Comparative study of hydrogen and methane production in the human colon using caecal and faecal homogenates

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Abstract

Rates of hydrogen and methane production were compared in caecal and faecal homogenates in six methane producers. Faecal homogenates produced hydrogen and methane in the absence of and after the addition of lactulose, whereas caecal homogenates produced hydrogen but little methane.

Hydrogen (H_2) and methane (CH_4) are produced during anaerobic bacterial activity in the human large intestine. Methane is absorbed in portal blood and excreted in breath, and pulmonary CH_4 excretion can be used as a simple indicator of CH_4 production in the colon.¹ In general, the population can be divided into two groups on the basis of CH_4 excretion – producers and non-producers of CH_4 .¹

In contrast to H_2 production, which rapidly fluctuates depending upon the availability of fermentable substrates, CH_4 excretion remains relatively constant throughout the day and is not influenced by meals.^{2,3} Similarly, ingestion of small doses (10 g) of lactulose, a non-absorbable disaccharide, does not increase breath CH_4 excretion.^{1,3} Larger amounts (20-33 g) of lactulose, however, can cause an appreciable increase in breath CH_4 excretion in CH_4 producers⁴⁻⁶ – subjects harbouring a sufficient count of methanogenic bacteria, mainly *Methanobrevibacter smithii*, which uses H_2 to reduce CO_2 to CH_4 .⁷ Methanogenic bacteria are strict anaerobes whose growth is favoured by a high pH and low dilution rates.^{8,9} These environmental conditions probably exist in the distal but not in the proximal part of the human large intestine.^{8,10,11} Moreover, during experiments aimed at determining the site of colonic CH_4 production, Bond *et al*¹ incidentally found that in one subject CH_4 production induced by in situ lactose infusion was occurring mainly distal to the colonic splenic flexure.

It was stated by Grimble in a recent issue of *Gut*,¹² that these data suggest that H_2 and CH_4 production may be separated by anatomy in the human large intestine and this prompted us to compare the rates of H_2 and CH_4 production from lactulose in human caecal and faecal samples.

Methods

SUBJECTS

Studies were carried out on six healthy male volunteers (aged 22-45 years) who were CH_4 producers (defined as producing at least one part

per million (ppm= μ l/l) of CH_4 above room air concentration).¹

CAECAL AND FAECAL SAMPLING

Caecal content was sampled in fasting subjects after intestinal intubation as described previously.¹³ Subjects were intubated with a double lumen tube led by a mercury bag that could be inflated with air to accelerate progression of the tube. When the bag reached the caecum, which was confirmed fluoroscopically, progression of the tube was stopped by deflating the bag. One lumen sealed with a three way stopcock was used to sample caecal content in a 50 ml sterile syringe after flushing the tube with 10 ml ultra-high purity nitrogen.

On the day elected for tube placement, stools were collected from each subject in a plastic bag containing an Anaerocult A (Merck, Darmstadt, Germany).

IN VITRO INCUBATION AND ANALYSIS

Caecal and faecal samples were processed immediately. Some 10 g were homogenised in 60 ml of 0.1 M anaerobic phosphate buffered saline (pH 7).¹⁴ Two 4 ml aliquots of caecal and faecal samples were then transferred to 50 ml sterile syringes. To one syringe each of treated caecal and faecal samples one ml of 1.25 g/100 ml solution of pure lactulose (Serva, Heidelberg, Germany) in 154 mM NaCl was added (experimental incubates), and the pH was measured with a pH meter (Radiometer, Copenhagen, Denmark). Then one ml of 154 mM NaCl was added to the other two syringes (control incubates). The contents of the syringes were mixed by shaking, flushed with 45 ml ultra-high purity nitrogen, and incubated at 37°C in a shaking water bath. Delays between all procedures were made as short as possible, and the total duration of handling time was about 15 minutes. After one hour of incubation the gas was recovered from both experimental and control incubates by displacement into another syringe that was equilibrated to room temperature before measurement of H_2 and CH_4 . The syringes were immersed in water at 100°C for five minutes, the final pH was determined, and the amounts of lactulose as fructose were measured by an enzymatic method (Boehringer, Mannheim, Germany). The H_2 and CH_4 concentrations were multiplied by the volume of gas in the syringes and expressed as μ l. In the caecal and faecal incubates with lactulose, the volumes of H_2 and CH_4 were recorded as the changes in μ l minus control values. The quantity of lactulose

