The effect and action mechanism of methane gas on ileal motor function

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The effect and action mechanism of methane gas on ileal motor function

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ABSTRACT

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Background: Methane has been associated with constipation-predominant irritable bowel syndrome, slowing intestinal transit time by augmenting contractile activity. However, the precise mechanism remains unclear. Therefore, the aim of this study is the mechanisms underlying the aforementioned actions of methane and whether such effects are mediated by nerve impulse or muscle contraction. **Materials and Methods**: The muscle strips of guinea pig ileum was connected to a transducer and measured amplitudes of contraction in response to electrical field stimulation (EFS; 1, 2, 8, 16 Hz) following methane infusion in the presence of tetradotoxin (TTX), atropine, guanethidine, or GR 113808 (5-HT_4 receptor antagonist). Then, calcium imaging study was performed using Oregon Green 488 BAPTA-1 AM in order to visualize changes in calcium fluorescence in response to EFS following methane infusion in the presence of TTX, atropine, or high K^+ solution. **Results**: Methane

significantly increased amplitudes of contraction ($P < 0.05$), while treatment with TTX abolished such contraction. Methane-induced increases in amplitude were inhibited when lower-frequency (1, 2 Hz) EFS was applied following atropine infusion ($P < 0.05$). Neither guanethidine nor GR 113808 significantly altered contraction amplitudes. Methane significantly increased calcium fluorescence, though this increase was attenuated following atropine infusion ($P < 0.05$). Although calcium fluorescence was increased by the high K^+ solution under pre-treatment with TTX, the intensity of the fluorescence was unchanged after methane infusion. **Conclusion:** The actions of methane on the intestine are affected by the cholinergic pathway of the enteric nervous system, rather than directly on the muscle. These results support the classification of methane as a gasotransmitter.

Keywords: methane, ileal motility, smooth muscle, calcium

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I. INTRODUCTION

Intestinal gases produced by enteric bacteria include carbon dioxide (CO_2), hydrogen sulfide (H_2S), hydrogen (H_2), and methane (CH_4). Anaerobic organisms participate in the process of digestion through fermentation in the large intestine and these microorganisms obtain their energy primarily by breaking down carbohydrates—mainly the undigested polysaccharide fraction. This process results in the generation of short-chain fatty acids, CO_2, H_2, and CH_4.[1,2] Gas production can induce in uncomfortable distension of the large intestine, known as bloating. Intestinal gas and bloating are among the most frequent gastrointestinal (GI) complaints.[3] A large amount of gases within the human body originate from gut bacteria. Bacteria can clearly be implicated in functional GI disorder, such as infectious diarrhea, small intestinal bacterial overgrowth (SIBO), and peptic ulcer disease.

CH_4 production has been related to such diseases as constipation predominant

irritable bowel syndrome (C-IBS), diverticulosis, and colon cancer.[4-6] At room temperature, CH_4 has typically been thought of as an inert gas, aside from the effects of gaseous distention.[7] CH_4 is produced using H_2 and CO_2 during methanogenesis performed mainly by a group of anaerobes called methanogens in the intestine.[1,2] CH_4 are mostly produced in the large intestine of normal subjects, during the partial or full fermentation of the undigested polysaccharide fraction of certain carbohydrates by the anaerobic flora called methanogens in the intestine.[8] The methanogens are a primitive, diverse group of microorganisms that belong to the Kingdom Archaea.[9] Methanogens have long been studied in ruminant species. A ruminant is a hoofed animal (such as cattle, sheep, and goats) that regurgitates its food, then chews the semi-digested food, before it makes its way to the rumen. The rumen is one of the four chambers of the ruminant fore-stomach. It is a complex anaerobic ecosystem in which the feed consumed by the animal undergoes fermentation.[10] In methanogenic individuals, methanogens range from 10^7 to 10^{10} per gram dry weight of feces.[11] Methanogens comprise of *Methaninobrevibacter smithii* (*M. smithii*) and *Methanospaera stadmagnae*. The predominant CH_4 producing organism in humans is *M. smithii*.[8,12] Methanogens in the rumen produce CH_4 from H_2 and CO_2. Methanogenesis is a unique metabolic process whereby CO_2 is reduced to CH_4 using H_2 produced by anaerobic bacterial fermentation as an electron donor. Through this procedure methanogens obtain energy to survive and they reduce the gas molar volume in the intestine since for the production of 1 mole of CH_4, 4 H_2 moles are consumed.[8] Although the procedure is mostly undertaken by methanogens, certain *Clostiridium* and *Bacteroides* species can also produce CH_4.[13]

Intestinal CH_4 can be excreted either in the flatus or exhaled after traversing the gut mucosa and entering the systemic circulation without any additional metabolism.[7] Twenty to fifty percent of the methane produced is excreted in the exhaled breath giving the opportunity for breath testing to become an indirect measurement of its production in the intestine.[14,15] Lactulose breath testing (LBT) measures methane and hydrogen in breath samples obtained at baseline and every 15 to 20 minutes after 10 grams lactulose ingestion until 180 minutes or even later, using gas chromatography. Methane breath testing shows 2 discrete patterns of the gas production after lactulose ingestion: (1) high baseline level and early rise in the breath methane, associated probably with SIBO production; and (2) late rise, corresponding to the arrival of lactulose at the left colon, where methanogens digest it in normal humans.[16] Previous data have suggested that CH_4 is associated with disorders of intestinal motility such as chronic constipation and C-IBS.[3,17-19] 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.[16] In patients with C-IBS, the amount of methane measured during the LBT correlates with the severity of constipation.[20] In addition, normalization of the breath test in IBS patients after antibiotics treatment correlated with resolution of bowel symptoms.[17] Conversely, methane production is relatively low in diarrheal diseases like Crohn's disease and ulcerative colitis.[5,21] In healthy individuals, CH_4 is primarily produced in the large intestine.[9] However, in patients with IBS and constipation, higher levels of CH_4 are present in the small intestine.[16,22] Individuals presenting with elevated levels of CH_4 on a LBT

exhibit delayed colonic transit, suggesting that the effect extends beyond the small bowel.[13,16] Increased CH_4 production as a result of altered gut flora is considered to be a pathophysiological component of IBS and is responsible for a variety of symptoms, including abdominal bloating, pain, and flatulence.[16,17,23] Excessive small bowel bacteria may serve in generating symptoms in patients with IBS.[3,17]

