

Invited Review**Enhancement of the intrinsic defecation reflex by mosapride, a 5-HT₄ agonist, in chronically lumbosacral denervated guinea pigs**

Yu KOJIMA^{1,2*}, Hisao FUJI¹, Renta KATSUI^{1,2},
Yoshiyuki NAKAJIMA¹ and Miyako TAKAKI²

¹*Departments of Surgery,*

²*Physiology II, Nara Medical University, 840 Shijo-cho, Kashihara 634-8521, Japan*

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Abstract

The defecation reflex is composed of rectal distension-evoked rectal (R-R) reflex contractions and synchronous internal anal sphincter (R-IAS) reflex relaxations in guinea pigs. These R-R and R-IAS reflexes are controlled via extrinsic sacral excitatory nerve pathway (pelvic nerves), lumbar inhibitory nerve pathways (colonic nerves) and by intrinsic cholinergic excitatory and nitrergic inhibitory nerve pathways. The effect of mosapride (a prokinetic benzamide) on the intrinsic reflexes, mediated via enteric 5-HT₄ receptors, was evaluated by measuring the mechanical activity of the rectum and IAS in anesthetized guinea pigs using an intrinsic R-R and R-IAS reflex model resulting from chronic (two to nine days) lumbosacral denervation (PITH). In this model, the myenteric plexus remains undamaged and the distribution of myenteric and intramuscular interstitial cells of Cajal is unchanged. Although R-R and R-IAS reflex patterns markedly changed, the reflex indices (reflex pressure or force curve-time integral) of both the R-R contractions and the synchronous R-IAS relaxations were unchanged. The frequency of the spontaneous R and IAS motility was also unchanged. Mosapride (0.1–1.0 mg/kg) dose-dependently increased both intrinsic R-R (maximum: 1.82) and R-IAS reflex indices (maximum: 2.76) from that of the control (1.0) 6–9 days following chronic PITH. The dose-response curve was similar to that in the intact guinea pig, and had shifted to the left from that in the guinea pig after acute PITH. A specific 5-HT₄ receptor antagonist, GR 113808 (1.0 mg/kg), decreased both reflex indices by approximately 50% and antagonized the effect of mosapride 1.0 mg/kg. This was quite different from the result in the intact guinea pig where GR 113808 (1.0 mg/kg) did not affect either of the reflex indices. The present results indicate that mosapride enhanced the intrinsic R-R and R-IAS reflexes and functionally compensated for the deprivation of extrinsic innervation. The actions of mosapride were mediated through endogenously active, intrinsic 5-HT₄ receptors which may be post-synaptically located in the myenteric plexus of the anorectum.

Key words: extrinsic reflex, internal anal sphincter, intrinsic reflex rectum, 5-HT₄ receptor

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Correspondence to: Miyako Takaki, Ph.D., Department of Physiology II, Nara Medical University, 840 Shijo-cho, Kashihara 634-8521, Japan

