Neurogastroenterology & Motility

Neurogastroenterol Motil (2012) **24,** 185–e92 doi: 10.1111/j.1365-2982.2011.01819.x

The effects of methane and hydrogen gases produced by enteric bacteria on ileal motility and colonic transit time

J. JAHNG,* I. S. JUNG,* E. J. CHOI,* J. L. CONKLIN† & H. PARK*

*Department of Internal Medicine, Gangnam Severance Hospital, Yonsei University, College of Medicine, Seoul, Korea
†Division of Gastroenterology, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Abstract

Background *Gases produced by intestinal flora may modulate intestinal motor function in healthy individuals as well as those with functional bowel disease. Methane, produced by enteric bacteria in the human gut, is associated with slowed intestinal transit and constipation. The effects of hydrogen, another main gas produced by bacterial fermentation in the gut, on small bowel and colonic motor function remains unrecognized. Therefore, we set out to investigate whether intestinal gases including methane and hydrogen could influence the small bowel motility and colonic transit.* ***Methods*** *Guinea pig ileum was placed in the peristaltic bath with tension transducers attached to measure velocity and amplitude of peristaltic contraction before and after the infusion of control, hydrogen, and methane gases. Also, changes in the intraluminal pressures were monitored before and after the gas infusions.* ***Key Results*** *Methane decreased peristaltic velocity and increased contraction amplitude significantly of guinea pig ileum ($P < 0.05$). The AUC of intraluminal pressure was significantly increased with methane in guinea pig ileum ($P < 0.05$). In a second experiment, guinea pig colon was placed in the peristaltic bath to measure transit time before and after control, hydrogen, methane, and methane-hydrogen mixture gas infusions. Hydrogen shortened colonic transit time by 47% in the proximal colon, and by 10% in the distal colon, when compared with baselines ($P < 0.05$).* ***Conclusions & Inferences*** *Methane delayed ileal peristaltic conduction velocity by augmenting contractility. Hydrogen shortened colonic transit, and that effect was more prominent in the proximal colon than distal colon.*

Keywords *colonic transit, hydrogen, ileal motility, methane.*

INTRODUCTION

Intestinal gases produced by enteric microflora are associated with bloating, abdominal pain, and change in bowel habit, which are the primary symptoms of the irritable bowel syndrome (IBS).[1,2] The irritable bowel syndrome is a widespread chronic disorder, which affects approximately 11–14% of the general populations. The proposed pathophysiologic mechanisms are varied and include the following: abnormalities in gastrointestinal motility, visceral hypersensitivity, alterations of the enteric nervous system and/or brain-gut axis, aberrant immune responses, and psychologic disorders.[3] In many cases, these pathogenic processes may be triggered by an intestinal infection to produce what is now called postinfectious IBS. A number of recent studies also implicate small intestinal bacterial overgrowth (SIBO) as a cause of IBS or constipation.[1,4] In a double blind study done by Pimentel *et al.*, 78% of IBS patients had a positive lactulose hydrogen breath test (LHBT).[5] Successful treatment of their IBS symptoms by an antibiotic was associated with normalization of the LHBT, strongly supporting the hypothesis that enteric flora participate in the genesis of IBS.[5]

Intestinal gases produced by enteric bacteria include methane (CH_4), hydrogen (H_2), hydrogen sulfide (H_2S), and carbon dioxide (CO_2).[6] They are formed as byproducts from bacterial fermentations of unabsorbed carbohydrates throughout the gastrointestinal tract.[7] There is, in fact, a strong association between constipation predominant IBS and excessively high

Address for Correspondence
Hyojin Park MD, PhD, Department of Internal Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, 211 Eunjuro, Gangnamgu, Seoul 135-720, Korea.
Tel: +82 2 2019 3318; fax: +82 2 3463 3882;
e-mail: hjpark21@yuhs.ac
Received: 29 July 2011
Accepted for publication: 20 October 2011

methane levels identified by the LHBT.[8] However, about 36% of healthy individuals without any gastrointestinal symptoms have methane gas identified by breath testing.[7] Several lines of evidence support the hypothesis that methane generated in the intestinal tract can alter intestinal motor function. Using animal and human models, Pimentel *et al.* demonstrated that CH_4 gas slows intestinal transit, augments reflex contraction of intestinal muscle, and alters intestinal motor function.[9]

How these enteric gases interact and where they are produced depend on various microenvironments in the bowel. How gases produced by the enteric flora modulate intestinal functions requires a great deal more study.

