A 5-HT₄ agonist, mosapride, enhances intrinsic rectorectal and rectoanal reflexes after removal of extrinsic nerves in guinea pigs

Yu Kojima,¹,² Tadashi Nakagawa,¹ Rentia Katsui,¹,² Hisao Fujii,² Yoshiyuki Nakajima,² and Miyako Takaki¹

¹Department of Physiology II and ²Department of Surgery, Nara Medical University, Kashihara, Japan

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Kojima, Yu, Tadashi Nakagawa, Rentia Katsui, Hisao Fujii, Yoshiyuki Nakajima, and Miyako Takaki. A 5-HT₄ agonist, mosapride, enhances intrinsic rectorectal and rectoanal reflexes after removal of extrinsic nerves in guinea pigs. Am J Physiol Gastrointest Liver Physiol 289: G351–G360, 2005. First published April 7, 2005; doi:10.1152/ajpgi.00532.2004.—Distension-evoked reflex of recto-rectal (R-R) contractions and rectointernal anal sphincter (R-IAS) relaxations can be generated in guinea pigs through an extrinsic sacral excitatory neural pathway (pelvic nerves) as well as intrinsic cholinergic excitatory and nitrergic inhibitory pathways. The aim of the present study was to create intrinsic R-R and R-IAS reflex models by pithing (destruction of the lumbar and sacral cords; PITH) and to evaluate whether the prokinetic benzamide mosapride, a 5-HT₄ receptor agonist, enhances these reflexes. The mechanical activities of the R-R and R-IAS were recorded in the anesthetized guinea pig on days 2–9 after PITH. Although the basal rectal pressure at distension after PITH was significantly lower than control, the reflex indexes of R-R contractions and synchronous R-IAS relaxations were unchanged between days 4 and 9 after PITH. The frequency of spontaneous rectal and IAS motility were also unchanged. Immunohistochemical studies revealed that the distribution of myenteric and intramuscular interstitial cells of Cajal (ICC) were not altered after PITH. Mosapride (0.1–1.0 mg/kg iv) dose-dependently increased both intrinsic R-R and R-IAS reflexes mediated via rectal (R-R) contractions and rectointernal anal sphincter (R-IAS) relaxations can be generated in guinea pigs through an extrinsic sacral excitatory neural pathway (pelvic nerves) as well as intrinsic cholinergic excitatory and nitrergic inhibitory pathways. The aims of the present study were to create intrinsic R-R and R-IAS reflex models by pithing (destruction of the lumbar and sacral cords; PITH) and to evaluate whether the prokinetic benzamide mosapride, a 5-HT₄ receptor agonist, enhances these reflexes. Mosapride moderately enhanced intrinsic R-R and R-IAS reflexes functionally compensated after depravation of extrinsic nerves, mediated through endogenously active intrinsic 5-HT₄ receptors.

Therefore, we have concluded 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 (27).

Recently, we (21) reported that a 5-HT₄ receptor agonist, mosapride, enhanced R-R and R-IAS reflex responses in intact guinea pigs in vivo without any effects on the R or IAS spontaneous motility. Although after acute PITH, mosapride moderately enhanced the intrinsic R-R and R-IAS reflex responses, the dose-response curve shifted right (21). Therefore, we speculated that the trauma caused by acute PITH might have attenuated the effect of mosapride, and we concluded that to circumvent the detrimental effect by the trauma, a chronic PITH model should be tested.

The aims of the present study were to create an intrinsic R-R and R-IAS reflex model by chronic PITH, and to evaluate whether the 5-HT₄ agonist mosapride enhances the intrinsic R-R and R-IAS reflexes induced by means of gradual and sustained rectal distension. We also evaluated whether mosapride enhances the respective spontaneous motility in the R and IAS in the same model. This guinea pig chronic PITH model has a severe urination disturbance but no marked defecation disturbance such as constipation. Mosapride moderately enhanced the intrinsic R-R and R-IAS reflexes mediated via 5-HT₄ receptors without any effects on the spontaneous motility in the R and IAS in this model.

