A 5-HT₄ agonist mosapride enhances rectorectal and rectoanal reflexes in guinea pigs

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The present results indicate that mosapride enhanced the R-R and R-IAS reflexes induced by mosapride 1.0 mg/kg iv. 1.88, respectively. A specific 5-HT4 receptor antagonist, GR 113808 (GR), SB 204070, and RS 39604 (5). The receptor is widely distributed in the central nervous system and peripheral tissues. In the gastrointestinal tract, stimulation of 5-HT4 receptors has a pronounced effect on smooth muscle tone, mucosal electrolyte secretion, and the peristaltic reflex (1, 4, 5, 7, 8). Since its discovery in 1988, significant advances have been made in our understanding of the physiology and pharmacology of 5-HT4 receptors. These advances have led to the development of several selective 5-HT4 receptor agonists and antagonists that may have therapeutic utility in the treatment of peripheral disorders such as irritable bowel syndrome, diarrhea, and constipation (5).

Sakurai-Yamashita et al. (14, 15) have recently reported that 5-HT4 receptors are distributed in the human sigmoid colon, guinea pig distal colon, and human rectum. They also recently reported that mosapride accelerated intestinal motor activity in parallel with increases in the ACh release from enteric cholinergic neurons within the dog small intestine in the intraadrenergically stimulated contractions of isolated longitudinal muscle strips. In vitro experiments, mosapride also enhances the electrically stimulated contractions of isolated longitudinal muscles with attached myenteric plexus of the guinea pig ileum via the 5-HT4 receptor (11). Furthermore, there have been recent reports showing that mosapride enhances the colonic motility and peristalsis in the guinea pig (6, 11) and the rat (8), although mosapride has been considered to be effective on the upper gastrointestinal tract and ineffective on the lower gastrointestinal tract (11).

The 5-HT4 receptor is a member of the seven-transmembrane-spanning G protein-coupled family of receptors. The 5-HT4 receptor is pharmacologically defined by selective agonists such as SC 53116 and RS 67506 and selective antagonists such as GR 113806 (GR), SB 204070, and RS 39604 (5). The receptor is widely distributed in the central nervous system and peripheral tissues. In the gastrointestinal tract, stimulation of 5-HT4 receptors has a pronounced effect on smooth muscle tone, mucosal electrolyte secretion, and the peristaltic reflex (1, 4, 5, 7, 8). Since its discovery in 1988, significant advances have been made in our understanding of the physiology and pharmacology of 5-HT4 receptors. These advances have led to the development of several selective 5-HT4 receptor agonists and antagonists that may have therapeutic utility in the treatment of peripheral disorders such as irritable bowel syndrome, diarrhea, and constipation (5).

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The aim of the present study was to evaluate whether a prokinetic benzamide, mosapride, enhances the R-R and R-IAS reflexes mediated through 5-HT4 receptors. The 5-HT4 receptor is a member of the seven-transmembrane-spanning G protein-coupled family of receptors. The 5-HT4 receptor is pharmacologically defined by selective agonists such as SC 53116 and RS 67506 and selective antagonists such as GR 113806 (GR), SB 204070, and RS 39604 (5). The receptor is widely distributed in the central nervous system and peripheral tissues. In the gastrointestinal tract, stimulation of 5-HT4 receptors has a pronounced effect on smooth muscle tone, mucosal electrolyte secretion, and the peristaltic reflex (1, 4, 5, 7, 8). Since its discovery in 1988, significant advances have been made in our understanding of the physiology and pharmacology of 5-HT4 receptors. These advances have led to the development of several selective 5-HT4 receptor agonists and antagonists that may have therapeutic utility in the treatment of peripheral disorders such as irritable bowel syndrome, diarrhea, and constipation (5).

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METHODS AND MATERIALS

Experimental procedures followed the guidelines of the local animal ethics committee. Experiments were performed on 29 male guinea pigs (body wt: 402 ± 42 g; 315–450 g) anesthetized with ethyl carbamate (0.7–1.0 g/kg ip) artificially ventilated via a trachea cannula and immobilized with gallamine (0.1 mg/kg iv). The level of anesthesia was intermittently tested after stopping the immobilization.

After laminectomy, the 1st through 4th lumbar cords and 1st through 3rd sacral cords were removed by inserting the needle into the vertebral canal at intervals of 25 min to exclude the extrinsic excitatory reflex through the pelvic nerves and the inhibitory reflex through the lumbar colonic nerves, while leaving intact the intrinsic (enteric) neural pathway. Hemostasis was obtained by inserting cotton wool into the vertebral canal.