On the basis of these findings, we hypothesized that intestinal gases produced by enteric bacteria such as methane or hydrogen may affect small and large bowel motility and intestinal transit. Firstly, we used a guinea pig model to study the effects of methane and hydrogen on small bowel motility and in specific, ileal motility. Secondly, the effects of methane, hydrogen and their mixture on intestinal transit were investigated in the proximal and distal colon. We speculated that intestinal gases have different effects between the proximal colon, which is mainly responsible for mixing ingested food contents, and the distal colon that functions as reservoir and evacuation of the feces.

MATERIALS AND METHODS

Animal preparation

Male Hartley guinea pigs (Charles River Laboratories, Inc. Wilmington, MA, USA) weighing 300 g were used. They were kept under conventional conditions in an environmentally controlled room (21 °C, 60% humidity, 12 : 12 h light-dark cycle). Guinea pigs were deprived of food, but not water, for a 24-h period before experiments. Guinea pigs were stunned by a blow on the head and exsanguinated by severing carotid arteries just before the laparotomy to harvest segments of distal ileum or colon. The experimental procedures were conducted in accordance with the guidelines of Yonsei University Animal Care and Use Committee.

Mechanical recordings and expermients

Ileal motility The experimental apparatus and methods described by Tonini *et al.*[10] and subsequently modified,[11–14] were used for evaluations in isolated intestinal tissues. Fifteen cm of distal ileum was carefully removed from a point, 5 cm proximal to the ileocecal valve. The harvested bowel was placed in an organ bath containing Krebs-Henseleit solution (K-H solution, in mmol L^{-1}: 138.5 Na^+, 4.6 K^+, 2.5 Ca^{2+}, 1.2 Mg^{2+}, 125 Cl^-, 21.9 $HCO3^-$, 1.2 $H_2PO_4^-$, 1.2 SO_4^-, and 11.5 glucose) at 37 °C and gassed constantly with 95% O_2–5% CO_2 (final pH 7.3–7.4). The length, width, and height of organ bath were 23.5 cm, 8.7 cm, and 5.5 cm containing 500 mL of K-H solution. Partial pressure of each gas perfused was maintained constantly throughout the experiment. Tissues were placed in the peristaltic bath, so that the oral-to-anal orientation was maintained.

Velocity and amplitude of peristaltic contraction The tissue was placed in the peristaltic tissue bath, taking care to maintain the oral-to-anal orientation intact. Force/tension transducers were connected at 2.5 cm intervals to the serosal surface of oral, mid, and anal segments of the intact ileum.[13] The transducers were positioned to record contractile activity of the circular muscle layer. Baseline tension was maintained at 1 g. After the tissue was equilibrated for 60 min with O_2–CO_2 mixture, the bath was gassed with control (95% O_2–5% CO_2), hydrogen (99.9% H_2), or methane (99.9% CH_4) gases using peristaltic pump (Masterflex 7523-30 with cartridge 3519-85; Cole-Palmer, Chicago, IL, USA) with a flow of 0.45 mL min^{-1}. The pH of the bath was constantly monitored and maintained. The velocity and amplitude of peristaltic contractions were measured before and after the infusion of gases. Peristaltic velocity was calculated by the time (seconds) required for the peristaltic wave to travel 5-cm distance.

Area under the curve (AUC) of intraluminal pressure A barometer was placed at the anal side to measure AUC of intraluminal pressure before and after the infusion of control, hydrogen, and methane gases.

