Comparison of Effect of Mosapride Citrate and Existing 5-HT4 Receptor Agonists on Gastrointestinal Motility In Vivo and In Vitro

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ABSTRACT
Mosapride citrate is a new gastroprokinetic agent that enhances the upper GI motility by stimulating 5-hydroxytryptamine4 (5-HT4) receptors. The purpose of this study was to compare the effects of mosapride and the existing 5-HT4 receptor agonists on GI motility in conscious dogs and on various probe organs. Mosapride is a selective 5-HT4 receptor agonist on GI motility in conscious dogs and on various 5-HT4 receptor-mediated responses in vitro. In conscious dogs with force transducers implanted, mosapride (0.3–3 mg/kg i.v.) stimulated the antral motility without affecting the colonic motility. However, cisapride, zacopride and BIMU 8 (0.1–1 mg/kg i.v.) stimulated both antral and colonic motility. The enhanced GI motility induced by mosapride or cisapride was antagonized by pretreatment with GR113808 (1 mg/kg bolus i.v., thereafter 1 mg/kg/hr infusion), a selective 5-HT4 receptor antagonist. In vitro, mosapride inhibited [3H]-GR113808 binding to 5-HT4 receptor sites of guinea pig striatum with an IC50 value of 113 nM. In addition, mosapride caused relaxation of the carbachol-precontracted rat esophagus, enhanced the electrically evoked contractions of guinea pig ileum and evoked the contractions of guinea pig distal colon with EC50 values of 208, 73, and 3029 nM, respectively; this indicates that mosapride has a low affinity for colon than for the rest of the GI tract. In contrast, cisapride, zacopride or BIMU 8 had similar potencies in all preparations examined. In conclusion, these studies indicate that mosapride selectively stimulates upper GI motility in vivo and in vitro. These results also suggest heterogeneity of 5-HT4 receptors in the GI tract.

The 5-HT4 receptors, first identified in fetal mouse collicular cell cultures and named by Dumuis et al. (1988), are positively coupled to adenylyl cyclase in brain tissue (Bockaert et al., 1990, 1992) and smooth muscle (Ford et al., 1992). 5-HT4 receptor-mediated functional responses have been detected in a variety of tissues, including the guinea pig ileum (Craig and Clarke, 1990) and colon (Elswood et al., 1991; Wardle and Sanger, 1993), rat esophagus (Baxter et al., 1991), sheep pulmonary vein (Cocks and Arnold, 1992) and both pig (Villalon et al., 1990, 1991) and human heart (Kau mann et al., 1990a, b). Agonists of the 5-HT4 receptors are currently known to include three structurally distinct chemical classes: indoles-based molecules, substituted benzamides and benzimidazolones. Among them, substituted benzamides such as metoclopramide, zacopride, cisapride and renzapride and benzimidazolone derivatives such as BIMU 1 and BIMU 8 are notable agonists at 5-HT4 receptors in GI tissues (Turconi et al., 1991; Rizzi et al., 1992; Briejer et al., 1995) and stimulate neurally mediated cholinergic contractions in the GI tract in vivo and in vitro (Ford and Clarke, 1993).

Mosapride citrate, a substituted benzamide, is a novel 5-HT4 receptor agonist. We have reported that mosapride increases the gastric emptying in rats, stimulates the gastric motor activity in conscious dogs and increases the electrically evoked contractions in isolated guinea pig ileum (Yoshida et al., 1989, 1991). In clinical studies, mosapride alleviates such dysfunctions in GI motility as nonulcer dyspepsia, gastroparesis, gastric stasis and gastroesophageal reflux disease (Kanazumi et al., 1991; Yoshida et al., 1993). Its prokinetic action derives from facilitating ACh release from neurons of the myenteric plexus via stimulation of 5-HT4 receptors (Yoshida et al., 1991, 1993). Moreover, receptor ligand binding studies demonstrated that mosapride showed no affinity for dopamine D2, adrenaline a1, adrenaline a2, 5-HT1 and 5-HT3 receptors except a weak affinity for 5-HT3, whereas other benzamides have high affinities for several of the existing 5-HT and other neurotransmitter receptors (Yoshida et al., 1989; Karasawa et al., 1990). For example, zacopride or BIMU 8, although they act as potent 5-HT4 receptor agonists, also possesses high affinity for 5-HT3 receptors (Turconi et al., 1991; Briejer et al., 1995). Cisapride is a nonselective 5-HT4 receptor agonist that possesses affinity for dopamine D2, 5-HT2 alpha-1 adrenoceptor and muscarinic receptors (Karasawa et al., 1990; Briejer et al., 1995). These findings indicate that mosapride would be a more selective 5-HT4 agonist than other benzamides and benzimidazolone. On the

