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Original

Effect of colonic distension on gastric adaptive relaxation in rats: barostatic evaluation using an orally introduced gastric balloon

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Abstract

While the gastrocolonic reflex has been known, the cologastric relationship has not been clarified especially with regard to gastric adaptive relaxation. Therefore, in this study we have examined the correlation between gastric adaptive relaxation and colonic distension. Male Sprague-Dawley rats were used after fasting for 18 hrs. Colonic distension was performed by injecting 2.2 ml of air into a colonic balloon inserted into the colon for 5 min in conscious state. After urethane anesthesia, gastric adaptive relaxation was investigated by using a slightly modified gastric balloon introduced into the stomach through the mouth. Gastric balloon volumes increased gradually just after an increment in the gastric balloon pressure (1 to 8 mmHg), and reached a plateau within 1 min. This increased volume was defined as gastric adaptive relaxation. In control rats, gastric adaptive relaxation increased with pressure increments in a pressure dependent manner. In the colon-distended rats, gastric adaptive relaxation increased also in a pressure dependent manner, but was significantly inhibited as compared with control at 8 mmHg ($P<0.05$). These findings show that colonic distension inhibits the gastric adaptive relaxation and suggests the existence of a cologastric relationship in rats.

Key words: gastric adaptive relaxation, stomach of rat, colonic distension, cologastric relationship

Introduction

Cannon and Lieb (1) described gastric adaptive relaxation. Gastric adaptive relaxation results in an increased volume in the gastric fundus. It is thought to be a vagally mediated reflex that occurs postprandially and results in reduction of the smooth muscle tone, providing a larger reservoir for the ingested meal (2).

In 2008, Boccia et al. (3) reported that the majority of children with functional dyspepsia were affected by functional constipation associated with delayed gastric emptying and found that normalization of bowel habit may improve gastric emptying as well as dyspeptic symptoms. In addition, van Hoek et al. (4) found that distension of the rectum influenced postprandial release of gut hormones involved in the regulation of gastro-

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intestinal motility in healthy subjects. These findings strongly suggest the existence of a cologastric relationship. Indeed, Ley et al. (5) reported that rectal distension inhibits gastric tone in a volume-dependent manner and impairs gastric accommodation in dogs, and suggested that the observed effect may not be mediated by a cholinergic pathway.

Barostat studies have been used for the investigation of gastric functions, such as changes in gastric pressure or adaptive relaxation of the stomach in clinical studies. On the contrary, in basic studies using experimental animals, gastric adaptive relaxation has been evaluated by measuring the changes in intra-gastric pressure by either surgically inserting the pressure transducer into the rat stomach (6–8), by connecting a pressure transducer to the isolated guinea pig stomach (9, 10) or by inserting the gastric balloon into the rat stomach via an incision in the fundus (11). In our previous report (12), we improved the method to investigate the gastric adaptive relaxation using a barostat without surgical intervention, because surgical procedures are likely to affect normal gastric physiological function in an *in vivo* study, and also because *in vitro* studies do not necessarily reflect the *in vivo* state.

Thus, in this study we investigated the existence of a cologastric relationship by observing the changes in gastric adaptive relaxation caused by colonic distension using our previously reported method (12).

Materials and Methods

The following animal studies were performed in accordance with the *Guiding Principles for the Care and Use of Laboratory Animals* approved by Meiji Co. Ltd.

Animals

Male Sprague-Dawley rats (230–280 g) were purchased from SLC (Shizuoka, Japan) and kept for 1 week in a room where the temperature and humidity were kept at 21 ± 2°C and 55 ± 15%, respectively. The animals were fasted for 18 h before each experiment, being held in mesh cages to prevent coprophagy, with free access to drinking water.

Gastric barostat studies

Gastric barostat studies were performed according to our previous report (12). Rats were anesthetized with urethane (1.2g/kg, i.p.). In this study, a slightly improved gastric balloon was used. A pair of polyvinyl tubes attached to a polyethylene bag (maximum volume 7 ml; 3 cm maximum diameter) was introduced through the mouth into the stomach as shown in Fig. 1. Five ml of air was injected into the gastric balloon from one of the gastric balloon tubes with the other side gastric balloon tube closed to allow placement of the gastric balloon within the stomach, after which the gastric balloon tubes were immediately opened to the air. After a 5 min recovery period, the tubes of the gastric balloon were connected to the barostat (Barostat Distender IIR, G&J Electronics, Toronto, Canada).