Colonic transit

The large bowel was carefully removed using aforementioned methods.[10–13] Ten cm of proximal colon at a point 3 cm distal to the ileo-cecal valve and 10 cm of distal colon at point 3 cm proximal to the rectum were retrieved. Harvested colon was placed in an organ bath containing K-H solution at 37 °C and gassed with 95% O_2–5% CO_2 (final pH 7.3–7.4). Partial pressure of each gas perfused was maintained constantly throughout the experiment. The tissue was oriented in the bath with care to localize the oral and aboral or anal sides of the tissue.

Colonic transit in the proximal and distal colon The methods employed to study colonic transit were those previously described by Kim *et al.*[14] Tissue was placed in the peristaltic bath, so that the oral-to-anal orientation was maintained. A scale with intervals of 2 cm was placed alongside the peristaltic bath to measure the time (s) it took for an artificial pellet (12 mm × 4 mm) to travel every 2-cm interval. After the proximal colon was equilibrated for 60 min with O_2–CO_2 mixture, the bath was gassed with control (95% O_2–5% CO_2), methane (99.9% CH_4), hydrogen (99.9% H_2), or 1 : 1 mixture of methane and hydrogen gases using a peristaltic pump (Masterflex 7523-30 with cartridge 3519-85; Cole-Palmer) at a rate of 0.25 mL min^{-1}. The pH of the bath was constantly monitored and maintained. The distal colon was prepared in the same manner, and control, methane, hydrogen, or methane-hydrogen mixtures gases were infused in a similar manner. Colonic transit time was measured before and after the infusion of each gas in the proximal and distal colon.

Data analysis and statistics

All measurements of contractile force, velocity of peristalsis, transit time, and intraluminal pressure were expressed as percentile change from the measured baseline values before the infusion of each gases. Paired Student's *t*-test was used for statistical analysis. All data were expressed a means ± SD, with statistical significance set at $P < 0.05$, and PASW (Predictive Analytics

Software) Statistics, release version 18.0.0 (SPSS Inc., Chicago, IL, USA) was used for statistical anaylsis.

RESULTS

Effects of control, hydrogen, and methane gases on the velocity of peristaltic contraction in the ileum

The peristaltic velocity was calculated by the time required for the peristaltic wave to travel 5 cm distance. The velocities of peristaltic contraction (% change) in the ileum after infusion of control, H_2, and CH_4 gases were 108.2 ± 15%, 120.3 ± 52.8%, and 79.6 ± 12.1% as compared with baseline. The velocity of peristaltic contraction decreased significantly after infusion of CH_4 ($P < 0.05$). The increase in velocity produced by H_2 was not statistically significant (Fig. 1).

Effects of control, hydrogen, and methane gases on the amplitude of peristaltic contraction in the ileum

The amplitude of peristaltic contraction did not change in the control group. However, the contraction amplitude significantly decreased in oral, mid and anal segments (86.6 ± 20.4%, 82.4 ± 5.8%, and 82.7 ± 5.5% of base line, respectively) after H_2 gas infusion ($P < 0.05$). They significantly increased in all oral, mid, and anal side (107.1 ± 4.3%, 112.7 ± 7.3, 116.7 ± 8.0% of baseline, respectively) after CH_4 gas infusion ($P < 0.05$) (Fig. 2).

Effects of control, hydrogen, and methane gases on AUC of intraluminal pressure in the ileum

The measured intraluminal pressure after infusion of CH_4 increased significantly in the ileum. The AUC for intraluminal pressures were 99.8 ± 4.4%, 64.1 ± 26.0%, and 137.8 ± 34.1% when compared with baseline, for control, H_2, and CH_4 gases, respectively. CH_4 gas significantly increased AUC for intraluminal pressure ($P < 0.05$). While H_2 gas decreased AUC for intraluminal pressure, the change was not statistically significant (Fig. 3).

