuclidinyl benzilate binding to muscarinic M$_2$ receptors (Yamamura and Snyder, 1974), [^{3}H]naloxone binding to opioid μ receptors (Chang and Cuatrecasas, 1979) and [^{3}H]flunitrazepam binding to benzodiazepine receptors (Damm et al., 1978).

2.3. Contraction of the isolated guinea pig distal colon

The distal portion of the colon was removed from a Hartley guinea pig (300–500 g). The colon was cleaned in fresh Krebs–bicarbonate buffer at room temperature and cut into approximately 20 mm segments. The segments were suspended longitudinally in organ bath containing Krebs–bicarbonate solution warmed to 37°C and equilibrated with 95% O_2/5% CO_2. Isometric contraction under a loading tension of 1 g was recorded. The agonists were applied cumulatively to the bath. For antagonist studies, antagonists were added to the bath 15 min before the application of the agonists. For the desensitization of 5-HT$_3$ receptors, 2-methyl-5-HT (30 μM) was added to the bath 30 min before the application of the agonists.

2.4. Short-circuit current (I_{sc}) response in the isolated guinea pig distal colon

The distal portion of the colon was removed from a Hartley guinea pig (350–550 g), and preparations of the mucosa were prepared by dissection of the muscle layers (Bunce et al., 1991). The tissues were then mounted in Ussing chambers (window area: 0.8 cm^2), bathed on both sides with Krebs–bicarbonate solution warmed to 37°C and equilibrated with 95% O_2/5% CO_2. These tissue preparations were short-circuited by use of a short-circuit current amplifier (Nihon Kohden CEZ-9100; Tokyo, Japan), and the I_{sc} was continuously recorded. Concentration–response curves of agonists were constructed in a cumulative manner. For antagonist studies, preparations were exposed to ramosetron for 30 min before the addition of the agonists.

2.5. Chronotropic responses in the isolated guinea pig right atrium

The right atrium was removed from a Hartley guinea pig (300–500 g), freed of connective tissue, and suspended at 1 g tension in organ bath containing Krebs–bicarbonate solution warmed to 37°C and equilibrated with 95% O_2/5% CO_2. The concentration–response curves were constructed in a cumulative manner. For antagonist stud-

ies, ramosetron was added to the bath 15 min before the application of the agonists.

2.6. Drugs

[^{3}H]Ramosetron HCl (78 Ci/mmol) was specially synthesized by Amersham International (Buckinghamshire, UK). YM-31636 (2-(1*H*-imidazol-4-ylmethyl)-8*H*-indeno[1,2-*d*]thiazole monofumarate), 2-methyl-5-HT, *m*-chlorophenylbiguanide, SB204070 ((1-butyl-4-piperidinyl)-methyl-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate hydrochloride) and ramosetron HCl were prepared by Yamanouchi Pharmaceutical. Methysergide was kindly supplied by Novartis Pharma (Basel, Switzerland). The other drugs used were 5-HT creatinine sulfate (Merck, Darmstadt, Germany), atropine sulfate (Wako, Osaka, Japan), tetrodotoxin (Sigma, St. Louis, MO, USA), acetylcholine (Daiichi Pharmaceutical, Tokyo, Japan), prostaglandin E_2 (Sigma) and Rhein (Extrasynthese, Genay, France). The composition of the Krebs–bicarbonate solution was (in mM): NaCl 118.2, KCl 4.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 24.8, $MgSO_4$ 1.2 and glucose 10.0. All drugs were dissolved in Krebs solution or binding assay buffer.

2.7. Statistical analysis

Data are expressed as the mean ± S.E.M. EC_{50} values were estimated by linear regression using the least-squares method. In the receptor binding studies, IC_{50} values were computed by logit–log analysis from the following equation:

$$\log\left[(B_o - B_i)/(B_i - B_n)\right] = n\left[\log(\text{antagonist concentration}) - \log(IC_{50})\right]$$

where B_o and B_i are binding in the absence and presence of the antagonists to be tested, respectively; B_n is nonspecific binding and n is the slope factor identical to the Hill coefficient. K_i values were calculated according to the following equation (Cheng and Prusoff, 1973):

$$K_i = IC_{50}/(1 - [L]/K_d)$$

where $[L]$ is the radioligand concentration and K_d is the dissociation constant of the radioligand. pK_i is the negative logarithm to base 10 of K_i.