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that disappeared after one hour of incubation was calculated by subtracting the postincubation value from the added amount of lactulose. Final results were expressed as $\mu\text{l H}_2$ and CH_4 per mg lactulose consumed.

Statistical analysis for significance was performed using Wilcoxon's signed rank test. Data are expressed as mean (SEM).

Results

In control incubates, the H_2 released by faecal homogenates tended to be lower than in caecal homogenates ($0.717 (0.446)$ v $3.560 (1.706)$ μl). Methane production occurred almost exclusively in faecal incubates at a rate significantly higher than in caecal homogenates ($5.658 (1.858)$ v $0.052 (0.008)$ μl , $p < 0.05$). The initial pH (6.6 v 7.2) and the final pH (6.7 v 7.0) did not vary significantly in caecal and faecal control incubates.

The percentage of lactulose consumed during the one hour incubation period was not significantly different in caecal and faecal homogenates ($80.5 (5.2)$ v $97.5 (1.5)$ %). The H_2 liberated by faecal homogenates from lactulose tended to be higher than in caecal homogenates. Methane production occurred almost exclusively in faecal incubates at a rate significantly higher than in caecal homogenates ($p < 0.05$) (Table). The initial pH (6.6 v 7.2) and the final pH (5.9 v 6.0) were not significantly different in caecal and faecal incubates.

Discussion

Although lactulose was extensively degraded in both caecal and faecal homogenates CH_4 production occurred only in faecal homogenates. The same result was obtained in control incubates. The extent to which the final pH of the incubates with lactulose changed did not explain the difference in CH_4 production between caecal and faecal homogenates. This finding seems to contrast with that of Perman and Modler,¹⁵ who concluded that CH_4 production is sharply pH-dependent. In their incubation experiments, in which CH_4 formation was inhibited, however, the pH of faecal incubates declined to 4.0, whereas it averaged 6.0 in our experimental conditions. In any case, the important point is that the pH of caecal incubates was not significantly lower than that of the faecal ones.

We did not measure other fermentation products, such as organic acids and total viable anaerobic and methanogenic bacterial concentrations in the faecal and caecal samples. Nevertheless, our results confirm data from an in vitro model mimicking the human colon⁸ and provide further evidence that methanogenesis and probably the growth of methanogenic bacteria are taking place distal to the right part of the large intestine where suitable environmental conditions would be present. This could explain why large oral loads of lactulose able to induce

Hydrogen and methane production ($\mu\text{l/mg}$ lactulose consumed) by caecal and faecal homogenates from six CH_4 producers after addition of lactulose (mean (SEM))

	Hydrogen	Methane
Caecal homogenates	0.892 (0.457)	0.002 (0.001)*
Faecal homogenates	1.206 (0.452)	2.607 (0.834)†

* v † $p < 0.05$.

diarrhoea with the presence of sugars in stools¹⁶ increase CH_4 excretion in CH_4 producers⁴⁻⁶ when a part of this sugar reaches the distal colon, whereas small doses of lactulose, which are completely degraded in the right colon,¹³ do not support CH_4 production. A few days²⁻⁵ are required to observe a sustained increase of CH_4 excretion in CH_4 producers consuming diets rich in xylan or pectin,¹⁷ which can escape fermentation in the right colon and reach the distal colon. As the colonic transit time of dietary fibres is slow, it is not surprising that these substrates do not increase CH_4 excretion a short time after their ingestion, and this probably explains why breath CH_4 excretion is not influenced by meals, unlike H_2 which is produced when fermentable substrates from meals are delivered to the right colon.

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