The effects of butyrate enemas on visceral perception in healthy volunteers


*TI Food and Nutrition, Wageningen, The Netherlands
†Division of Gastroenterology and Hepatology, Department of Internal Medicine, Maastricht University, Nutrim, Maastricht, The Netherlands
‡Department of Population Genetics, Genomics and Bioinformatics, Maastricht University, Maastricht, The Netherlands
§TNO Quality of Life, Department of Biosciences, Zeist, The Netherlands
¶School of Health and Medical Sciences, Örebro University, Örebro, Sweden

Abstract Fermentation of dietary fibres by colonic microbes leads to the production of short chain fatty acids (mainly propionate, butyrate and acetate), which are utilized by the colonic mucosa. Previous studies showed positive effects of butyrate on parameters of oxidative stress, inflammation and apoptosis. Recent studies in rats, however, showed that butyrate increased visceral sensitivity. The aim of this study was to determine the effects of physiologically relevant concentrations of butyrate on visceral perception in healthy human subjects. Eleven healthy volunteers participated in this randomized double-blind, placebo controlled cross-over study. The study consisted of three periods of 1 week each, in which the volunteers daily self-administered rectal enemas containing 100, 50 mmol L\(^{-1}\) butyrate, or placebo (saline) prior to sleeping. A rectal barostat measurement was performed at the start and the end of each test period for the measurement of pain, urge and discomfort. Butyrate treatment resulted in a dose-dependent reduction of pain, urge and discomfort throughout the entire pressure range of the protocol. At a pressure of 4 mmHg, 50 and 100 mmol L\(^{-1}\) butyrate concentrations resulted in a 23.9% and 42.1% reduction of pain scores, respectively, and the discomfort scores decreased by 44.2% and 69.0% respectively. At a pressure of 67 mmHg, 50 and 100 mmol L\(^{-1}\) of butyrate decreased the pain scores by 23.8% and 42%, respectively, and discomfort scores 1.9% and 5.2% respectively. Colonic administration of butyrate, at physiologically relevant concentrations, dose-dependently decreases visceral sensitivity in healthy volunteers.

Keywords barostat, butyrate, enema, humans, rectal, visceral perception.

INTRODUCTION

The human large intestine harbours a complex diversity of micro-organisms, which exert both positive and negative effects on gut physiology. Short chain fatty acids (SCFAs) are derived from microbial fermentation of undigested dietary fibres in the colon. Most saccharolytic fermentation occurs in the proximal colon whereas in the distal colon the depletion of fermentable carbohydrates leads to a switch towards proteolytic fermentation, which is less favourable due to the formation of toxic end products (such as ammonia, sulphur compounds, indoles and phenolic compounds). The amount of SCFAs (mainly acetate, propionate and butyrate) produced in the colon depends on the site of fermentation, the diet and the composition of the microbiota, and can provide up to 5–15% of the substrates for human energy production.\(^1\)

Among the different SCFAs, butyrate is known to modulate numerous processes.\(^2\) Increased colonic butyrate formation has often been proposed as one of the protective mechanisms of high fibre diets.\(^3,4,5\) Butyrate is the major energy source for colonocytes\(^6\) and may act as a signal metabolite affecting epithelial cell proliferation, apoptosis and differentiation.\(^7\) There
is evidence that butyrate beneficially affects several inflammatory parameters such as cytokines and myeloperoxidase activity, primarily via inhibition of nuclear factor kappa B activation. Furthermore, butyrate stimulates intestinal mucus production, thereby supporting the mucosal barrier function, increases anti-oxidant capacity, increases mucosal blood flow, and may decrease colonic epithelial permeability.11,12

Although there is ample evidence for these beneficial effects from in vitro and animal models, limited studies have confirmed these effects in vivo in humans. Previously, positive effects of butyrate on inflammation in active distal ulcerative colitis have been reported. Paradoxically, two studies in rats showed that butyrate instillation in the colon increased visceral sensitivity and maintained an increased visceral sensitivity after trinitrobenzene sulphonic acid (TNBS) induced colitis. It is not known whether a comparable effect of butyrate administration on visceral sensitivity can be observed in human subjects. The aim of this study therefore is to evaluate the effects of rectally administered butyrate, in a concentration that can be reached by consumption of a high fibre diet, on visceral perception in healthy volunteers.