METHODS AND MATERIALS

Experimental procedures followed the regulations of the animal care and use committee of Nara Medical University. After laminecotomy under anesthesia with Nembutal (40 mg/kg ip) in 35 male guinea pigs, the L1–L4 segments and S1–S3 segments were gently removed by inserting a needle into the vertebral canal at an interval of 25 min to disrupt the extrinsic excitatory reflex involving the pelvic nerves and the inhibitory reflex involving the lumbar colonic nerves (PITH), while leaving intact the intrinsic (enteric) neural pathway. Hemostasis was obtained by inserting cotton wool into the vertebral canal. After the surgery, the animals were carefully monitored for 9 days, and urine was expressed by manual bladder compression.

Experiments were performed on 38 male guinea pigs (including 10 intact guinea pigs and 28 guinea pigs that underwent chronic PITH) (body wt: 422 ± 39 g; range, 356–508 g) anesthetized with ethyl carbamate (0.7–1.0 g/kg ip), artificially ventilated via a tracheal cannula and immobilized with gallamine (0.1 mg/kg iv). The level of anesthesia was intermittently tested after stopping the immobilization.

Rectal motility was recorded with a warm water-filled balloon 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. During experiments, the tubing was loosely fixed to a metal rod to prevent evaporation 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 the balloon itself did not generate any pressure due to the elastic properties of the balloon when <2.0 ml of water was 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 (21, 27). This rectal distension method simulated the physiological distension of the rectum by feces (21, 27). 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 70–180 mmHg in intact guinea pigs. This volume is the same as the previous one (21, 27) and corresponds to two pieces of fresh feces.

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 needles were horizontally fixed and inserted into the anus 0.5 cm oral to the anal margin. 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 was upward and 1-g weights were suspended on it. The transducer is similar to that used by Mizutani and Nakayama (13) 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 (21, 27) validated that this transducer records motility of the IAS independently of rectal motility.

The trial for control reflex response was repeated three times in each experiment. A reproducible reflex response was obtained in intact and chronically pithed animals 2, 4, 6, and 9 days after PITH throughout the experiments by the present protocol without any interventions. After the control reflex response became stable, mosapride or GR-113808 was injected intravenously to each guinea pig. Five to thirty min after each drug application, one to two trials of rectal distension for 5 min at 20-min intervals was performed. The reflex response was selected from one to two trials and evaluated by mean amplitude, frequency, and “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. Body temperature was maintained normal at 36–37°C with a heating pad.

**Reflex index.** All data were acquired into a personal computer (Fujitsu, Tokyo, Japan) through an A/D converter (Digidata model 1322A, Axon Instruments, Foster City, CA) at 166.7 Hz and filtered at 10 Hz with Axoscope 7 (Axon Instruments, Foster City, CA). The R-R reflex area (area under the pressure-time curve of R-R responses minus nonreflex area) and R-IAS reflex area (area over the force-time curve of R-IAS responses minus nonreflex area) (Fig. 1, Ref. 27) were calculated with Origin 6.1J (OriginLab, Northhampton, MA). In each R-R and R-IAS response, the area of the TTX-insensitive first phasic response was excluded from the reflex area (21, 27). Reflex area is expressed as positive values for rectal contractions and IAS relaxations. For example, when the amplitude of the reflex response increases associated with the decreased frequency of the response, the resulting reflex area may not change. However, the measurement of the reflex area could detect any changes either in amplitude, duration, or frequency of the reflex response. The reflex index is expressed as a relative ratio to the control reflex area (1.0) (21, 27).

**Drugs.** 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-113808 (Wako Pure Chemical Industries), and TTX (Sankyo, Tokyo, Japan). Mosapride and GR-113808 were dissolved in a solution containing 50% DMSO and injected intravenously; thus final DMSO concentration was <1%.