Several data has suggested that methane and slowed intestinal motility coexist or that the abundance of methane is secondary to impaired intestinal motility. Researchers[24] showed in a three-part translational and human study that: (1) in a canine study, CH_4 infusion through fistulae slows small intestinal transit time by 59%, while CH_4 infused into the distal small intestine slows transit time in the proximal intestine. (2) methane augmented ileal circular muscle contractile activity in a guinea pig ileum experiment; and (3) IBS methane producers had significantly higher fasting and post-prandial motility indices, as well as, isolated fasting contractions during antro-duodenal manometry compared to IBS H_2 producers. The consistency of the motility findings in response to methane in the animal models and in human subjects provided for the first time experimental evidence that methane may not be an inert intestinal gas, but it can modulate to the neuromuscular function of the intestinal tract. The evidence for rather causative association of methane with delayed intestinal motility was further augmented by an experiment that aimed to further explore possible effects of gases produced in the intestine in the ileum and colon motility. Another researchers[25] showed in experiment using guinea pig ileal, right and left colon segments in a peristaltic tissue bath that: (1) CH_4 significantly decreases peristaltic velocity, increases the amplitude of contractions, and increases

the area under the curve (AUC) of intraluminal pressure in guinea pig ileum. (2) colonic transit was shortened by hydrogen infusion, but this effect was diminished when methane was additionally pumped; these effects being more prominent in the right colon. Although there are limitations to mimic the physiological status of the intestinal environment, these two translational studies provide experimental evidence to support a direct action of methane to delay intestinal transit time.

Intestinal transit time is a physiological marker of intestinal motility. It is difficult to combine the results because it can be measured by different ways such as stool frequency, whole gut, orocecal and colonic transit time. There are studies that due to different estimation of intestinal motility-using the motility index.[24,26] Previous studies showed that methane breath test positivity was related to higher fasting motility index, while the study of Pimentel et al.[24] showed a higher postprandial motility index in methane producing subjects, as well. There are prior studies revealing that the prevalence of methane producers was higher in the slow transit (75% and 58.8%) compared to the normal transit (44% and 12.2%) chronic constipated patients and to the healthy controls (28% and 13.3%), respectively.[27,28] Therefore, data support that production of methane as determined by breath testing, is associated with delayed intestinal transit. In addition, on the basis of experiments using isolated ileum segments, these results suggest that the action of CH_4 may be associated with a topical effect through the enteric nerve system rather than through the brain-gut axis.

However, the mechanisms of transit delay induced by methane are still unclear. No published translational study has explored the underlying mechanism by which

CH_4 affects intestinal motility. Therefore, the aim of this study was to investigate the effect of CH_4 on intestinal motility and to determine whether the underlying mechanism of action is mediated by nerve or muscle in the guinea pig ileum.

II. MATERIALS AND METHODS

1. Tissue preparation

Male Hartley guinea pigs (Orient Bio Inc., Gyeonggi-do, Korea) weighing 300–350 g were obtained for the present study. Guinea pigs were stunned by a blow on the occipital region of the head and then sacrificed by severing the carotid arteries just prior to laparotomy in order to harvest segments of the distal ileum. About 15 cm of distal ileum was removed from a point 5 cm proximal to the ileocecal valve. The harvested bowel was placed in an organ bath containing Krebs-Henseleit (K-H) solution. The mucosal and submucosal layers were carefully removed using microsurgical instruments under light microscopy in order to expose the circular muscle of the ileum. Rectangular muscle strips with a length of 10 mm and a width of 3 mm were then obtained. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (2013-0275), Department of Laboratory Animal Resources, Yonsei Biomedical Research Institute, Yonsei University College of Medicine.

2. Tissue bath technique and electrical field stimulation protocol

Two separate experiments were conducted in order to determine the mechanism by which CH_4 contributes to changes in intestinal motor function.

First, a muscle strip of 3 mm in width and 10 mm in length was placed in a tissue bath, and the bathing solution was changed with fresh K-H solution. One side was

connected to a platinum hook (electrode), and the other end was attached to a fixed hook connected via thread to a force/tension transducer (BIOPAC TSD 105; BIOPAC Systems, Inc., Santa Barbara, CA, USA). The activity of the muscle was analyzed using a computerized integration system (BIOPAC MP 100; BIOPAC Systems, Inc.). Baseline tension was maintained at 1 g, and the isometric force was recorded using a physiograph (Grass, Quincy, MA, USA). After the tissue was equilibrated for 60 min with an O_2–CO_2 mixture, the bath was gassed with control (95% O_2–5% CO_2) or CH_4 (99.9% CH_4) gases using a peristaltic pump (LEGATO 100; KD Scientific Inc., Holliston, MA, USA).

Electrical field stimulation (EFS) was used to estimate the contractile activity (amplitude) of the circular muscle of the ileum. The muscle strip was connected to the electrode for electric stimulation during infusion of CH_4. The stimulation using an electric stimulator (Grass, Quincy, MA, USA) was transferred to the electrode. The EFS was generated by stimuli of 0.5 ms duration at 60 V during 10 s, applied at frequencies of 1, 2, 8, and 16 Hz.

To assess neural control of ileal muscle contraction, tetradotoxin (TTX) 10^{-6} M was applied to the tissue bath solution either before or after CH_4 infusion. To evaluate the influence of cholinergic and adrenergic activity, atropine 5 x 10^{-5} M or guanethidine 5 x 10^{-5} M, respectively, were added to the chamber. To create non-adrenergic/non-cholinergic (NANC) conditions via inhibition of cholinergic and adrenergic neurotransmission, a mixture of atropine 5 x 10^{-5} M and guanethidine 5 x 10^{-5} M was used. To examine the effects of the serotonergic neural pathway, we performed the same experiment using GR 113808 10^{-6} M (selective 5-HT_4 receptor

antagonist). Following an equilibrium period of at least 15 minutes, we then performed EFS using an interval of at least one minute following the administration of each drug.

3. Calcium imaging

Second, based on the results of the tissue bath study, we performed additional experiments to identify alterations in calcium signaling in the nerve plexus of ileal smooth muscle. We used a confocal inverted microscope (Nikon Eclipse T 1; Nikon Instruments Inc., Melville, NY) to verify calcium fluorescence and ordered a specific chamber suitable to microscope in order to maintain the same conditions as in the tissue bath experiment. This chamber is made of acryl and is 100 mm x 60 mm x 25 mm in size and contains a square-shaped hole (50 mm x 25 mm x 10 mm) in the center for attaching platinum plates and fixing tissue samples. The ileal muscle strip was prepared as mentioned previously, and electrical stimulation was transferred to the platinum plates (electrode) during CH_4 infusion. The muscle strip was maintained at 37°C and gassed continuously with a mixture of 95% O_2 and 5% CO_2 with K-H solution through the central hole of the chamber.

