Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity

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THE DISCOVERY THAT a gas, nitric oxide (NO), produced by endothelial cells controls vascular smooth muscle tone was a major advancement in the understanding of gaseous cell signaling (11). NO was later shown to facilitate numerous physiological processes in the body, including neural control of gastrointestinal motor functions. More importantly, aberrations in NO production can mimic clinical gastrointestinal disease in humans, such as esophageal spasm (15, 29) and achalasia (26). Two other endogenously produced gases, carbon monoxide (9) and hydrogen sulfide (H2S) (52), are biologically active; however, in the case of H2S, its role in gastrointestinal neuromuscular processes has not been well characterized. This small but growing list of gaseous transmitters raises the possibility that other gaseous mediators of biological processes may exist.

Several gases are liberated in large quantities by fermentation of food from bacteria colonizing the intestinal tract. The three most common of these exogenous gases are carbon dioxide, hydrogen, and methane (14, 16). Apart from the physiological role of gas buildup in the hollow viscous of the intestine, there is little direct evidence that any of these gases contribute to intestinal physiology or pathology.

Methane gas is produced by enteric bacteria in 30–62% of humans (1, 3, 6, 8, 17, 24, 36). Although the large quantities of methane produced in the gut are thought to be inert (51), three independent groups (6, 24, 46) reported slower intestinal transit in subjects with known intestinal fermentation producing methane compared with nonmethane producers. This association between methane and transit has never been investigated. In two studies of irritable bowel syndrome (IBS) subjects, our group (34, 35) reported that the presence of methane in expired breath after lactulose ingestion is universally associated with the constipation-predominant subgroup of IBS. Most of these subjects have an early rise in breath methane levels after lactulose, suggesting that the carbohydrate is being fermented to methane in the small intestine.

Based on these observations, we tested the hypothesis that methane slows intestinal transit by altering intestinal neuromuscular function. We tested this hypothesis is three ways. First, we used a well-characterized canine model (18, 20) to study the effects of methane on small bowel transit. Second, the effect of methane on neuromuscular function was explored using a physiological model of peristalsis in the guinea pig ileum (42). Finally, antroduodenal manometry findings were compared between methane- and nonmethane-producing IBS subjects.

METHODS

Methane and Transit in an In Vivo Canine Model

Animal preparations. In five mongrel dogs, two chronic small intestinal fistulas were each created surgically. Dogs averaged 25 kg in weight. The fistulas were located ~10 cm (distal to the bile and pancreatic ducts) and ~160 cm (midgut fistula) from the pylorus (18, 20). Cannulas were placed into each fistula and sutured in place to prevent rotation. A recovery period of 4 wk followed surgery so that testing was initiated only after normal feeding behaviors were well established. With this preparation, dogs remained healthy for more than 12 mo of observation.

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Experimental preparations. Dogs were deprived of food, but not water, for an 18-h period before experiments. Thirty minutes before the start of each experiment, both intestinal cannulas were uncerrou 90 min perfusion of buffer, so that a Foley catheter could be placed into the distal limb of the duodenal and the midgut fistulas. By inflating the Foley balloon with 10 ml of water, we achieved a water-tight seal at each fistula (18, 20). The output of each fistula was allowed to drain freely by gravity via the catheter. With this method, the proximal (between fistulas) and distal (beyond midgut fistula) halves of the gut were compartmentalized. Phosphate buffer (pH 7.0) alone was delivered at 2 ml/min for 90 min into the proximal half of gut via the catheter in the duodenal fistula (18, 20). To test for the effect of gas on transit, room air or methane (Air Liquide, Houston, TX) was delivered into the distal half of the gut via the Foley catheter in the midgut fistula at 2 ml/min for 90 min. This rate of methane delivery was selected because it achieved a methane concentration (50 ppm) in the exhaled breath of the dogs within the range observed in patients with IBS who have methane production.