Phone: +81-744-29-8829 Fax: +81-744-23-4696 e-mail: mtakaki@naramed-u.ac.jp

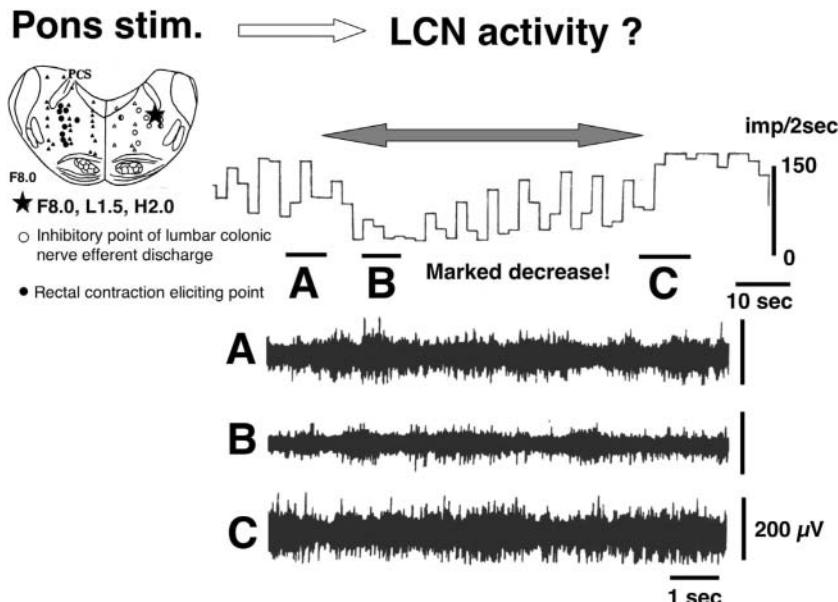


Fig. 1. Effect of stimulation of the pontine lateral reticular formation (PS) after L4 transection (dissection of pelvic afferents) on lumbar colonic nerve efferent discharge. PS markedly inhibited the lumbar colonic efferent discharge. Inhibitory points of lumbar colonic efferent discharge distribute diffusively in the pontine lateral reticular formation. Rectal contraction eliciting points showed a similar distribution. An example of stimulating points, showed by solid star (F8.0, L1.5, H2.0) is localized at 8.0 mm frontal and 1.5 mm lateral from the obex and 2.0 mm deeper from the dorsal surface of the pons. The traces A, B and C show periods of lumbar colonic efferent discharge corresponding to A, B and C of the frequency histogram (uppermost trace): A, before PS; B, maximal inhibition during PS; C, after PS. Parameters of stimulation: 0.3 ms, 0.3 mA, 50 Hz.

Introduction

We have previously reported that gradual and subsequent sustained rectal distension evoked rectal (R-R) cholinergic reflex contractions and the synchronous internal anal sphincter (R-IAS) nitrenergic reflex relaxation in a reproducible manner (Yamanouchi *et al.*, 2002). These reflexes are composed of both extrinsic and intrinsic components. Firstly, the extrinsic component which involves the lumbar inhibitory reflex through the lumbar colonic nerves, that is subject to supraspinal inhibition from the pons, together with the extrinsic sacral excitatory reflex through the pelvic nerves. In fact, stimulation of the pontine lateral reticular formation inhibited lumbar colonic nerve efferent discharge (Fig. 1). Pelvic afferent stimulation also inhibited lumbar colonic nerve efferent discharge mediated via the pons. Nevertheless, the extrinsic lumbar inhibitory reflex suppresses the extrinsic sacral R-R and R-IAS reflexes (Takaki *et al.*, 1980; 1983; 1985; 1987). Secondly, the intrinsic component involves both cholinergic excitatory and nitrenergic inhibitory reflexes (Yamanouchi *et al.*, 2002). Since defecation involves both of these intrinsic and extrinsic reflexes affecting the anorectum (Matsufuji *et al.*, 1998), our

experimental model is suitable for the evaluation of the effect of drugs on these defecation pathways.

To exclude the influence of both supraspinal control and extrinsic nervous control, we have adopted a procedure which involves the acute destruction of both the lumbar and sacral spinal cord (PITH). The trauma possibly caused by acute PITH *per se*, however, might have interfered with the experimental outcome. In fact, acute PITH reduced the R-R and R-IAS reflex indices to approximately 40% and 60% of that in control, respectively. We had previously thought that this indicated that 40% of the rectal contraction is due to the intrinsic (enteric) excitatory nerve-mediated reflex response while 60% of the IAS relaxation is due to the intrinsic inhibitory nerve-mediated reflex response (Yamanouchi *et al.*, 2002). However, four to nine days after chronic PITH, the reflex indices of R-R reflex contractions and synchronous R-IAS reflex relaxations were unchanged although the reflex pattern had markedly changed. Therefore, it is considered that the trauma caused by acute PITH *per se* might have continued for two days and have decreased the R-R and R-IAS reflex indices. The chronic PITH model should enable the determination of the actual contribution of the enteric nervous system to both the R-R and R-IAS reflexes, although some remodeling of the enteric nervous system may occur to compensate for the lack of extrinsic nerve input.

Chronic PITH model

Experimental procedures followed the guidelines of the local animal ethics committee. After laminectomy under anesthesia with Nembutal 40 mg/kg *i.p.*, the 1st to 4th lumbar spinal cord and the 1st to 3rd sacral spinal cord was gently and slowly removed by inserting a needle into the vertebral canal to remove 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. After surgery, guinea pigs were kept carefully for two to nine days, using manual bladder compression for urination, because of the interruption of the normal bladder control (Kojima *et al.*, 2005).