The pressure inside the gastric balloon was increased stepwise from 1 through 2, 4 and 8 mmHg, at 1 min intervals. The volume of the gastric balloon increased sharply with each change in pressure. The gastric balloon volume increased gradually just after the change of pressure and reached a plateau after about 1 min following the change of pressure. The increased volume was defined as gastric adaptive relaxation.

After each barostat study, the position of the balloon was checked by laparotomy. If the position of the balloon was not appropriate, the obtained datum was deleted.

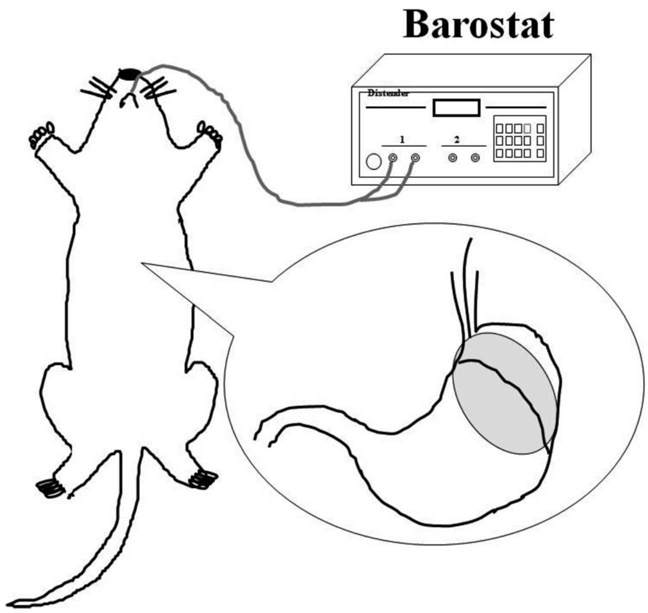

Fig. 1. Schematic representation of the experimental set-up. The gastric balloon was introduced through the mouth into the stomach in anesthetized rats without surgery and placed between the fundus and fore-stomach as shown as the shaded oval in the figure.

Effect of colonic distension on the gastric adaptive relaxation

The colonic balloon whose volume was 5 ml (Star Medical, Tokyo) was intubated into the colon at a distance of 8 cm from the anus. A 2.2 ml volume of air was blown into the colonic balloon and maintained there for 5 min. After releasing the pressure, the colonic balloon was pulled out. After pulling out the colonic balloon, the rat was immediately anesthetized. Five min later, the gastric balloon was introduced into the stomach and the gastric barostat study performed. In control rats, sham intubation of the colonic balloon was performed.

The final volume of the gastric balloon was measured after each pressure loading and the effect of colonic distension was evaluated.

In the present study, we distended the colon at a distance of 8 cm from the anus. This position was selected because this position influenced gastric emptying by inducing colitis (preliminary study). The volume of 2.2 ml was determined not to cause bleeding from the colonic mucosa.

Time course of the study

The conscious rats were held gently by hand and the colonic balloon was introduced into the colon and pulled out after 5 min. Just after colonic distension, the rats were immediately anesthetized by urethane and a gastric balloon introduced 5 min after anesthesia. The Barostat study was started 5 min after gastric balloon intubation. It takes 15 min from the colonic distension until the start of the barostat study.

In this study, we distended the colon in the conscious state, because we expected nervous reflexes would influence gastric accommodation. However, the barostat study could not be done in the conscious state. The rats were anesthetized using urethane just after the colonic distension so that the effect of colonic distension would not fade away.

Data analysis

All results are presented as the mean ± S.E.M. Statistical analyses were performed by using Stat View, Version 5.0.0.0 (SAS Institute Inc., USA), and P values <0.05 (Two-way repeated measures analysis of variance (ANOVA), Bonferroni post test and Student's t-test) were considered to be statistically significant.

Results

Gastric barostat studies

With the increment in gastric balloon pressure, gastric adaptive relaxation increased in a pressure-dependent manner as shown in Fig. 2.

Effect of colonic distension on the gastric accmmodation

In control rats, gastric adaptive relaxation increased in a pressure-dependent manner with the increment in gastric balloon pressure as shown in Fig. 3. On the contrary, in the colonic distended rats, a significant decrease in the volume of gastric adaptive relaxation was observed with a significant difference observed at 8 mmHg, although gastric adaptive relaxation increased also in a pressure-dependent manner (Fig. 3).

Fig. 4 shows the final volume of the gastric balloon after the 8 mmHg pressure loading. While a lower final volume was observed as a result of colonic distension as compared with control, there was no significant difference observed (Fig. 4).

