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

INTRODUCTION
IBS is a functional bowel disorder, and its major clinical symptom is disordered defecation associated with abdominal pain/discomfort[1,2]. The disordered defecation can be diarrhea or constipation, or an alternating bowel habit from one to the other over time[3]. 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[4]. It has been suggested that the disordered defecation in IBS patients results from abnormal motor function of the colon[5-44]. 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[45]. Interacting mainly with the neurokinin-1 receptor (NK,R), SP can both stimulate and inhibit bowel motility by direct activation of the muscle cells and stimulation of enteric neural circuits[46]. Thus, it is highly conceivable that alterations in the NK,R-mediated signaling can cause bowel dysmotility. Indeed, pathophysiological involvement of NK,R has been shown in inflammation and stress-induced colonic dysmotility[1-14].

Considering the importance of the NK,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,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 subcutaneous injection of acetic acid-induced colitis[15]. 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,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[16]. 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% O2 and 50 mL/L CO2) 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-1,500, 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.
The Krebs solution contained (in mmol/L) 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, and 11 glucose. The Ca²⁺-free PSS contained (in mmol/L) 135 NaCl, 5 KCl, 1.2 MgCl₂, 10 glucose, and 10 HEPES (adjusted to pH 7.4 with Tris). PSS contained (in mmol/L) 135 NaCl, 5 KCl, 2 CaCl₂, 10 glucose, and 10 HEPES. [Sar⁹,Met(O)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), N⁷-nitro-L-arginine methyl ester (L-NAME), dithioerythritol, trypsin inhibitor.

**Statistical analysis**

Data were expressed as mean±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 NK,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 with wet weight of the colonic segment) between normal and IBS rat colons (329±31 mmHg/g vs 326±37 mmHg/g, P>0.95, n = 10).

The frequency of the spontaneous phasic contraction was 0.79±0.08 beat per minute (BPM) (n = 10) in normal and 0.77±0.07 BPM (n = 10) in IBS rat colon. The amplitude of the spontaneous contraction was 13.1±1.6% KCl and 13.3±1.2% KCl in normal rat colon and IBS rat colon, respectively (P>0.9).

**Effect of NK,R agonist on the motor activities of isolated colon**

As shown in Figure 2A, a selective NK,R agonist, [Sar⁹,Met(O)¹¹] substance P (SP), contracted the circular muscle of an isolated distal colonic segment. The contractile effect of [Sar⁹, Met(O)¹¹] SP was more prominent in IBS rat colon than that in normal rat colon at 10 and 30 mmol/L (P<0.05).

Blocking the enteric neurotransmission with TTX (1 μmol/L) increased the resting mean intraluminal pressure by 9.7±1.1% KCl (n = 8) in normal rat colon and by 8.0±1.7% KCl (n = 7) in IBS rat colon (P = 0.4). The contractile effect of [Sar⁹,Met(O)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 [Sar⁹,Met(O)¹¹]-SP was increased to 19.0±2.2% KCl (P = 0.01 vs 11.2±0.9% KCl) and to 33.5±3.8% KCl (P<0.01 vs 16.3±0.9% KCl) at 10 and 30 mmol/L, respectively (Figure 2C, which implied that an inhibitory neural component was involved in the NK,R agonist-induced contraction in IBS rat colon.

**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.
normal rat colon. In IBS rat colon (n = 7), the contraction at each concentration of [Sar₉, Met(O₂)¹¹]-SP was not significantly changed by TTX pretreatment (Figure 2D).

**Effect of NK₁R 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). [Sar₉, Met(O₂)¹¹]-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 [Sar₉, Met(O₂)¹¹]-SP was not different between the two groups (Figures 2E-G).