3. Results

3.1. Binding studies

The affinities of YM-31636, 5-HT, 2-methyl-5-HT and *m*-chlorophenylbiguanide for cloned human 5-HT_{3A} receptors are shown in Table 1. YM-31636 had a pK_i value of 9.67 vs. ramosetron and pK_i values for other 5-HT_3 receptor agonists were less than 7. Additionally, YM-31636 was quite selective, showing very low affinities for 5-HT_1, 5-HT_2, adrenalin α_1, α_2, β, dopamine D_1, D_2, histamine H_1, muscarinic M_2, μ-opioid and benzodiazepine receptors, with pK_i values ranging from 5.89 for α_2-adrenoceptors to less than 5 for the other receptors (Table 2).

Table 1
Affinities of 5-HT_3 receptor agonists for cloned human 5-HT_{3A} receptor

Compounds	pK_i	n_H
YM-31636	9.67 ± 0.05	0.89 ± 0.19
5-HT	6.47 ± 0.17	0.81 ± 0.07
2-Methyl-5-HT	6.18 ± 0.09	1.10 ± 0.13
m-Chlorophenylbiguanide	6.65 ± 0.07	0.91 ± 0.09

Each value represents mean ± S.E.M. ($n = 3$).
Radioligand used is [^{3}H]ramosetron.

3.2. Contraction of guinea pig distal colon

YM-31636, 5-HT, 2-methyl-5-HT, and *m*-chlorophenylbiguanide caused concentration-dependent contractions of guinea pig colon preparations, with EC_{50} values of 0.12 (0.087–0.17), 3.1 (2.6–3.8), 4.8 (4.2–5.7) and 13 (4.5–21) μM, respectively (Fig. 2). The intrinsic activities of these agonists were 0.90, 1.0, 1.0 and 0.76, respectively. The contractions induced by these agonists were antagonized by ramosetron (0.3 μM), a potent and selective 5-HT_3 receptor antagonist (data not shown). On the other hand, the contractile responses to YM-31636, 5-HT and 2-methyl-5-HT were not inhibited by methysergide, which blocks 5-HT_1 and 5-HT_2 receptors (Apperley et al., 1980; Leff and Martin, 1986), and SB204070, a selective 5-HT_4 receptor antagonist (Bingham et al., 1995). In addition, contractile responses to these agonists were inhibited by pretreatment of atropine (1 μM), tetrodotoxin (1 μM), and desensitization of 5-HT_3 receptors by 2-methyl-5-HT (30 μM) (data not shown). YM-31636 showed no antagonistic activity against 5-HT-induced contraction (data not shown).

3.3. I_{sc} in guinea pig distal colon

Secretagogues, including 5-HT (0.1–100 μM), prostaglandin E_2 (0.01–30 μM), acetylcholine (0.1–3000 μM) and rhein (1–300 μM), concentration-dependently increased I_{sc} in guinea pig colonic mucosa (Fig. 3A). The EC_{50} values for 5-HT, prostaglandin E_2, acetylcholine and rhein were 1.7 (0.55–2.8), 1.5 (0.54–2.4), 20 (5.2–30) and 70 (50–80) μM, respectively. The maximal responses to 5-HT, prostaglandin E_2, acetylcholine and rhein were 301.4 ± 47.1, 193.1 ± 15.7, 510.3 ± 53.7 and 438.8 ± 56.6 $\mu A/cm^2$, respectively.

The 5-HT receptor agonists, YM-31636 (0.01–10 μM), 5-HT (0.1–100 μM), 2-methyl-5-HT (0.1–100 μM) and *m*-chlorophenylbiguanide (1–300 μM) concentration-dependently increased I_{sc} in guinea pig colonic mucosa (Fig. 3B). The EC_{50} values for YM-31636, 5-HT, 2-methyl-5-HT

