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Butyrate specifically modulates *MUC* gene expression in intestinal epithelial goblet cells deprived of glucose

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Gaudier, E., A. Jarry, H. M. Blottière, P. de Coppet, M. P. Buisine, J. P. Aubert, C. Laboisse, C. Cherbut, and C. Hoebler. Butyrate specifically modulates *MUC* gene expression in intestinal epithelial goblet cells deprived of glucose. *Am J Physiol Gastrointest Liver Physiol* 287: G1168–G1174, 2004. First published August 12, 2004; doi:10.1152/ajpgi.00219.2004.—The mucus layer covering the gastrointestinal mucosa is considered the first line of defense against aggressions arising from the luminal content. It is mainly composed of high molecular weight glycoproteins called mucins. Butyrate, a short-chain fatty acid produced during carbohydrate fermentation, has been shown to increase mucin secretion. The aim of this study was to test *1*) whether butyrate regulates the expression of various *MUC* genes, which are coding for protein backbones of mucins, and *2*) whether this effect depends on butyrate status as the major energy source of colonocytes. Butyrate was provided at the apical side of human polarized colonic goblet cell line HT29-Cl.16E in glucose-rich or glucose-deprived medium. In glucose-rich medium, butyrate significantly increased *MUC3* and *MUC5B* expression (1.6-fold basal level for both genes), tended to decrease *MUC5AC* expression, and had no effect on *MUC2* expression. In glucose-deprived medium, i.e., when butyrate was the only energy source available, *MUC3* and *MUC5B* increase persisted, whereas *MUC5AC* expression was significantly enhanced (3.7-fold basal level) and *MUC2* expression was strikingly increased (23-fold basal level). Together, our findings show that butyrate is able to upregulate colonic mucins at the transcriptional level and even better when it is the major energy source of the cells. Thus the metabolism of butyrate in colonocytes is closely linked to some of its gene-regulating effects. The distinct effects of butyrate according to the different *MUC* genes could influence the composition and properties of the mucus gel and thus its protective function.

mucin; short-chain fatty acids; energy source; human colonic cell line

THE MUCUS LAYER, COVERING the gastrointestinal mucosa, is considered the first line of defense against mechanical, chemical, or microbiological aggressions arising from the luminal contents (14). Mucus is mostly composed of mucins, i.e., glycoproteins of high molecular weight, whose protein backbones are encoded by *MUC* genes. So far, at least 15 different *MUC* genes have been identified in humans (15, 32). In the large intestinal mucosa, the main *MUC* genes are *MUC2*, and to a lesser extent *MUC1*, *MUC3*, and *MUC4*. *MUC2* codes for the main secreted mucin in the colon, whereas *MUC1*, *MUC3*, and *MUC4* mainly code for membrane-located mucins but also present splicing variants coding for secreted mucins (50). Apart from their gel-forming protective function, some membrane-linked mucins, such as MUC1 (22) and MUC4 (12), exhibit specific functions in adhesion and cell signaling.

MUC gene expression is altered in many colonic diseases. *MUC2* is overexpressed in mucinous colorectal carcinoma, whereas its expression is particularly low in nonmucinous carcinoma (17, 43). *MUC5AC* and *MUC6* expressions are abnormally induced in colon adenoma (6, 9). Aberrant expression of *MUC* genes (8) as well as modifications of their transcription (34, 45) have also been observed in inflammatory bowel disease. In addition, the thickness of the mucus layer is reduced in ulcerative colitis (33). On the other hand, mice knockout for *Muc2* gene spontaneously develop adenocarcinoma in both small and large intestine (48).

In the colon, the mucus layer is in direct contact with carbohydrate fermentation end products, the short-chain fatty acids (SCFA). It has been reported that SCFA stimulate mucin discharge in rats (36). Among three main SCFA produced in the human colon (i.e., acetate, propionate, and butyrate) butyrate appears to be the most effective in modulating mucin synthesis and release (5, 16, 39). Interestingly, a recent study (2) points to *MUC* gene expression as a target of SCFA in goblet cells. Our hypothesis was that SCFA could act at the earliest step of mucin synthesis (i.e., on *MUC* gene expression) and that their effect could be different according to the *MUC* gene studied. Indeed, butyrate is known to modulate the expression of numerous genes mainly involved in cell proliferation and apoptosis (3, 7, 18, 27, 40, 44). In addition, we assumed that butyrate was more active because of its specific utilization by the colonic epithelium. Indeed, butyrate is extensively and preferentially oxidized by the colonic epithelium to whom it provides 70% of its oxidization energy (35).