**Immunohistochemistry.** For c-Kit and protein gene product (PGP) 9.5 immunohistochemistry, the whole mount preparations of R and IAS, where the mucosa was completely removed and the circular muscle layer was partially removed, were fixed in acetone (4°C, 1 h). After fixation, preparations were washed for 30 min in PBS (0.1 M, pH 7.4). Nonspecific antibody binding was reduced by incubation for
12 h in 10% normal goat serum in PBS, containing 0.3% (vol/vol) Triton X-100 at room temperature. Tissues were incubated 48 h at 4°C with a rat monoclonal antibody raised against c-Kit protein (ACK545, 5 μg/ml in PBS; BD Biosciences, San Jose, CA) and with a rabbit polyclonal antibody raised against human brain PGP 9.5 (5 μg/ml in PBS; Chemicon International, Temecula, CA). Immunoreactivity for Kit was detected using conjugated secondary antibody (Alexa Flour 488 goat anti-rat; Molecular Probes, Eugene, OR; 1:200 in PBS for 48 h in the dark at room temperature) and that for PGP 9.5 was detected using Texas Red-conjugated secondary antibody (Texas Red goat anti-rabbit; Pharmacia, Aurora, OH; 1:100 in PBS for 48 h in the dark at room temperature). Tissues were examined with an MRC 600 (Bio-Rad, Hercules, CA) confocal microscope. Confocal micrographs are digital composites of Z-series scans of 10–15 optical sections through a depth of 100–150 μm. Final images were constructed with Comos software (Bio-Rad).

Statistical significance of differences between means was estimated by Student’s nonpaired or paired t-test, or by one-way ANOVA and were followed by multiple comparisons by Bonferroni’s or Dunnett’s post hoc test. A P value of <0.05 was considered statistically significant.

RESULTS

Changes in intrinsic R-R and R-IAS reflexes 2–9 days after chronic PITH. As we have previously reported, acute PITH attenuated the intrinsic (enteric) R-R and R-IAS reflexes (reflex indexes: 0.37 ± 0.17 and 0.59 ± 0.21, respectively, in 6 guinea pigs) (27). Examples of intrinsic reflexes on days 2–9 after PITH are shown in Fig. 1. The initial transient increase in rectal intraluminal pressure, induced by gradual distension, did not elicit reflex response and was largely TTX-insensitive. This phase is excluded from reflex area. The subsequent, sustained rectal distension evoked reflex responses superimposed on a sustained, passively generated pressure of 80–100 mmHg. On day 2 after PITH, the R-R reflex was attenuated, although many small waves were observed (Fig. 1B). These small waves were likely to be distension-evoked neurogenic phasic activity, because these waves in R and IAS were synchronous and abolished by TTX. During days 4–9 after PITH, typical R-R and R-IAS reflexes were observed, but the reflex pattern gradually changed; there was a decrease in frequency associated with an increase in amplitude (Fig. 1, C–E; Fig. 2). Frequency was significantly decreased relative to control reflexes on day 9 after PITH (Fig. 2). In contrast, the amplitude was significantly increased on days 6 and 9 after PITH (Fig. 2).

Mean basal pressure during rectal distension gradually decreased, reaching significance on the days 6 and 9 after PITH (Fig. 3).

The reflex index is expressed as a relative ratio to the control reflex area (1.0) (27). The mean reflex index in R-R was significantly decreased on day 2 after PITH and returned to the control level on day 4 after PITH. The mean reflex index in R-IAS was unchanged between days 4 and 9 after PITH. The mean reflex index in R-IAS was unchanged throughout days 2–9 after PITH (Fig. 4).

Changes in spontaneous motility in R and IAS without rectal distension. Spontaneous motility was observed in R and IAS without rectal distension from days 2–9 after PITH. Representative recordings in R and IAS obtained on day 2 after PITH, before and after TTX administration, are shown in Fig. 5A. Although these motilities are almost mirror images and the frequency and amplitude were decreased after treatment with TTX (Fig. 5B), significant differences in the frequency of R and IAS spontaneous motility due to TTX treatment were only detected on the day 6 after PITH and in the frequency of IAS in controls (Fig. 6). As TTX significantly decreased the frequency in IAS but not R, it is possible that the frequency of IAS is constitutively increased by neuronal mechanism.

Immunoreactivity for PGP 9.5 and c-Kit. Representative images of immunostaining for PGP 9.5 in R myenteric plexus (MP) before and after PITH are shown in Fig. 7. No marked changes of MP were observed in R after PITH, compared with control. Representative images of immunostaining for c-Kit in intramuscular ICC (ICC-IM) and myenteric ICC (ICC-MY) in day after PITH.
R and IAS in control and pithed preparations are shown in Fig. 8. No differences in the distribution of ICC-IM and ICC-MY were observed between R and IAS. It appeared that there were no marked differences in codistribution of MP and ICC-MY in R between control and day 9 after PITH.