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; GMC, giant migrating contractions; LMMP, longitudinal muscle myenteric plexus.
other hand, the prokinetic effect of mosapride on GI motor activity was somewhat different from that of cisapride. Specifically, mosapride selectively enhanced the motor activity of the upper GI tract, such as of the stomach and duodenum, whereas cisapride stimulated the motor activity in all sites of the GI tract from the stomach to the colon in conscious dogs (Yoshida et al., 1991). At the present time, the mechanisms underlying the different effects of mosapride and cisapride on lower GI motor activity remain obscure. Moreover, there are no reports on whether the existing 5-HT₄ agonists, such as zacopride and BIMU 8, can stimulate upper and/or lower GI motor activity in dogs. Therefore, we felt it necessary to compare more precisely the GI motor activity of mosapride and that of the existing 5-HT₄ agonists cisapride, zacopride and BIMU 8 in the dog. We further determined the relative activities of these agents at 5-HT₄ receptor in GI tract tissues: the rat esophagus, the guinea pig ileum and the guinea pig colon.

**Materials and Methods**

**Animals.** Beagle dogs (Nihon Nohsan Kohgyo Inc., Yokohama, Japan) of both sexes weighing 9 to 13 kg, male rats of Jcl SD strain (Nihon Clea Inc., Osaka, Japan) weighing 180 to 300 g and guinea pigs of the Hartley strain (Nihon SLC Inc., Shizuka, Japan) weighing 200 to 400 g were used.

The dogs were individually housed in experimental cages and given dog food (20 g of dry weight per kilogram of body weight, Oriental Yeast, Tokyo, Japan) at 10:00 A.M. daily. The composition of dog food was protein 25.7%, fat 8.6%, carbohydrates 47.3% and minerals 4.3%. Water was available ad libitum. The rats and guinea pigs were housed, with free access to food and water, in a room kept at 22–24°C under a 12-hr light-dark cycle. The experiments were carried out at a room temperature of 22–24°C.

**GI motor activity in conscious dogs.** Five healthy beagle dogs of both sexes were anesthetized with pentobarbital sodium (30 mg/kg i.v.), and the abdominal cavity was opened under sterile conditions. Extraluminal force transducers (F-121S, Star Medical, Tokyo, Japan) were sutured onto the seromuscular layer of the gastric antrum, 3 cm proximal to the pyloric ring, and in the ascending colon, 10 cm and 20 cm distal to the ileo-colonic junction, respectively. The lead wires of the transducers from the abdominal cavity were brought out through a skin incision made between the scapulae. The outer ends of the lead wires were sutured onto the skin adjacent to the skin incision. A Silastic tube (Fr. size 6.5, Dow Corning, Midland, MI) was placed into the superior vena cava through the vein as a route for the i.v. injection of test drugs, and the tube was sutured onto the adjacent skin. After the operation, a jacket protector was placed on the dog to protect the lead wires and the Silastic tube.

GI motor activity was recorded on a polygraph system (RTA-1200M, Nihon Kohden Kohgyo, Tokyo, Japan) by connecting the lead wires of the transducers with cables from the amplifiers (AP-100P, Nihon Kohden Kohgyo) under the protective jacket as reported previously (Itoh et al., 1977). The transducers were implanted in the gastric antrum, 3 cm proximal to the pyloric ring, and in the ascending colon, 10 cm and 20 cm distal to the ileo-colonic junction, respectively. The lead wires of the transducers from the abdominal cavity were brought out through a skin incision made between the scapulae. The outer ends of the lead wires were sutured onto the skin adjacent to the skin incision. A Silastic tube (Fr. size 6.5, Dow Corning, Midland, MI) was placed into the superior vena cava through the vein as a route for the i.v. injection of test drugs, and the tube was sutured onto the adjacent skin. After the operation, a jacket protector was placed on the dog to protect the lead wires and the Silastic tube.

GR113808, a 5-HT₄ receptor antagonist (1 mg/kg bolus i.v., thereafter 1 mg/kg/hr infusion), was administered, and then mosapride or cisapride was i.v. administered 10 min after the start of the infusion of GR113808.

To measure motor activity quantitatively, the motor index was determined. The signals from the force transducers implanted in the gastric antrum and ascending colon were analyzed by our own system controlled by a computer (PC-9801RX, NEC, Inc., Tokyo, Japan). The motor index given by the processing system corresponded to the measurements of the area surrounded by the contraction wave and base line, i.e., the product of the amplitude (voltage) and the time in minutes over a fixed period. The motor index for the 30-min period after the administration of test drugs is expressed as a percentage of that for the 30-min period before administration.