The aim of this study was to determine the effects of butyrate and the other main SCFA on various *MUC* gene expressions in the HT29-Cl.16E cell line, and to assess whether manipulation of the energy source of the culture medium can influence these effects. We used the human colonic goblet cell line HT29-Cl.16E (4) because this clonal derivative of HT29 displays a homogeneous and stable differentiated phenotype of mucus-secreting cells. HT29-Cl.16E cells, which can be maintained as polarized monolayers on permeable filters, have been validated as a good model for deciphering the regulation of mucin secretion (19). In addition, the *MUC* gene expression profile of HT29-Cl.16E cells includes *MUC2* and *MUC3*, the major mucins expressed in the human colon (4, 19, 47). The

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O-glycosylation profile of their mucins has also been characterized as a rather colonic profile (11).

MATERIALS AND METHODS

Cell culture. HT29-Cl.16E cell line (4), a clonal derivative of the HT29 human colonic cell line (49) was routinely cultured in DMEM 25 mM glucose with glutamine without sodium pyruvate (Invitrogen, Cergy-Pontoise, France) supplemented with 10% heat-inactivated FCS (D. Dutscher, Brumath, France) in 25-cm^2 flasks (Costar, Brumath, France). The culture medium was changed daily; cells were passaged every week and seeded at a density of 40,000 cells/cm^2.

Cell treatments. Cells were used at late postconfluence (*day 18*) when they were fully differentiated and secreted mucus. They were then seeded and cultured for 8 days at high density on permeable filters (12-well Transwell Clear, 0.4-μm porosity, Costar, Brumath, France) and formed polarized monolayers, secreting mucins into the apical compartment. Treatments with 2 or 5 mM butyrate, 2 mM propionate, 6 mM acetate (Sigma, St. Quentin Fallavier, France), 1 μM trichostatin A (TSA) (Upstate Biotechnology, Euromedex, Mundolsheim, France), or 10^{-5} M forskolin (Calbiochem, Meudon, France) were performed during 1, 3, 8, and 24 h. SCFA and TSA were added to the apical compartment; forskolin was added to both the apical and basolateral compartments. Adding phosphoric acid 2.5 M to the medium performed the adjustment of pH to 6.4. Glucose-deprived medium was obtained by adding 10% of dialyzed FCS to DMEM without glucose (Invitrogen) and applied to cells in both compartments.

Assessment of cell death by lactate dehydrogenase activity measurement. Lactate dehydrogenase (LDH) activity was assessed by using a standardized kinetic determination (Enzyline LDH kit; Biomerieux, Marcy-l'Etoile, France). LDH activity was measured in both floating dead cells and viable adherent cells. Floating cells released into the culture medium were carefully collected by centrifugation (240 *g* for 10 min at 4°C), and the LDH content from the pellets, referred to as LDH_p, was used as an index of apoptotic cell death (13, 21). The LDH released into the culture medium of extracellular LDH (LDH_e) was used as an index of necrotic cell death. The LDH present in the adherent, viable cells was referred to as intracellular LDH (LDH_i). The percentage of apoptotic and necrotic cell death was calculated as follows: %cell death = $(LDH_p + LDH_e)/(LDH_p + LDH_e + LDH_i) \times 100$.

RT-PCR analysis of MUC gene expression. RNA was extracted from HT29-Cl.16E using RNeasy mini kit (Qiagen, Courtaboeuf, France) and submitted to DNase digestion following the manufacturer's instructions. Reverse transcriptase was performed by using Advantage RT-for-PCR kit (Clontech Laboratories, Palo Alto, CA). cDNA were then amplified by using *Taq* DNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany). Denaturation for 4 min at 94°C was followed by 26 to 30 cycles composed of 30 s denaturation at 94°C, 30 s hybridization at 60°C, and 1-min elongation at 72°C. The last cycle was followed by 7-min final elongation at 72°C. Oligonucleotide (MWG Biotech, Ebersberg, Germany) sequences used for the amplification of each gene are shown in Table 1. PCR products were run on 1.5% agarose gel. A semiquantitative analysis of *MUC* gene expression normalized to GAPDH expression was performed by densitometry (NIH Image 1.62). *MUC* gene RT-PCR products were normalized to GAPDH RT-PCR products, the arbitrary value of 1 being assigned to this ratio for control cells. *MUC2*, *MUC3*, *MUC5AC*, and *MUC5B* only were analyzed, because *MUC1*, *MUC4*, and *MUC6* were not expressed nor induced by SCFA in the HT29-Cl.16E cell line.