Discussion

Functional dyspepsia is the presence of symptoms thought to originate from the gastro-duodenal region, in the absence of organic, systemic, or metabolic disease that could explain the symptoms (13). In patients with irritable bowel syndrome, postprandial worsening of symptoms is frequently reported (14, 15). The mechanism behind this has largely been attributed to an exaggerated motor response of the colon after a meal as compared with healthy people (16, 17). These findings show the existence of a gastrocolonic reflex.

On the other hand, Boccia et al. (3) reported that the majority of children with functional dyspepsia were affected by functional constipation associated with delayed gastric emptying, and that normalization of their bowel habits improved gastric emptying as well as the dyspeptic symptoms. Martínez et al. (18) reported that distension of the proximal colon significantly inhibited gastric emptying by 82% and 34% as measured 30 and 60 min after the distension, respectively, in Wistar Kyoto rats as compared with control rats. These findings strongly show that the colonic distension delays gastric emptying and suggest the existence of cologastric relationship.

Sanaka et al. (19) reported that the delay of gastric emptying may be related to the gastric adaptive relaxation observed during proton pump inhibitor therapy, suggesting that the enhancement of gastric adaptive relaxation induces the delay of gastric emptying. Although there are many reports showing the correlation between the colonic distension and gastric emptying, Ley et al. (5) reported a correlation between rectal distension and gastric accommodation in dogs. Thus in the present study, we aimed to clarify the correlation between colonic distension and gastric accommodation using barostat studies in rats.

In this study we have used our method to investigate the gastric adaptive relaxation using a modified gastric balloon introduced via the mouth without surgical intervention. In this way there should be minimal effect on gastric physiological function although under anesthesia. We observed gastric adaptive relaxation with each

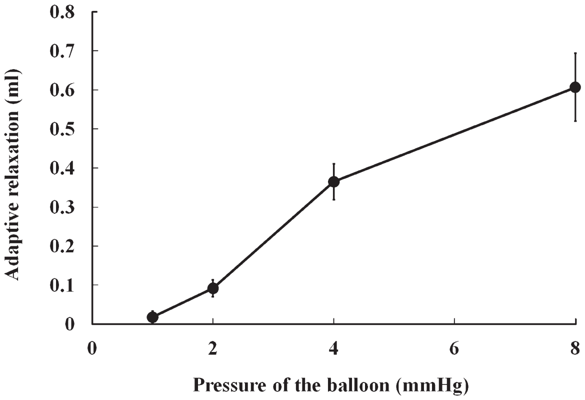

Fig. 2. Effect of gastric balloon pressure on the gastric adaptive relaxation. With increments in gastric balloon pressure, gastric adaptive relaxation increased in a pressure-dependent manner. Symbols represent the mean and standard error of the mean (SEM) of 6 rats.

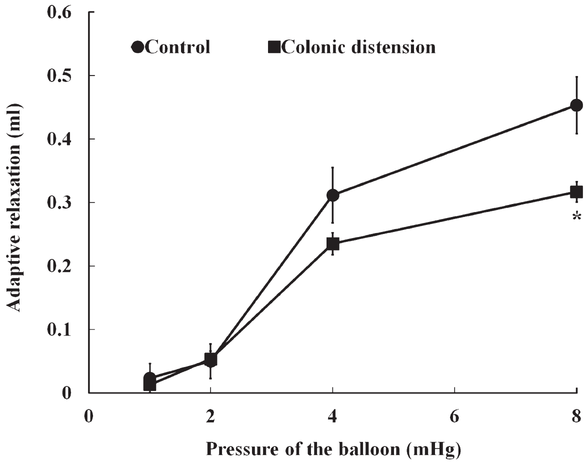

Fig. 3. The effect of the colonic distension on the gastric adaptive relaxation. Symbols represent the mean and standard error of the mean (SEM) of 6 rats. *; Significant difference from the control group (P<0.05).

