

The Selective 5-Hydroxytryptamine (5-HT)₄-Receptor Agonist RS67506 Enhances Lower Intestinal Propulsion in Mice

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ABSTRACT—Interactions of gastrointestinal prokinetic benzamides with 5-hydroxytryptamine (5-HT)₃ and 5-HT₄ receptors and the relation to their effects on gastrointestinal propulsion were investigated. Renzapride and zacopride potently inhibited 5-HT₃-receptor-mediated contractions in the guinea pig colon, whereas RS67506 (1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-(2-methyl sulphonylamino)ethyl-4-piperidinyl]-1-propanone hydrochloride), a selective 5-HT₄-receptor agonist, showed no inhibition. RS67506, renzapride and zacopride all exerted 5-HT₄ receptor-mediated relaxation in the carbachol-precontracted rat oesophagus. In mice, RS67506 shortened the whole gut transit time, whereas renzapride and zacopride were reported to prolong it. Gastrointestinal prokinetic benzamides, which are selective for 5-HT₄-receptor agonistic over 5-HT₃-receptor antagonistic action, may be useful in treating gastrointestinal disorders associated with impaired lower intestinal propulsion such as constipation.

Keywords: 5-HT₃ receptor, 5-HT₄ receptor, RS67506

Both 5-hydroxytryptamine (5-HT)₃ and 5-HT₄ receptors have been shown to exist in the mammalian gastrointestinal tract where they seem to be involved in the control of gastrointestinal propulsion (1, 2). Although the blockade of 5-HT₃ receptors enhances (3) or fails to affect (4) gastroduodenal propulsion, it inhibits lower intestinal propulsion (2). The activation of 5-HT₄ receptors facilitates acetylcholine release from enteric nerve terminals and leads to the enhancement of gastrointestinal propulsion (1). A series of compounds, which are chemically related to metoclopramide and enhance gastrointestinal propulsion and coordinated motility, have been called gastrointestinal prokinetic benzamides. Most gastrointestinal prokinetic benzamides, including renzapride and zacopride, possess 5-HT₃ receptor antagonistic as well as 5-HT₄-receptor agonistic activity (5). To date, the facilitatory efficacy of the benzamides on gastroduodenal propulsion has been well-established, but that on lower intestinal propulsion is still obscure (5). It is possible that the poorly defined efficacy results from their potent 5-HT₃-receptor antagonistic activity, namely, that their simultaneous blockade of 5-HT₃ receptors offsets their 5-HT₄-receptor-mediated facilitatory effect on lower intestinal propulsion. It is of interest whether a gastrointestinal prokinetic benzamide, which is selective for 5-HT₄-

receptor agonistic over 5-HT₃-receptor antagonistic action, can enhance gastroduodenal as well as lower intestinal propulsion. Recently, Eglen et al. introduced the novel gastrointestinal prokinetic benzamide RS67506 that possesses a potent 5-HT₄-receptor agonistic but no 5-HT₃-receptor antagonistic action (6). In addition, it is also reported that RS67506 shows a high affinity for the 5-HT₄ receptor but has no or minor affinity for other 5-HT and a wide range of non-5-HT receptors (6). We previously showed that renzapride and zacopride prolonged whole gut transit time in mice (7).

In the present study, we assessed the 5-HT₄-receptor agonistic and 5-HT₃-receptor antagonistic effects of RS67506, renzapride and zacopride in the rat esophagus and guinea pig colon, respectively. In addition, the effect of RS67506 on whole gut propulsion was investigated in mice by a simple method in which the time taken for excretion of the head of an orally administered non-absorbable marker (whole gut transit time) was measured.

All experiments were performed according to the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical Co., Ltd.

Male Wistar rats (210–280 g) were used in the esophagus functional study, which was performed according to a previously reported method (8). Briefly, a tunica mus-

cularis mucosa preparation (about 20-mm-long) was prepared and vertically suspended in an organ bath containing Krebs-bicarbonate solution warmed to 37°C and aerated with a mixture of 95% O₂ and 5% CO₂. Tissues were attached to an isometric force-displacement transducer (SB-1T; Nihon Kohden, Tokyo) connected to an ink oscillograph (SS-100F; Sekonic, Tokyo) through a carrier amplifier (AP-621G, Nihon Kohden), placed under a resting tension of 0.5 g. The preparations were induced to contract with 3×10^{-6} M carbachol, and a cumulative concentration-relaxation response curve to test drug was constructed. The tissues were washed with Krebs-bicarbonate solution and exposed to 10^{-5} M 5-methoxytryptamine to selectively desensitize 5-HT₄-receptor-mediated relaxation. Induction of contraction by carbachol and the concentration response curve for test drug were repeated 30 min after the exposure to 5-methoxytryptamine. Responses were measured as a decrease in isometric tension and are expressed as percentage relaxation of the carbachol-induced tone. The difference between responses in the presence and absence of 5-methoxytryptamine in the same preparation was considered as the 5-HT₄-receptor-mediated response.