Quantification of SCFA uptake by the cells. SCFA were analyzed by gas-liquid chromatography (20) on supernatants of thawed apical and basolateral medium centrifuged at 8,000 *g* for 10 min. 4-Methylvaleric acid was used as an internal standard. The uptake of SCFA was calculated by subtracting the remaining SCFA in apical and basolateral medium to the initial amount given to the cells.

Statistical analysis. MUC gene expression was given as means ± SE, and the modulator effects of the treatments were analyzed with a Student's *t*-test compared with control cells. The percentages of cell death assessed by LDH activity measurements, as well as amounts of SCFA used by the cells, were given as means ± SE, and the effects of treatments or of glucose presence or absence were assessed by one-way ANOVA. For all statistical analyses, differences were considered as significant for $P < 0.05$.

RESULTS

Time and dose-dependent effects of butyrate on MUC gene expression in the mucus-secreting cell line HT29-Cl.16E. To analyze the effect of butyrate on *MUC* gene expression, we cultured the mucus-secreting cells HT29-Cl.16E without or with 2 and 5 mM butyrate for different time periods. Cells were also treated with forskolin (10^{-5} M) and used as a positive control of *MUC2* gene induction (19). RT-PCR analysis of *MUC2*, *MUC3*, *MUC5AC*, and *MUC5B* mRNA levels showed that treatment with 2 and 5 mM butyrate for 24 h significantly enhanced *MUC3* expression compared with control cells (1.6- and 1.9-fold basal level, $P = 0.016$ and $P = 0.0013$, respectively). In addition, *MUC5B* gene expression was significantly increased on 2 mM butyrate treatment (1.6-fold basal expression, $P = 0.0105$), whereas *MUC2* and *MUC5AC* gene expression were not significantly modified (Fig. 1).

A kinetic study showed that the significant increase in *MUC* gene expression on butyrate treatment was observed after 24-h incubation, but not at the earlier time points tested (1, 3, and 8 h, data not shown).

Comparison between butyrate and other SCFA effects on MUC gene expression. We compared the effect of 2-mM butyrate incubation for 24 h with those of propionate (2 mM), acetate (6 mM), or a mixture of the three SCFA. These SCFA

Table 1. *Oligonucleotides used for RT-PCR analysis of MUC gene expression*

Gene	Sequence of PCR Primers	Access Number	Amplified Fragment	Cycle Number
MUC2	Forward: 5′-CTGCACCAAGACCGTCCTCATG-3′ Reverse: 5′-GCAAGGACTGAACAAAGACTCAGA-3′	L21998	401 bp	27
MUC3	Forward: 5′-AGTCCACGTTGACCACCACTGCC-3′ Reverse: 5′-TGTTCACATCCTGGCTGGCG-3′	AF113616	522 bp	30
MUC5AC	Forward: 5′-TGATCATCCAGCAGCAGGGCT-3′ Reverse: 5′-CCGAGCTCAGAGGACATATGGG-3′	AJ001402	409 bp	26
MUC5B	Forward: 5′-CTGCGAGACCGAGGTCAACATC-3′ Reverse: 5′-TGGGCAGCAGGAGCACGGAG-3′	Y09788	451 bp	27
GAPDH	Forward: 5′-TGAAGGTCGGAGTCAACGGATTTGG-3′ Reverse: 5′-CATGTGGGCCATGAGGTCCACCAC-3′	AF261085	923 bp	20

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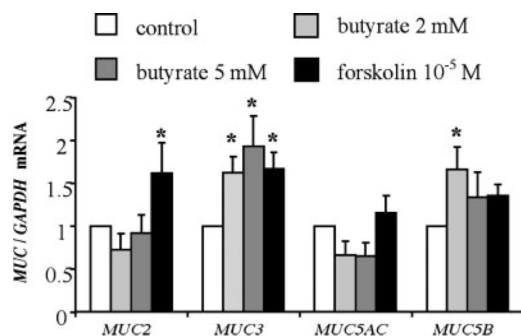

Fig. 1. Butyrate effects on *MUC* gene expression. HT29-Cl.16E monolayers cultured on Transwell filters were treated for 24 h with butyrate (2 or 5 mM) or forskolin (10^{-5} M) as described in MATERIALS AND METHODS. A semiquantitative RT-PCR analysis for *MUC2*, *MUC3*, *MUC5AC*,and *MUC5B* was performed. Data are expressed as *MUC* RT-PCR products normalized to GAPDH products, the arbitrary value of 1 being assigned to this ratio for control cells. Graphs represent means ± SE of 5 separate experiments. $*P < 0.05$ relative to control cells.