Measurement of intestinal transit. Sixty minutes after the start of the 90-min perfusion of buffer, ~20 μCi 99m-Tc-diethylenetriaminepentaacetate was delivered as a bolus into the duodenal fistula to begin measurement of intestinal transit. One-milliliter samples were collected every 5 min from the output of the midgut fistula over 30 min. The radioactivity in each sample was measured in a gamma well counter to determine intestinal transit. The total radioactivity in the 99m-Tc bolus delivered into the small intestine was determined by counting a matched dose of 99m-Tc. After correcting all counts to time zero, we calculated intestinal transit as the cumulative percent recovery of 99m-Tc-diethylenetriaminepentaacetate over the 30-min collection period.

Methane and Guinea Pig Ileal Contractile Responses

Animals. Albino guinea pigs (200–400 g) were euthanized via CO2 asphyxiation. Laparotomy was then performed, and 3 cm of distal ileum were removed at a point 10 cm proximal to the ileocecal valve. The lumen was exposed by longitudinally cutting the mesenteric insertion. The tissue was then pinned serosa side down at its length in situ in an organ bath (Radnoti Glass, Monrovia, CA) containing modified Kreb solution (in mM: 118 NaCl, 4.8 KCl, 1.2 KH2PO4, 2.5 CaCl2, 25 NaHCO3, and 11 dextrose) at 37°C and gassed with 95% O2:5% CO2 at a rate necessary to maintain the pH at 7.4. The tissue was oriented in the bath with care to localize the oral and aboral sides of the tissue. The bath was then equilibrated for 30 min. The procedures used in this study were approved by the Institutional Animal Care and Use Committee at Cedars-Sinai Medical Center (Los Angeles, CA).

Orad contractile response. In these experiments, the tissue was pinned in the bath such that the 1.5 cm of tissue on the aboral end was pinned on both longitudinal tissue borders. On the oral side, the tissue was only pinned on one longitudinal border. A suture was placed through the mucosal edge at the midpoint along the unpinned longitudinal border. The suture was connected to a strain gauge (FT-103; Grass Telefactor, West Warwick, RI) via a pulley. The tissue was stretched until it generated 0.25 g of resting force. After the tissue was equilibrated in the bath gassed with the O2:CO2 mixture, 2, 4, 6, 8, and 10 brush strokes were applied to the mucosa 1.5 cm aboral of the suture insertion site. The tissue was allowed to rest for 7–10 min between stroke sets.

At the conclusion of the 10-stroke set, the bath was then gassed with 99.9% ultrapure methane (CH4; Air Liquide). The rate at which methane was bubbled into the bath remained constant, and the O2:CO2 flow was adjusted to ensure a pH of 7.4. In a preliminary experiment, methane was infused into the bath at various rates, and aliquots of Krebs were siphoned from the bath, equilibrated with room air head gas, and the head gas was analyzed. All rates of methane gassing resulted in identical head gas concentrations of 980–1,010 ppm. After a 10-min gassing period, the brush stroke sequence was repeated and contractile responses were measured.

Aborad contractile response. In the next phase of experiments, the ileal tissue was reversed in the bath such that the oral side was pinned on both sides and the aboral side only on one. The study of the aboral side of the reflex used a new set of guinea pigs from the oral experiments since the length of study would result in tissue fatigue. The suture was attached to the free edge of the aboral side and to the strain gauge as above. In this experiment, 0.5 g of tension was placed on the tissue to evaluate contractile response. Once again, 2, 4, 6, 8, and 10 brush stroke sets were applied to the tissue, this time 1.5 cm oral to the suture, with relaxation or contraction being measured. After completion of this sequence, the bath was gassed with CH4; after 10 min of equilibration, the sequence of brush strokes was repeated.

Methane and Small Bowel Motility in Humans

Patient population. Between 2001 and 2003, subjects referred for evaluation of IBS underwent a lactulose breath test to determine the presence of bacterial overgrowth. Subjects with an abnormal breath test then had routine small bowel manometry recordings in an attempt to identify factors contributing to the breath test abnormalities. In a retrospective review, consecutive subjects with methane and contemporary sex-matched IBS subjects with hydrogen on lactulose breath test who also had a small bowel manometry were eligible for inclusion in the study. Subjects were excluded if they had a history of small bowel obstruction, inflammatory bowel disease, celiac disease, narcotic use, autoimmune disease, diabetes, or previous bowel resection. The study was approved by the Cedars-Sinai Medical Center Institutional Review Board.