Oregon Green 488 BAPTA-1 AM was chosen as the Ca^{2+} indicator for the present study. Prior to initiation of the experiment, we removed the Oregon Green 488 BAPTA-1 AM from the freezer (-20°C) and allowed it to thaw at room temperature. We then took 1 ml of PSS in a test-tube and placed it in a water-bath, which was bubbled with 95% O_2 and 5% CO_2 at a rate of around 1 bubble per second. The

prepared ileal muscle strip was washed well using PSS every 10 min. Then the muscle strip was placed in the chamber with 125 μl of 10 μM Oregon Green 488 BAPTA-1 AM and the pre-warmed, bubbled PSS for 2 hours of incubation. We then rinsed the tissue in bubbled PSS three times for 5 minutes each. The tissue samples were then viewed under a confocal microscope at a laser power 488 nm and an objective magnification of 20x.

To identify the influence of neural activity on the contraction of muscle strips, we performed additional experiments using TTX 10^{-6} M, atropine 5 x 10^{-5} M, and 25 mmol of a high K^+ solution.

4. Solutions and drugs

The composition of the K-H solution was as follows, in mmol L^{-1}: 138.5 Na^+, 4.6 K^+, 2.5 Ca^{2+}, 1.2 Mg^{2+}, 125 Cl^-, 21.9 HCO_3^-, 1.2 $H_2PO_4^-$, 1.2 SO_4^-, and 11.5 glucose at 37°C, which was gassed constantly with a mixture of 5% CO_2 and 95% O_2 (pH 7.3-7.4). The high concentration K^+ solution (25 mmol), for which we adjusted the concentration of K^+ to 25 mmol by lowering the concentration of Na^+, was used to induce muscle contraction. TTX, atropine sulfate (a muscarinic receptor antagonist), and guanethidine (a norepinephrine release inhibitor) from Sigma-Aldrich (St. Louis, MO, USA), and GR113808 (a selective 5-HT_4 antagonist) from Tocris Cookson (Ellisville, MO, USA) were purchased. For Ca^{2+} imaging, Oregon Green 488 BAPTA-1 AM was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

5. Statistical analysis

Measurement of amplitude was expressed as percentile change from the measured baseline values (control), and the effects of CH_4 and drugs were expressed as % change from control. To standardize data, we defined "control at 1 Hz of EFS" as 100%. In addition, we analyzed image sequences from the calcium imaging experiment using MetaMorph analytic software (Molecular Devices, Sunnyvale, CA, USA). A Linear mixed model was used for a repeated measures covariance pattern model within the same sample. Two fixed effects were included: one addressing the within group and one addressing the within Hz (1, 2, 8, 16). Possible differences in group across Hz were analyzed according to group and Hz interactions. Bonferroni method was considered as post-hoc analysis. The estimation method used was the restricted maximum likelihood estimation to produce unbiased estimators. Statistical analyses were conducted with use of the SAS (version 9.2, SAS Inc., Cary, NC, USA). All data were expressed as means ± SE, and "N" values indicate the number of tissue samples. A *P*-value of < 0.05 was considered statistically significant.

III. RESULTS

1. Effects of CH_4 on the amplitude of ileal muscle contraction

The amplitude of contraction significantly increased following CH_4 infusion at 1, 2, 8, and 16 Hz of EFS (Fig. 1). As compared with the control, the percentage of change in contractile amplitude was 182.7 ± 20.1%, 208.6 ± 29.6%, 415.8 ± 58.9%, and 677.1 ± 93.7% at 1, 2, 8, and 16 Hz, respectively ($P < 0.05$, N=8). These results accord with those obtained in previous study.[25]

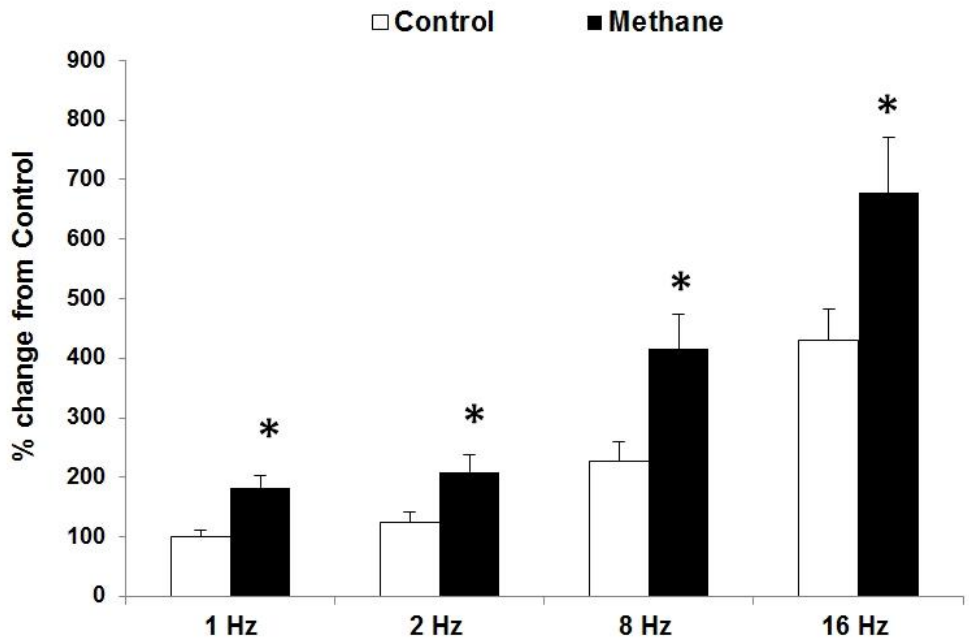


Figure 1. The amplitude of ileal contraction following the infusion of methane under electrical field stimulation (EFS). The amplitude of contraction significantly increased following CH_4 infusion at 1, 2, 8, and 16 Hz of EFS. Data are expressed as mean ± SE % change compared with control. *$P < 0.05$ (N=8).

2. Neural effect of CH_4 on ileal muscle

To evaluate the effects of CH_4 on muscle contraction through neural pathway, TTX was used for a block of neurotransmitter release. This treatment totally abolished contractions induced by CH_4, indicating that contractile responses elicited by CH_4 on ileal muscle are mediated by a neural pathway (Fig. 2).