concentrations were chosen to mimic the levels of 60% acetate, 20% propionate, and 20% butyrate found in the human colonic lumen. Acetate and propionate had no effect on *MUC3* and *MUC5B* expression (Fig. 2). The SCFA mixture tended to stimulate *MUC3* gene expression (1.5-fold basal level) although this effect was not statistically significant ($P = 0.06$). The expression of *MUC2* was not modified, whatever the SCFA tested. *MUC5AC* expression was significantly decreased by acetate ($P = 0.0376$) and almost significantly decreased by the SCFA mixture ($P = 0.0537$).

After 24-h incubation, the levels of SCFA uptake by HT29-Cl.16E cells reached 55% of butyrate, 59% of acetate, and 33% of propionate they received, (Table 2). When a mixture of the three SCFA was used, butyrate and acetate uptake remained stable, whereas propionate uptake was significantly inhibited ($P = 0.016$), reaching only 5%.

Influence of the culture medium pH on the butyrate-induced effects on MUC gene expression. In standard cell culture conditions, the medium is buffered to reach a pH of 7.4, whereas in the colonic lumen, fermentation leads to pH values ≤6 (23). Moreover, it has been shown that butyrate uptake by Caco-2 cells within 1 h is stimulated by lowering extracellular

Table 2. *Uptake of SCFA by HT29-C116E cells during 24 h*

	Amount of SCFA	
	Given in 0.5 ml	Uptake in 24 h
Butyrate (2 mM)	1.03±0.10	0.56±0.03
Propionate (2 mM)	0.95±0.03	0.35±0.10
Acetate (6 mM)	2.70±0.58	1.61±0.09
Mix:		
Butyrate (2 mM)	0.99±0.15	0.56±0.03
Propionate (2 mM)	0.95±0.08	0.05±0.06
Acetate (6 mM)	2.77±0.47	1.42±0.19

Values are means ± SE in micromoles of 3 separate experiments. Short-chain fatty acid (SCFA) uptake by the cells was measured as described in MATERIALS AND METHODS.

pH (42). We aimed at determining whether lowering extracellular pH modified butyrate modulation of *MUC* gene expression. Lowering the pH to 6.4 increased *MUC3* ($P = 0.0007$) but not *MUC5B* basal expression, and it did not change the stimulatory effect of butyrate (2 mM for 24 h) compared with control cells at pH 7.4 (Fig. 3). *MUC2* and *MUC5AC* gene expression of control and butyrate-treated cells was not altered by an acidic pH (6.4) (data not shown). In addition, lowering the pH did not change the total uptake of butyrate by HT29-Cl.16E cells during 24 h incubation (Table 3).

Influence of glucose deprivation on butyrate effect on MUC gene expression. In standard culture conditions, cell lines are maintained in high glucose medium (25 mM), whereas in the colonic epithelium, glucose reaches the cells only through the 5 mM systemic circulation. We aimed at determining whether butyrate effects on *MUC* gene expression, which we observed in glucose-rich medium, were maintained in glucose-deprived medium. Glucose deprivation did not lead to cell death of untreated cells. However, it increased cell death under butyrate treatment (from 6.4 to 10.5%, $P = 0.0194$), but cell death remained acceptable, because it never exceeded 11% (Fig. 4). The stimulatory effect of butyrate on *MUC3* and *MUC5B* gene expression was maintained (1.9- and 1.6-fold control level, $P = 0.007$ and $P = 0.0036$, respectively) (Fig. 5). However, *MUC5AC* and above all *MUC2* gene expression were significantly enhanced by 2 mM butyrate exposure in the glucose-free medium (3.7- and 23-fold basal level, $P = 0.0123$ and $P <$