Male Hartley guinea pigs (600–950 g) were used in the colon functional study, which was performed according to a previously reported method (9). Briefly, a 20-mm segment of descending colon was excised and was vertically suspended in an organ bath containing Krebs-bicarbonate solution warmed to 37°C and aerated with a mixture of 95% O₂ and 5% CO₂. Tissues were attached to an isometric force-displacement transducer (SB-1T) connected to an ink oscillograph (SS-100F) through a carrier amplifier (AP-621G), placed under a resting tension of 1 g. A cumulative concentration-response curve for 5-HT was constructed. The tissues were washed with Krebs-bicarbonate solution and exposed to test drugs. The concentration-response curve for 5-HT was repeated 30 min after the exposure to test drugs.

Male ICR mice (24–29 g) were used in the whole gut transit test as previously described (7). Briefly, each mouse, which was not deprived of food before the experiment, was transferred to an individual cage. Carmine employed as a marker was orally administered to each mouse at 0.3 ml (3 g of carmine in 50 ml of 0.5% methylcellulose). The time taken for excretion of the head of the orally administered marker was measured. The endpoint was taken as the first appearance of one colored (red) pellet, based on visual observation. Each mouse was subcutaneously treated with either vehicle (10 ml/kg, s.c.) or a single dose of test drug just before administration of marker.

Male ICR mice (24–29 g) were used in the upper gastrointestinal transit test. After oral administration of 0.3

ml of marker (as used in the whole gut transit test), the mouse was returned to the individual cage. At 20 min after the administration of marker, they were sacrificed by cervical dislocation, the abdomen was opened and the intestine was removed from the pyloric junction to the caecal end. The distance traveled by the head of the marker and the total length of the intestine were measured. Upper gastrointestinal transit was expressed as the percentage of the distance traveled by the head of the marker relative to the total length of the small intestine. Each mouse was subcutaneously treated with either saline or a single dose of test drug 30 min before the administration of marker.

The potency of agonists in the rat esophagus was expressed as the pEC₅₀ value, and that of antagonists in the guinea pig colon was expressed as the pA₂ value. All experimental data were expressed as the mean ± S.E.M. or mean with 95% confidence limits of the number (n) of observations. Group data of upper gastrointestinal and whole gut transit were compared by analysis of variance followed by Dunnett's multiple range test. Probability values less than 0.05 were considered significant.

RS67506 (1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-(2-methylsulphonylamino)ethyl-4-piperidinyl]-1-propanone hydrochloride), GR 113808 ([1-[2(methylsulphonylamino)ethyl]4-piperidinyl]methyl-1-methyl-indole-3-carboxylate), ramosetron (YM060) hydrochloride, renzapride hydrochloride and *R,S*-zacopride fumarate were all prepared by Yamanouchi Pharmaceutical Co., Ltd. (Tsukuba). Methysergide hydrogen maleate was kindly donated by Sandoz, Ltd. (Basle, Switzerland). Carmine and carbachol (carbamylocholine chloride) were purchased from Wako Pure Chemical Industries (Osaka). 5-HT creatinine sulfate and 5-methoxy-tryptamine hydrochloride were purchased from E. Merck (Darmstadt, Germany) and Fluka AG (Buchs, Switzerland), respectively.

The cumulative administration of 5-HT (3×10^{-9} – 10^{-6} M) caused 5-HT₄ receptor-mediated relaxant responses in a dose-dependent manner in rat isolated esophageal tunica muscularis mucosae (Table 1). RS67506, renzapride and zacopride also exerted 5-HT₄ receptor-mediated relaxant responses, with the following rank order of agonist potency: RS67506 > renzapride > zacopride. All these benzamides acted as partial agonists with respect to 5-HT (Table 1).