Effects of control, methane, hydrogen, and methane-hydrogen mixture gases on the colonic transit in the proximal and distal colon

The colonic transit time (% change compared with baseline) measured in the proximal colon after infusion of control, H_2, CH_4, and CH_4-H_2 mixtures gases were 101.7 ± 53.6%, 53.5 ± 36.4%, 100.6 ± 43.5%, and 187.2 ± 107.1%, respectively. H_2 gas infusion significantly shortened colonic transit ($P < 0.05$) (Fig. 4). The colonic transit time measured in the distal colon after infusion of control, H_2, CH_4, and CH_4-H_2 mixtures gases were 98.4 ± 2.4%, 90.0 ± 8.1%, 97.8 ± 9.3%, and 108.1 ± 17.3, respectively. H_2 gas also significantly shortened colonic transit in the distal colonic segment ($P < 0.05$) (Fig. 5). However, this effect was not demonstrated when H_2 was mixed in a 1 : 1 ratio with CH_4 gases.

DISCUSSION

The relationship between abnormal methane production discovered on breath testing and constipation dominant IBS, was demonstrated by previous studies.[1,5,7,8] However, there are few translational studies exploring the mechanisms by which methane may cause constipation.[9,15]

About 50% of the CH_4 produced in the gastrointestinal tract is absorbed and excreted in expired air. There appears to be no catabolism of this gas by other colonic

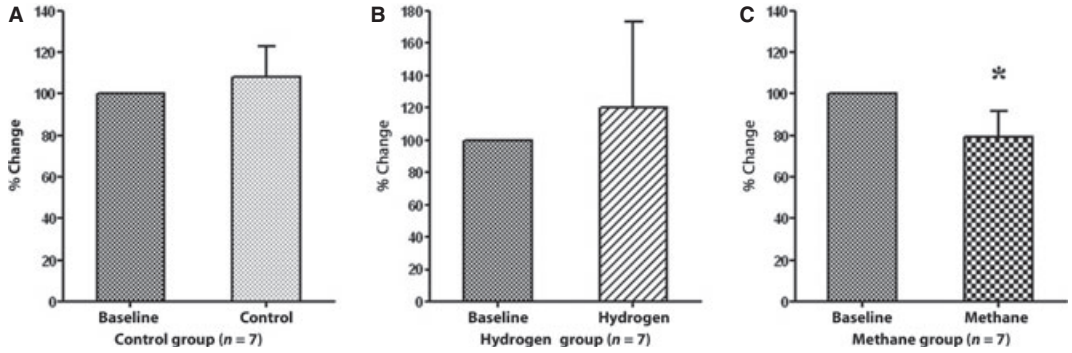

Figure 1 The velocity of peristaltic contraction in the ileum before and after infusion of (A) control, (B) hydrogen, and (C) methane gases. Data are expressed as mean ± SD % change compared with baseline. *$P < 0.05$.

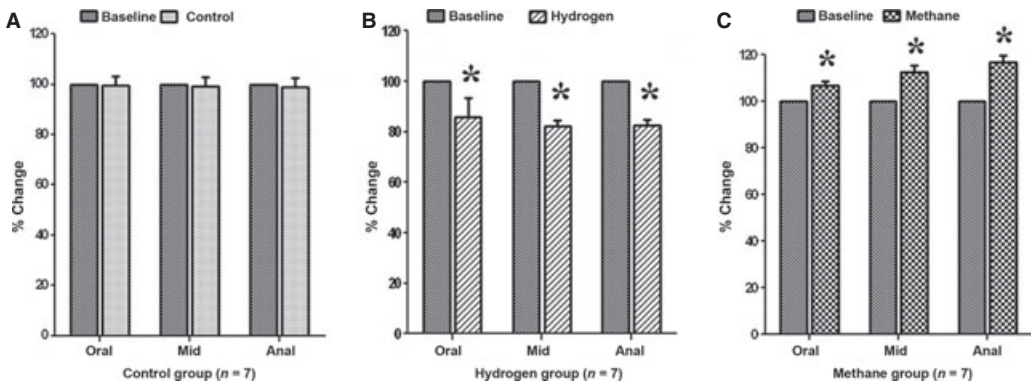

Figure 2 The amplitude of peristaltic contraction in the ileum before and after infusion of (A) control, (B) hydrogen, and (C) methane gases. Data are expressed as mean ± SD % change compared with baseline. *$P < 0.05$.