Antroduodenal manometry. For the small bowel manometry, patients presented to the gastrointestinal laboratory having fasted from midnight. After the nasal passage of the patient was anesthetized with 2% xylcaine gel (ASTRA USA, Westborough, MA), an eight-channel water-perfused small bowel manometry catheter (5 cm spacing) was placed transnasally into the stomach. Subsequently, the catheter was advanced via fluoroscopy into the small bowel such that five recording channels were in the small bowel with the distal channel at the ligament of Treitz and three channels in the antrum.

Once the catheter was in place, it was attached to an Arndorfer perfusion pump (Arndorfer Medical Specialties, Greendale, WI) with pressure transducers continuously recording pressure via Medtronic Polygraph and Polygram software (Medtronic Functional Diagnostics, Shoreview, MN). Subjects underwent 4 h of fasting recording. Subsequently, all subjects received a standard test meal followed by additional minutes of postprandial recording. The motility index was then determined for the fasting and postprandial periods (4). In addition, the number of phase III events and the number of motor contractions greater than 20 mmHg were counted for each tracing. Both of these were done by an investigator blinded to the presence or absence of methane in the subject.

Lactulose breath testing. All subjects must have had a lactulose breath test within 3 mo of the time of manometry. The breath test was performed by having the subjects fast for 12 h. Breath samples were then taken at baseline and every 15 min after the ingestion of 10 g of lactulose (Constulose; Alpharma USPD, Baltimore, MD) for 180 min. The breath was collected as an end-expiratory sample using the Quintron gas collection bag valve system (Quintron Instrument, Milwaukee, Wl). Breath samples were then analyzed for hydrogen and methane (in ppm) using a Quintron SC gas chromatograph (Quintron Instrument). The data were plotted graphically against time. From these data, the maximum hydrogen and methane levels as well as area under the curve of the breath test profile for each subject were determined.
Data Analysis

Statistical analysis. In the canine experiments demonstrating intestinal transit, the percent recovery of tracer at 30 min was compared between dogs infused with room air and methane using a paired $t$-test. In the studies of ileal contraction, forces of the ileal contractions were plotted against the intensity of brush strokes. The contraction forces (in g) were compared between simple bath oxygenation and the addition of CH$_4$ for both the orad and aborad compartments using a paired $t$-test. For the human study on small intestinal motility, the motility index and small bowel contraction frequencies were compared between methane- and nonmethane-producing subjects using a Student’s $t$-test. All data were expressed as means ± SD, with significance set at $P < 0.05$ in a two-tailed analysis.

RESULTS

Effect of Exogenous Methane on Transit in Dog Small Intestine

In the first phase of experimentation, five dogs were surgically outfitted with the intestinal fistulae outlined above. Subsequently, when transit was measured with and without methane in random order methane produced a reduction in transit (Fig. 1). Luminal methane infusion reduced radioactive marker recovery in all five dogs compared with room air by an average of 59% ($P < 0.0001$).

Effect of Exogenous Methane on Contractile Forces in Guinea Pig Ileum

In the second phase of experimentation, the effect of methane on the contractile response from mechanical stimulation of the guinea pig ileum was explored. After excision, mounting, and equilibration of the segment of ileum in the organ bath, the sets of brush strokes were applied. In the first component of study, stroking the mucosa produced contraction orad to the stimulus ($n = 8$), but the stimulus response was flat (Fig. 2A). Exposing the tissue to methane increased the force of contractions in response to mucosal stimulation (Fig. 2A). The change was statistically significant with 4, 8, and 10 strokes ($P < 0.05$). To better define the overall increase in contractile response and tone, the area under the curve produced by circular muscle contraction was studied. The area under the curve for the period of 10 s immediately after the brush strokes was also significantly higher after the tissue was exposed to methane (Fig. 2B). An example of the exaggerated contractile response is shown in Fig. 3.