**Effects of mosapride on the intrinsic R-R and the R-IAS reflex responses after chronic PITH.** Representative recordings of R-R and R-IAS reflex responses on day 9 after PITH before intravenous mosapride and after successive mosapride (0.1–1.0 mg/kg) iv are shown in Fig. 9. The R-R reflex was composed of a single phasic contraction and the R-IAS reflex was composed of a simultaneous single phasic relaxation. Mosapride 0.1 mg/kg caused a twofold increase in the frequency of the R-R and R-IAS reflex responses. This result suggested that the amount of feces evacuated would be twofold, since we have observed that one R-R reflex response evacuates the balloon inserted into the rectum from the anus when the tubing is not fixed to a metal rod. Subsequent administration of mosapride (0.5 and 1.0 mg/kg) increased amplitudes of both the R-R and R-IAS reflex responses without any effect on basal spontaneous motility. Mosapride (5.0 mg/kg) did not exert an additional effect (data not shown).

Mosapride (0.1–5.0 mg/kg) did not affect the mean reflex indexes of R-R and R-IAS at time points of days 2–4 after PITH (1.0; n = 7; data not shown). However, mosapride (0.1–1.0 mg/kg) caused dose-dependent increases in both the mean R-R and R-IAS reflex indexes during days 6–9 after PITH (n = 7). Mosapride at 0.5 and 1.0 mg/kg significantly increased R-R reflex index (P < 0.05) to 1.71 ± 0.81 and 1.82 ± 0.61, and significantly increased the R-IAS reflex index (P < 0.05) to 1.96 ± 1.55 and 2.75 ± 1.45 (Fig. 10).

The 5-HT4 antagonist, GR-113808 (1.0 mg/kg iv) (3) did not affect the mean reflex indexes of R-R and R-IAS 2–4 days after PITH (1.0)(n = 3)(data not shown). Representative recordings of the R-R reflex contractions and the R-IAS reflex relaxations before GR-113808 (1.0 mg/kg iv), after GR-113808, and after additional mosapride (1.0 mg/kg) on day 9 after PITH are shown in Fig. 11. The R-R reflex was composed of single intense phasic contraction and the R-IAS reflex was composed of simultaneous single intense phasic relaxation (Fig. 11A). The 5-HT4 receptor antagonist, GR-113808 (1.0 mg/kg) partially suppressed the R-R and R-IAS reflexes (Fig. 11B) without any effect on the spontaneous motility in the R and IAS. This contrasts with the result in intact guinea pigs (21). In the presence of GR-113808 (1.0 mg/kg), mosapride (1.0 mg/kg) did not enhance these reflexes (Fig. 11C), indicating that GR-113808 (1.0 mg/kg) completely antagonized the enhancement of the R-R and R-IAS reflexes caused by mosapride (1.0 mg/kg). This antagonism (1.0 mg/kg

Fig. 3. Summarized data of rectal basal pressure during distension in control, and on days 2–9 after PITH. *P < 0.01 vs. control. #P < 0.05 vs. 2nd day after PITH.

Fig. 4. Summarized data of R-R (A) and R-IAS (B) reflexes evaluated by reflex index in the same guinea pigs (n = 7 each) in control and on days 2–9 after PITH. *P < 0.005 vs. control. #P < 0.005 vs. 4th day after PITH.
GR-113808 (1.0 mg/kg) reduced the amplitudes of the R-R and R-IAS reflexes to 86.3 ± 6.7 and 73.1 ± 17.7% of control values, respectively, and reduced the frequency to 66.7 ± 28.9% of control (duration was unaffected). GR-113808 (1.0 mg/kg) reduced the amplitudes of the R-R and R-IAS reflexes to 86.3 ± 6.7 and 73.1 ± 17.7% of control values, respectively, and reduced the frequency to 66.7 ± 28.9% of control (duration was unaffected).
reduced R-R and R-IAS reflex indexes to 0.52 ± 0.18 and 0.47 ± 0.24, respectively. Subsequent administration of mosapride (1.0 mg/kg) did not reduce either the amplitude, frequency, or R-R (0.50 ± 0.26) and R-IAS reflex indexes (0.44 ± 0.36).