Rectal motility was recorded with a warm water-filled balloon that was attached to flexible polyethylene tubing connected to a pressure transducer. The 1.5-cm-long balloon was introduced into the rectum 4 cm oral to the anus. In experiments, the tubing was loosely fixed to a metal rod to prevent evacuation of the balloon through the anus. To record the basal rectal motility, 0.05 ml of water had been infused into the balloon. We confirmed that the balloon itself did not generate any pressure, due to the elastic properties of the balloon, when fewer than 2.0 ml of water were infused. Gradual and sustained rectal distension at each interval of 20 min was performed by continuously infusing 0.6 ml of warm water into the balloon at the rate of 1.5 ml/min for 24 s and by clamping the infusion tube for 4 min and 36 s (total 5 min) as previously reported (22). This rectal distension method simulated the physiological distension of the rectum by feces (22). The rectal distension did not affect the systemic blood pressure, indicating that the stimulus was nonnociceptive. During infusion of water into the balloon up to 0.6 ml for 24 s, no reflex response was evoked. Subsequent sustained rectal distension evoked rectal reflex responses superimposed on a sustained, passively generated pressure of 55–75 mmHg. This volume is the same as the previous one (16) and corresponds to two pieces of fresh feces (22).

The motility of the IAS was recorded with a custom-made strain gauge force transducer composed of a pair of needles and a base. The needles were horizontally fixed and inserted into the anus 0.5-cm oral to the anal margin. The needles are fixed on the base where two strain gauges (120 ± 0.5 Ω each) forming a half bridge are mounted, as previously shown (22). The needles move from the left to the right according to the IAS motility, and this moves strain gauges. At calibration, this transducer was vertically fixed at the base so that the needle on either side is upward and 1-g weights are suspended on it. The transducer is similar to that used by Mizutani and Nakayama (12) to measure the motility of the canine IAS but modified for use in guinea pigs. Force generated by strain in this transducer is linear between 0 and 1.0 mm. We have previously validated that this transducer records motility of the IAS independently of rectal motility (22).

The trial for control reflex response was repeated three times in each experiment. A reproducible reflex response was obtained in normal animals throughout the experiments by the present protocol without any interventions. After the control reflex response became stable, mosapride or GR (or cisapride) was injected intravenously into each guinea pig. Five to thirty minutes after each drug application, one to two trials of rectal distension for 5 min at 20-min intervals were performed. The reflex response showing two to three rectal contractions and IAS relaxations during a rectal distension was selected from one to two trials and evaluated by “reflex index.” Mean systemic arterial blood pressure was maintained between 100 and 150 mmHg throughout the experiment, and Po2, PCO2, and pH were maintained within the physiological range by changing the tidal volume and rate of artificial ventilation. The body temperature was maintained normal at 36–37°C with a heating pad.

Reflex index. We digitized the observed curves of each R-R and R-IAS response and calculated the area under the pressure-time curve of R-R responses (area under the curve [AUC]) and the area over the AOC (or under) the force-time curve of R-IAS responses with a computer-operated scanner-digitizer (Macintosh and Flexi-Trace, Three’s Company, Tokyo, Japan). The baseline was drawn on the basal pressure in R-R reflex response curve or basal force level in R-IAS reflex response curve to obtain the nonreflex area. We also digitized and calculated each nonreflex area with the same computer-operated scanner-digitizer. In each R-R and R-IAS response, the area of the tetrodotoxin-insensitive 1st phasic response was measured as nonreflex area. We finally obtained the “reflex area” by subtracting nonreflex area from AUC or AOC, as previously explained in detail (22). Reflex areas are expressed as positive values for rectal contractions and IAS relaxations. The measurement of the reflex area reflects any changes in amplitude, duration, or frequency of the reflex-mediated response. The reflex index is expressed as a relative ratio to the control reflex area (equal to 1.0) (22).

Drug used. The following drugs were used: mosapride citrate (kindly donated by Dainippon Pharmaceutical, Osaka, Japan), gallamine triethiodide (Sigma, St. Louis, MO), ethyl carbamate (Wako Pure Chemical Industries, Osaka, Japan), GR (Wako Pure Chemical Industries), and cisapride (extracted from Acenalin, Kyowa Hakko Kogyo, Tokyo, Japan). Mosapride, GR, and cisapride were dissolved in a solution containing 50% DMSO and injected intravenously, and thus final DMSO concentration was <1%.