Table 2
Receptor binding profile of YM-31636

Receptor	[^{3}H]Ligand	Tissue	K_i (nM)
5-HT$_3$	GR65630	Rabbit ileum	0.20 ± 0.02
5-HT$_1$	5-HT	Rat brain	> 10,000
5-HT$_2$	Ketanserin	Rat brain	> 10,000
α_1-Adrenoceptor	Prazosin	Rat brain	> 10,000
α_2-Adrenoceptor	Rauwolsine	Rat brain	1300 ± 80
β-Adrenoceptor	Dihydroalprenolol	Rat brain	> 10,000
Dopamine D$_1$	SCH23390	Rat striatum	> 10,000
Dopamine D$_2$	Raclopride	Rat striatum	> 10,000
Histamine H$_1$	Pyrilamine	Guinea-pig brain	> 10,000
Muscarine M$_2$	Quinuclidinyl benzilate	Rat brain	> 10,000
Opioid μ	Naloxone	Rat brain	> 10,000
Benzodiazepine	Flunitrazepam	Rat brain	> 10,000

Each value represents mean ± S.E.M. ($n = 3$).

and *m*-chlorophenylbiguanide were 0.16 (0.058–0.26), 2.1 (0.74–3.5), 10 (5.3–20) and 30 (20–40) μM, respectively. As compared with 5-HT, the intrinsic activities of YM-31636, 2-methyl-5-HT and *m*-chlorophenylbiguanide were 0.19, 0.97 and 0.22, respectively. The I_{sc} response to 2-methyl-5-HT or YM-31636 was completely antagonized by ramosetron (0.3 μM) (data not shown).

3.4. Chronotropic responses in guinea pig right atria

The resting rate of the isolated guinea pig right atria was 150.9 ± 4.9 beats/min ($n = 11$). YM-31636, 5-HT and 2-methyl-5-HT induced a positive chronotropic response in the atria with EC$_{50}$ values of 0.099 (0.054–0.15), 3.9 (2.5–5.4) and 11 (9.2–13) μM, respectively (Fig. 4). The intrinsic activities of these agonists were 0.23, 1.0 and

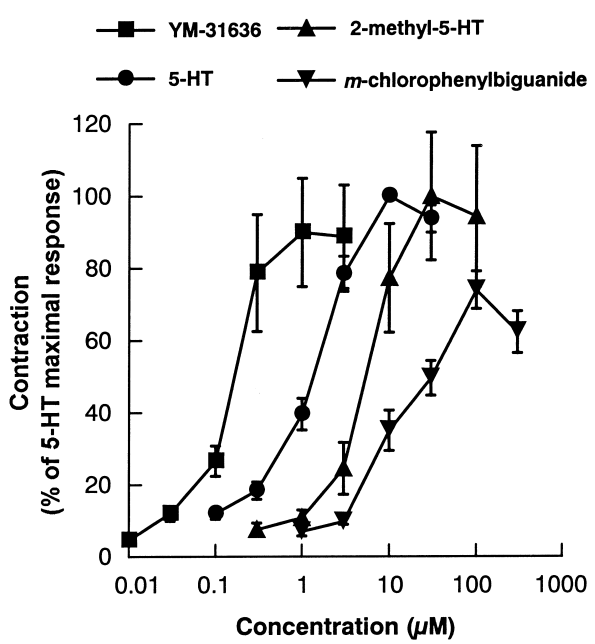

Fig. 2. The effects of YM-31636, 5-HT, 2-methyl-5-HT and *m*-chlorophenylbiguanide ($n = 6$) on contraction of isolated guinea pig distal colon. The segments of the distal colon approximately 20 mm long were suspended longitudinally in organ bath containing Krebs–bicarbonate solution, and isometric contractions were recorded. Concentration–response curves were constructed in a cumulative manner. Each point represents the mean ± S.E.M.