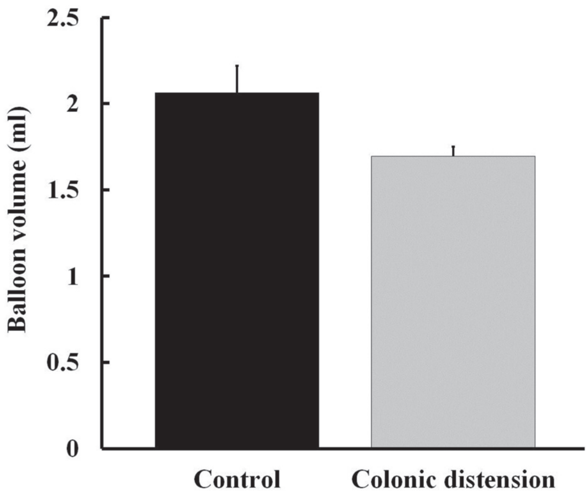

Fig. 4. The effect of the colonic distension on the gastric balloon volume after 8 mmHg loading. Columns represent the mean and standard error of the mean (SEM) of 6 rats. No significant difference was observed between two groups.

pressure increment as shown in Fig. 3. This finding is in accordance with our previous report (12). With colonic distension, a significantly reduced gastric adaptive relaxation was observed by increasing the gastric balloon pressure. In addition, the final volume of the gastric balloon at 8 mmHg showed a lower value as compared with control rats, although no significant difference was observed. These findings show that the colonic distension inhibits the gastric adaptive relaxation in our experimental condition.

On the mechanism of gastric adaptive relaxation, nitric oxide (NO) plays an important role as reported by many investigators (20–22). We also reported (12) that NO is involved in the gastric adaptive relaxation using N^{ω}-Nitro-L-arginine methyl ester (L-NAME) which inhibits NO synthase non-selectively (23) and capsaicin, which releases NO and calcitonin gene-related peptide (24). Güal et al. (25) found that sex steroids have a modulatory role on the feedback control of gastric motility induced by noxious colonic distension. Gué et al. (26) reported that fedotozine, a proton pump inhibitor (PPI), acts through kappa receptors to block the colonic distension-induced delay on gastric motility and emptying, and found that the cologastric reflex involves nicotinic ganglionic receptors but not adrenergic pathway and 5-hydroxytryptamine 3 (5-HT_3) receptors. Sanaka et al. (19) found a delayed gastric emptying using the same PPI, and suggested that this delay may be caused by the enhancement of gastric adaptive relaxation, although no mechanism was clarified. In this study we have observed an inhibition of gastric adaptive relaxation as a result of colonic distension. If colonic distension induced inhibition of gastric emptying as observed in many reports, the enhancement of gastric adaptive relaxation would have been expected in the present study, but this did not occur under our experimental condition. In the present study, the distension position was in the middle of the colon. The middle colon appears to be innervated by the pelvic nerve. Therefore, the present cologastric relationship may be induced by hormonal effects, because the barostat study was performed 15 min after the colonic distension. With regard to these points, further studies would be needed to clarify the correlation between gastric emptying, gastric adaptive relaxation and colonic distension, and the mechanisms involved.

In conclusion, the results obtained in the present study show that colonic distension inhibits gastric adaptive relaxation through a cologastric relationship.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Cannon WB, Lieb CW. The receptive relaxation of the stomach. Am J Physiol. 1911; 29: 267–73.
2. Choung RS, Talley NJ. Novel mechanisms in functional dyspepsia. World J Gastroenterol. 2006; 12(5): 673–7.
3. Boccia G, Buonavolontà R, Coccorullo P, Manguso F, Fuiano L, Staiano A. Dyspeptic symptoms in children: the result of a constipation-induced cologastric brake? Clin Gastroenterol Hepatol. 2008; 6(5): 556–60.
4. van Hoek F, Mollen RM, Hopman WP, Kuijpers HH, Jansen JB. Effect of rectal distension on gallbladder emptying and circulating gut hormones. Eur J Clin Invest. 2000; 30(11): 988–94.
5. Lei Y, Zhu H, Xing J, Chen JD. Rectal distension modulates canine gastric tone and accommodation. Dig Dis Sci. 2005; 50(11): 2134–40.
6. Desai KM, Sessa WC, Vane JR. Involvement of nitric oxide in the reflex relaxation of the stomach to accommodate food or fluid. Nature. 1991; 351(6326): 477–9.
7. Desai KM, Zembowicz A, Sessa WC, Vane JR. Nitroxergic nerves mediate vagally induced relaxation in the isolated stomach of the guinea pig. Proc Natl Acad Sci USA. 1991; 88(24): 11490–94.
8. Hayakawa T, Arakawa T, Kase Y, Akiyama S, Ishige A, Takeda S, Sasaki H, Uno H, Fukuda T, Higuchi K, Kobayashi K. Liu-Jun-Zi-Tang, a kampo medicine, promotes adaptive relaxation in isolated guinea pig stomachs. Drugs Exp Clin Res. 1999; 25(5): 211–8.
9. Takahashi T, Owyang C. Characterization of vagal pathways mediating gastric accommodation reflex in rats. J Physiol (Lond). 1997; 504(Pt 2): 479–88.
10. Takahashi T, Nakamura K, Itoh H, Sima AA, Owyang C. Impaired expression of nitric oxide synthase

in the gastric myenteric plexus of spontaneously diabetic rats. Gastroenterology. 1997; 113(5): 1535–44.