**Figure 2** Contractile effect of NK₁R 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 [Sar₉, Met(O₂)¹¹]-SP was higher than that of normal rat colon. aP<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 [Sar₉, Met(O₂)¹¹]-SP-induced contraction; C and D: TTX increased the [Sar₉, Met(O₂)¹¹]-SP-induced contraction in normal rat colon but not in IBS rat colon. bP<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 [Sar₉, Met(O₂)¹¹]-SP (right). Bar = 30 μm. G: Dose-response plot showing the contractile effect of [Sar₉, Met(O₂)¹¹]-SP on the isolated colonic myocytes. The [Sar₉, Met(O₂)¹¹]-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 [Sar₉, Met(O₂)¹¹]-SP. bP<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).
Effect of NK,R agonist on the motor activities of isolated colonic sphincter 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% KC1 (n = 7) in normal rat colon and by 12.9±3.4% KC1 (n = 6) in IBS rat colon (P = 0.55). In normal rat colon (n = 7), the contractile effect of [Sar9,Met(O2)11]-SP was increased by the pretreatment of L-NAME to 17.2±2.2% KC1 (P = 0.01 vs 11.2±0.9% KC1), and to 22.1±1.5% KC1 (P < 0.01 vs 16.3±0.9% KC1) at 10 and 30 mmol/L, respectively. On the other hand, L-NAME was ineffective to augment the contractile effect of [Sar9,Met(O2)11]-SP in IBS rat colon (Figure 2H, 2I).

DISCUSSION

In the present study, we found that the NK,R-mediated colonic motor response was altered in a rat model of IBS. A selective NK,R agonist [Sar9,Met(O2)11]-SP contracted IBS rat colon more potently than normal rat colon. Because SP could stimulate both intestinal smooth muscle cells and enteric inhibitory nerves,[9] we hypothesized that the higher contractile sensitivity of IBS rat colon to the NK,R agonist resulted from increased contractile response of muscle cells, and/or decreased response of enteric inhibitory nerves to the NK,R agonist. Our results support the second hypothesis. In normal rat colon, the contractile effect of [Sar9,Met(O2)11]-SP was enhanced by a neurotransmitter blocker TTX, whereas in IBS rat colon was not significantly changed by TTX. Furthermore, the [Sar9,Met(O2)11]-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 [Sar9,Met(O2)11]-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,R-mediated control of peristalsis in isolated guinea-pig ileum[10] and in isolated rabbit distal colon[11]. Therefore, we supposed that nitrergic inhibitory nerves were the inhibitory neural components activated by [Sar9,Met(O2)11]-SP. Expectedly, we found that the [Sar9,Met(O2)11]-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,R-mediated contraction in IBS rat colon results from the decreased NK,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[20,21]. In addition, there have been studies reporting that dysfunctions of enteric nitrergic nerves in animals with gut inflammation. Researchers have shown the decreased NOS-immunoreactivities in TNBS-induced colitic rats[21], the reduced activity and synthesis of nNOS in DSS-induced colitic rats[22], and the diminished NO-mediated relaxation in nematode-infected mice[24]. Thus, it seems likely that alterations of enteric nitrergic neural function by colitis can persist in the colon of IBS rat colon. Putting these lines of evidence together, it can be concluded that altered nitrergic inhibitory neural components rather than increased contractile response of muscle cells.

In conclusion, the present results indicate that the NK,R-mediated contraction is exaggerated in the colon of IBS model. The higher contractile sensitivity of IBS rat colon to the NK,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 disorders of defecation in IBS patients, especially who develop IBS after intestinal inflammation, might be related to the alterations in the NK,R-mediated control of bowel motility.

REFERENCES

1 Camilleri M, Heading RC, Thompson WG. Clinical perspectives, mechanisms, diagnosis and management of irritable bowel syndrome. Aliment Pharmacol Ther 2002; 16: 1407-1430
2 Dressman DA. Review article: an integrated approach to the irritable bowel syndrome. Aliment Pharmacol Ther 1999; 13: 3-14
3 Wood JD. Neuropathophysiology of irritable bowel syndrome. J Clin Gastroenterol 2002; 35: S11-S22
4 Clemens CH, Samsom M, Van Berge Henegouwen GP, Smout AJ. Abnormalities of left colonic tone in ambulant nonconstipated patients with irritable bowel syndrome. Dig Dis Sci 2003; 48: 74-82
5 Chaudhary NA, Truelove SC. Human colonic motility. A comparative study of normal subjects, patients with ulcerative colitis, and patients with the irritable colon syndrome. Gastroenterology 1968; 54: 777-uppl:778
6 Chey WY, Jin HO, Lee MH, Sun SW, Lee KY. Colonic motility abnormality in patients with irritable bowel syndrome exhibiting abdominal pain and diarrhea. Am J Gastroenterol 2001; 96: 1499-1506
7 Connell AM. Intestinal motility and the irritable bowel. Postgrad Med J 1984; 60: 791-796
8 Vassallo MJ, Camilleri M, Phillips SF, Steadman CJ, Talley NJ, Hanson RB, Haddad AC. Colonic tone and motility in patients with irritable bowel syndrome. Mayo Clin Proc 1992; 67: 725-731
9 Holzer P, Holzer-Petsche U. Tachykinins in the gut. Part I. Expression, release and motor function. Pharmacol Ther 1997; 73: 173-217
10 Scherer U, Drack E, Halter F. Substance P activates rat colonic motility via excitatory and inhibitory neural pathways and direct action on muscles. J Pharmacol Exp Ther 1994; 271: 7-13
11 Castagliuolo I, Lamont JT, Qiu B, Fleming SM, Bhaskar KR, Niklasson ST, Kornetsky C, Pothoulakis C. Acute stress causes mucin release from rat colon: role of corticotropin releasing factor and mast cells. Am J Physiol 1996; 271: G884-G892
12 Di Sebastiano P, Grossi L, Di Mola FF, Angelucci D, Frisell H, Marzio L, Innocenti P, Buchler MW. SR140333, a Substance P receptor antagonist, influences morphological and motor changes in rat experimental colitis. Dig Dis Sci 1999; 44: 439-444
13 Ikeda K, Miyata K, Orita A, Kubota H, Yamada T, Tomioka...
K. RP67580, a neurokinin1 receptor antagonist, decreased restraint stress-induced defecation in rat. *Neurosci Lett* 1995; 198: 103-106