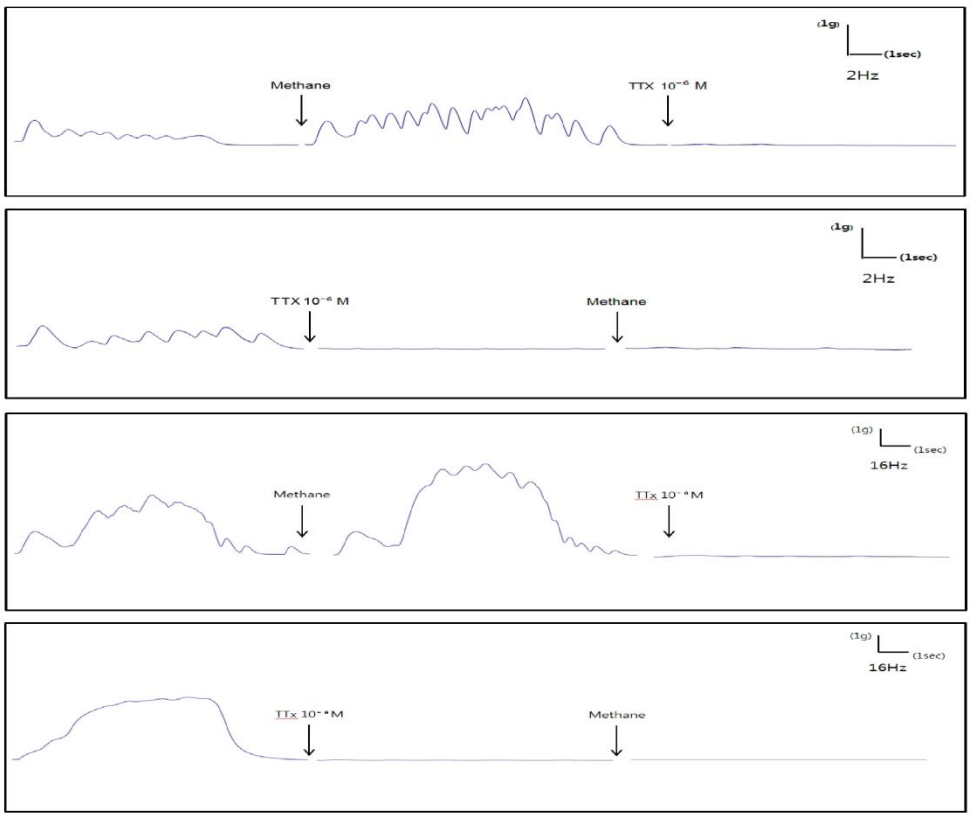
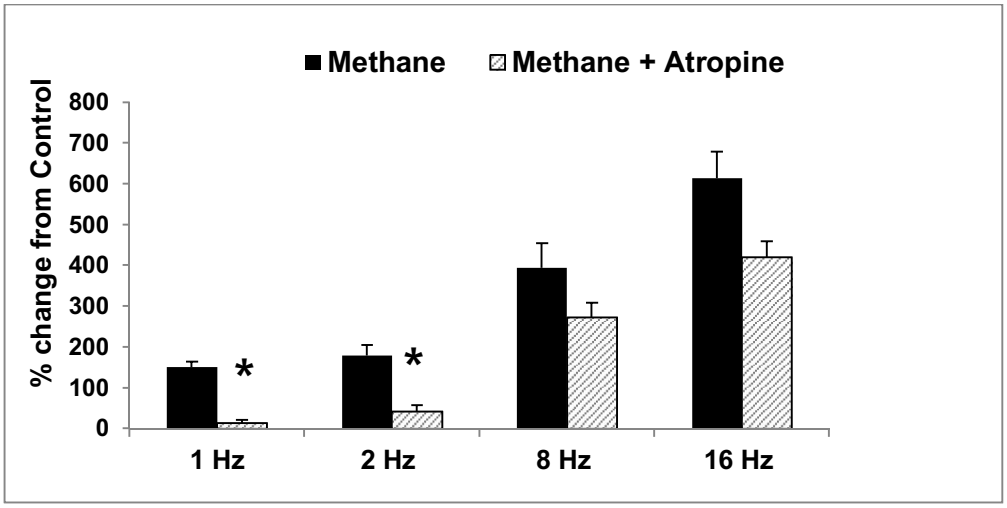


Figure 2. Mechanical tracking showing the effect of tetradotoxin (TTX) on ileal muscle. TTX infusion totally abolished contractions induced by CH_4.

The anticholinergic atropine 5 x 10^{-5} M inhibited increases in amplitude elicited by CH_4 at 1 and 2 Hz only (Fig. 3-A). The percentage of amplitude induced by CH_4 infusion was 151.5 ± 12.8% at 1 Hz and 179.4 ± 25.9% at 2 Hz. As compared with the CH_4 group, the degree of the inhibition by atropine was 13.5 ± 7.7% at 1 Hz and 41.7 ± 15.5% at 2 Hz ($P < 0.05$, N=6). Under NANC conditions (a mixture of atropine 5 x 10^{-5} M and guanethidine 5 x 10^{-5} M), the amplitude of contraction elicited by CH_4 infusion was inhibited at lower frequencies (1, 2 Hz) (Fig. 3-B). Compared with the CH_4 group, the degree of a decrease was from 143.1 ± 26.7 to 34.8 ± 37.7% at 1 Hz and from 190.7 ± 35.1 to. 71.7 ± 49.6% at 2 Hz ($P < 0.05$, N=6).

(A)



(B)

