

# Increase in neurokinin-1 receptor-mediated colonic motor response in a rat model of irritable bowel syndrome

Jun-Ho La, Tae-Wan Kim, Tae-Sik Sung, Hyn-Ju Kim, Jeom-Yong Kim, Il-Suk Yang

**Jun-Ho La, Tae-Sik Sung, Hyn-Ju Kim, Il-Suk Yang**, Department of Physiology, College of Veterinary Medicine, Seoul National University, Republic of Korea

**Tae-Wan Kim**, Department of Physiology, College of Veterinary Medicine, Kyungpook National University, Republic of Korea

**Jeom-Yong Kim**, Institute of Bioscience and Biotechnology, Daewoong Pharm Co. Ltd. Republic of Korea

**Correspondence to:** Il-Suk Yang, D.V.M. Ph.D., Department of Physiology, College of Veterinary Medicine, Seoul National University, San 56-1 Sillim-Dong, Kwanak-Gu, Seoul, 151-742, Republic of Korea. isyang@snu.ac.kr

**Telephone:** +82-2-880-1261 **Fax:** +82-2-885-2732

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## Abstract

**AIM:** Irritable bowel syndrome (IBS) is a functional bowel disorder. Its major symptom is bowel dysmotility, yet the mechanism of the symptom is poorly understood. Since the neurokinin-1 receptor (NK<sub>1</sub>R)-mediated signaling in the gut is important in the control of normal bowel motor function, we aimed to investigate whether the NK<sub>1</sub>R-mediated bowel motor function was altered in IBS, using a rat IBS model that was previously reported to show colonic dysmotility in response to restraint stress.

**METHODS:** IBS symptoms were produced in male Sprague-Dawley rats by inducing colitis with acetic acid. Rats were left to recover from colitis for 6 d, and used for experiments 7 d post-induction of colitis. Motor activities of distal colon were recorded *in vitro*.

**RESULTS:** The contractile sensitivity of isolated colon to a NK<sub>1</sub>R agonist [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-substance P (1-30 nmol/L) was higher in IBS rats than that in normal rats. After the enteric neurotransmission was blocked by tetrodotoxin (TTX, 1 μmol/L), the contractile sensitivity to the NK<sub>1</sub>R agonist was increased in normal colon but not in IBS rat colon. The NK<sub>1</sub>R agonist-induced contraction was not different between the two groups when the agonist was challenged to the TTX-treated colon or the isolated colonic myocytes. A nitric oxide synthase inhibitor N<sup>ω</sup>-nitro-L-arginine methyl ester (L-NAME, 100 μmol/L) augmented the NK<sub>1</sub>R agonist-induced contraction only in normal rat colon.

**CONCLUSION:** These results suggest that the NK<sub>1</sub>R-mediated colonic motor response is increased in IBS rats, due to the decrease in the nitrergic inhibitory neural component.

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**Key words:** Irritable bowel syndrome; Neurokinin-1 receptor

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## INTRODUCTION

IBS is a functional bowel disorder, and its major clinical symptom is disordered defecation associated with abdominal pain/discomfort<sup>[1,2]</sup>. The disordered defecation can be diarrhea or constipation, or an alternating bowel habit from one to the other over time<sup>[3]</sup>. Based on the disordered defecation patterns, patients diagnosed with IBS have been divided into different subtypes such as diarrhea-predominant IBS or constipation-predominant IBS<sup>[4]</sup>. It has been suggested that the disordered defecation in IBS patients results from abnormal motor function of the colon<sup>[5-8]</sup>. However, the mechanisms underlying the disordered defecation in IBS are still poorly understood.

Researchers have consistently reported that substance P (SP) is an important enteric transmitter in the control of bowel motility<sup>[9]</sup>. Interacting mainly with the neurokinin-1 receptor (NK<sub>1</sub>R), SP can both stimulate and inhibit bowel motility by direct activation of the muscle cells and stimulation of enteric neural circuits<sup>[10]</sup>. Thus, it is highly conceivable that alterations in the NK<sub>1</sub>R-mediated signaling can cause bowel dysmotility. Indeed, pathophysiological involvement of NK<sub>1</sub>R has been shown in inflammation or stress-induced colonic dysmotility<sup>[11-14]</sup>.