**Isolated rat thoracic esophageal muscularis mucosae.** Rats were killed by a blow on the head, and the most distal 2 cm of the esophagus was removed. The esophageal segments were prepared as described by Baxter et al. (1991). Briefly, the external muscularis propria, containing the outer longitudinal and circular muscle layers of the esophagus, was carefully removed in order to isolate the smooth muscle of the tunica muscularis mucosae. The preparations were suspended longitudinally under an initial tension of approximately 1 g in modified Krebs-Henseleit solution at 37°C and saturated with 95% O₂ and 5% CO₂. The ionic composition of the Krebs-Henseleit solution (mM) was NaCl 118, KCl 4.75, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25 and glucose 10. This solution routinely contained alapraproclate hydrochloride (1 μM) and corticosterone (30 μM) to prevent tissue uptake of 5-HT, methysergide (1 μM) to block 5-HT₁ receptors and pargyline (100 μM) to prevent oxidation of 5-HT by monoamine oxidase. Tissues were left to equilibrate with Krebs-Henseleit solution for 60 min (with washing every 15 min) before starting the experiment. Responses were recorded isometrically through a force displacement transducer (SB-IT, Nihon Kohden Kohgyo) coupled to a chart recorder (Servocoder SR 6221, GRAPHTEC, Tokyo, Japan).

The preparations were contracted by addition of a submaximal concentration of carbachol (3 μM) to the bathing solution. Upon establishment of a stable contraction, a cumulative concentration-effect curve for relaxation to 5-HT was constructed. After the construction of the control curve, the tissues were washed with fresh modified Krebs-Henseleit solution and allowed to recover for 60 min before recontracting with carbachol. In agonist studies, potency relative to 5-HT was calculated from experiments in which two concentration-effect curves were constructed in the same preparation: the first to 5-HT itself and the second to a test agonist. In antagonist studies, a control concentration-effect curve to an agonist was constructed. The antagonist was incubated with the tissue for 45 min, and the second concentration-effect curve was constructed in the presence of the antagonist.

**Isolated guinea pig distal colon.** Guinea pigs were killed by a blow on the head, and distal colon (approximately 7–8 cm from the anus) was removed and placed in De Jalon’s solution of the following composition (mM): KCl 5.6, CaCl₂ 0.5, NaHCO₃ 6.0, NaCl 155, glucose 2.8. Sections of longitudinal muscle with myenteric plexus (LMMP; 2–3 cm in length) were dissected as previously described (Wardle and Sanger, 1993). The preparations were suspended longitudinally under an initial tension of approximately 1 g in modified De Jalon’s solution at 37°C saturated with 95% O₂ and 5% CO₂ and containing granisetron (1 μM) and methiothepin (100 nM) to inhibit responses mediated by 5-HT₂ receptors and by 5-HT₁-like and 5-HT₂ receptors respectively. Responses were recorded through an isotonic transducer (TD-112S, Nihon Kohden Kohgyo) and displayed on a chart recorder (Servocoder SR 6221, GRAPHTEC). The preparations were allowed to stabilize for 45 min (washing every 15 min) before the experiment was started.

The preparations were first exposed to a supramaximal concentration of carbachol hydrochloride (1 μM) to establish the maximal responses evoked by each tissue. Then tissues were exposed to 5-HT (1 μM) four or five times until consistent responses were obtained. Agonist concentration-response curves were constructed noncumulatively by adding increasing concentrations of agonist at 15-min intervals. The agonist was left in contact with the tissue until a maximal response was obtained (usually 30 sec). In each tissue, two or three agonist concentration-response curves were constructed. In all experiments, the first curve was constructed to 5-HT itself, and
the second curve was constructed to a test agonist. For antagonist experiments, the third curve was constructed to a test agonist in the presence of the appropriate concentration of antagonist added immediately after completion of the second curve. In all experiments, a minimum of 45 min was left between successive curves. Responses were expressed as a percentage of the maximal 5-HT-evoked contraction in the first concentration-response curve in each tissue.

**Electrically evoked contractions of the guinea pig ileum.** Guinea pigs were killed by a blow on the head. The distal ileum was removed at least 10 cm proximal to the cecum, and the longitudinal muscles with the myenteric plexus (2–3 cm in length) were prepared. The preparations were set up in a 10-ml organ bath containing Krebs-Henseleit solution. The solution was maintained at 37°C and saturated with 95% O2 and 5% CO2. A tension of 1 g was applied, and the response was recorded isometrically through a force displacement transducer. The preparations were suspended between two parallel platinum wire electrodes and stimulated at supramaximal voltage with 0.2-Hz square-wave pulses (1 msec in duration) from an electrical stimulator (SEN-1101, Nihon Kohden Kohgyo). According to the method of Craig and Clarke (1990), the preparation was incubated with phenoxbenzamine (3 × 10−7 M) for 30 min and washed out several times. Thereafter, the stimulus voltage was adjusted to the submaximally effective voltage that induced approximately 50% of the maximal twitch response (approximately 20–35 V). After obtaining stable responses, we applied 5-HT or agonists cumulatively by increasing the concentration of the drugs, without washing between concentrations. For cumulative dosing, a new concentration was applied at the moment of maximal effect of the previous concentration. Only one concentration-response curve was constructed for each preparation. The effect of the test drug was expressed as percentage recovery of the twitch response.