5-HT induced concentration-dependent contractions of the isolated guinea pig colon with an pEC₅₀ value of 5.5 (5.3–5.9). The concentration-contraction curve was monophasic. The selective 5-HT₃-receptor antagonist ramosetron caused a parallel, rightward shift of the curve (Table 2). In contrast, methysergide (a 5-HT₁ and 5-HT₂ antagonist) at 10^{-5} M or GR 113808 (a 5-HT₄-receptor antagonist) at 10^{-7} M had no effect on the curve (data not shown). Because RS67506 itself caused contractions at

Table 1. The agonistic potency and intrinsic activity at 5-HT₄ receptors in isolated rat esophageal tunica muscularis mucosae

Compounds	pEC ₅₀	Intrinsic activity (relative to 5-HT)	No. of preparations
5-HT	7.3 (7.1–7.4)	1.0	8
RS67506	7.5 (7.3–7.7)	0.6	5
Renzapride	7.4 (7.3–7.5)	0.9	4
Zacopride	6.5 (6.4–6.7)	0.6	4

Values are the mean with 95% confidence limits of the stated number of observations.

Table 2. 5-HT₃-receptor antagonistic potency of ramosetron, RS67506, renzapride and zacopride in inhibiting contractions produced by 5-HT in isolated guinea pig distal colon

Compounds	pA ₂	Slope ^b	No. of preparations
Ramosetron	8.9 (8.6–9.5)	1.1 (0.9–1.1)	12
RS67506	No inhibition ^a	—	12
Renzapride	6.6 (6.4–6.8)	1.1 (0.8–1.4)	12
Zacopride	7.8 (7.6–8.3)	1.1 (0.7–1.6)	12

Values are the mean with 95% confidence limits of the stated number of observations. ^aRS67506 showed no inhibitory effect at concentrations up to 10⁻⁵ M. ^bSlopes were determined by Schild analysis, and none of them were different from unity.

3×10^{-5} , the highest test concentration was set to 10⁻⁵ M. RS67506, renzapride and zacopride did not have any effect on basal contraction at any tested concentration. RS67506 showed no inhibitory effect on the concentration-response curve to 5-HT at concentrations up to 10⁻⁵ M, whereas renzapride and zacopride caused a parallel rightward shift of the concentration-response curve to 5-HT (Table 2).

RS67506 at 0.3–3 mg/kg, s.c. dose-dependently shortened the whole gut transit time. The effect of RS67506 was significant relative to that of vehicle at doses of 1 mg/kg, s.c. or higher (Fig. 1A). RS67506 had no significant effect on upper gastrointestinal transit at doses up to 3 mg/kg, s.c. (Fig. 1B).

The result of the functional test in the isolated rat esophagus is consistent with that of Eglen et al. (6) who reported that RS67506, renzapride and zacopride produced relaxation of the isolated rat esophagus, a response that was antagonized by GR 113808, and tachycardia in the anesthetized micropig (a well-characterized 5-HT₄-receptor-mediated response) with the following rank order of agonist potency: RS67506 > renzapride > zacopride.

In the guinea pig colon, renzapride and zacopride showed a potent 5-HT₃-receptor antagonistic activity, in accordance with the result of Schiavone et al. (10) who