MATERIALS AND METHODS

Subjects

Eleven healthy volunteers (three male and eight female) were included in this randomized, double-blind, placebo-controlled cross-over study. None of the subjects had a history of gastrointestinal disease or previous abdominal surgery. The study was approved by the Medical Ethics Committee of University Hospital Maastricht and conducted in full accordance with the principles of the Declaration of Helsinki (52nd WMA General Assembly, Edinburgh, Scotland, Oct 2000). All volunteers gave their written informed consent prior to participation. The study has been registered in the US National Library of Medicine (http://www.clinicaltrials.gov, NCT 00726817).

Study design

The study consisted of three periods of 1 week each, with a wash-out period of 2 weeks in between the test weeks. The volunteers self-administered rectal enemas (enema bottles were kindly provided by Tramedico Holding B.V., Weesp, The Netherlands) containing a 60 mL solution of either butyrate (100 or 50 mmol L–1) or placebo (saline) once daily just prior to sleeping. During the first 20 min after enema instillation, volunteers were instructed to stay in a left lateral supine position. The enemas were made isotonic with sodium chloride and had a pH of 7. Barostat measurements to assess visceral perception were performed at the start and at the end of each test week (Fig. 1). Compliance of the enema administration and complaints were monitored using a daily questionnaire.

Barostat protocol

After arrival in the hospital, the volunteers self-administered a rectal enema containing 60 mL of saline to clean the rectum. Five minutes thereafter, subjects were instructed to void rectal contents.

Subsequently, the volunteers laid down on a bed in a left lateral supine position and remained in this position during the entire test procedure. A commercially available barostat balloon (Mai Scientific, Mississauga, ON, Canada, part: C7-2CB-R) was lubricated with KY gel (Johnsson & Johnsson, Langhorne, PA, USA) and inserted rectally 3 cm proximal to the anal sphincter. After a 5-min habituation period, the balloon was attached to the barostat equipment (Distender II, G&J Electronics, Toronto, ON, Canada) and the barostat procedure was started. The controlled balloon distensions were programmed using the standard software package of the barostat equipment [Protocol Plus Deluxe, version 6.7; G&J Electronics].

The barostat protocol consisted of four subprotocols, each designed for the measurement of specific parameters of interest.

Balloon unfolding This part of the protocol consisted of one single distension at a balloon pressure of 20 mmHg for 1 min, to ensure that the balloon was placed correctly without folds that may impair the airflow.

Minimal distension pressure The second part of the protocol consisted of a staircase distension protocol with pressure steps of 1 mmHg with a duration of 30 s each and a range from 0 to 20 mmHg. The minimal distension pressure (MDP), which is the minimal balloon pressure needed to overcome the intra-abdominal pressure, was defined as the first pressure at which respiratory curves were present in the volume recording of the balloon. The entire protocol was performed up to the 20 mmHg pressure in all subjects as a sensitization step prior to the compliance and perception measurements. The obtained MDP value was set to zero as a reference point. During this protocol, the volunteers were asked to report the moment at which they could sense the balloon for the first time. This pressure was defined as the threshold for first sensation.

Compliance Directly after finishing the MDP measurements, the third part of the protocol was initiated. This part of the protocol, designed for compliance measurement, which represents the elasticity of the rectum in mL mmHg–1, consisted of a staircase distension protocol with pressure steps of 3 mmHg with a duration of 30 s each and a range of 0–53 mmHg.
Visceral perception Subsequently, the distension protocol of the visceral perception measurements was initiated. This protocol consisted of semi-random distensions (at 4, 13, 10, 19, 16, 25, 22, 31, 28, 37, 34, 40, 49, 46, 55, 52, 61, 58, 67, 64, 71 mmHg above MDP respectively) with a duration of 1 min each, interspaced with 30-s intervals at MDP. Thirty seconds after the start of each distension, volunteers scored the sensation of pain and discomfort on a 10-cm visual analogue scale (VAS) and urge on a six-point scale (0 = no feeling, 1 = just sensible, 2 = clearly sensible/light urge, 3 = normal urge, 4 = strong urge/have to run to toilet, 5 = maximum/stop) represented by six buttons on an electronic control panel (distender II perception panel), which was directly linked to the barostat equipment. The procedure was stopped when the maximum score for pain, urge or discomfort was reached.