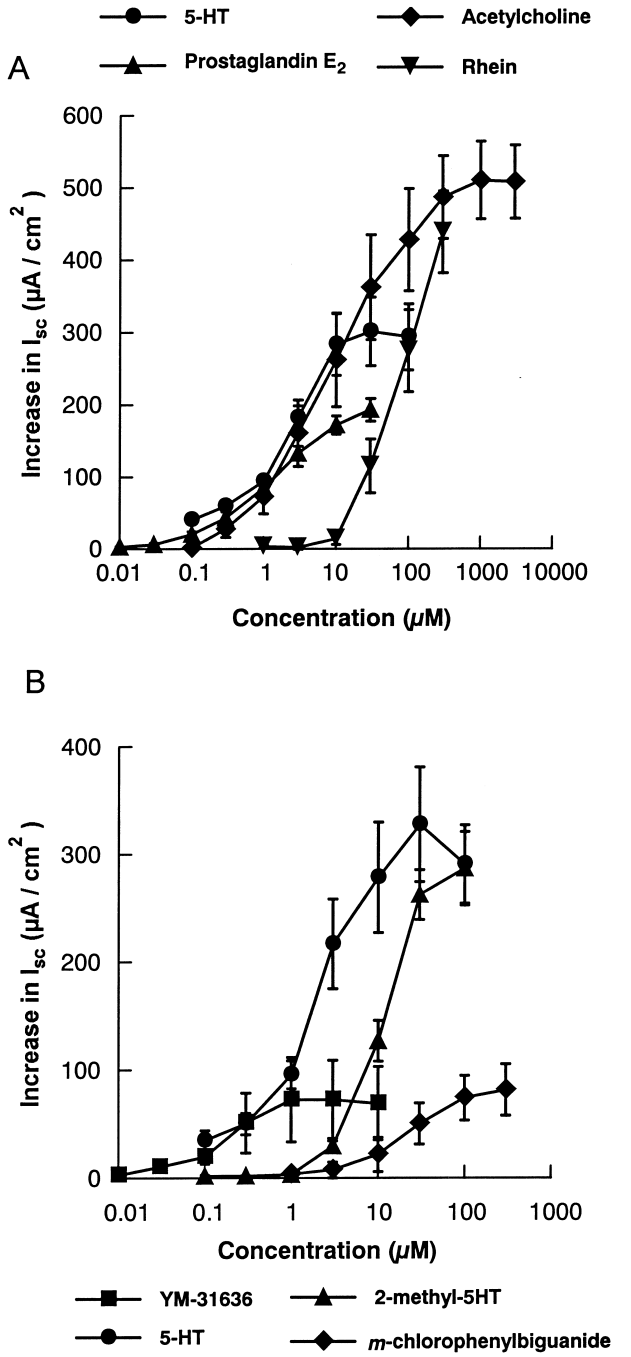

Fig. 3. The short-circuit current (I_{sc}) responses to (A) 5-HT, prostaglandin E$_2$, acetylcholine, rhein ($n = 8$), and (B) YM-31636, 5-HT, 2-methyl-5-HT and *m*-chlorophenylbiguanide ($n = 7$) exhibited by isolated guinea pig colonic mucosa. Preparations of the mucosa from the distal colon were mounted in Ussing chambers (window area 0.8 cm^2), immersed in Krebs–bicarbonate solution. Preparations were short-circuited and the I_{sc} was continuously recorded. Concentration–response curves were constructed in a cumulative manner. Each point represents the mean ± S.E.M.

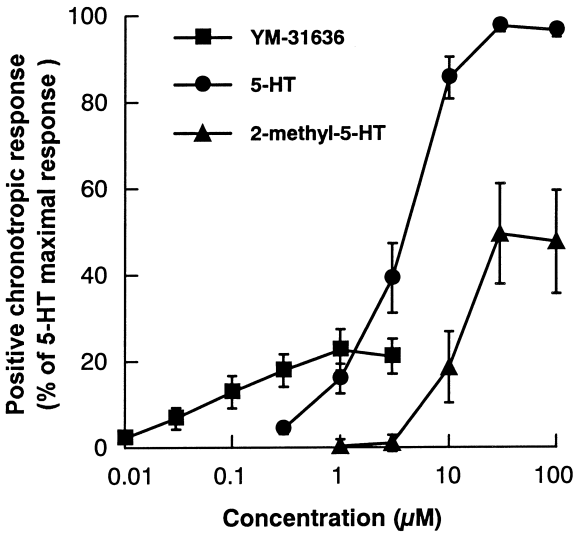

Fig. 4. The positive chronotropic effects of YM-31636 ($n = 7$), 5-HT ($n = 7$) and 2-methyl-5-HT ($n = 4$) on isolated guinea pig right atria. The preparations were suspended in organ baths containing Krebs–bicarbonate solution. The concentration–response curves were constructed in a cumulative manner. Each point represents the mean ± S.E.M.

0.50, respectively. These tachycardiac effects were antagonized by ramosetron (0.3 μM) (data not shown).