Statistical significance of differences between means was estimated by one-way ANOVA and followed by multiple comparisons by Fisher’s post hoc test. A P value of <0.05 was considered statistically significant.

RESULTS

The representative sets of tracings of the R-R and the R-IAS reflex responses before and after successive injections of mosapride (0.1–1.0 mg/kg iv) are shown in Fig. 1. The initial transient increase in rectal intraluminal pressure induced by gradual distension did not elicit reflex response and was tetrodotoxin insensitive. This phase is excluded from reflex area. The subsequent, sustained rectal distension evoked reflex responses superimposed on a sustained, passively generated pressure of 55–75 mmHg. The R-R reflex was composed of three phasic contractions, and the R-IAS reflex was composed of three simultaneous phasic relaxations. Mosapride (0.1 mg/kg) increased frequency in R-R and R-IAS reflex responses (Fig. 1B). The subsequent mosapride (0.5 and 1.0 mg/kg) further increased frequency in both the R-R and R-IAS reflex responses without any effect on basal motility (Fig. 1, C and D). Four examples showed similar results. The other three examples showed increased amplitude, and the remaining four examples showed both increased
frequency and amplitude in R-R and R-IAS reflex responses.

The summarized data on the effects of successive mosapride intravenous injections on R-R and R-IAS reflexes evaluated by the reflex index in the same guinea pigs \((n = 12)\) are shown in Fig. 2. The reflex index in the control was expressed as 1.0. Mosapride \((0.1-1.0 \text{ mg/kg})\) increased dose dependently both R-R and R-IAS reflex indexes. Mosapride at 0.5 and 1.0 mg/kg significantly increased R-R reflex index \((P < 0.05)\) to 1.64 ± 0.66 and 1.92 ± 1.01 and significantly increased R-IAS reflex index \((P < 0.05)\) to 1.56 ± 0.59 and 1.88 ± 1.01.

Each representative set of tracings of the R-R reflex contractions and the R-IAS reflex relaxations before and after intravenous GR \((1.0 \text{ mg/kg})\) is shown in Fig. 3. The R-R reflex was composed of two phasic contractions, and the R-IAS reflex was composed of two simultaneous phasic relaxations (Fig. 3A). A 5-HT4 receptor antagonist, GR \((1.0 \text{ mg/kg})\) \((3)\) did not show any effect on the R-R and R-IAS reflexes (Fig. 3B) without any effect on the spontaneous motility in the rectum and IAS (data not shown). In the presence of 1.0 mg/kg GR, 1.0 mg/kg mosapride did not enhance these reflexes at all (Fig. 3C), indicating that 1.0 mg/kg GR antagonized the enhancement of the R-R and R-IAS reflexes caused by 1.0 mg/kg mosapride.

The summarized data of the antagonizing effects of 1.0 mg/kg GR on the effect of 1.0 mg/kg mosapride on R-R and R-IAS reflexes evaluated by the reflex index in the same guinea pigs \((n = 5)\) are shown in Fig. 4. The reflex index in the control was expressed as 1.0. GR \((1.0 \text{ mg/kg})\) did not affect either R-R or R-IAS reflex index. In the presence of 1.0 mg/kg GR, 1.0 mg/kg mosapride did not affect either R-R reflex index \((0.94 ± 0.11)\) or R-IAS reflex index \((1.13 ± 0.15)\).

Intrinsic R-R and R-IAS reflexes. As Yamanouchi et al. \((22)\) previously reported, pithing the 1st through 4th lumbar cords and 1st through 3rd sacral cords (PITH) attenuated the intrinsic (enteric) R-R and R-IAS reflex (reflex indexes: 0.37 ± 0.17 and 0.59 ± 0.21, respectively, in 6 guinea pigs). As an example shown in Fig. 5A, PITH largely decreased typical R-R and R-IAS reflexes without any effect on basal motility. Mosapride \((2.0-5.0 \text{ mg/kg})\) enhanced frequency and ampli-
tude in the intrinsic R-R reflex contraction and R-IAS reflex relaxation without any effect on basal motility. The other six examples showed similar results; mosapride increased frequency \((n = 4)\), amplitude \((n = 1)\), and both \((n = 1)\).