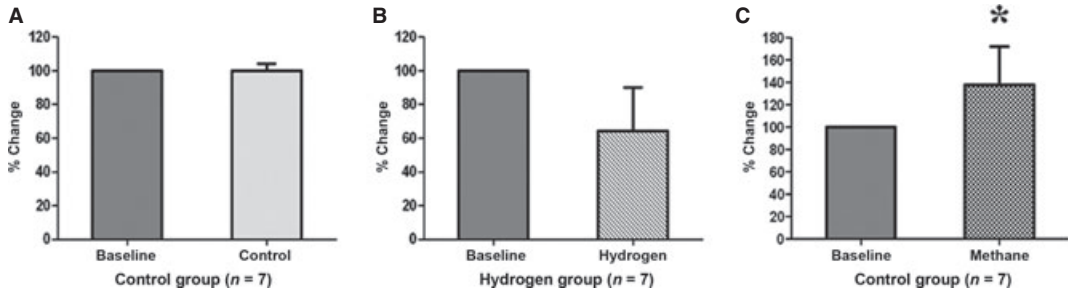

Figure 3 The effects of (A) control, (B) hydrogen, and (C) methane gases on the AUC of intraluminal pressure in the ileum. Data are expressed as mean ± SD % change compared with baseline. *$P < 0.05$.

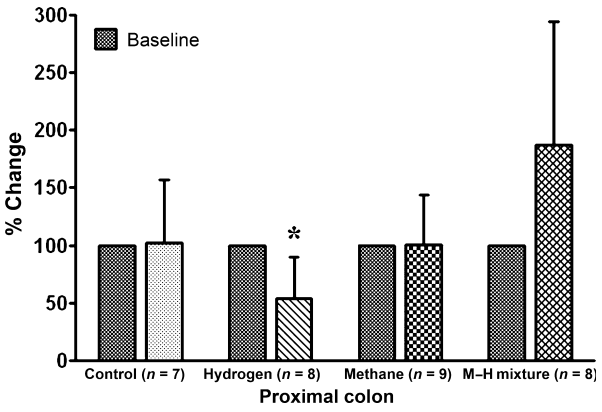

Figure 4 The transit time in the proximal colon before and after infusion of control, hydrogen, methane, and methane-hydrogen mixture gases. M-H = methane-hydrogen. Data are expressed as mean ± SD % change compared with baseline. *$P < 0.05$.

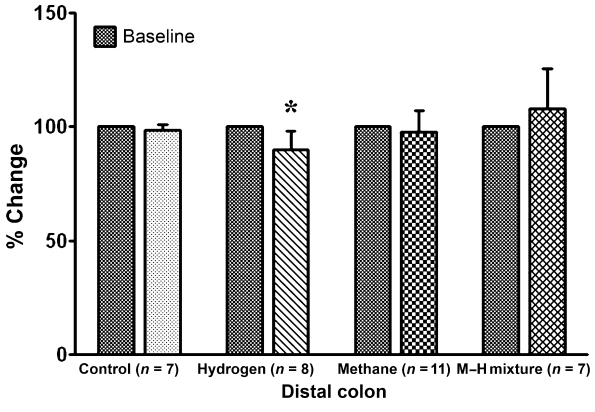

Figure 5 The transit time in the distal colon before and after infusion of control, hydrogen, methane, and methane-hydrogen mixture gases. M-H = methane-hydrogen. Data are expressed as mean ± SD % change compared with baseline. *$P < 0.05$.