Under control conditions, stroking the mucosa produced contraction aborad to the stimulus. Gassing the bath with methane again significantly increased the amplitude of contraction (Fig. 4A). In addition, the area under the curve for 10 s after stimulation was increased by methane (Fig. 4B).

Association Between Endogenous Methane and Motility Patterns in IBS Subjects

After retrospective review, 11 methane-producing and 12 sex-matched hydrogen-producing subjects meeting Rome I criteria for IBS who had antroduodenal manometry were identified. One subject had <10 min of postprandial recording (hydrogen producer) due to meal intolerance and was not included in the postprandial analysis. Of the subjects analyzed, the mean age of methane-producing subjects was 47 ± 6 yr, which was significantly older than the mean age of 38 ± 12 yr in hydrogen producers ($P < 0.05$).

After evaluation of the small bowel motility tracings of these IBS subjects, the fasting motility index in methane-producing subjects was noted to be significantly higher (1,851 ± 861) compared with hydrogen-producing subjects (1,199 ± 301) ($P < 0.05$) (Fig. 5). Similarly, in the postprandial period, the...
motility index was also increased in methane producers compared with hydrogen producers (545 ± 207 and 335 ± 74 in methane and hydrogen producers, respectively) (P < 0.05) (Fig. 6). When the number of contractions was compared, methane producers also had a larger number of isolated contractions in the fasting period. Over a 180-min fasting period, there were 155 ± 51 contractions in the methane group and 104 ± 29 contractions in the hydrogen group. Finally, the frequency of phase III activity fronts was no different between methane- and hydrogen-producing IBS subjects.

DISCUSSION

We have previously demonstrated that subjects with IBS have a propensity for abnormal findings on the lactulose breath test, suggesting bacterial overgrowth (33, 34), although our group (33) initially failed to explain the constipation subgroup of IBS (49). Our group (34, 35) subsequently found that, when methane is the bacterial fermentation product, these IBS subjects almost universally suffer from constipation. Despite this association, any causal relationship between methane production and constipation remained unexplored. In this translational study, we show for the first time that methane gas affects small intestinal neuromuscular function. Specifically, methane slows small intestinal transit in an in vivo dog model, it augments ileal circular muscle contraction produced by mechanical mucosal stimulation, and its presence during lactulose breath testing in humans with IBS is associated with an increase in the small bowel motility index.

As the role of gut ecology in chronic gastrointestinal disorders evolves, new evidence suggests that enteric bacteria play a role in functional bowel diseases such as IBS. Research has confidently demonstrated that an acute intestinal infection can trigger IBS in a substantial subset of patients (10), and IBS symptoms persist in one-third of patients after acute gastroenteritis (30) despite documented clearance of the inciting organism. These observations, however, fail to explain why persistent symptoms sometimes follow acute gastrointestinal infections. Some studies suggest that it relates to persistent inflammatory changes (7, 45). Our recent work (34, 35) suggests that excessive small bowel bacteria may play a role in generating symptoms in IBS subjects. Based on an indirect measure for the presence of enteric bacteria (lactulose breath testing) (32, 40), we have shown that a large proportion of subjects with IBS have test results suggesting the presence small intestinal bacterial overgrowth (33, 34). In a well-controlled double-blind study, normalization of the breath test in IBS subjects after antibiotic treatment correlates with near complete resolution of altered bowel symptoms (34). Because conventional thinking suggests that bacterial overgrowth is a condition manifesting primarily as diarrhea (40), the varied symptoms of IBS, specifically the constipation-predominant variant, initially defined explanation based on a bacterial theory. In subsequent analyses, however, our group (34, 35) found that methane-producing IBS subjects are universally constipation predominant.