The summarized data on the effects of successive mosapride intravenous injections on the intrinsic R-R and R-IAS reflexes evaluated by the reflex index in the same guinea pigs \((n = 7)\) are shown in Fig. 6. The reflex index in the control after PITH was expressed as 1.0. Mosapride \((0.1–1.0 \text{ mg/kg})\) increased dose dependently both R-R and R-IAS reflex indexes but not significantly. Mosapride at 2.0 and 5.0 mg/kg significantly increased the intrinsic R-R reflex index to \(1.64 \pm 0.60\) \((P < 0.05)\) and \(2.22 \pm 0.88\) \((P < 0.001)\) and increased R-IAS reflex index to \(1.44 \pm 0.80\) \((P = 0.28)\) and \(2.32 \pm 1.58\) \((P < 0.005)\).

Another 5-HT\(_4\) receptor agonist, cisapride \((0.5–1.0 \text{ mg/kg})\), increased dose dependently both R-R and R-IAS reflexes (Fig. 7), but at a higher dose than 2.0–5.0 mg/kg, cisapride decreased both R-R and R-IAS reflexes (data not shown). The summarized data on the effects of successive cisapride intravenous injections on the intrinsic R-R and R-IAS reflexes evaluated by the reflex index in the same guinea pigs \((n = 5)\) are shown in Fig. 8. Cisapride \((0.5–1.0 \text{ mg/kg})\) dose dependently increased both R-R and R-IAS reflexes. At the dose of 1.0 mg/kg, cisapride increased R-R reflex significantly and maximally increased R-IAS reflex but not significantly.

**DISCUSSION**

The most important finding in the present study was that a prokinetic benzamide, mosapride, moderately enhanced both the rectal reflex contraction (cholinergic) \((22)\) and IAS reflex relaxation (nitrergic) \((22)\) during the gradual, sustained rectal distension mediated through 5-HT\(_4\) receptors located possibly in the enteric nerve pathway. This new finding suggests that the activation of 5-HT\(_4\) receptors plays an important role in integrative control of the defecation reflex composed of the R-R reflex and the R-IAS reflex in the guinea pig.

5-HT\(_4\) receptor and its agonist and antagonist. Mosapride did not affect the spontaneous motility of the rectum and IAS but only enhanced the neural reflex response in the rectum and IAS. Although recent reports \((14, 15)\) were not able to clearly demonstrate whether 5-HT\(_4\) receptors are located in the smooth muscle or enteric nervous system, the densities of 5-HT\(_4\) receptors were markedly higher in the myenteric and submucosal plexus than in the muscle layers. It is conceivable that the motor activity of circular muscle profoundly contributes to R-R and R-IAS reflex responses. Therefore, the increased motor activity of
circular muscle is probably due to 5-HT4 receptors located on the myenteric plexus (9). Our present results showed that mosapride only enhances the incidence of both reflex responses even after PITH, in a synchronous way in the two reflex pathways, without any effects on spontaneous motor activity. This fact strongly suggests that 5-HT4 receptors are located perhaps on the nerve terminals in myenteric ganglia impinging on myenteric motor neurons. This mechanism is very likely to be an underlying mechanism for the effect of cisapride (21).

A selective 5-HT4 receptor antagonist (3), GR, did not affect either the R-R or R-IAS reflex responses but antagonized the facilitating effect of mosapride on the reflex responses without any effect on the spontaneous motility in the rectum and IAS. These results support the idea that endogenous 5-HT plays no role in the R-R and R-IAS reflexes. It is conceivable that mosapride enhances the R-R and R-IAS reflexes mediated via extrinsic and/or intrinsic neural 5-HT4 receptors. As shown in dog gastrointestinal tissues, 5-HT4 receptor-mediated excitatory effects on intestinal motor activity are associated with increases in ACh release (9). The enhancement of R-R reflex contraction may be due to facilitation on the activity of cholinergic motoneurons (22) mediated via 5-HT4 receptors. The enhancement of R-IAS reflex relaxation may be due to facilitation on the activity of cholinergic interneurons mediated via 5-HT4 receptors and due to consequent facilitation of final nitrergic inhibitory motor nerve activities (22).