**[3H]-GR113808 binding in guinea pig brain.** Guinea pigs were decapitated and the brain removed and dissected. Tissue was used immediately.

For the binding assay, as reported by Grossman et al. (1993), striatal brain tissue was placed in 15 volumes of HEPES buffer (50 mM, pH 7.4, 4°C) and was homogenized in a Teflon homogenizer and subsequently centrifuged at 48,000 g and 4°C for 10 min. The resulting pellet was resuspended in HEPES buffer to make a homogenate at 30 mg/ml. All determinations were performed in duplicate. Assay tubes contained 400 μl HEPES buffer (65 mM, pH 7.4, 4°C); 100 μl of a solution of either a competing agent (for drug competition studies), 5-HT to give a final concentration of 30 μM (to determine nonspecific binding) or deionized water (for determination of total binding); 400 μl of [3H]-GR113808 in HEPES buffer (50 mM pH 7.4, 4°C) and 100 μl of tissue preparation. [3H]-GR113808 in HEPES buffer was used to give final concentrations of 0.004 to 1.5 nM for saturation analysis and 0.1 nM for drug competition studies.

For both saturation analysis and drug competition studies, assay tubes were incubated at 37°C for 30 min, and the reaction was terminated by rapid vacuum filtration and washout (3 × 4 ml) with ice-cold Tris-HCl buffer (pH 7.7) through Whatman GF/B filter paper using a Brandel Cell Harvester (Brandel Inc. MB-48, Gaithersburg, MD). Filters were presoaked in a solution of polyethyleneimine (0.1%) to reduce filter binding. Filters were shaken vigorously with 10 ml of ACS-II scintillation cocktail, and the radioactivity in the filters were counted using a scintillation counter (ACS-II, Amersham-Japan Int'l PLC, Tokyo, Japan).

**Statistics.** Differences from the control group that were statistically significant were identified by means of the YUKMS statistical analysis program (Yukms Co., Tokyo, Japan) using Student’s t test for paired data (motor index). The EC50 (relaxation in esophagus, contraction in distal colon and electrically evoked contraction in ileum) and IC50 (binding in brain) values, the concentrations causing 50% of the maximum observed for each response, were determined by linear regression analysis. The pA2 value and slope were calculated by the method of Arunlakshana and Schild (1959).

**Drugs.** The drugs used in the experiments were mosapride ([±]-4-amino-5-chloro-2-ethoxy-N-(4-(fluorobenzyl)-2-morpholino)imethyl)benzamidinate citrate), GR113808, (±)zacopride hydrochloride, BIMU 8 hydrochloride, granisetron hydrochloride and alaprocate hydrochloride, which were synthesized at our laboratories; cisapride (extracted from Acatalin, Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan), 5-HT creatine sulfate (E. Merck Darmstadt, Germany); methysergide maleate (Research Biochemical Inc., Nattrix, MA); carbachol hydrochloride and pargyline hydrochloride (Sigma Chemical Co., St. Louis, MO) and methysergide maleate (Sandoz Ltd., Basel, Switzerland).

Mospadride and cisapride were dissolved in a solution containing 1% lactic acid or 0.1% dimethyl sulfoxide (DMSO). The other drugs were dissolved in saline or in deionized water.

**Results**

**In Vivo Studies.**

Effects of 5-HT4 receptor agonists on antral and colonic motor activity in conscious dogs. In the postprandial state, the antral motor activity was characterized by regular contractile activity and remained constant for at least a few hours after feeding without interdigestive migrating contractures occurring. On the other hand, the colonic motor activity was characterized by colonic migrating or nonmigrating motor complexes and GMCs (Sarna et al., 1984; Karaus and Sarna, 1987).