Methane production in humans is believed to be exclusively due to methane generation in the gut. This methane production is limited to only a few species of bacteria. Two of these methanogens, Methanobrevibacter smithii (27) and Methanobacterium ruminatum (31), are only found in the left colon where methane production is thought to occur in 54% of normal subjects (24). Specific species of Bacteroides and Clostridium that reside in the gut can also liberate CH4 (25) and could easily be part of the small intestinal flora. There is some evidence for this. The study of methane using breath testing has demonstrated two discrete patterns of methane production after lactulose ingestion (6). One type of methane response is a high baseline level and an early rise in breath.
methane within 120 min after lactulose ingestion. A second type is a rise in methane beyond 600 min after lactulose. Methane detection beyond 600 min most likely represents lactulose arrival in the left colon, whereas production before 120 min seems more compatible with a small bowel source because it is implausible for lactulose to reach the left colon in this short period of time. Consistent with this interpretation, another study reports a rise in methane by 90 min after lactulose ingestion (36).

Although methane is widely considered to be inert, there are clues that it may affect intestinal function. In a study by Cloarec et al. (6), slower intestinal transit was observed in methane producers. In this study, healthy volunteers who produced methane on breath test had an orocecal transit time (based on rise in hydrogen during lactulose breath test) of 111 min compared with 68 min in subjects not producing methane. Supporting this association, Stephan et al. (46) similarly reported a whole gut transit time of 84.6 h in methane producers compared with 48.6 h in nonmethane producers. These studies simply described an association but provided no concept of a cause and effect relationship between methane and transit.

Could methane gas slow transit? The idea that gasses participate in physiological and pathophysiological processes is not novel. For example, NO is well established as a signaling molecule that controls myriad physiological processes. It and some products of its metabolism are important mediators of inflammatory processes that cause cellular and tissue injury. Bacterial fermentation of carbohydrate in the gut generates copious amounts of several gasses: H₂, CO₂, H₂S, and CH₄. Each of these is a small molecule that can easily traverse plasma membranes and move down its concentration gradient into tissues. In fact, the cecal wall of the mouse is supersaturated with CO₂ produced by intraluminal bacteria (38). This means that small molecules generated by bacterial fermentation in the intestinal lumen are likely to have local access to all components of the intestinal wall, including its neuromuscular apparatus. If this is so, any biologically active gas generated within the intestinal lumen may modulate intestinal function. This appears to be the case for CO₂, as it stimulates the absorption of Na⁺ by colonic and ruminal epithelia (5). H₂S is also biologically active. Although it seems to affect neuronal and smooth muscle function (2), there is as yet little evidence that its luminal generation affects intestinal function (50).

Our data support the hypothesis that methane slows transit by altering intestinal motor function, but how? In our dog studies, infusing methane into the distal small bowel slowed transit in a proximal intestinal segment. This implies that methane activates a reflex pathway that produces slowing in the proximal intestinal segment. This reflex is not likely to be triggered by mechanical stimulation produced by gaseous distension of the intestine because transit was not slowed when room air was infused at the same rate. There is already a well-established reflex pathway by which transit in the proximal gut is slowed by ileal contents in the form of fat (19). This phenomenon, called the ileal brake, is a complex reflex that depends on serotonin, opiate, β-adrenergic, and peptide YY signaling systems (21–23, 53). Discovery of such a mechanism for methane-induced slowing of transit would change our way of thinking about the control of intestinal motor function.

We subsequently used the isolated guinea pig ileum to determine whether methane had local effects on signaling systems controlling intestinal motor function. For this, we used a well-established model for studying reflex control of intestinal neuromuscular function. In this model, stimulating sensory neurons sensitive to stretch, mucosal deformation, or substances in the intestinal lumen trigger contractile reflexes. Sensory neurons synapse with interneurons that ascend or descend within the intestinal wall to communicate with motoneurons supplying the intestinal musculature. Brush strokes of the ileal mucosa or stretching the ileal wall generates circular and longitudinal muscle contraction orad and aborad to the stimulus (42–44). In our experiments, stroking the mucosa also produced circular muscle contraction orad and aborad to the stimulus, but the stimulus response relationship was rather flat. This flat response has also been reported by Monro et al. (28). When methane was gassed into the bath, it augmented the contractions on both sides of the stimulus. Because methane diffuses freely through membranes, it could affect any of the neuromuscular elements participating in the reflex. Although much work is needed to isolate this effect more conclusively, an effect on membrane channels would be an important future research area. Other hydrocarbon gases (similar to methane), such as halothane, have known demonstrable effects on smooth and striated muscle function (12, 37, 41).