DISCUSSION

The most important finding in the present study was that although the intrinsic R-R reflex and R-IAS reflex indexes 6–9 days after chronic PITH were unchanged, they were associated with dramatically increased amplitude and decreased frequency in the reflex responses. Furthermore, our data indicate that endogenously active 5-HT4 receptors located in the enteric nerve pathway augment R-R and R-IAS reflexes, whereas this is not the case in intact guinea pigs (21). This novel finding suggests that the activation of intrinsic 5-HT4 receptors plays an important role in integrative control of the defecation reflex composed of the intrinsic R-R and R-IAS reflexes in the chronically pithed guinea pigs, which have a urination disturbance but no apparent defecation disturbance. Chronically pithed animals have no apparent defecation disturbance means that the extrinsic

Fig. 7. Immunostaining for protein gene product (PGP) 9.5 in R myenteric plexus (MP) in control (A), and on day 4 (B) and day 9 (C) after PITH. A-a: corresponding to a in A. c-Kit-positive myenteric interstitial cells of Cajal (ICC-MY) and PGP 9.5-positive MP are merged.

Fig. 8. Immunostaining for c-Kit in intramuscular ICC (ICC-IM) and ICC-MY in R and IAS in control (A), and on day 4 after PITH (B) and day 9 after PITH (C). C-a: corresponding to ICC-MY in R (a in C). c-Kit-positive ICC-MY and PGP 9.5-positive MP in R are merged.
component of R-R, and R-IAS has no a major role in the defecation in contrast to acute pithed animals.

The R-R reflex and R-IAS reflexes. In the chronic PITH model, 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 guinea pig large intestine (2), we have previously (27) revealed that major parts of distension-induced rectal contractions and IAS relaxations involve both intrinsic and extrinsic nerve-mediated reflex responses. Although defecation involves these intrinsic and extrinsic reflexes affecting the anorectum (11), the experimental model used here is suitable to test the specific effect of drugs on the intrinsic defecation pathways.

Rationality of reflex index for evaluating the effect of drugs on R-R and R-IAS reflexes. In the present study, a reflex index was adopted to quantitatively evaluate reflex-mediated rectal contractions and IAS relaxations, as previously proposed (21, 27). While the reflex frequency was decreased and the mean reflex amplitude was increased, the reflex responses are composed of various wave patterns, so that the simple evaluation of either the amplitude or frequency of each wave was not adequate to evaluate the intrinsic R-R and R-IAS reflexes. 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 intrinsic reflex responses.

Extrinsic and intrinsic reflexes. Based on the findings that acute PITH left the R-R and R-IAS reflex indexes at ~40 and 60%, we have concluded that 40% of the rectal contraction corresponds to the intrinsic (enteric) excitatory nerve-mediated reflex response and the remaining 60% of the IAS relaxation corresponds to the intrinsic inhibitory nerve-mediated reflex response (27). However, the trauma caused by PITH may reduce the reflex response. Because on day 2 after PITH, only the R-R reflex index significantly decreased relative to control (intact guinea pigs), it appears that the recovery of R-R reflex from the trauma caused by PITH is slower than R-IAS reflex.

In the chronic PITH model, the R-R and R-IAS reflex indexes remained unchanged except for the R-R reflex on day 2, although the reflex response pattern and rectal basal pressure largely changed during 6–9 days after PITH; large amplitude and low frequency in the reflex response and low rectal basal pressure were observed. We could not detect any changes of immunohistochemistry for MP in R after chronic PITH; however, the intrinsic neural reflexes may have fully compensated the deficiency of the extrinsic neural reflexes mediated via enhancement of intrinsic neural activity during 6–9 days after PITH. Further studies are needed to know the underlying mechanisms for this compensation.
Spontaneous motility and ICC. Present results suggest the possibility that spontaneous motility in the guinea pig IAS is modulated by neuronal mechanisms as in the canine rectoanal region where is innervated by extrinsic nerves (26). Similar distribution of ICC-MY and ICC-IM observed in R and IAS, was unchanged after chronic PITH. Similar frequency of spontaneous motility observed in R and IAS after chronic PITH (extrinsic denervation) was almost unchanged after TTX (intrinsic denervation). These results suggested that the consecutive distribution of ICC-MY and/or ICC-IM throughout the rectoanal region determines the frequency of spontaneous motility in the guinea pig rectoanal region, differently from the canine rectoanal region (6, 14). Although the importance of ICC distributed in IAS for evoking R-IAS reflex has been reported in human diseased subjects (17), the present study did not reveal the importance of ICC on R-R and R-IAS reflex responses.