Statistics
The pain and discomfort data were analysed using a Gaussian nonlinear regression, a random effect, and an autocorrelation. Urge was scored on an ordinal six-point scale and was analysed using a combination of a logistic distribution [parameterized as a proportional-odds] and a gamma distribution [to introduce a series dependence]. The mean regression was imposed through the time variable to follow a logistic (‘S-shape’) curve. The model included pressure, MDP, first sensation, procedure (treated or not), and carry over effect as explanatory variables. The inference criterion used for comparing the models is their ability to predict the observed data, i.e. models are compared directly through their minimized minus log-likelihood. When the numbers of parameters in models differed, they were penalized by adding the number of estimated parameters, a form of the Akaike information criterion (AIC). The variable under consideration was found to be affected by butyrate if the AIC decreased compared to the model not containing the treatment. For each variable of interest, the dose as a continuous variable was then added to the model. Effects were considered significant if the AIC decreased and the confidence intervals did not overlap. The effect of butyrate on first sensation thresholds was considered significant when the confidence intervals of the threshold difference did not include zero.

RESULTS
Eleven volunteers were enrolled in the study. One volunteer was excluded from further participation in the study due to non-compliance, which was assessed on basis of a questionnaire and the returned enema bottles. No side effects of the enema use were reported and no carry-over was found between the three test conditions.

Butyrate treatment resulted in a significant reduction in pain, urge and discomfort scores over the entire pressure range of the protocol in a dose-dependent way (Figs 2 and 3 and Table 1). In Fig. 2, also the confidence intervals for the pain scores are shown, demonstrating a significant effect of butyrate over the entire pressure range. The confidence intervals for the discomfort scores were too small for graphical representation and therefore presented in Table 2, at three chosen points in the pressure range of the protocol (at 4, 22 and 40 mmHg above MDP). The urge scores, shown in Table 1, also differed significantly over the entire pressure range. Due to the ordinal [six-point scale] characteristics, it was not possible to provide confidence intervals of this parameter. For each dose of butyrate, the urge score with the highest likelihood to be scored at each pressure step has been presented.

The pressure threshold for first sensation was higher after the placebo treatment compared to baseline [increase of 3 mmHg; CI: 0.87–5.12 mmHg]. Compared to placebo, both 50 and 100 mmol L\(^{-1}\) of butyrate

© 2009 Blackwell Publishing Ltd
administration significantly reduced the threshold (reduction of 2.7, CI: 0.11–5.33 mmHg and 4.9, CI: 2.18–7.65 mmHg respectively).

Only the highest dosage of butyrate increased the dynamic compliance, when measured at a pressure of 12 mmHg, which was in the linear part of the graph (Fig. 4). At a pressure of 12 mmHg, the volume increased from 200 mL (CI: 197–203 mL) after placebo and 50 mmol L\(^{-1}\) butyrate to 215 mL (CI: 212–218 mL) after 100 mmol L\(^{-1}\).

DISCUSSION

The aim of the present study was to evaluate the physiological effects of butyrate on visceral perception in healthy subjects. The study showed that 1-week rectal butyrate administration decreased the perception of pain, urge and discomfort compared to placebo in a dose-dependent fashion. Compliance of the rectal wall increased significantly after 100 mmol L\(^{-1}\) butyrate treatment.

The present results indicating a significant decrease in visceral perception are in contrast with previous findings from rat studies, in which butyrate prolonged visceral hypersensitivity in TNBS-treated rats and induced visceral hypersensitivity in control animals.\(^{16,17}\) Although the perception scores were decreased due to the butyrate treatment, no conclusions about the putative underlying mechanisms can be drawn as we did not take biopsy samples for histological, biochemical and genetic analysis.

The decrease in visceral perception due to butyrate treatment could be via modulation of 5-hydroxytryptamine (5-HT or serotonin) activity. 5-HT is a neurotransmitter that is found predominantly in the gastrointestinal tract. SCFAs are known to stimulate intraluminal 5-HT release in rat colon, resulting in an increase in motility.\(^{22}\) Previous studies in humans have shown that an increased 5-HT release leads to an increased compliance and a decrease in visceral perception.\(^{18}\) 5-HT exerts its actions by an activation of specific serotonin receptors, of which 5-HT\(_3\) and 5-HT\(_4\) are known to induce colonic contractions leading to an increased motility\(^{23}\) and some evidence exists for a neuroprotective effect of 5-HT\(_4\) receptor stimulation.\(^{24}\)