14 Okano S, Nagaya H, Ikeura Y, Natsugari H, Inatomi N. Effects of TAK-637, a novel neurokinin-1 receptor antagonist, on colonic function in vivo. *J Pharmacol Exp Ther* 2001; 298: 559-564

15 La JH, Kim TW, Sung TS, Kang JW, Kim HJ, Yang IS. Visceral hypersensitivity and altered colonic motility after subsidence of inflammation in a rat model of colitis. *World J Gastroenterol* 2003; 9: 2791-2795

16 Coupar IM, Liu L. A simple method for measuring the effects of drugs on intestinal longitudinal and circular muscle. *J Pharmacol Toxicol Methods* 1996; 36: 147-154

17 Isenberg G, Klockner U. Calcium tolerant ventricular myocytes prepared by preincubation in a “KB medium”. *Pflugers Arch* 1982; 395: 6-18

18 Holzer P. Involvement of nitric oxide in the substance P-induced inhibition of intestinal peristalsis. *Neuroreport* 1997; 8: 2857-2860

19 Onori L, Aggio A, Taddei G, Loreto MF, Cicciocoppo R, Vicini R, Tonini M. Peristalsis regulation by tachykinin NK1 receptors in the rabbit isolated distal colon. *Am J Physiol Gastrointest Liver Physiol* 2003; 285: G325-G331

20 Sanovic S, Lamb DP, Blennerhassett MG. Damage to the enteric nervous system in experimental colitis. *Am J Pathol* 1999; 155: 1051-1057

21 Tornbloh H, Lindberg G, Nyberg B, Veress B. Full thickness biopsy of the jejunum reveals inflammation and enteric neuropathy in irritable bowel syndrome. *Gastroenterology* 2002; 123: 1972-1979

22 Miampamba M, Sharkey KA. Temporal distribution of neuronal and inducible nitric oxide synthase and nitrotyrosine during colitis in rats. *Neuropathol Motil* 1999; 11: 193-206

23 Mizuta Y, Isomoto H, Takahashi T. Impaired nitrergic innervation in rat colitis induced by dextran sulfate sodium. *Gastroenterology* 2000; 118: 714-723

24 Barbara G, Vallance BA, Collins SM. Persistent intestinal neuromuscular dysfunction after acute nematode infection in mice. *Gastroenterology* 1997; 113: 1224-1232

25 Middleton SJ, Cuthbert AW, Shorthouse M, Hunter JO. Nitric oxide affects mammalian distal colonic smooth muscle by tonic neural inhibition. *Br J Pharmacol* 1993; 108: 974-979

26 Mule F, D’Angelo S, Amato A, Contino I, Serio R. Modulation by nitric oxide of spontaneous mechanical activity in rat proximal colon. *J Auton Pharmacol* 1999; 19: 1-6

27 Mule F, D’Angelo S, Tabacchi G, Serio R. Involvement of tachykinin NK2 receptors in the modulation of spontaneous motility in rat proximal colon. *Neuropathol Motil* 2000; 12: 459-466

Edited by Wang XL