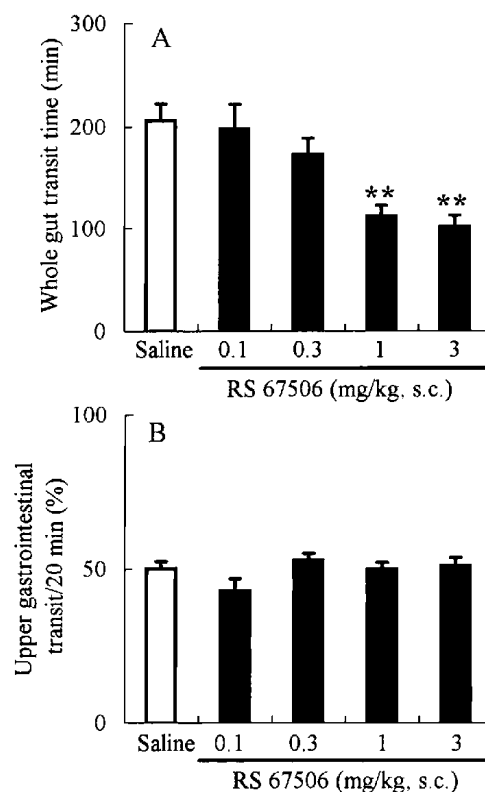


Fig. 1. Effect of RS67506 on gastrointestinal transit in ICR mice. In the whole gut transit test (A), the time taken for excretion of the head of an orally administered marker (whole gut transit time) was measured, and RS67506 or vehicle was subcutaneously administered just before marker administration. In the upper gastrointestinal transit test (B), upper gastrointestinal transit was expressed as a percentage of the distance traveled by an orally administered marker relative to the total length of the small intestine over 20 min after marker administration, and RS67506 or vehicle was subcutaneously administered 30 min before marker administration. Each column and vertical bar represent the mean ± S.E.M. of 8 mice. ***P* < 0.01, significant difference from saline treatment.

reported that renzapride and zacopride inhibited the von Bezold-Jarisch reflex in rats, also a well-characterized 5-HT₃-receptor-mediated bradycardiac response, with ED₅₀ values of 12.4 and 0.4 µg/kg, i.v., respectively. In contrast, RS67506 showed no inhibitory effect in this preparation, indicating that, unlike renzapride and zacopride, RS67506 has no 5-HT₃-receptor antagonistic activity. Based on these results, it is indicated that RS67506 is a gastrointestinal prokinetic benzamide that is selective for 5-HT₄-receptor agonistic over 5-HT₃-receptor antagonistic action, whereas renzapride and zacopride are non-selective. This is also substantiated by the previous results of radioligand receptor binding studies: Affinity of RS67506 for the 5-HT₄ receptor in the guinea pig striatum (pK_i = 8.8) is about 3 log units higher than that for the 5-HT₃ receptor in the NG 108-15 cells (pK_i = 5.6) (6), whereas renzapride and zacopride have higher

affinity for the 5-HT₃ receptor in the NG 108-15 cells (K_i = 2.8 and 0.33 nM, respectively) than the 5-HT₄ receptor in the guinea pig caudate nucleus (K_i = 138 and 444 nM, respectively) (11).

In mice, RS67506 shortened the whole gut transit time. Because RS67506 did not affect upper gastrointestinal transit at doses that significantly shortened whole gut transit time, it is likely that the site at which it enhances propulsive activity is the lower intestine (caecum and colon). Kadowaki et al. reported that the mixed 5-HT₃- and 5-HT₄-receptor antagonist FK1052 but not selective 5-HT₃-receptor antagonists reduced 5-HT-induced acceleration of colonic transit in rats (12). Also, Banner et al. found that a highly selective and in vivo active 5-HT₄-receptor antagonist SB204070 reduced 5-hydroxytryptophan (a precursor of 5-HT)-induced increase of fecal output in mice (13). Furthermore, in the whole gut and upper gastrointestinal transit test in mice, SB204070 prolonged whole gut transit time but did not affect upper gastrointestinal transit (Y. Nagakura et al., unpublished data). These evidence suggest that 5-HT₄ receptors are involved in the control of lower intestinal propulsion and that 5-HT₄-receptor agonists have a potential ability to enhance lower intestinal propulsion. It is possible that RS67506 stimulated lower intestinal propulsion by activating 5-HT₄ receptors in the present study. In contrast to the stimulatory effect of RS67506, we previously reported that renzapride and zacopride prolonged the lower intestinal propulsion in the same mouse model (7). Their inhibitory effect seemed to be mediated through the blockade of 5-HT₃ receptors because their potency in the whole gut transit test correlated with that in the blocking of 5-HT₃ receptors in the Bezold-Jarisch reflex test in rats.

Taken together, the effect of gastrointestinal prokinetic benzamides on lower intestinal propulsion in mice may depend on the selectivity for 5-HT₄-receptor agonistic over 5-HT₃-receptor antagonistic action. It has recently been reported that a 5-HT₄-receptor agonist SDZ HTF 919, which has a slight affinity for the 5-HT₃ receptor, reduces colonic transit time in both the pre-clinical (mice) and clinical (healthy subjects) models (14, 15). Their and our results raise the new possibility that gastrointestinal prokinetic benzamides, which are selective for 5-HT₄-receptor agonistic over 5-HT₃-receptor antagonistic action, may surpass existing gastrointestinal prokinetic benzamides in facilitating lower intestinal propulsion and in treating patients with impaired lower intestinal propulsion such as constipation.