As shown in figure 1, mosapride (0.3–3 mg/kg i.v.) dose-dependently increased the antral motor activity in conscious dogs, and the maximal effect of mosapride (3 mg/kg) on antral motor activity was equal to that of cisapride (1 mg/kg). The antral motor activity-stimulating effect of cisapride (1

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**Fig. 1.** Effect of i.v. administered mosapride, cisapride, zacopride and BIMU 8 on antral motor activity in the postprandial state in conscious dogs. The motor index for the 30-min period after i.v. administration of test drugs is expressed as a percentage of that for the 30-min period before administration. Each point represents the mean with the S.E.M. in 4 to 5 dogs. *P < .05; **P < .01, significant differences from the solvent treatment. (Student’s t test).
mg/kg) lasted longer than that of mosapride (3 mg/kg). Even at high dose (3 mg/kg), mosapride did not affect the colonic motor activity during the measurement of GI motor activity. However, cisapride (0.1–1 mg/kg), zacopride (0.1–1 mg/kg) and BIMU 8 (0.1–1 mg/kg) stimulated both antral and colonic motor activity (figs. 1 and 2). Figures 3 and 4 show the typical tracings of the effects of mosapride, cisapride, zacopride and BIMU 8. The stimulating effects of these drugs on colonic motor activity were characterized by GMC-like patterns that have large-amplitude contractions with rapid propagating velocity. The effects of cisapride (1 mg/kg) and zacopride (1 mg/kg) were maintained for 60 min, whereas the effect of BIMU 8 (1 mg/kg) disappeared immediately (figs. 3 and 4). In addition, some dogs examined defecated after the i.v. administration of cisapride (1 mg/kg) and zacopride (1 mg/kg).

**Effects of mosapride and cisapride on antral and colonic motor activity under treatment with GR113808 in conscious dogs.** As shown in figures 5 and 6, GR113808 alone (1 mg/kg i.v., thereafter 1 mg/kg/hr i.v. infusion for 10 min), a selective 5-HT4 receptor antagonist, did not affect either basal antral or colonic motor activity. Mosapride (3 mg/kg i.v.) and cisapride (1 mg/kg i.v.), when administered 10 min after the start of GR113808 infusion, did not stimulate either antral or colonic motor activity under treatment with GR113808 (figs. 5 and 6). The enhanced antral or colonic motor activity induced by these drugs was antagonized by treatment with GR113808 in dogs.

**In Vitro Studies**

**Effects of 5-HT4 receptor agonists on carbachol-induced contractions of rat thoracic esophageal muscularis mucosae.** A submaximal concentration of 3 μM carbachol produced a well-maintained contraction for at least 60 min. The cumulative addition of 5-HT to the carbachol-contracted rat esophagus caused a concentration-dependent relaxation with an EC50 value of 5.21 ± 0.52 nM (n = 24). The maximal relaxation with 5-HT occurred at 1 μM. Mosapride, cisapride, zacopride and BIMU 8 also caused a concentration-dependent relaxation with EC50 values of 208.4 ± 33.8 (n = 6), 39.1 ± 3.4 (n = 6), 317.2 ± 106.9 (n = 8) and 31.5 ± 3.8 (n = 4) nM, respectively, although they were less potent agonists than 5-HT and were markedly slower to obtain steady state than 5-HT (fig. 7; table 1). Compared with 5-HT, these agents behaved as full agonists. Addition of GR113808 (10−3 to 10−7 M) to the bathing medium did not cause relaxation of the esophagus preparation. GR113808 (10−9 to 10−7 M) caused a parallel rightward displacement of the concentration-response curve to 5-HT and mosapride (data not shown). Estimates of the pA2 values for GR113808 against 5-HT and mosapride are 8.85 ± 0.06 (Schild plots slope, 1.28 ± 0.05) and 8.72 ± 0.28 (Schild plots slope, 0.83 ± 0.09), respectively.

**Effects of 5-HT4 receptor agonists on the contractions of isolated guinea pig distal colon.** In the guinea pig distal colon, 5-HT evoked monophasic concentration-dependent contractions with an EC50 value of 13.8 ± 2.1 nM (n = 10). Cisapride and zacopride were full agonists with EC50 values of 31.5 ± 2.9 (n = 5) and 265 ± 39.0 (n = 6) nM, respectively. Mosapride and BIMU 8 were partial agonists relative to 5-HT with intrinsic activities of 0.82 and 0.69, respectively. The EC50 values of mosapride and BIMU 8 were estimated to be 3029 ± 591 (n = 13) and 175 ± 37.9 (n = 11) nM, respectively (fig. 8; table 1).

In the presence of increasing concentrations of GR113808, the concentration-response curves to 5-HT and mosapride were displaced to the right in a concentration-dependent manner (data not shown). The estimate of the pA2 value for GR113808 against 5-HT was 10.17 ± 0.17 (Schild plots slope, 1.15 ± 0.16). However, rightward displacements of the concentration-response curve to mosapride were associated with a reduction in the maximal response with a concentration ratio of 3.16 yielding estimated pKp values of 9.20 ± 0.19.