Changes in intrinsic R-R and R-IAS reflexes two to nine days after chronic PITH

Acute PITH attenuated the intrinsic (enteric) R-R and R-IAS reflexes (reflex indices: 0.37 ± 0.17 and 0.59 ± 0.21 respectively in six guinea pigs) from those in the control ($= 1.0$) (Yamanouchi *et al.*, 2002). On the 2nd day after PITH, while the R-R reflex was attenuated, many small waves were observed. These small waves were likely to be indicative of rectal distension-evoked neurogenic phasic activity, because these waves in the rectum and IAS were synchronous and abolished by a neuron blocker, tetrodotoxin (TTX) (Kojima *et al.*, 2005). However, if the recovery from the surgical trauma in the ICC is faster than that in enteric neurons, it may be that the interstitial cells of Cajal (ICC) contributed to the generation of these small waves, because the normal distribution of myenteric (ICC-MY) and intramuscular ICC (ICC-IM) in the anorectum was unchanged after chronic PITH (Fig. 2). The attenuation of both

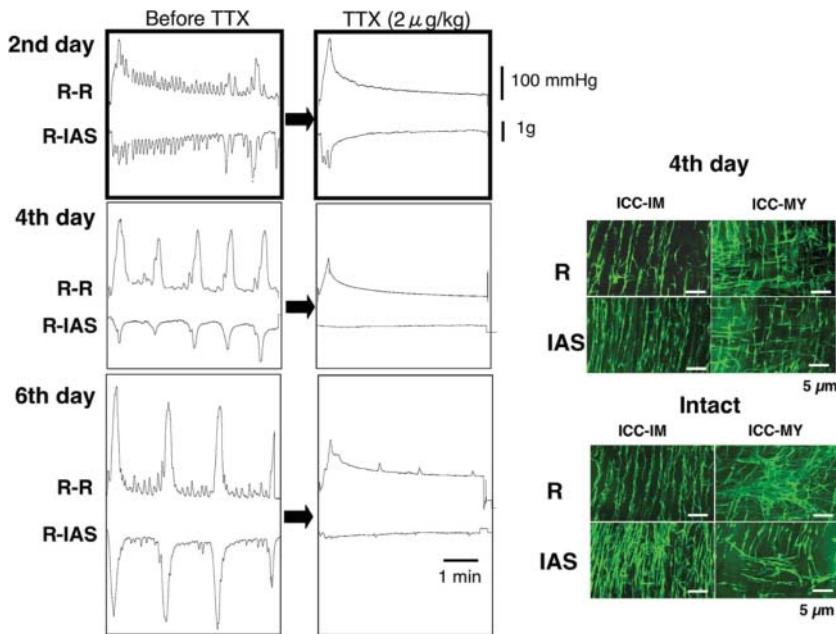


Fig. 2. Representative tracings of the recto-rectal (R-R) reflex response and the recto-internal anal sphincter (R-IAS) reflex response following the destruction of the lumbar and sacral cords (PITH) at 2, 4 and 6 days following the PITH procedure in each guinea pig. Rectal distension was performed for 5 min at 20-min intervals. The amplitude of the reflex response increased and its frequency decreased between 4 and 6 days following PITH. The reflex response was abolished by tetrodotoxin (TTX). On the 2nd day following PITH, small amplitude and high frequency contractions were observed. These contractions were also abolished by TTX. Immunostaining for c-Kit in intramuscular ICC (ICC-IM) and myenteric ICC (ICC-MY) in the rectum (R) and internal anal sphincter (IAS) on 4th day after PITH (upper panel) and at an intact state (lower panel). No differences in ICC-IM and ICC-MY were observed between that of the control and that observed on the 4th day following the PITH procedure.

the R-R and R-IAS reflexes on the 2nd day following PITH, was possibly due to trauma of the enteric neurons as a result of the PITH procedure (Fig. 2). Between the 4th to 9th day following PITH, typical R-R and R-IAS reflexes were observed, but the reflex pattern gradually and markedly changed; there was a decrease in frequency associated with an increase in amplitude (Fig. 2) (Kojima *et al.*, 2005).

5-HT receptors

There is an extensive distribution of 5-HT receptors in both the central nervous system and in peripheral tissues. A number of sub-types of 5-HT receptors are known to be expressed by enteric neurons, including the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT₃, and 5-HT₄ sub-types (Liu *et al.*, 2005). In addition, the 5-HT_{1P} sub-type has also been identified in the enteric nervous system (ENS) (Takaki *et al.*, 1985; Branchek *et al.*, 1988; Mawe *et al.*, 1986). However, although this receptor sub-type has been proposed to be identical to the 5-HT₄ receptor (Grider *et al.*, 1996),

it is thought to be unrelated to the 5-HT₄ receptor by others (Galligan *et al.*, 1996; 2003; Gershon, 2004). Either way, both of these receptor types are important because each has been implicated in the initiation of peristaltic reflexes (Gershon, 2004; Grider *et al.*, 1998; Jin *et al.*, 1999).