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REFERENCES

- 1 Tonini M, Rizzi CA, Manzo L and Onori L: Novel enteric 5-HT₄ receptors and gastrointestinal prokinetic action. *Pharmacol Res* **24**, 5–14 (1991)
- 2 Talley NJ: Review article: 5-Hydroxytryptamine agonists and antagonists in the modulation of gastrointestinal motility and sensation: clinical implications. *Aliment Pharmacol Ther* **6**, 273–289 (1992)
- 3 Costall B, Gunning SJ, Naylor RJ and Tyers MB: The effects of GR 38032F, novel 5-HT₃ receptor antagonist on gastric emptying in the guinea pig. *Br J Pharmacol* **91**, 263–264 (1987)
- 4 Stacher G, Bergmann H, Schneider C, Steiner-Mittelbach G, Gaupmann G, Steinringer H, Abatzi TA and Stacher-Janotta G: Effects of the 5-HT₃ receptor antagonist ICS 205-930 on fat-delayed gastric emptying and antral motor activity. *Br J Clin Pharmacol* **30**, 41–48 (1990)
- 5 Briejer MR, Akkermans LMA and Schuurkes JAJ: Gastrointestinal prokinetic benzamides: The pharmacology underlying stimulation of motility. *Pharmacol Rev* **47**, 631–651 (1995)
- 6 Eglen RM, Bonhaus DW, Leung JE and Clark RD: Pharmacological characterization of two novel and potent 5-HT₄ receptor agonists, RS 67333 and RS 67506, in vitro and in vivo. *Br J Pharmacol* **115**, 1387–1392 (1995)
- 7 Nagakura Y, Naitoh Y, Kamato T, Yamano M and Miyata K: Compounds possessing 5-HT₃ receptor antagonistic activity inhibit intestinal propulsion in mice. *Eur J Pharmacol* **311**, 67–72 (1996)
- 8 Miyata K, Yamano M, Kamato T and Akuzawa S: Effect of serotonin (5-HT)₃-receptor antagonists YM060, YM114 (KAE-393), ondansetron and granisetron on 5-HT₄ receptors and gastric emptying in rodents. *Jpn J Pharmacol* **69**, 205–214 (1995)
- 9 Miyata K, Kamato T, Nishida A, Ito H, Katsuyama Y, Iwai A, Yuki H, Yamano M, Tsutsumi R, Ohta M, Takeda M and Honda K: Pharmacological profile of (R)-5-[(methyl-3-indolyl)carbonyl]-4,5,6,7-tetrahydro-1H-benzimidazol hydrochloride (YM060), a potent and selective 5-hydroxytryptamine₃ receptor antagonist, and its enantiomer in the isolated tissue. *J Pharmacol Exp Ther* **259**, 15–21 (1991)
- 10 Schiavone A, Volonte M and Micheletti R: The gastrointestinal motor effect of benzamide derivatives is unrelated to 5-HT₃ receptor blockade. *Eur J Pharmacol* **187**, 323–329 (1990)
- 11 Schiavi GB, Brunet S, Rizzi CA and Ladinsky H: Identification of serotonin 5-HT₄ recognition sites in the porcine caudate nucleus by radioligand binding. *Neuropharmacology* **33**, 543–549 (1994)
- 12 Kadowaki M, Nagakura Y, Tomoi M, Mori J and Kohsaka N: Effect of FK1052, a potent 5-hydroxytryptamine₃ and 5-hydroxytryptamine₄ receptor dual antagonist, on colonic function in vivo. *J Pharmacol Exp Ther* **266**, 74–80 (1993)
- 13 Banner SE, Smith MI, Bywater D, Gaster LM and Sanger GJ: Increased defecation caused by 5-HT₄ receptor activation in the mouse. *Eur J Pharmacol* **308**, 181–186 (1996)
- 14 Appel S, Buchheit KH, Kumle A and Thebault JJ: Preclinical and clinical effects of SDZ HTF 919, a new prokinetic drug on colonic transit. *Naunyn Schmiedebergs Arch Pharmacol* **353**, Supp R143 (1996)
- 15 Pfannkuche HJ, Buchheit KH, Buhl T, Gamse R, Hoyer D and Mattes H: Substituted carbazimidamides, a new class of potent and selective 5-HT₄ receptor agonists and antagonists. *Naunyn Schmiedebergs Arch Pharmacol* **353**, Supp R90 (1996)