**Effects of 5-HT4 receptor agonists on electrically evoked contractions of isolated guinea pig ileum.** When cumulatively applied, 5-HT concentration-relatedly enhanced the twitch response to electrical field stimulation in guinea pig ileum with an EC50 value of 8.6 ± 3.7 (n = 5) nM. Other 5-HT4 receptor agonists also enhanced the twitch response. The EC50 values of mosapride, cisapride, zacopride and BIMU 8 were 73.2 ± 28.3 (n = 5), 47.6 ± 15.6 (n = 6), 69.0 ± 21.4 (n = 8) and 66.3 ± 10.2 (n = 4) nM, respectively (table 1). Zacopride was a full agonist, whereas mosapride, cisapride and BIMU 8 were partial agonists related to 5-HT with intrinsic activities of 0.58, 0.78 and 0.49, respectively.

**Effects of 5-HT4 receptor agonists on [3H]-GR113808 binding in guinea pig brain homogenate.** The equilibrium saturation studies on the specific binding of [3H]-GR113808 revealed a single saturable site of high affinity in homogenates of guinea pig striatum (KD = 0.162 ± 0.005 nM, Bmax = 62.5 ± 1.9 fmol/mg protein). Non-specific binding increased linearly with increasing ligand concentration. 5-HT displaced [3H]-GR113808 binding to the 5-HT4 receptors in a concentration-dependent manner with an IC50 value of 35.8 ± 3.3 nM. Mosapride, cisapride, zacopride and BIMU

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**Fig. 2.** Effect of i.v. administered mosapride, cisapride, zacopride and BIMU 8 on colonic motor activity in the postprandial state in conscious dogs. The motor index for the 30-min period after i.v. administration of test drugs is expressed as a percentage of that for the 30-min period before administration. Each point represents the mean with the S.E.M. in 4 to 5 dogs. *P < .05; **P < .01, significant differences from the solvent treatment. (Student’s t test).
8 also inhibited the specific binding of [3H]-GR113808 with IC_{50} values of 113 ± 14 (n = 3), 23.0 ± 3.1 (n = 3), 197 ± 12 (n = 3) and 12.6 ± 0.9 (n = 3), respectively (table 1).

Discussion
The present study demonstrates that mosapride selectively enhanced the upper GI motility in conscious dogs through the stimulation of 5-HT_{4} receptors. To clarify this selectivity further, we compared the effects of mosapride with those of other existing 5-HT_{4} receptor agonists—cisapride, zacopride and BIMU 8—on antral and colonic motor activity in conscious dogs.

Colonic motor activity in dogs consists of rhythmically occurring contractile states that have been called migrating and nonmigrating motor complexes. The main propulsive force in the canine colon appears to be a function of large-amplitude GMCs. It is well known that GMCs occur during defecation and are associated with mass movement (Karaus and Sarna, 1987; Shibata et al., 1993). In the present study, cisapride, zacopride and BIMU 8 administered by the i.v.
route caused the GMC-like patterns, which have large-amplitude contractions with rapid propagating velocity. In addition, these drugs caused the defecation associated with the occurrence of GMC-like patterns in some dogs. These observations with cisapride were consistent with those obtained by Lee et al. (1984) and by Sarna and Otterson (1992). However, the colonic motility-stimulating effects of cisapride, zacopride and BIMU 8 had a short duration of action. In contrast, these drugs at doses 5 to 10 times lower than those that stimulated colonic motility produced strong and long-lasting contractions in gastric antrum, which indicates a greater sensitivity in antrum than in colon. On the other hand, mosapride even at doses 10 times higher than those that enhanced antral motility failed to produce colonic contractions in any animals. Accordingly, these comparative data in dogs with four 5-HT4 receptor agonists indicate that mosapride selectively enhances the antral motility and that the other 5-HT4 receptor agonists enhance both antral and colonic motility. Our findings of different effects on colonic motility among 5-HT4 receptor agonists suggest that the 5-HT4 receptors involved in colonic motility may be different from those involved in antral motility.

We have previously reported that the antral motor activity-stimulating effect of mosapride in dogs is decreased after desensitization of 5-HT receptors, which suggests that 5-HT

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**Fig. 6.** Effect of mosapride and cisapride on antral and colonic motor activity under treatment with GR113808 in the postprandial state in conscious dogs. A) Effect of mosapride on antral motor activity. B) Effect of cisapride on antral and colonic motor activity. Mosapride (3 mg/kg i.v.) or cisapride (1 mg/kg i.v.) was given 10 min after GR113808 administration (1 mg/kg i.v., thereafter 1 mg/kg/hr infusion for 20 min). The control motor index during a 10-min period was expressed as 100%. The second (GR113808 alone) and third (mosapride alone and its combination with GR113808) indices were expressed as a percentage of the control motor index. Each point represents the mean with the S.E.M. in 3 to 5 dogs. *P < .05; **P < .01, significant differences from the solvent treatment. (Student’s t test).
receptors are involved in the stimulating effect of mosapride (Yoshida et al., 1991). In addition, the high-dose infusion of tropisetron, a nonselective 5-HT4 receptor antagonist, was found to antagonize the stimulating effect of mosapride on antral motor activity (Yoshida and Itoh, 1994). However, in in vitro studies, tropisetron has been reported to possess high 5-HT4 receptor antagonistic activity and direct ion channel and muscarinic cholinergic blocking activity in addition to 5-HT3 receptor antagonistic activity (Scholtysik et al., 1988; Bockaert et al., 1992). We therefore felt it necessary to examine the interaction between mosapride and more selective 5-HT4 receptor antagonists.