Considering the importance of the NK<sub>1</sub>R-mediated signaling in normal bowel motility, one can hypothesize that the disordered defecation in IBS might be related to a disturbance in the NK<sub>1</sub>R-mediated control of colonic motility. We aimed to test this hypothesis using an animal model of IBS. Previously we reported that rats developed IBS symptoms after subsidence of acetic acid-induced colitis<sup>[15]</sup>. This animal model showed a visceral hypersensitivity and an altered defecation pattern in the absence of histological and biochemical signs of intestinal inflammation. In the colon of this rat model of IBS, we investigated whether the NK<sub>1</sub>R-mediated motor response was altered.

## MATERIALS AND METHODS

### **Experimental animals and induction of IBS**

Male Sprague-Dawley rats, weighing 270-310 g, were housed in stainless steel hanging cages in a colony room maintained under a 12 h light/dark cycle with a room temperature of 22±1 °C and a humidity of 65-70%. Water and food were available *ad libitum*. IBS symptoms were produced as described previously<sup>[15]</sup>. Briefly, colitis was induced by intracolonic instillation of 1 mL 4% acetic acid. Control animals received saline instead of acetic acid. Rats were left to recover from colitis for 6 d, and used for experiments 7 d post-induction of colitis.

### **Recording of colonic motor activities**

**Motor activity of isolated colonic segment** On the day of experiments, rats were killed by cervical dislocation, and a 2 cm distal colonic segment was removed. The segment was suspended in a 20 mL organ bath containing oxygenated (95% O<sub>2</sub> and 50 mL/L CO<sub>2</sub>) Krebs solution maintained at 37 °C. The distal end of the segment was tied around the mouth of J-tube that was connected via a 3-way connector to a syringe and to a pressure transducer (RP-1500, Narco Bio-systems Inc., USA). The proximal end of the segment was ligated with a thread that was connected to an isometric force displacement transducer

(FT-03, Grass-Telefactor, USA). The signals from both transducers were acquired by PowerLab/400 (AD Instruments, Castle Hill, Australia) and recorded on an IBM-compatible computer.

Initial 1-g tension was loaded on the colonic segment and the lumen of the segment was filled with a 0.2 mL Krebs solution per 1 cm length of the segment. The mechanical activities of the colonic segment were detected as changes in intraluminal pressure. This parameter was reported to reflect the motor activity of intestinal circular muscles<sup>[16]</sup>. After a 60-min equilibration period, drugs were cumulatively administered into the bath with a 5-min exposure time at each concentration. The effect of a drug on the colonic motor activity was quantified by measuring the mean intraluminal pressure at a given concentration. The mean intraluminal pressure was digitally calculated by dividing an integral value of pressure (area under the pressure trace) by the number of data points (tracing time). At the end of each experiment, the tonic contraction by KCl (60 mmol/L) was measured to normalize the motor activity of the isolated colon at each concentration of drugs (% of the maximal amplitude of the KCl-induced tonic contraction, % KCl).

### Contractility of isolated colonic smooth muscle cells

Smooth muscle layers from the colon were isolated, cut into small pieces and placed in nominal  $\text{Ca}^{2+}$ -free physiological salt solution ( $\text{Ca}^{2+}$ -free PSS). These segments were incubated in a medium modified from Kraft-Brühe (KB) medium<sup>[17]</sup> for 30-60 min at room temperature. They were then incubated for 20-30 min at 37 °C in digestion medium ( $\text{Ca}^{2+}$ -free PSS) containing 1.5 mg/mL collagenase type 2, 2.0 mg/mL trypsin inhibitor, 2.0 mg/mL bovine serum albumin and 0.5 mg/mL dithioerythritol. After digestion, the supernatant was discarded, the softened muscle segments were transferred again into modified KB medium, and single cells were dispersed by gentle agitation with a wide-bore glass pipette. Isolated colonic myocytes were kept in modified KB medium at 4 °C until use. All experiments were carried out at room temperature within 12 h of harvesting cells.

Isolated colonic myocytes were transferred to a stage on an inverted microscope (Olympus CK2, Japan) and allowed to stick lightly to the glass coverslip bottom of a small chamber for 10 min. The cells were then perfused with physiological salt solution (PSS) to remove cellular debris. Single smooth muscle cells were identified and cell image was digitally captured using CCD camera (TMC-7, PULNiX Inc., USA) at 0.05 frame per second (fps) rate. Cell length was measured using software for image analysis. Cell contraction was expressed as a percent decrease in cell length by a drug from control length (the length of cells before the application of drugs).