Neither mosapride nor GR-113808 affected spontaneous motility in R and IAS after chronic PITH like after acute PITH (21), suggesting a lesser possibility for the presence of endogenously active 5-HT₄ receptors on ICC in the rectoanal region.

5-HT₄ receptor and its agonist and antagonist in intrinsic reflexes. The 5-HT₄ receptor is pharmacologically defined by other selective agonists, such as SC-53116 and RS-67506, and selective antagonists, such as SB-204070 and RS-39604 (3, 5).

In the gastrointestinal tract, stimulation of 5-HT₄ receptors has pronounced effects on smooth muscle tone and motility, mucosal electrolyte secretion, and the peristaltic reflex (1, 4, 5, 7–9, 12). Furthermore, a partial 5-HT₄ receptor agonist, tegaserod stimulates canine colonic transit (16) and orocecal and colonic transits in patients with constipation-predominant irritable bowel syndrome (18). However, we selected mosapride as a 5-HT₄ receptor agonist and GR-113808 as the 5-HT₄ receptor antagonist, because we have previously well-investigated antagonism of mosapride by GR-113808 in intact and acute PITH guinea pig model (21).

After acute PITH, the R-R and R-IAS reflexes have been decreased by 60 and 40% (27) and the dose-effect curve of mosapride on the R-R and R-IAS reflexes shifted rightward (21). On days 4–9 after chronic PITH, the R-R and R-IAS reflexes existed almost unchanged. On days 6–9 after chronic PITH, mosapride moderately enhanced the intrinsic R-R and R-IAS reflex responses, but the dose-effect curve was the same as that in intact guinea pigs. Therefore, the right shift of the dose-effect curve obtained in guinea pigs that underwent acute PITH (19) may be due to the trauma caused by acute PITH and no compensations for the deficiency of the extrinsic neural reflexes by the intrinsic neural reflexes.

In the present study, we fully investigated the effects of mosapride and GR-113808 in chronic PITH model associated with urination disturbance and without defecation disturbance.
On days 6–9 after chronic PITH, mosapride moderately enhanced the intrinsic R-R and R-IAS reflex responses and the selective 5-HT4 receptor antagonist (3) GR-113808 reduced the reflex responses, suggesting that 5-HT4 receptors are endogenously active in the enteric nervous system, activated by extrinsic nerve deprivation at least for 5 days after chronic PITH. The present result contrasts with our previous results in guinea pigs with intact extrinsic nerves; GR-113808 did not affect either the R-R or R-IAS reflex responses, but antagonized the facilitating effect of mosapride on the reflex responses (21).

Mosapride did not affect the spontaneous motility of the R and IAS, but enhanced the intrinsic neural reflex response in the R and IAS of the guinea pig chronic PITH model. Recent reports have revealed that the densities of 5-HT4 receptors are markedly higher in the myenteric and submucosal plexus than in the muscle layers (19, 20). 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 (10). Our present results showed that mosapride only enhances the incidence of both reflex responses even after chronic PITH, by the way synchronous 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 but not on ICC-MY and/or ICC-IM.

As shown in dog gastrointestinal tissues, 5-HT4 receptor mediated excitatory effects on intestinal motor activity are associated with increases in ACh release (10). The enhancement of R-R reflex contraction may be due to facilitation on the activity of intrinsic cholinergic motor neurons mediated via 5-HT4 receptors (21, 27). The enhancement of R-IAS reflex relaxation may be due to facilitation on the activity of intrinsic cholinergic interneurons mediated via 5-HT4 receptors and due to consequent facilitation of final intrinsic nitrergic inhibitory motor nerve activities (27).

In conclusion, the findings reported here indicate that the intrinsic R-R and R-IAS reflexes are functionally compensated after chronic deprivation of extrinsic autonomic nerves and that mosapride moderately enhanced the intrinsic R-R and R-IAS reflexes mediated through endogenously active enteric neural 5-HT4 receptors in the guinea pig model with chronic spinal cord injury. We propose that the present model is appropriate for exploring a better pharmacotherapy for the defecation function, corresponding to time-course changes in patients with acute and chronic spinal cord injury.

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