Recently, potent and selective 5-HT4 receptor antagonists have been introduced, such as SDZ 205-557 (Buchheit et al., 1992; Schiavone et al., 1994) and GR113808 (Eglen et al., 1993; Gale et al., 1992; Schiavone et al., 1995). Therefore, we decided to investigate whether a highly selective and potent 5-HT4 receptor antagonist, GR113808, can antagonize the gastroprokinetic action of mosapride. In the present experiments, GR113808 alone, when infused, did not affect the antral motor activity in dogs. Under these conditions, the infusion of GR113808 was found to antagonize the stimulating effects of mosapride on antral motor activity in dogs. Similarly, Rizzi et al. (1994) reported that cisapride and BIMU 1, but not ondansetron, accelerated gastricemptying of a liquid meal in conscious dogs and that these effects could be blocked by DAU 6285, another 5-HT4 receptor antagonist. Furthermore, Fankhauser et al. (1994) demonstrated that SDZ 205-557 inhibited the stimulatory effect of zacopride on antral, duodenal and jejunal myoelectrical activity in anesthetized dogs. These reports and our data indicate that the mechanism underlying the stimulation of gastric and small intestinal motility in dogs by prokinetic agents is 5-HT4 receptor activation that involves cholinergic nerves. Previously, there was little evidence that the colonic motility-stimulating effects of prokinetic agents could be mediated by activation of 5-HT4 receptors. In the present study, GR113808 antagonized the stimulating effect of cisapride on colonic as well as antral motility in dogs. These findings strongly suggested that the stimulation of GI motility by prokinetic 5-HT4 receptor agonist is derived from the 5-HT4 receptor activation, confirming the existence of 5-HT4 receptors in the canine GI tract. However, the mechanism underlying the different profile of the effect of mosapride and other 5-HT4 receptor agonists on colonic motility remains unclear at present.

To confirm the lack of effect of mosapride on canine colonic motility further, we compared the in vitro effects of mosapride using guinea pig colon and ileum and rat esophagus with those of cisapride, zacopride and BIMU 8. In addition, we investigated the affinity of these drugs for 5-HT4 receptors using [3H]GR113808 ligand. In the present experiments, 5-HT and 5-HT4 receptor agonists caused relaxation of the carbachol-precontracted rat esophagus, stimulated electrically evoked contractions of guinea pig isolated ileum, induced contractions of guinea pig isolated distal colon and displaced [3H]GR113808 at the binding sites in guinea pig striatum homogenates. The responses of four different preparations to these drugs are well documented, and it is widely accepted that these responses are mediated largely via 5-HT4 receptors (Craig and Clarke, 1990; Baxter et al., 1991; Grossman et al., 1993; Wardle and Sanger, 1993).

As summarized in table 1, the estimated EC50 values of 5-HT, cisapride, zacopride and BIMU 8 correlated well with functional data on three GI preparations and binding affinities. The rank order of these reference drugs in each model was also consistent with previous reports (Craig and Clarke, 1990; Baxter et al., 1991; Turconi et al., 1991; Wardle and Sanger, 1993). Interestingly, this study revealed unexpected GI tissue selectivity to mosapride. Specifically, mosapride had a low potency in guinea pig colon compared with the other GI tissue and binding affinities. The potency of mosapride.

| TABLE 1 Summary of comparative agonist potency in the rat esophagus, guinea pig ileum, guinea pig distal colon and guinea pig striatum |
|---------------------|---------------------|---------------------|---------------------|
|                      | [3H]GR113808 Binding of Guinea Pig Brain | Relaxation of Rat Esophageal Muscularis Mucosa | Electrically Evoked Contraction of Guinea Pig Ileum | Contraction of Guinea Pig Colon |
|                      | IC50 (nM) | n | EC50 (nM) | n | EC50 (nM) | n | EC50 (nM) | n |
| Mosapride            | 112.9 ± 13.8 | 3 | 208.4 ± 33.8 | 6 | 73.2 ± 28.3 | 5 | 3028.7 ± 591.0 | 13 |
| Cisapride            | 23.0 ± 3.1  | 3 | 39.1 ± 3.4  | 6 | 47.6 ± 15.6 | 6 | 31.5 ± 2.9  | 5  |
| Zacopride            | 196.7 ± 11.9 | 3 | 317.2 ± 106.9 | 8 | 69.0 ± 21.4 | 8 | 265.0 ± 39.0 | 6  |
| BIMU 8               | 12.6 ± 0.9  | 3 | 31.5 ± 3.8  | 4 | 66.3 ± 12.0 | 4 | 174.7 ± 37.9 | 11 |
| 5-HT                 | 35.8 ± 3.3  | 3 | 5.2 ± 0.5   | 24 | 8.6 ± 3.7  | 5 | 13.8 ± 2.1  | 10 |

Values are expressed as mean ± S.E.M.