The effect of serotonin on visceral sensitivity is not clear but an increased compliance has been reported in literature, suggesting that sensation thresholds may be higher when serotonin is released.\(^{25–27}\)

An indirect effect of the butyrate-mediated 5-HT release could be through sensitization of transient
receptor potential vanilloid 1 (TRPV1) receptors in the colonic mucosa. TRPV1 receptors are responsible for pain transduction to the central nervous system and stimulation of these receptors by butyrate or 5-HT could lead to an increase in pain sensation. Moreover, increased TRPV1 receptor expression in irritable bowel syndrome (IBS) was found to correlate with abdominal pain. Overstimulation or repetitive stimulation of this receptor, however, is known to desensitize afferent neurons as has been shown for capsaicin. As butyrate-induced serotonin release could trigger TRPV1, it may well be that desensitization of TRPV1 after 1 week of butyrate administration occurs. These effects are in concordance with the effects of butyrate on perception scores and compliance in this study.

Another mechanism by which butyrate could affect visceral perception is via inhibition of histone deacetylation. Several of these inhibitors, valproate, butyrate and trichostatin A, have previously been reported to induce microglial apoptosis and to reduce inflammation-induced neurotoxicity in rat tissue, which may affect visceral perception. Those findings, together with the previously reported observation that valproate is effective in pain reduction, suggest that butyrate exerts its effect on visceral sensitivity in part via its histone deacetylation inhibiting capacity.

These possible mechanisms underlying the effect of butyrate on visceral perception do not fully explain the difference between the results found in rats and humans. However, the differences may in part be explained by the concentration differences or differences between 5-HT receptor subtypes and their precise function and location in both species. Furthermore, the methods for the visceral perception measurements in rats (behavioural changes and abdominal contractions) and humans (VAS or ordinal scales) are different, which makes a good comparison of the results difficult. The subjects of this study were well instructed and underwent a dummy barostat measurement at the screening to ensure a minimal level of uncertainty and anxiety. This may further explain the difference between well-instructed volunteers and rats, as fear and anxiety have been reported to influence pain scores.

The applied barostat methodology is a generally accepted and validated method to measure visceral perception in humans. The pressures that were induced during the semi-random staircase protocol for visceral perception were corrected for the MDP. In this study, the MDP value was determined by increasing the pressure gradually until respiratory waves were visible in the volume curve of the barostat apparatus. Although we are aware of alternative methods to estimate MDP, we consider this approach as most reliable as it is based on physiological differences between subjects and conditions rather than an arbitrary cut-off.

In a previous pilot study, the length of the wash-out period of 2 weeks was determined and found to be sufficient (data not shown). This was confirmed by this study as no carry-over effect of butyrate and placebo was found.

In this study, the butyrate and placebo were delivered by enemas, which provide a reliable and non-invasive tool to deliver a substance to the distal colon. Although this way of delivery was considered most appropriate, a placebo effect was found on the parameter ‘first sensation’, which may have been caused by the use of enemas. None of the other parameters was significantly affected by placebo administration.

The results of this study show a remarkable improvement of parameters of visceral perception and suggest a possible beneficial effect of butyrate in disorders, which are associated with visceral hypersensitivity such as IBS. This is in line with previous findings of Kilkens et al., which demonstrate a serotonin-dependent difference on compliance between IBS patients and controls. More research is needed to unravel the mechanisms of action by which butyrate decreases visceral perception. Furthermore, the results of this study should be confirmed by an oral-intake study with non-digestible fibres, which lead to a fermentation-mediated increase in colonic butyrate levels.

In conclusion, intraluminal administration of a physiologically relevant dose of butyrate into the distal colon, increases compliance and decreases pain, urge and discomfort measured with a rectal barostat procedure in healthy subjects. This provides a basis for future trials with dietary modulation resulting in intracolonic butyrate production in both healthy and IBS subjects.

ACKNOWLEDGMENTS AND DISCLOSURES

This study was funded by the Top Institute for Food and Nutrition (TIFN), Wageningen, The Netherlands. The authors had complete access to the data supporting this publication. An abstract of this study was accepted for an oral presentation at the NGM 2008 in Lucern.

COMPETING INTERESTS

The authors have no competing interests.
REFERENCES

1 Bergman EN. Energy contributions of volatile fatty acids from the gastro-intestinal tract in various species. Physiol Rev 1990; 70: 567–90.