11. Ozaki N, Bielefeldt K, Sengupta JN, Gebhart GF. Models of gastric hyperalgesia in the rat. Am J Physiol. 2002; 283(3): G666–76.
12. Uchida M, Shimizu K. Evaluation of adaptive relaxation of the rat stomach using an orally inserted balloon instead of surgical intervention by demonstrating the effects of capsaicin and N^{ω}-nitro-L-arginine methylester. J Smooth Muscle Res. 2012; 48(4): 97–104.
13. Tack J, Talley NJ, Camilleri M, Holtmann G, Hu P, Malagelada JR, Stanghellini V. Functional gastroduodenal disorders. Gastroenterology. 2006; 130(5): 1466–79.
14. Ragnarsson G, Bodemar G. Pain is temporally related to eating but not to defaecation in the irritable bowel syndrome (IBS): patients' description of diarrhea, constipation and symptom variation during a prospective 6-week study. Eur J Gastroenterol Hepatol. 1998; 10(5): 415–21.
15. Simrén M, Månsson A, Langkilde AM, Svedlund J, Abrahamsson H, Bengtsson U, Björnsson ES. Food-related gastrointestinal symptoms in the irritable bowel syndrome. Digestion. 2001; 63(2): 108–15.
16. Narducci F, Bassotti G, Granata MT, Pelli MA, Gaburri M, Palumbo R, Morelli A. Colonic motility and gastric emptying in patients with irritable bowel syndrome: effect of pretreatment with octylonium bromide. Dig Dis Sci. 1986; 31(3): 241–6.
17. Sullivan MA, Cohen S, Snape WJ Jr. Colonic myoelectrical activity in irritable-bowel syndrome: effect of eating and anticholinergics. N Engl J Med. 1978; 298(16): 878–83.
18. Martínez V, Ryttinger M, Kjerling M, Astin-Nielsen M. Characterisation of colonic accommodation in Wistar Kyoto rats with impaired gastric accommodation. Naunyn Schmiedebergs Arch Pharmacol. 2007; 376(3): 205–16.
19. Sanaka M, Yamamoto T, Kuyama Y. Effects of proton pump inhibitors on gastric emptying: a systematic review. Dig Dis Sci. 2010; 55(9): 2431–40.
20. Currò D, Ipavec V, Preziosi P. Neurotransmitters of the non-adrenergic non-cholinergic relaxation of proximal stomach. Eur Rev Med Pharmacol Sci. 2008; 12 Suppl 1: 53–62.
21. Mihara H, Suzuki N, Yamawaki H, Tominaga M, Sugiyama T. TRPV2 ion channels expressed in inhibitory motor neurons of gastric myenteric plexus contribute to gastric adaptive relaxation and gastric emptying in mice. Am J Physiol Gastrointest Liver Physiol. 2013; 304(3): G235–40.
22. Matsumoto Y, Ito M, Tsuge M, Matsuo T, Tanaka S, Haruma K, Chayama K. Ecabet sodium induces neuronal nitric oxide synthase-derived nitric oxide synthesis and gastric adaptive relaxation in the human stomach. J Gastroenterol. 2009; 44(11): 1118–24.
23. Bishop-Bailey D, Larkin SW, Warner TD, Chen G, Mitchell JA. Characterization of the induction of nitric oxide synthase and cyclo-oxygenase in rat aorta in organ culture. Br J Pharmacol. 1997; 121(1): 125–33.
24. Lefebvre RA, De Beurme FA, Sas S. Relaxant effect of capsaicin in the rat gastric fundus. Eur J Pharmacol. 1991; 195(1): 131–7.
25. Güal O, Bozkurt A, Deniz M, Sungur M, Yeğen BC. Effect of sex steroids on colonic distension-induced delay of gastric emptying in rats. J Gastroenterol Hepatol. 2004; 19(9): 975–81.
26. Gué M, Junien JL, Buéno L. The kappa agonist fedotozine modulates colonic distension-induced inhibition of gastric motility and emptying in dogs. Gastroenterology. 1994; 107(5): 1327–34.