### Solutions and chemicals

The Krebs solution contained (in mmol/L) 118 NaCl, 4.7 KCl,

2.5  $\text{CaCl}_2$ , 1.2  $\text{MgSO}_4$ , 1.2  $\text{KH}_2\text{PO}_4$ , 25  $\text{NaHCO}_3$ , and 11 glucose. The  $\text{Ca}^{2+}$ -free PSS contained (in mmol/L) 135 NaCl, 5 KCl, 1.2  $\text{MgCl}_2$ , 10 glucose, and 10 HEPES (adjusted to pH 7.4 with Tris). PSS contained (in mmol/L) 135 NaCl, 5 KCl, 2  $\text{CaCl}_2$ , 10 glucose, and 10 HEPES.  $[\text{Sar}^9, \text{Met}(\text{O}_2)^{11}]$ -substance P (SP) was purchased from Tocris Cookson (Avonmouth, UK). Collagenase type 2 was purchased from Worthington (Lakewood, NJ, USA). All the following chemicals were purchased from Sigma (St. Louis, MO, USA): tetrodotoxin (TTX),  $\text{N}^{\omega}$ -nitro-L-arginine methyl ester (L-NAME), dithioerythritol, trypsin inhibitor.

### Statistical analysis

Data were expressed as mean $\pm$ SE with *n*, the number of animals. Unpaired Student's *t*-test was used for statistical comparison (at  $P < 0.05$  significance level). In case of analyzing the effect of a  $\text{NK}_1\text{R}$  agonist on normal and IBS colon before and after TTX-treatment, the significance level was adjusted using Bonferroni procedure.

## RESULTS

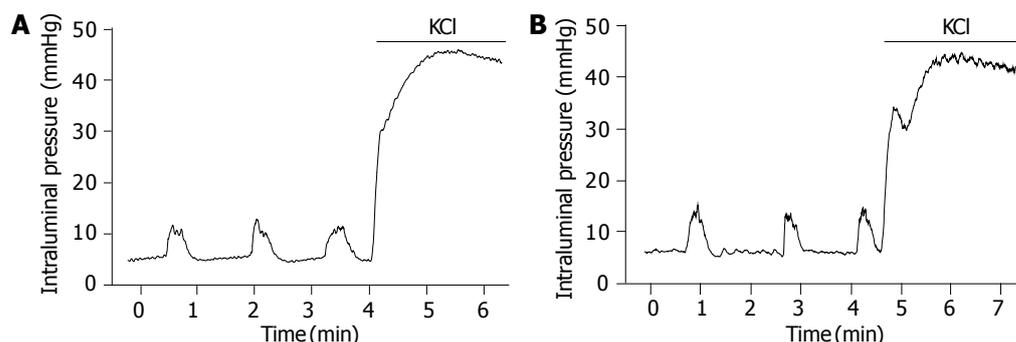
### Spontaneous motor activities of isolated colon

The isolated colonic segments showed spontaneous motor activities in rest, and tonically contracted under a high KCl (60 mmol/L) solution (Figure 1). There was no difference in the KCl-induced contraction (normalized by dividing by wet weight of the colonic segment) between normal and IBS rat colons ( $329 \pm 31$  mmHg/g vs  $326 \pm 37$  mmHg/g,  $P > 0.95$ ,  $n = 10$ ). The frequency of the spontaneous phasic contraction was  $0.79 \pm 0.08$  beat per minute (BPM) ( $n = 10$ ) in normal and  $0.77 \pm 0.07$  BPM ( $n = 10$ ) in IBS rat colon. The amplitude of the spontaneous contraction was  $13.1 \pm 1.6\%$  KCl and  $13.3 \pm 1.2\%$  KCl in normal rat colon and IBS rat colon, respectively ( $P > 0.9$ ).