Since the discovery of 5-HT₄ receptors in 1988, significant advances have been made in our understanding of their physiology and pharmacology. The 5-HT₄ receptor is a member of the seven transmembrane spanning G-protein-coupled family of receptors (Liu *et al.*, 2005). The 5-HT₄ receptor is thought to be coupled by Gs to the stimulation of adenylyl cyclase, increase in cAMP, and activation of PKA (Bender *et al.*, 2000; Bockaert *et al.*, 1990; Liu *et al.*, 2005). The 5-HT₄ receptor is pharmacologically defined by selective agonists such as SC 53116 and RS 67506, and selective antagonists such as GR 113808, SB 204070 and RS 39604 (Gale *et al.*, 1994; Hegde *et al.*, 1996). In the gastrointestinal tract, stimulation of 5-HT₄ receptors has a pronounced effect on smooth muscle tone, mucosal electrolyte secretion, and on the peristaltic reflex (Costall *et al.*, 1993; Grider *et al.*, 1998; Hegde *et al.* 1996; Jin *et al.*, 1999; Kadowaki *et al.*, 2002). Sakurai-Yamashita *et al.* (1999, 2000) have recently reported that 5-HT₄ receptors are distributed in the human sigmoid colon, guinea pig distal colon and human rectum.

Makimoto *et al.* (2002) also recently reported that mosapride, a prokinetic benzamide which is known to be a specific enteric 5-HT₄ agonist, accelerated intestinal motor activity in parallel with increases in the release of ACh from enteric cholinergic neurons within the dog small intestine in the whole animal, which were thus mediated via 5-HT₄ receptors. *In vitro* experiments have also shown that mosapride enhances the electrically stimulated contractions of isolated guinea-pig ileal longitudinal smooth muscle with attached myenteric plexus via 5-HT₄ receptors (Mine *et al.*, 1997). Furthermore, there are several papers showing that mosapride enhances colonic motility and peristalsis in both the guinea pig (Inui *et al.*, 2002; Mine *et al.*, 1997) and rat (Kadowaki *et al.*, 2002). On the basis of these results, it would be expected that mosapride would have an effect on the motility of regions of the lower gastrointestinal tract such as the rectum and the IAS.

A specific enteric 5-HT₄ receptor agonist, mosapride, enhances both R-R and R-IAS reflexes in the chronic PITH model

Mosapride (0.1–1.0 mg/kg *i.v.*) dose-dependently increased both intrinsic R-R and R-IAS reflex indices above control levels, six to nine days following PITH. The dose-response curve was similar to that in the intact guinea pig (Shimatani *et al.*, 2003). However, marked differences between the intact and chronic PITH model guinea-pigs were found as follows: a specific 5-HT₄ receptor antagonist, GR 113808 (1.0 mg/kg) decreased the R-R and R-IAS reflex indices by approximately 50% and antagonized the effect of mosapride (1.0 mg/kg) in the chronic PITH model whereas GR 113808 (1.0 mg/kg) did not affect the both reflex indices in the intact model. The results indicated that mosapride enhanced the intrinsic R-R and R-IAS reflexes which were thus remodeled to compensate for the deficiency in extrinsic nerve function, mediated through endogenously active, intrinsic 5-HT₄ receptors that may be post-synaptically located in the myenteric plexus of the anorectum (Fig. 3).