bacteria or host cells.[16] On the other hand, H_2, the most commonly assessed intestinal gas by breath testing, is metabolized efficiently by other bacteria such that only a small percentage of its total production is excreted in expired air.[16] Hydrogen sulfide is yet another gaseous byproduct of enteric microbial metabolism, which is now known to be produced by mammalian cells as well. While it is toxic in high concentrations, H_2S also appears to function as a modulator of neuronal and smooth muscle function, and under certain circumstances as a cytoprotective and anti-inflammatory agent. Abundant H_2 gas in the

bowel promotes H_2 consumption by methanogenic and sulfate-reducing bacteria, which contributes to overproduction of CH_4 and H_2S.[16]

Enteric bacteria in the human gut like *Methanobrevibacter smithii*, *Methanobacterium ruminatum*[17] produce methane by fermentation of carbohydrates substrates. These bacterial species are harbored mainly in the left colon where methane production is believed to occur in normal subjects. Other anaerobic species of *Bacteroides* and *Clostridium* can also produce methane,[18] and these bacteria can easily be part of small intestinal microflora. Hydrogen is another important byproduct of carbohydrate fermentation in the human gut.[19] It is eliminated by flatus, absorption into the systemic circulation with subsequent respiratory excretion, and metabolism by colonic bacteria.[20] About 14% of total hydrogen gas produced was excreted through the lungs and that breath hydrogen excreted correlated quite well with total hydrogen production.[16] Another study showed that hydrogen production varied from 25% to 60% depending on the hydrogen production rates.[20] The remaining hydrogen is utilized by methanogens to produce methane ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$), sulfate-reducing bacteria to produce hydrogen sulfide ($4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$), and acetogenic bacteria to produce acetate ($4H_2 + 2CO_2 \rightarrow CH3COO^- + H^+ + 2H_2O$). Four mol of hydrogen are converted to 1 mol of methane, hydrogen, or acetate. This means that there is a competition between methanogens and sulfate-reducing bacteria for hydrogen.[16,21] These are both important mechanisms for safely and effectively eliminating luminal hydrogen. In the human gut, sulfate-reducing bacteria outcompete methanogenic bacteria for hydrogen, because they have a greater affinity and thermodynamical stability for hydrogen than the methanogenic bacteria.[16,20–24]

Many evidences indicate that methane production is indeed related to constipation and constipation predominant IBS.[1,4,5,8,9] Cloarec *et al.* observed that patients who have methane in the exhaled air during the lactulose hydrogen breath test have a delayed orocecal transit (111 min) than those with no methane (68 min), even in healthy volunteers.[25] In patients with constipation predominant IBS, the amount of methane measured during the lactulose breath test correlates with the severity of constipation.[26] In addition, normalization of the breath test in IBS patients after antibiotics treatment correlated with resolution of bowel symptoms.[5] Other supportive studies demonstrated that methane production is largely detectable in children with constipation and encopresis,[27,28] and adults with diverticulosis.[17,29] Conversely, methane production is relatively low in diarrheal diseases like Crohn's disease and ulcerative colitis.[8,30]

In our study in the ileum, methane decreased the peristaltic velocity, increased the amplitude of peristaltic contractions, and increased the AUC of intraluminal pressure. These results are consistent with the study by Pimentel *et al.*, which showed ineffective transfer of food materials from oral to the aboral side and causing of delayed small intestinal transit.[9] In case of hydrogen, this effect seems to be reversed. Peristaltic velocity increased, but without statistical significance, whereas peristaltic contraction amplitude decreased significantly. Further data are needed to confirm the effects of hydrogen on ileal transit.

The only previous study correlating small bowel motility with intestinal gas was that by Pimentel *et al.*[9] In that study, small intestinal fistulae were created in a dog model to permit measurement of intestinal transit during infusion of methane or room air into a segment of small bowel. Methane infusion induced slowing of transit by an average of 59%, whereas room air had little effect.