4. Discussion

In the present study, YM-31636 showed extremely higher affinity for cloned human 5-HT_3 receptors than other 5-HT_3 receptor agonists, and showed 5-HT_3 receptor agonistic activities with higher potencies than other 5-HT_3 receptor agonists in guinea pig tissue preparations. Additionally, YM-31636 had very low affinities for other receptors. These results indicate that YM-31636 is a potent and selective 5-HT_3 receptor agonist. In the isolated guinea pig distal colon, YM-31636 showed no antagonistic activity against 5-HT-induced contraction. In addition, concentration range and EC_{50} values of YM-31636 in three guinea pig tissue preparations were almost the same. Taken together, we consider that YM-31636 had no antagonistic activity in guinea pigs.

Although YM-31636 showed potent 5-HT_3 receptor agonistic activities in isolated guinea pig distal colon and right atria, the intrinsic activities in each tissue are different. YM-31636 was almost full agonist in inducing contraction of the distal colon. In contrast, it showed partial agonistic activities in increasing I_{sc} of the distal colon, and in positive chronotropic effect in the right atria. Three causes are possibly considered about the differences in intrinsic activities of YM-31636. One is that YM-31636 induced contraction of the distal colon by stimulation of not only 5-HT_3 receptor but also other receptors. It has been shown that 5-HT-induced contraction of the guinea pig distal colon was mediated through 5-HT_3, 5-HT_4 and 5-HT_1-like receptors (Woollard et al., 1994). In the present study, however, the contractile response to YM-31636 were not affected by methysergide, which blocks 5-HT_1 and 5-HT_2 receptors (Apperley et al., 1980; Leff and Martin, 1986), and SB204070, a selective 5-HT_4 receptor antagonist (Bingham et al., 1995). In addition, contractile response to YM-31636 was inhibited by pretreatment of atropine, tetrodotoxin, and desensitization of 5-HT_3 receptors by 2-methyl-5-HT, similarly to the contractile responses to 5-HT and 2-methyl-5-HT. Moreover, YM-31636 showed very low affinities for receptors other than the 5-HT_3 receptor in receptor binding assay. Taken together, it is indicated that YM-31636 selectively stimulated 5-HT_3 receptor and induced contraction of the guinea pig distal colon.

The second possible cause of the differences of intrinsic activities of YM-31636 is that the different types of 5-HT_3 receptor exist in the distal colon and right atria. Several studies demonstrated the difference in intrinsic activities of agonists in some tissues or cells (Mochizuki et al., 2000). Apparent splice variants of the murine 5-HT_3 receptor subunit termed the 5-HT_3 R–A and 5-HT_3 R–As have been reported (Hope et al., 1993; Downie et al., 1994) and 5-HT_3 R–As showed a lower maximum current during activation by 2-methyl-5-HT when expressed in *Xenopus* oocytes. Recently, the existence of a new human 5-HT_3 receptor subunit class, 5-HT_{3B}, was reported, and *m*-chlorophenylbiguanide was more efficacious as an activator of heteromeric assemblies of 5-HT_{3A} and 5-HT_{3B} subunits than homomeric receptors (Davies et al., 1999). In addition, the intrinsic activities of 2-methyl-5-HT and *m*-chlorophenylbiguanide were reported to be different in cloned 5-HT_{3A} receptors of various species (Belelli et al., 1995; Miyake et al., 1995; Mair et al., 1998; Niemeyer and Lummis, 1998; Davies et al., 1999; Mochizuki et al., 2000). These findings suggest the possibility that different types of 5-HT_3 receptor exist in the different tissues.

Thirdly, the difference in intrinsic activities might be due to tissue differences in the number of 5-HT_3 receptors or some other conditions. 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). Further studies are necessary to confirm the cause of the differences in intrinsic activities of YM-31636.

As previously mentioned, YM-31636 is a potent 5-HT_3 receptor agonist acting on neuronal 5-HT_3 receptors and induces contraction of distal colon smooth muscle tissue with high intrinsic activity, while increases I_{sc} in the colonic mucosa with low efficacy. These results indicate that YM-31636 potently stimulates the motility and mildly evokes water secretion in the distal colon. This is a good profile for a drug that treats constipation. In contrast, 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). Although promising, further in vivo studies are necessary to clarify the pharmacological profile of YM-31636 for treating constipation.

In conclusion, YM-31636 is a potent and selective 5-HT_3 receptor agonist, and holds promise as a treatment for constipation.

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