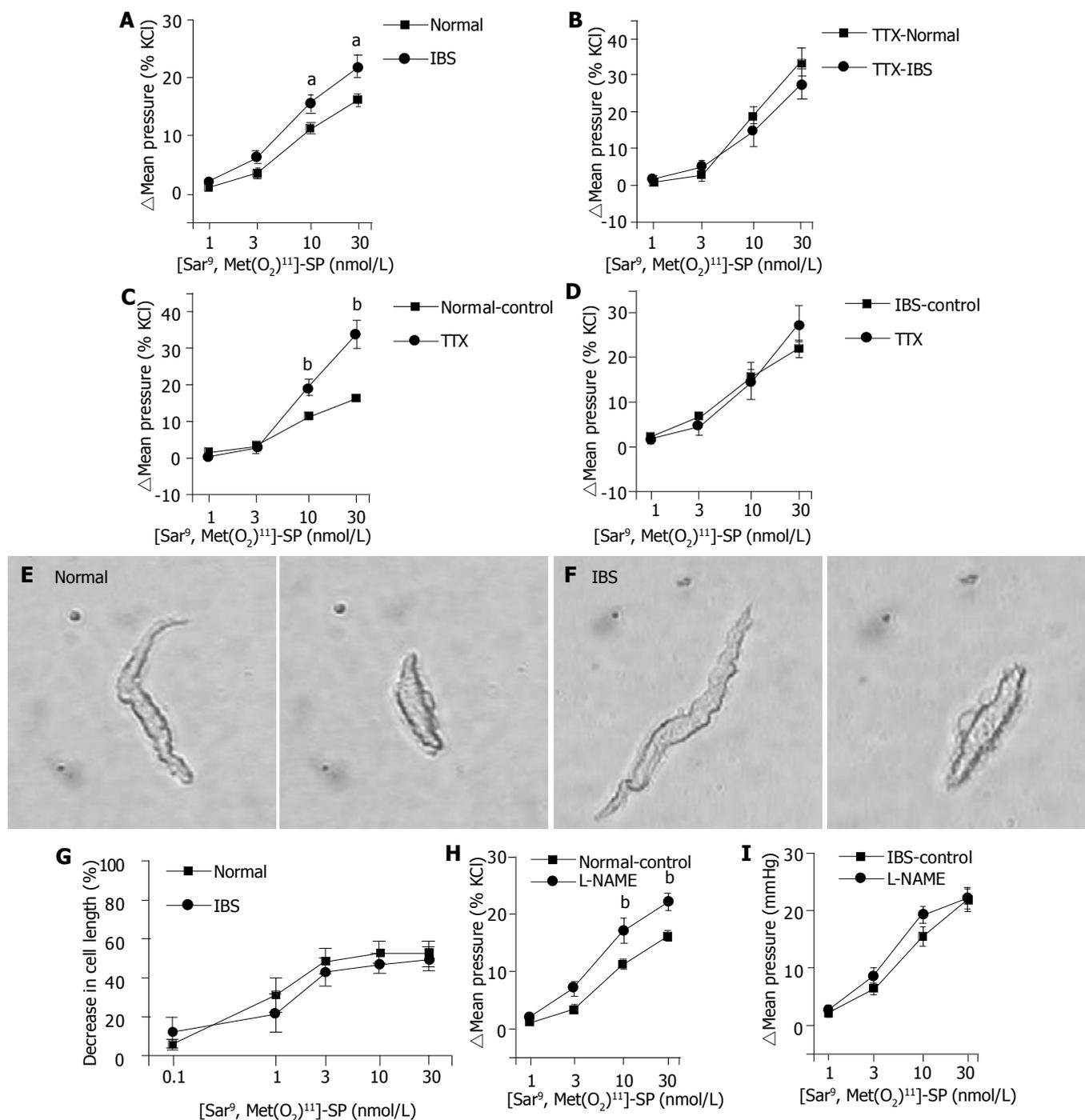
### Effect of $\text{NK}_1\text{R}$ agonist on the motor activities of isolated colon

As shown in Figure 2A, a selective  $\text{NK}_1\text{R}$  agonist,  $[\text{Sar}^9, \text{Met}(\text{O}_2)^{11}]$ -substance P (SP), contracted the circular muscle of an isolated distal colonic segment. The contractile effect of  $[\text{Sar}^9, \text{Met}(\text{O}_2)^{11}]$ -SP was more prominent in IBS rat colon than that in normal rat colon at 10 and 30 nmol/L ( $P < 0.05$ ).