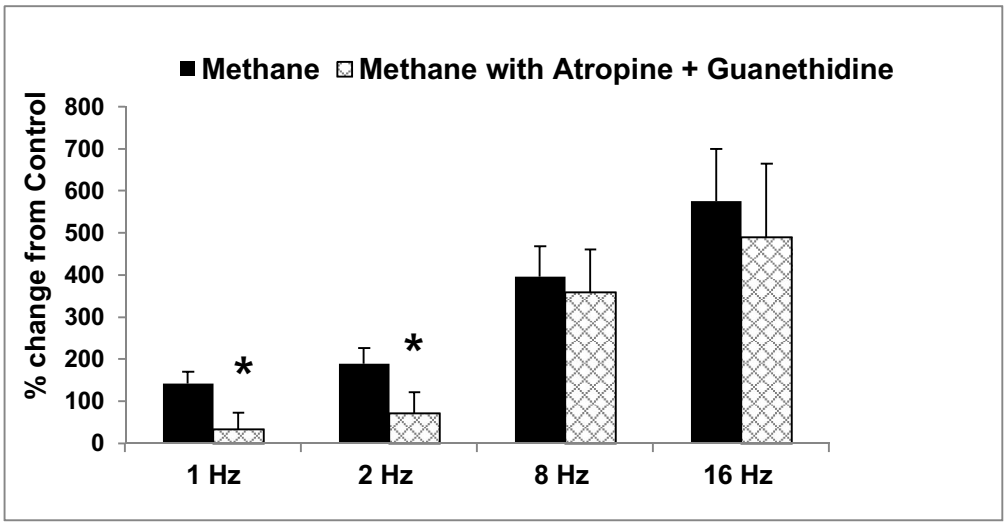
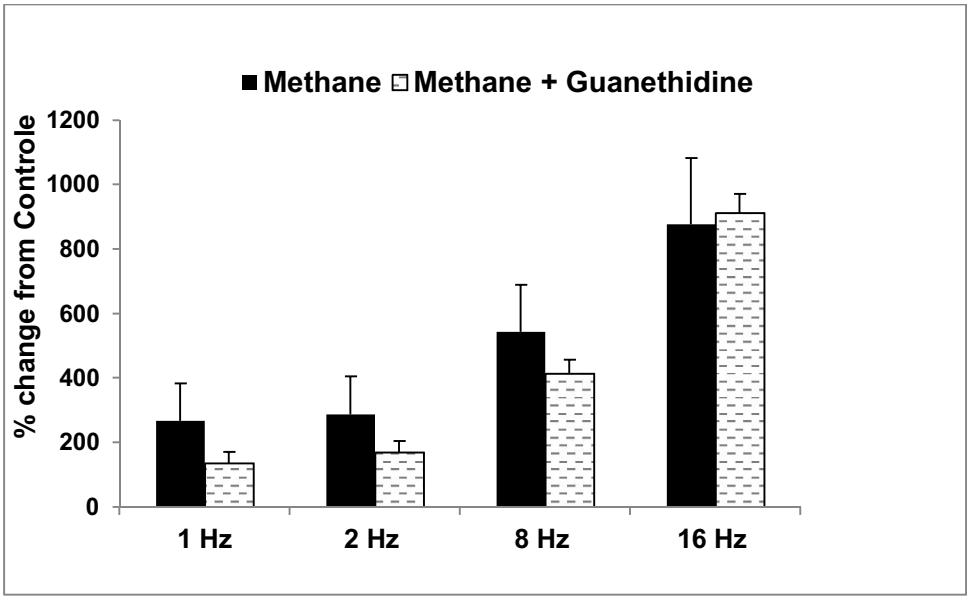


Figure 3. The effect of atropine (A) and non-adrenergic/non-cholinergic (NANC) conditions (B) on ileal contraction under electrical field stimulation. The anticholinergic, atropine inhibited increases in amplitude elicited by CH_4 at 1 and 2 Hz only. Under NANC conditions, the amplitude of contraction elicited by CH_4 infusion was inhibited at lower frequencies (1, 2 Hz). *$P < 0.05$ (N=6)

In contrast, guanethidine 5 x 10^{-5} M and GR 113808 10^{-6} M did not significantly affect the amplitude of contraction (Fig. 4-A, B). These results suggest that adrenergic or serotonergic pathways are not involved in the ileal muscle contractions induced by CH_4.

(A)



(B)

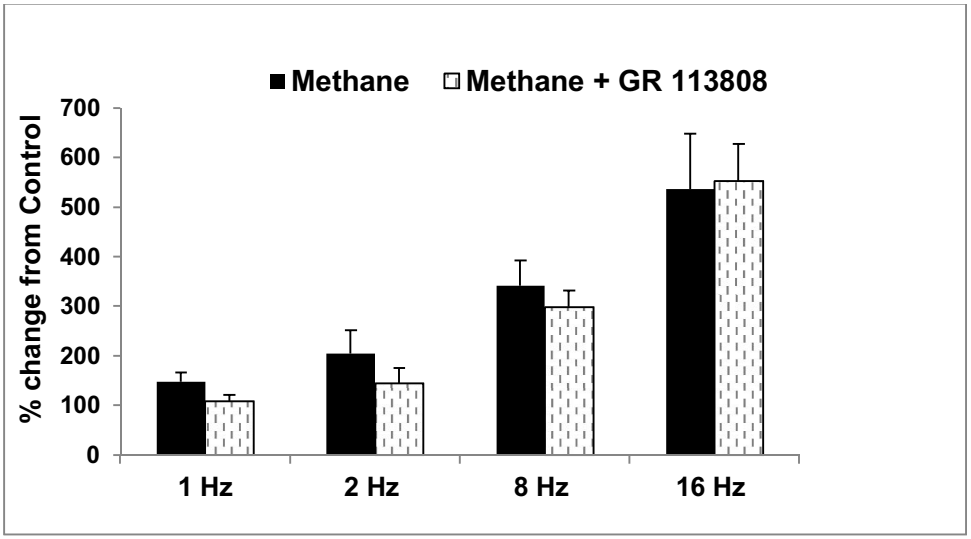


Figure 4. The effect of guanethidine (A) and GR 113808 (B) on ileal contraction under electrical field stimulation. These results do not show significantly changes in the amplitude of contraction. (N=6)

3. Calcium imaging

The contraction of smooth muscle depends on an increase in the concentration of intracellular calcium ions. Thus, we observed a change in calcium fluorescence during infusion of CH_4 and of certain drugs. In this experiment, using the same tissue bath method, ileal muscle specimens were placed between the two platinum plates in a chamber filled with the K-H solution.

Compared with the control conditions, calcium fluorescence increased following CH_4 infusion under EFS (Fig. 5-A, B) at all frequencies. The result of calcium fluorescence was 2.63 ± 0.22 at 1 Hz, 2.88 ± 0.20 at 2 Hz, 2.70 ± 0.15 at 8 Hz, and 2.60 ± 0.15 at 16 Hz ($P < 0.05$, N=6).