Although the data above suggest that methane augments small intestinal motor function, this basic work needs transla-
tion back to human patients with IBS through which this concept of methane began (34, 35). Intestinal motility is often described using single values such as the motility index (14) as a gross measure of forces (resistive and propulsive) in the small intestine. However, the pattern of motility is also an important determinant of flow (39, 47, 48). In the guinea pig experiments, methane augments contractions that are both oral and aboral to a stimulus. This suggests that methane may trigger nonpropulsive or segmental contractions, and this change in pattern may be responsible for the slowing effect of intestinal transit seen in the dog experiments (Fig. 1). The very localized events seen in the guinea pig are more difficult to evaluate in vivo, and the motility index is a surrogate of overall activity. Our initial observation of early methane production in IBS subjects (34, 35) with constipation led to the pursuit of methane as a factor influencing small intestinal motility. In the third phase of our investigation, antroduodenal manometry was used to evaluate the relationship between endogenous methane production and small bowel motor function in these humans. Through this work, we found a significant increase in the motility index and contractile activity of the small bowel in methane-producing IBS subjects compared with nonmethane-producing IBS subjects. The consistencies in the response to methane between dogs, guinea pigs, and humans provide strong support for the contribution of methane in the constipation symptoms of IBS. In gnotobiotic mice, methane production by mixed anaerobic bacteria is an important determinant of flow (39, 47, 48). In the guinea pig experiments, methane augments contractions that are both oral and aboral to a stimulus. This suggests that methane may trigger nonpropulsive or segmental contractions, and this change in pattern may be responsible for the slowing effect of intestinal transit seen in the dog experiments (Fig. 1). The very localized events seen in the guinea pig are more difficult to evaluate in vivo, and the motility index is a surrogate of overall activity. Our initial observation of early methane production in IBS subjects (34, 35) with constipation led to the pursuit of methane as a factor influencing small intestinal motility. In the third phase of our investigation, antroduodenal manometry was used to evaluate the relationship between endogenous methane production and small bowel motor function in these humans. Through this work, we found a significant increase in the motility index and contractile activity of the small bowel in methane-producing IBS subjects compared with nonmethane-producing IBS subjects. The consistencies in the response to methane between dogs, guinea pigs, and humans provide strong support for the contribution of methane in the constipation symptoms of IBS.

Despite the observed response to methane in the small bowel seen in this study, the perception is that constipation is a reflection of colonic dysfunction. This has not been studied in vitro with methane. The present study looks primarily at small bowel motility. The reason for this choice is the ease of determining transit in the small bowel coupled with continuous infusion of methane. To simultaneously infuse methane into the colon of an animal and establish transit are more difficult. Nevertheless, Stephan et al. (46) have already demonstrated that humans with methane on lactulose breath test have delayed colonic (whole gut) transit, suggesting that the effect extends beyond the small bowel. The second reason for testing the small bowel is to connect the theme of bacterial overgrowth as a potential mechanism for IBS in general. Obviously, further work is needed to determine whether methane exerts a similar effect on colonic function in vitro.

In conclusion, our studies demonstrate that methane slows small bowel transit and augments contractile activity in the small bowel. In addition, small intestinal contractile activity is increased in IBS patients who produce methane. It is possible that methane predisposes to constipation because it promotes segmental (nonpropagating contractions). Obviously, further investigation is needed to elucidate the physiological mechanisms by which methane modulates intestinal motor function and to determine whether manipulation of methane production may affect intestinal transit in humans.

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REFERENCES