Blocking the enteric neurotransmission with TTX (1  $\mu\text{mol/L}$ ) increased the resting mean intraluminal pressure by  $9.7 \pm 1.1\%$  KCl ( $n = 8$ ) in normal rat colon and by  $8.0 \pm 1.7\%$  KCl ( $n = 7$ ) in IBS rat colon ( $P = 0.4$ ). The contractile effect of  $[\text{Sar}^9, \text{Met}(\text{O}_2)^{11}]$ -SP was not different between the two groups under the presence of TTX (Figure 2B). In the TTX-pretreated normal rat colon ( $n = 8$ ), the contractile effect of  $[\text{Sar}^9, \text{Met}(\text{O}_2)^{11}]$ -SP was increased to  $19.0 \pm 2.2\%$  KCl ( $P < 0.01$  vs  $11.2 \pm 0.9\%$  KCl) and to  $33.5 \pm 3.8\%$  KCl ( $P < 0.01$  vs  $16.3 \pm 0.9\%$  KCl) at 10 and 30 nmol/L, respectively (Figure 2C), which implied that an inhibitory neural component was involved in the  $\text{NK}_1\text{R}$  agonist-induced contraction in



**Figure 1** Motor activities of circular muscle in isolated colonic segments. Motor activities of circular muscle were measured as changes in intraluminal pressure in (A) normal rat colon and (B) IBS rat colon. No difference was observed between groups in the spontaneous rhythmic phasic contraction and in the KCl (60 mmol/L)-induced tonic contraction.



**Figure 2** Contractile effect of NK<sub>1</sub>R agonist on isolated distal colonic segments, isolated colonic myocytes, and NOS inhibitor-pretreated isolated colonic segments. **A:** The contractile sensitivity of IBS rat colon to [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP was higher than that of normal rat colon. <sup>a</sup>*P*<0.05 *vs* normal by Student's *t*-test with Bonferroni correction; **B:** Under the presence of TTX (1 μmol/L), no statistical difference was detected between groups in the [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP-induced contraction; **C** and **D:** TTX increased the [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP-induced contraction in normal rat colon but not in IBS rat colon. <sup>b</sup>*P*<0.01 *vs* control by Student's *t*-test with Bonferroni correction (*n* = 12 in normal control, 9 in IBS control, 8 in TTX-normal, 7 in TTX-IBS); **E** and **F:** Photographs of myocytes in normal and IBS groups under control condition (left), and under the presence of 30 nmol/L [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP (right). Bar = 30 μm. **G:** Dose-response plot showing the contractile effect of [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP on the isolated colonic myocytes. The [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP-induced contraction was measured as a percent decrease in cell length (*n* = 7 in normal, 8 in IBS); **H** and **I:** Normal and IBS rat colonic segments were incubated with a NOS inhibitor L-NAME (0.1 mmol/L) for 10 min before the cumulative administration of [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP. <sup>b</sup>*P*<0.01 *vs* control by Student's *t*-test (**H:** *n* = 12 in control, 7 in L-NAME. **I:** *n* = 9 in control, 6 in L-NAME).

normal rat colon. In IBS rat colon (*n* = 7), the contraction at each concentration of [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP was not significantly changed by TTX pretreatment (Figure 2D).

#### Effect of NK<sub>1</sub>R agonist on the motor activities of isolated colonic myocytes

The initial length of isolated colonic myocytes was 77.8±3.2 μm

(*n* = 7) and 70.5±2.7 μm (*n* = 8) in normal and IBS groups, respectively (*P*>0.09). [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP concentration-dependently decreased the length of isolated muscle cells. At the highest concentration (30 nmol/L), the cell length was decreased by 52.3±6.4% in normal group and 50.1±6.0% in IBS group. The response of muscle cells to [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP was not different between the two groups (Figures 2E-G).

### Effect of NK<sub>1</sub>R agonist on the motor activities of isolated colon under the presence of NOS inhibitor

Pretreatment of a NOS inhibitor L-NAME (0.1 mmol/L) increased the resting mean intraluminal pressure by 10.7±1.5% KCl ( $n=7$ ) in normal rat colon and by 12.9±3.4% KCl ( $n=6$ ) in IBS rat colon ( $P=0.55$ ). In normal rat colon ( $n=7$ ), the contractile effect of [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP was increased by the pretreatment of L-NAME to 17.2±2.2% KCl ( $P<0.01$  vs 11.2±0.9% KCl), and to 22.1±1.5% KCl ( $P<0.01$  vs 16.3±0.9% KCl) at 10 and 30 nmol/L, respectively. On the other hand, L-NAME was ineffective to augment the contractile effect of [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP in IBS rat colon (Figure 2H, 2I).