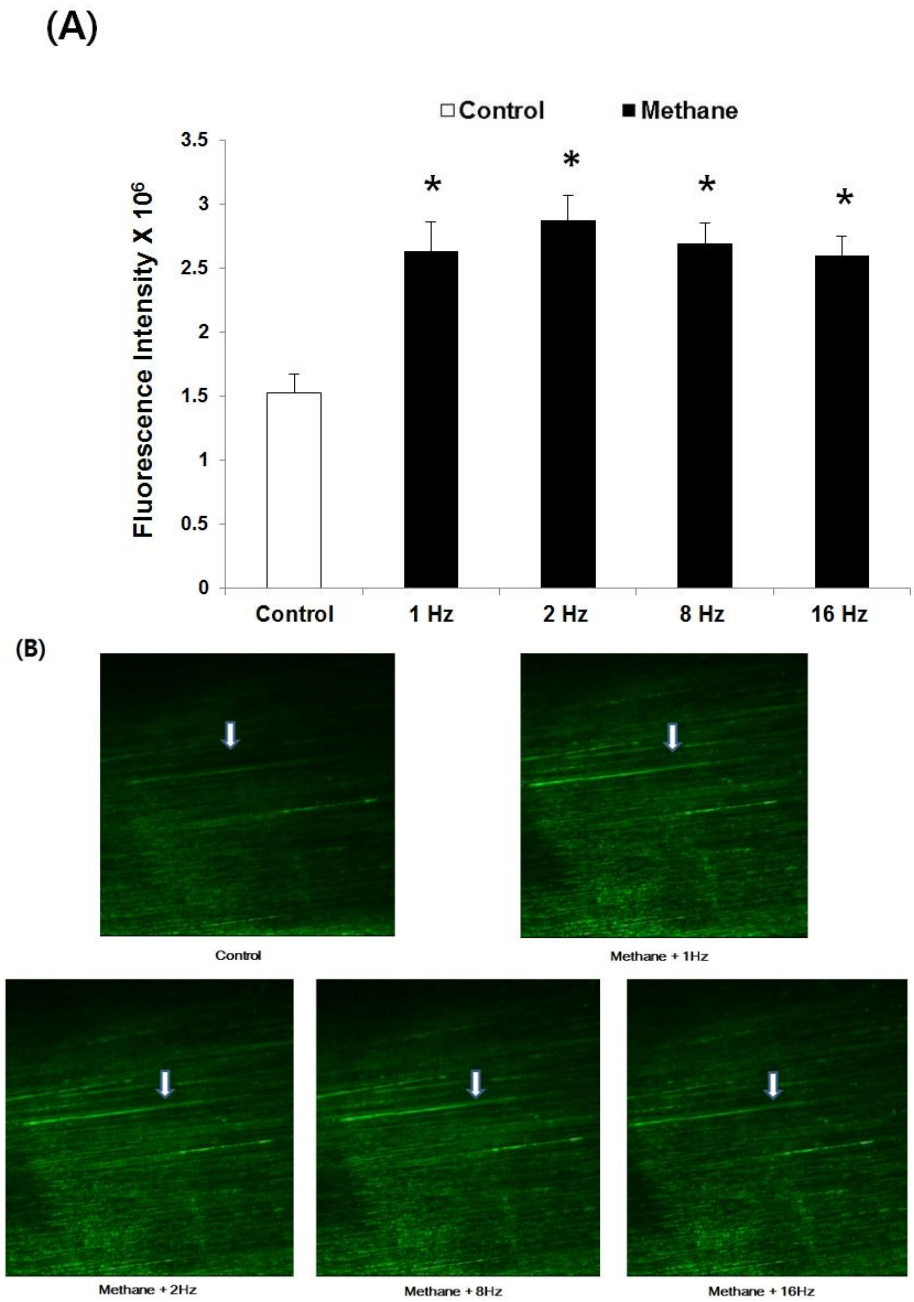


Figure 5. The effect of methane (A) as indicated by calcium fluorescence (B) on ileal contraction under electrical field stimulation (EFS). The calcium fluorescence increased following CH_4 infusion under EFS at all frequencies. *$P < 0.05$ (N=6)

This increase was significantly attenuated following atropine infusion (Fig. 6-A, B). As compared with the CH_4 group, the changes of calcium fluorescence were from 2.66 ± 0.27 to 1.50 ± 0.21 at 1 Hz, from 3.02 ± 0.30 to 1.49 ± 0.23 at 2 Hz, from 2.76 ± 0.28 to 1.52 ± 0.23 at 8 Hz, and from 2.69 ± 0.29 to 1.64± 0.35 at 16 Hz ($P < 0.05$, N=6).