Another 5-HT4 agonist, cisapride (20), also exerted moderate facilitatory effects on R-R and R-IAS reflex responses similar to mosapride, although the effective dose of cisapride is a little higher than mosapride. This may be related to the difference of binding affinity for 5-HT4 receptor from that of mosapride. Mosapride has no binding affinity for the dopamine D2 receptor, 5-HT1 receptor, 5-HT2 receptor, and α-adrenoceptor (23, 24). Furthermore, cisapride reversely depressed the R-R

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**Fig. 5.** Representative sets of tracings of the R-R reflex and R-IAS reflex before mosapride after pithing 1st-4th lumbar cords and 1st-3rd sacral cords (PITH; A), after 0.1 (B), 0.5 (C), 1.0 (D), 2.0 (E), and 5.0 mg/kg iv mosapride (F) in 1 guinea pig. Each drug was given 5 min before recording the reflex response (●).

**Fig. 6.** Summarized data of effects of mosapride on intrinsic R-R reflex index (A) and intrinsic R-IAS reflex index (B) in the same guinea pigs after pithing 1st-4th lumbar cords and 1st-3rd sacral cords (PITH; n = 7). *P < 0.05 vs. control. **P < 0.005 vs. control. ***P < 0.001 vs. control.
and R-IAS reflex responses at higher doses (2.0–5.0 mg/kg). This might be related to the inhibitory action of cisapride as a 5-HT$_3$ antagonist (13, 20).

The R-R and R-IAS reflexes. Gradual, sustained rectal distension, as reported in our recent study (22), simulating the physiological distension of the rectum by feces was a more physiological stimulus than those in our previous studies, where prompt, sustained rectal distension (16, 19) or electrical stimulation of the pons (17) or pelvic afferent nerves (18) was used. In the present study, we were able to induce reproducible rectal contractions and simultaneous IAS relaxations by means of the rectal distension. Although spontaneous slowly migrating motor activity involving both excitatory and inhibitory enteric neurons has recently been observed in the guinea pig large intestine (2), we have already revealed that major parts of rectal contractions and IAS relaxations elicited by the rectal distension are intrinsic and extrinsic nerve-mediated reflex responses. Because defecation involves these intrinsic and extrinsic reflexes affecting the anorectum (10), this experimental preparation is a suitable model to test the effect of drugs on the defecation pathways.

Rationality of reflex index for evaluating the effect of drugs on R-R and R-IAS reflexes. In the present study, we adopted a reflex index to quantitatively evaluate reflex-mediated rectal contractions and IAS relaxations and contractions as previously proposed (22). The reflex responses are composed of various wave patterns, so that the simple evaluation of either the amplitude or frequency of each wave is not appropriate for evaluating the R-R and R-IAS reflexes. Furthermore, mosapride increased either the frequency or amplitude or both of the reflex responses. Therefore, the reflex index corresponding to the power-evacuating fecal contents would be appropriate for quantitatively evaluating the total drug actions on either the frequency or amplitude or both of the reflex responses.

Fig. 7. Representative sets of tracings of the R-R reflex and R-IAS reflex before cisapride (A) and after 0.1 (B), 0.5 (C), and 1.0 mg/kg cisapride (D) in 1 guinea pig. Each drug was given 5 min before recording the reflex response (●).

Fig. 8. Summarized data of effects of successive cisapride doses on R-R (A) and R-IAS (B) reflexes evaluated by the reflex index in the same guinea pigs (n = 5). *P < 0.05 vs. control.
Extrinsic sacral excitatory and lumbar inhibitory reflexes and intrinsic excitatory and inhibitory reflexes. PITH left the rectal and IAS reflex indexes at ~40 and 60%, indicating that 40% of the rectal contraction corresponds to the intrinsic (enteric) excitatory nerve-mediated reflex response and 60% of the IAS relaxation corresponds to the intrinsic inhibitory nerve-mediated reflex response (22).

Mosapride moderately enhanced the intrinsic R-R and R-IAS reflex responses, but the dose-response curve shifted rightward, suggesting one possibility that sites other than the enteric nervous system might be activated by mosapride or another possibility that the trauma caused by PITH per se might attenuate the effect of mosapride. Further studies in chronically pithed guinea pigs are needed to clearly elucidate the reason for this result.

In conclusion, the findings reported here indicate that 1) mosapride moderately enhanced the R-R and R-IAS reflex responses induced by rectal distension mediated through neutral 5-HT1 receptors; and 2) mosapride also enhanced the intrinsic R-R and R-IAS reflex responses, although the effective dose is rather high compared with that in the guinea pig with the intact spinal-intestinal pathways. We conclude that the present experimental preparation is a good model for exploring an ideal pharmacotherapy for the defecation disturbance such as constipation.

REFERENCES