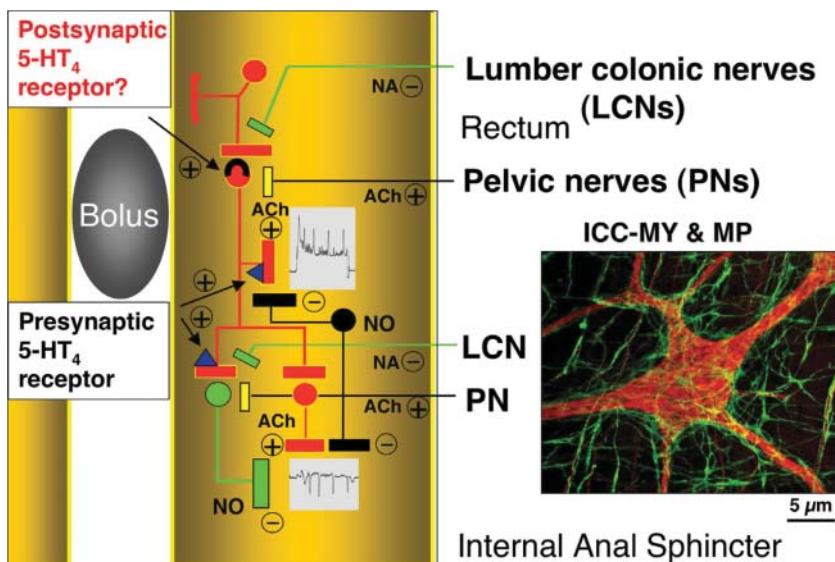


Fig. 3. Schematic illustration of the neural circuits responsible for the intrinsic defecation reflex following chronic destruction of the lumbar and sacral spinal cord (PITH) (left panel). The intrinsic defecation reflex is composed of rectal cholinergic excitatory motor nerves and internal anal sphincteric nitrergic inhibitory motor nerves. Presynaptic 5-HT₄ receptors are located on interneurons or motor neurons in intact guinea pigs. Between 6–9 days following PITH, postsynaptic 5-HT₄ receptors may express in the enteric nervous system, in addition to presynaptic 5-HT₄ receptors. The co-distribution of myenteric interstitial cells of Cajal (ICC-MY) and myenteric plexus (MP) in the rectum did not differ between 4th and 9th day following PITH (right panel). NA: noradrenaline, Ach: acetylcholine, NO: nitric oxide.

Mosapride only enhanced the neural reflex response in the rectum and IAS in guinea pigs even after chronic PITH (Kojima *et al.*, 2005). A recent report was able to clearly demonstrate that 5-HT₄ receptors are abundantly located in the enteric nervous system in the colon of guinea pig (Kim H.S. and Park H., personal communication, 2006). The densities of 5-HT₄ receptors were markedly higher in the myenteric and submucosal plexus than in the muscle layers and mucosa in the mouse bowel (Liu *et al.*, 2005).

Mosapride did not affect the spontaneous motility of the rectum and IAS, although a recent study by Liu *et al.* (2005) reported that 5-HT₄ receptors were also expressed in the mouse ileum ICC-MY.

Our results showing that mosapride synchronously enhances both reflex responses after chronic PITH, without any effects on spontaneous motor activity, strongly suggests that 5-HT₄ receptors are located on nerve terminals impinging on myenteric motor neurons of the myenteric ganglia (Fig. 3) but not on ICC-MY and/or ICC-IM, although Liu *et al.* (2005) revealed the presence of 5-HT₄ receptors on ICC-MY in the mouse ileum.

Changes in spontaneous motility in R and IAS without rectal distension

Spontaneous motility was observed in both the R and IAS without rectal distension from two to nine days following the PITH procedure. Although these motilities are almost the mirror image of each other, and the frequency and amplitude of both were decreased after treatment with TTX, significant differences in the frequency of R and IAS spontaneous motility due to TTX treatment were only detected on the 6th day after PITH, and in the frequency of IAS in controls. As TTX significantly decreased the frequency in IAS but not R, it is possible that the frequency of IAS is constitutively increased by a neuronal mechanism.

Immunoreactivity for PGP 9.5 and c-Kit

No marked changes in the myenteric plexus (MP) of the rectum were observed after PITH, compared to the control. No differences in the distribution of ICC-IM and ICC-MY were observed between the rectum and IAS. It appeared that there were no marked differences in the co-distribution of the MP and ICC-MY in the rectum between that of the control and that of the 9th day after PITH.

Conclusion

The findings reported here indicate that (1) the intrinsic R-R and R-IAS reflexes are functionally compensated after deprivation of autonomic extrinsic nerves and (2) mosapride moderately enhanced the intrinsic R-R and R-IAS reflexes mediated through endogenously active enteric neural 5-HT₄ receptors, possibly postsynaptic 5-HT₄ receptors. In addition, we propose that the present experimental procedure is a good model for exploring the ideal pharmacotherapy for the disturbance of defecation following spinal cord injury.

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