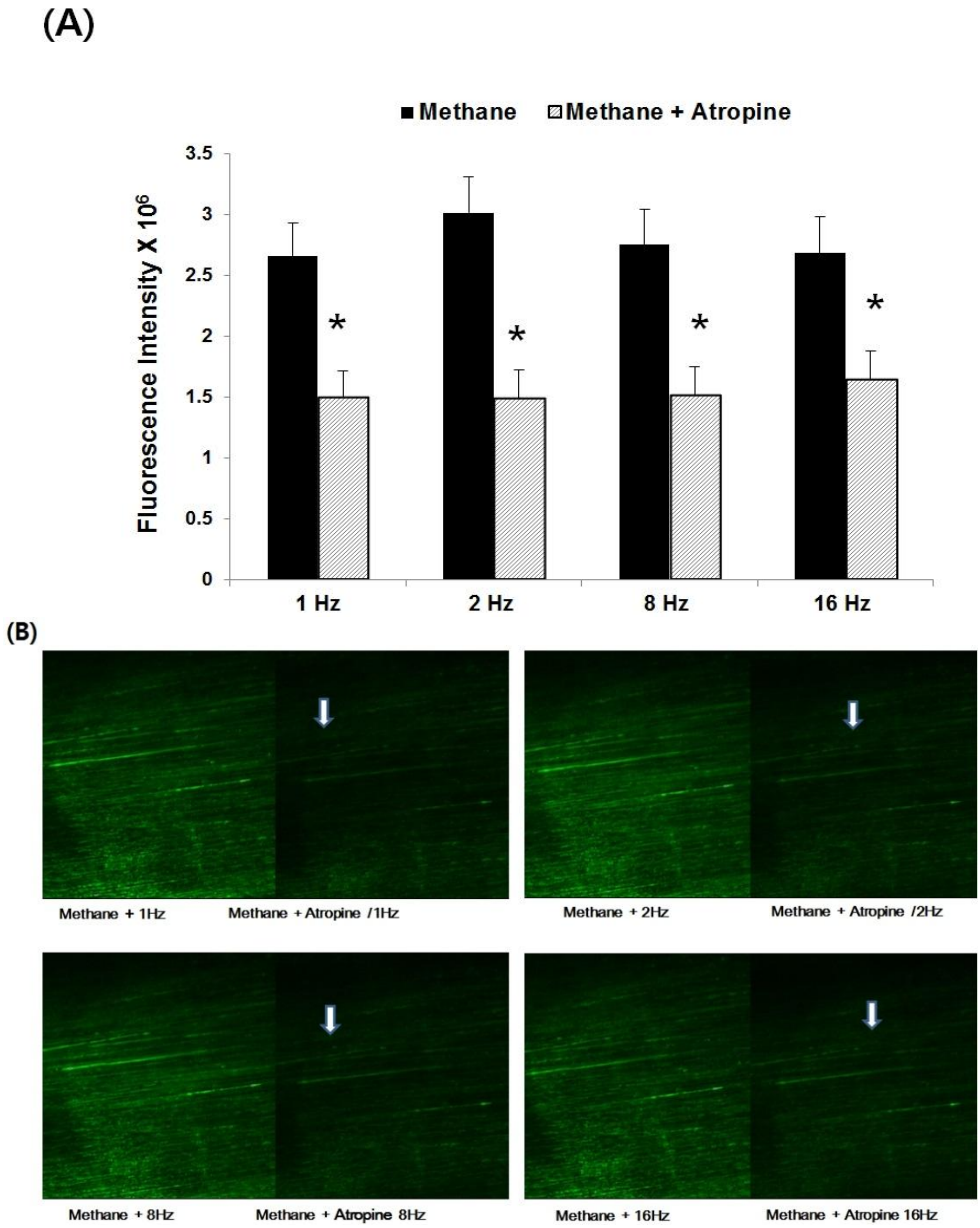


Figure 6. The effect of atropine (A) as indicated by calcium fluorescence (B) on ileal contraction under electrical field stimulation. An increased calcium signal by induced CH_4 was significantly attenuated following atropine infusion. *$P < 0.05$ (N=6)

High K^+ solution excites nerve and muscle. Figure 7 was showed a change of calcium fluorescence under high K^+ solution. Calcium fluorescence decreased after the addition of TTX, though the fluorescence increased again following the addition of the high K^+ solution ($P < 0.05$, N=6). However, high K^+ solution-enhanced calcium fluorescence remained unchanged following CH_4 infusion (P=0.229, N=6).

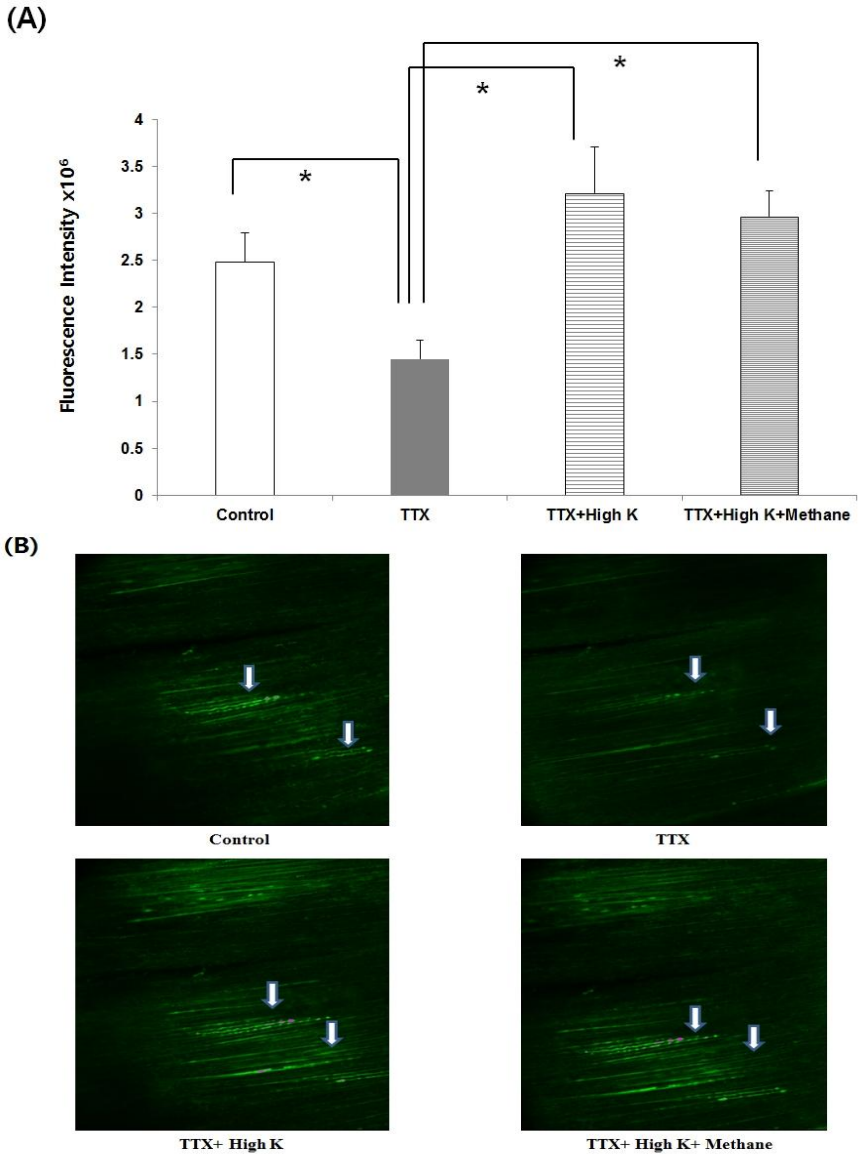


Figure 7. The effect of high potassium solution (A) as indicated by calcium fluorescence (B) on ileal contraction. Calcium fluorescence also decreased after the addition of tetradotoxin, though the fluorescence increased again following the addition of the high K^+ solution. Then, high K^+ solution-enhanced calcium fluorescence remained unchanged following CH_4 infusion. *$P < 0.05$ (N=6)

IV. DISCUSSION

CH_4 delayed the contraction velocity of ileal peristalsis and increased the amplitude of peristaltic contractions when CH_4 was infused to ileal muscle strips in a peristaltic bath in a previous study.[25] These results are consistent with those obtained by Pimentel et al., who reported ineffective transfer of food materials by augmenting ileal circular muscle contractile activity through segmental (non-propagating) contractions. In addition, they noted a higher fasting and postprandial motility index in patients with who produce excess CH_4.[24]

C-IBS has been associated with alterations in gut microbiota, such as increased *M.smithii,*[22] and alterations in the normal gut flora have further been suggested as etiologic factors in functional GI disorders.[29,30] Qualitative or quantitative alterations of microbiota in the small bowel may lead to clinical features such as small intestinal bacterial overgrowth (SIBO).[31] In SIBO, a change in the number and type of bacteria results in intestinal motor dysfunction and may be an underlying cause of IBS symptoms.[19,32,33] Researchers have also noted that methanogenesis is more common in constipating conditions, and that CH_4 levels measured using LBT are higher in C-IBS.[34-37] CH_4 is excreted either in flatus or breath and traverses the intestinal mucosa and is absorbed into the systemic circulation.[7] Because approximately 20–50% of CH_4 produced by anaerobic fermentation in intestine is excreted in the exhaled breath,[14,15] indirect measurement of CH_4 production rates provides a basis for LBT, which is a non-invasive and easy method for measuring the concentration of CH_4. Moreover, treatment with antibiotics has been associated

with normalization of the breath test and improvement in symptoms of IBS and constipation.[38-40]