The role of hydrogen in human gut and pathogenesis of bowel disease such IBS or gastrointestinal related disorders has not been clearly elucidated, but there might be a correlation between accelerated transit causing diarrhea and hydrogen production. Findings in our study suggest that hydrogen gas might be involved in relatively fast small intestinal motility and colonic transit, but this warrants further study and verification.

Our study with guinea pig colon demonstrated that hydrogen gas affected colon more prominently than methane gas. Hydrogen gas shortened colonic transit by 47% when compared with the baseline in the proximal colon, and by 10% when compared with the baseline in the distal colon. The reason for this difference in transit according the location is not certain, but if guinea pig exhibits regional differences in colonic flora such as in human, responsiveness may be dependent on the gas normally produced in each region. The different functions of proximal and distal colon that are back and forth movement of digested food *vs* storage and evacuation of fecal materials.

There are limitations of animal study and perfusion of CH_4 or H_2 gases to generate the actual physiologic setting of small bowel or colonic environments. Which one is adequate concentration to mimic physiologic setting still remains unknown. However, previous study had reported constant concentration of 980–1,010 ppm of infused gas and constant pH in the organ bath regardless of the rate of flow with pure CH_4.[9] We performed preliminary experiment in likewise manner and came up with same results with

CH_4 and H_2 gases. When dealing with harvested tissues in motility study, there are always issues of ischemic injuries after extraction of the tissues. In our experiment, bowel segments showed ischemic signs for around 1 hour after the harvest. In consequence, every experiment was carried within an hour of the extraction with measuring baseline data before CH_4 or H_2 gas infusion and after gas infusion. The reason for measuring control as well as baseline data was to cope with inter-variability between specimens and minimize flow and pressure effects of perfused gases. The combination of CH_4-H_2 gas was not studied in the small intestine which was done initially. However, after confirming the effects of CH_4 and H_2 gases in the small intestine, we wanted to see how the mixtures of two gases affected in the colon, but the results showed varied data.

Mechanisms of CH_4 and H_2 gases affecting bowel motility and transit are not fully understood. We may suspect that this will be through enteric nervous system rather than brain-gut axis as the experiments were carried out in isolated bowel specimens. The reason why shortened colonic transit caused by H_2 gas is diminished when H_2 is mixed with CH_4 is yet to be studied. Other observational studies suggest that the type of IBS from which a patient suffers may depend upon the relative luminal concentrations of these gasses. In addition, the marked temporal variability in IBS symptoms may result from changes in relative gas concentrations over time. However, more study is needed to verify this hypothesis.

In conclusion, our study demonstrated that CH_4 gas delayed the contraction velocity of ileal peristalsis and increased the amplitude of peristaltic contractions. This is consistent with previous report that CH_4 slows intestinal transit.[9] In addition, H_2 gas shortened colonic transit in the proximal and distal colon. The effect of H_2 gas was more prominent in the proximal colon than in the distal colon. Our results raise the interesting possibility that gases produced by the intestinal flora might modulate gastrointestinal motor functions in health and disease.

ACKNOWLEDGMENTS

This study was sponsored by grants from the Korean Society of Neurogastroenterology and Motility. This study was presented as oral presentation in 2009 Korean Society of Neurogastroenterology and Motility Meeting. The authors have no competing interests.

AUTHOR CONTRIBUTIONS

JJ was responsible for study concept and design, acquisition and analysis of data, and drafting the manuscript; ISJ carried out experimental design, acquisition, and analysis of data; EJC was in charge of mice handling, experimental design; JLC drafted the manuscript, and provided critical revision; HP was the corresponding author, and was responsible for study concept and design, critical revision, and study supervision.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. The actual traces of intraluminal pressure curve of guinea pig ileum before and after infusion of methane and hydrogen gases.

Figure S2. The methods used in this study were adopted and adjusted from previously confirmed and published papers (reference 10–14).

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