### DISCUSSION

In the present study, we found that the NK<sub>1</sub>R-mediated colonic motor response was altered in a rat model of IBS. A selective NK<sub>1</sub>R agonist [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP contracted IBS rat colon more potently than normal rat colon. Because SP could stimulate both intestinal smooth muscle cells and enteric inhibitory nerves<sup>[10]</sup>, we hypothesized that the higher contractile sensitivity of IBS rat colon to the NK<sub>1</sub>R agonist resulted from increased contractile response of muscle cells, and/or decreased response of enteric inhibitory nerves to the NK<sub>1</sub>R agonist. Our results support the second hypothesis. In normal rat colon, the contractile effect of [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP was enhanced by a neurotransmission blocker TTX, whereas that in IBS rat colon was not significantly changed by TTX. Furthermore, the [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP-induced contraction was not different between the two groups when the agonist was challenged to the TTX-treated isolated colon or directly to the isolated myocytes. These data indicate that the higher contractile sensitivity of IBS rat colon to [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP results from the decreased enteric inhibitory neural components rather than the increased contractile response of muscle cells.

Recently, enteric nitrergic inhibitory nerves were reported to participate in the NK<sub>1</sub>R-mediated control of peristalsis in isolated guinea-pig ileum<sup>[18]</sup> and in isolated rabbit distal colon<sup>[19]</sup>. Therefore, we supposed that nitrergic inhibitory nerves were the inhibitory neural components activated by [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP. Expectedly, we found that the [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-SP-induced contraction was augmented by the suppression of nitrergic inhibitory transmission with L-NAME in normal rat colon but not in IBS rat colon. Putting these lines of evidence together, it can be concluded that the increased NK<sub>1</sub>R-mediated contraction in IBS rat colon results from the decreased NK<sub>1</sub>R-mediated activation of enteric nitrergic inhibitory nerves.

Considering that the IBS rats used in this study developed IBS symptoms after subsidence of colitis, it is worthy to mention that intestinal inflammation could induce profound changes in enteric nerves, which might persist long after the inflammation subsided<sup>[20,21]</sup>. In addition, there have been studies reporting the dysfunction of enteric nitrergic nerves in animals with gut inflammation. Researchers have shown the decreased nNOS-immunoreactivities in TNBS-induced colitic rats<sup>[22]</sup>, the reduced activity and synthesis of nNOS in DSS-induced colitic rats<sup>[23]</sup>, and the diminished NO-mediated relaxation in nematode-infected mice<sup>[24]</sup>. Thus, it seems likely that alterations of enteric nitrergic neural function by colitis can persist in the colon of IBS rats, causing a higher contractile sensitivity of IBS colon to the NK<sub>1</sub>R agonist.

Since nitrergic nerves are tonically active in rat colon<sup>[25,26]</sup>, one would expect that dysfunction of enteric nitrergic nerves results in alterations of the resting colonic motility. However, we observed that the resting motility of the isolated colon was not different between normal group and IBS group (Figure 1). Moreover, the extent of the increase in the resting colonic motor

activities by a NOS inhibitor was similar in the two groups, suggesting that the tonic nitrergic inhibition of resting motility is maintained in IBS rat colon. This seems incompatible with our aforementioned conclusion that the decreased enteric nitrergic inhibitory neural components in IBS rat colon causes the increased NK<sub>1</sub>R-mediated colonic motor response. One of the possible explanations for this discrepancy is that the NK<sub>1</sub>R-mediated signaling pathways do not modulate the tonic inhibitory action of nitrergic nerves and hence have no influence on the resting colonic motility. Supporting this notion, Mule *et al*<sup>[27]</sup> reported that the resting spontaneous motility of rat colon was not affected by a NK<sub>1</sub>R antagonist but inhibited by several NK<sub>2</sub>R antagonists, indicating that the NK<sub>1</sub>R-mediated signaling pathways do not contribute to the control of the resting motility in rat colon.

In conclusion, the present results indicate that the NK<sub>1</sub>R-mediated contraction is exaggerated in the colon of rat IBS model. The higher contractile sensitivity of IBS rat colon to the NK<sub>1</sub>R agonist appears to result from the decreased enteric nitrergic inhibitory neural components rather than the increased contractile response of muscle cells. These results suggest that disordered defecation in IBS patients, especially who develop IBS after intestinal inflammation, might be related to the alterations in the NK<sub>1</sub>R-mediated control of bowel motility.

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