Fig. 8. Concentration-effect curves to 5-HT, mosapride, cisapride, zacopride and BIMU 8 in guinea pig distal colon LMMP. Experiments were carried out in the presence of methiothepin (100 nM) and granisetron (1 μM). Each point is the mean of 5 to 13 observations calculated as a percentage of the control 5-HT maximum.
Mosaipride Stimulates Upper GI Motility

In conclusion, the results of this study show that mosapride selectively stimulated upper GI motility in vivo and in vitro compared with other 5-HT4 receptor agonists. They also indicate that these different effects of 5-HT4 receptor agonists on GI motility may be based on the existence of heterogeneity of 5-HT4 receptors in the GI tract.

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References


KANAZUMI, T., NARANO, H., MATSUBI, Y., ISHIKAWA, H., SHIMIZU, R., PARK, S. AND

pride in guinea pig isolated colon was 15 to 40-fold lower than that in other GI tissue. The results were consistent with those for the experiments in which canine colonic motility was studied in vivo. In addition, the intrinsic activity of mosapride was less than that observed for 5-HT or cisapride, which indicates that it may act as a partial agonist. Furthermore, in antagonist experiments, the contractile response to mosapride in isolated guinea pig colon was found to be unsurmountably antagonized by GR113808. Specifically, under treatment with 3 × 10−6 M GR113808, the concentration-effect curve of mosapride was displaced to the right with a decrease in the maximal response. The phenomenon was different from that of 5-HT, which showed surmountable antagonism, yielding a slope of 1.15, which was not significantly different from unity. On the other hand, in rat esophagus, GR113808 was a surmountable antagonist of 5-HT or mosapride with the same pA2 value as has been reported previously (Gale et al., 1994). Similarly, the displacement isotherms observed in radioligand binding studies using 3H[GR113808 exhibited a Hill coefficient (0.98) not significantly different from unity, which suggests an interaction at a single site. These observations imply that both 5-HT and mosapride act at the same 5-HT4 receptor site in rat esophagus and guinea pig striatum, but not in guinea pig colon. Kaumann et al. (1991) demonstrated that order of agonists potency for tachycardia, which mediated putative cardiac 5-HT4 receptors, was different from that in CNS such as mouse colliculi neurons. Furthermore, Leung et al. (1996) suggested that 5-HT4 receptors in the guinea pig colon and rat esophagus can be operationally distinguished. Recently, molecular biological studies reported that 5-HT4 receptors consist of two splice variants, 5-HT4L and 5-HT4S, which differ in the length and sequence of their carboxy termini (Gerald et al., 1995). Therefore, heterogeneity may exist among 5-HT4 receptors, and mosapride may act on different 5-HT4 receptor subtypes. However, McLean and Coupar (1995) reported that a similar receptor mediates the 5-HT4 receptor-induced effects in human colon, guinea pig ileum and rat esophagus. Thus, further studies are needed to elucidate the 5-HT4 receptor coupling mechanisms between the tissues or the differences in molecular structure among the 5-HT4 receptors.

Recently, many investigators suggested that prokinetic benzamides could interfere with the release of noncholinergic substances through 5-HT4 receptor-independent mechanisms (Briejer et al., 1995). Schuurkes (1992) demonstrated that cisapride enhances the field stimulation-induced relaxation under nonadrenergic, noncholinergic conditions, which suggests the enhancing release of an inhibitory neurotransmitter. Similarly, in human colon, 5-HT and existing 5-HT4 agonists caused inhibition of spontaneous contractions through an ACh-independent mechanism (McLean and Coupar, 1996). Furthermore, De Ridder and Schuurkes (1992) reported that cisapride enhanced electrical field stimulation-induced cholinergic contractions of the dog gastric antrum and suggested that the effects of cisapride were not mediated by 5-HT1,2,3,4 receptors. From these findings, the differential effects of mosapride in antrum and colon may be due to species differences among dog, guinea pig and human and to mechanisms additional to (or even other than) 5-HT4 receptors stimulation as well as other species variants of the 5-HT4 receptors.


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