How then does methane affect intestinal motor function? The mechanism by which methane affects bowel motility is not fully understood. To our knowledge, the present study is the first to investigate mechanisms underlying the actions of methane on intestinal motility. The present results suggest a correlation between these actions and specific neural pathways. Increased contractile amplitude following the infusion of CH_4 was abolished before and after the injection of TTX, which blocks neurotransmission, suggesting that neural pathways are more relevant than directly muscle contraction. Increased amplitudes due to CH_4 infusion were inhibited at lower frequencies (1, 2 Hz) when atropine was administered alone ($p < 0.05$). Otherwise, no significant changes were observed for any of the remaining frequencies in the presence of guanethidine. Such results may indicate that CH_4 is associated with cholinergic rather than adrenergic pathways. Amplitude of contraction was also inhibited at lower frequencies under NANC conditions. This change may be the effect of atropine because of considering the result of single administration of guanethidine, a norepinephrine release inhibitor. Although some researchers have reported a correlation between IBS and serotonin levels in both animal and human studies, administration of GR 113808, a selective 5-HT_4 antagonist, resulted in no statistically significant differences.[41-43]

Since calcium is a major signaling and regulatory molecule in smooth muscle cells, a calcium imaging analysis was also performed. The calcium response visualized by imaging intact tissues simultaneously using confocal microscopy,

together with the development of calcium indicators.[44,45] This Ca^{2+} imaging technique has previously been used to study the excitability of myenteric neurons and nerve fibers in response to EFS in intact tissues from the guinea pig ileum.[45,46] Electrical stimulation in a multilayer muscle preparation allows for the quantification of neuronal activation in the aboral and oral direction, demonstrating calcium transit in neurons.[47] In the present study, in accordance with the outcomes of the tissue bath experiment, increased calcium fluorescence following an infusion of CH_4 decreased following the addition of TTX, and the fluorescence also decreased following a single administration of atropine, though only at lower frequencies (1 and 2 Hz) of EFS ($p < 0.05$), suggesting CH_4 is mediated by neural pathways, and not by muscle activity.

An additional experiment was conducted using a high-K^+ solution to further examine the correlation of CH_4 with neural pathways. When a high-K^+ solution was applied after neuromuscular transmission was blocked using TTX, increased calcium fluorescence was again observed ($p < 0.05$). However, no statistically significant difference was observed following additional infusion of CH_4. High-K^+ solution excite both nerves and muscles, and therefore this result further supports the notion that CH_4 acts on the enteric neurones, rather than directly on the muscle.

Given the inhibition of ileal muscle contraction following methane infusion in the presence of TTX and atropine, it is possible to regard methane as a novel neurotransmitter. The term “gasotransmitter” was coined in 2002 and has been used to describe such molecules as nitric oxide (NO), CO, and H_2S.[48] Although CO_2 is the most obvious, other gases are found and can have a role in the regulation of human

biologic systems. The first gasotransmitter discovered was NO. NO, which is released by the endothelial cells of both arteries and veins, plays a key role in controlling vascular tone.[49] H_2S is also produced by humans and is found to have physiological activity on smooth muscle.[48] Considering its gaseous nature, high membrane permeability, endogenous production and catabolism in mammalian cells, and the biological and cellular effects induced by its exogenous factors, CH_4 is also a potential candidate as a gasotransmitter.[50]

V. CONCLUSION

In conclusion, this study demonstrates that CH_4 increases the contractile amplitude of ileal muscle, and that these actions are influenced by neural pathways. Although it is difficult to infer *in vivo* responses from the findings of *in vitro* studies, these results may be an important step in understanding the main characteristics of the mechanism underlying the actions of CH_4 on intestinal motility. These results may contribute to the verification of the hypothesis that CH_4 modulates intestinal motor function in certain GI diseases, potentially providing an experimental background for the development of new drugs for IBS.

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ABSTRACT (IN KOREAN)

메탄 가스가 회장 운동에 미치는 영향과 작용 기전

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배경 및 목적: 장내 세균에 의해 생성된 메탄 가스 (CH_4)는 장 운동에 영향을 미치게 되는데, 본 교실의 선행 연구에서 메탄 가스는 회장의 수축압(contraction amplitude)을 증가시키고 연동운동속도 (peristaltic conduction velocity)를 지연시켜 소장의 운동 기능을 저하 시킴을 확인하였다. 그러나 이에 대한 기전이 밝혀져 있지 않아서, 본 연구에서는 장내 세균에 의해 생성되는 메탄 가스의 회장 운동에 미치는 영향에 대한 작용 기전을 알아보고자 한다.

방법: 회장 근육 절편을 이용한 장력 실험을 통하여 수축압을 측정한다. 전장자극(electrical field stimulation, EFS) 하에서 tetradotoxin (TTX), atropine, guanethidine, GR 113808을 추가 투약하여 메탄 가스의 영향을 관찰한다. 이후 형광현미경을 이용하여 전장자극 하에 tetradotoxin, atropine 그리고 고칼륨용액 (high K+ solution)을 투약한 후 메탄 가스를 주입하였을 때 calcium signal의 변화를 살펴본다.

결과: 메탄가스는 수축압을 증가시키며 (p <0.05), TTX 투약은 수축을 사라지게 하였다. 이러한 결과로 메탄가스에 의한 변화는 신경성 매개임을 추측하였다. 이후 몇 가지 종류의 시약을 투여하여 자세한 신경성 매개 여부를 확인하였다. 메탄 가스에 의해 증가된 수축강도는 atropine을 투약에 의해 억제된다 (p <0.05). Guanethidine과 GR 113808 투약에서는

유의한 변화를 보이지 않았다. Calcium imaging study에서도 메탄 가스 투여 시 칼슘 신호 (calcium fluorescence)가 증가하고 atropine 투여 시에는 감소하였다 (p <0.05). 칼슘 신호는 고칼륨용액의 투여 시 증가하였으나 이후 메탄 가스를 추가 투여하였을 때에는 칼슘 신호의 강도의 변화는 없었다 (p <0.05).

결론: 장 내의 메탄가스는 신경성 통로, 특히 콜린성 통로와 연관성이 있는 것으로 보여진다. 이러한 결과로 메탄은 또 다른 gasotransmitter로 작용할 수 있음을 제안하는 바이다.

핵심되는 말: 메탄가스, 회장 운동, 평활근, 칼슘