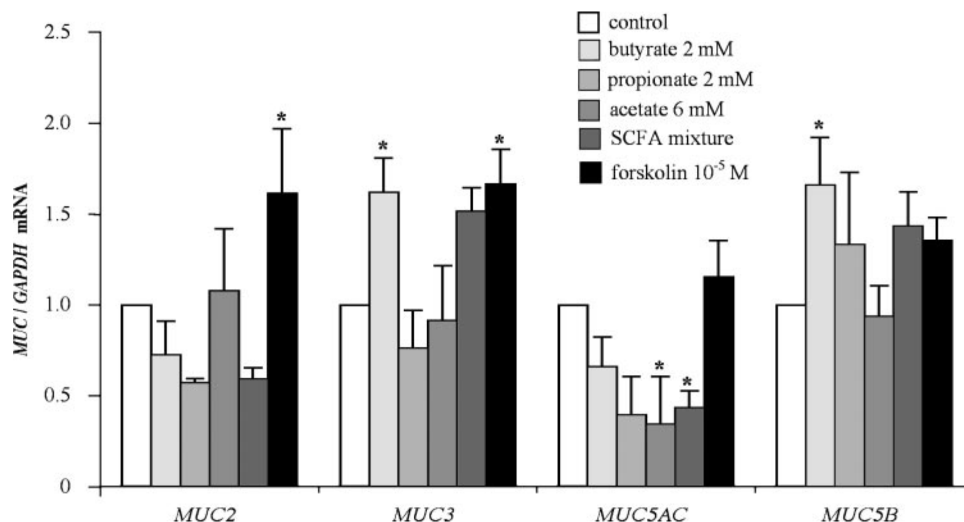

Fig. 2. Comparison of the effects of various short-chain fatty acids (SCFA) on *MUC* gene expression. HT29-Cl.16E were treated and processed as mentioned in the legend of Fig. 1. Graphs represent means ± SE of 3 separate experiments. $*P < 0.05$ relative to control cells.

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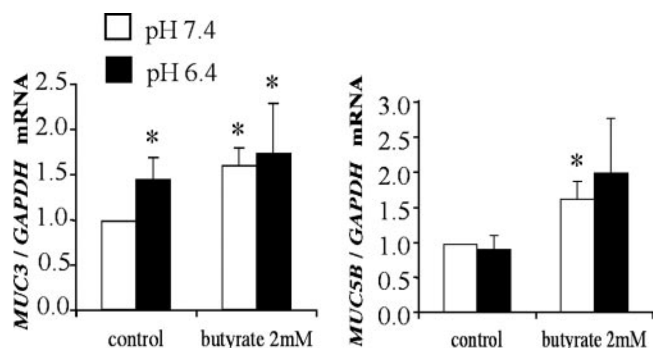

Fig. 3. Influence of the extracellular pH on the effect of butyrate on *MUC3* and *MUC5B* gene expression. Graphs represent values of 3 separate experiments ± SE. $*P < 0.05$ relative to control cells at pH 7.4.

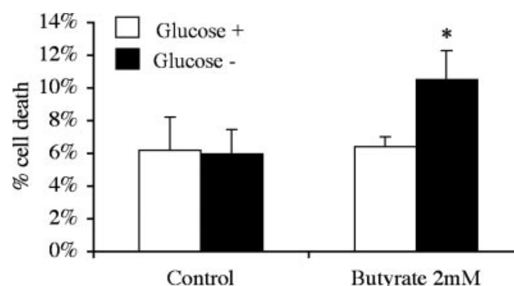

Fig. 4. Cell death in HT29-Cl.16E maintained in the presence or absence of glucose. Cells were incubated or not for 24 h with 2 mM butyrate in glucose-rich (glucose +) or glucose-free (glucose −) culture medium. Percent cell death was assessed by LDH measurements as described in MATERIALS AND METHODS. Values are means ± SE of 3 separate experiments. $*P < 0.05$ relative to control cells in glucose-rich medium.

0.0001, respectively). These effects were not reproduced by 2 mM glucose. Propionate or acetate still had no effect on *MUC* gene expression in the glucose-free medium (data not shown). Moreover, glucose deprivation significantly increased butyrate uptake by the cells in 24 h ($P = 0.0407$), because it reached 81% of the total amount provided to the cells compared with 55% in the glucose-containing medium (Table 4).

Comparison between TSA and butyrate effects on MUC gene expression. It is well established that butyrate modulates the expression of numerous genes by inhibiting histone deacetylase (HDAC) activity (10, 41). We thus tested the effect of TSA, a specific HDAC inhibitor, on *MUC* gene expression. In glucose-deprived medium, TSA stimulated *MUC3* gene expression in the same range as butyrate (1.7-fold control level, $P < 0.0001$) (Fig. 6); however, *MUC2, MUC5AC, MUC5B* gene expressions were unaltered (data not shown).

DISCUSSION

This study is the first demonstration that butyrate modulates *MUC* gene expression in human colonic goblet cells, specifically compared with other SCFA. This modulation varies according to the *MUC* gene studied, and is strongly dependent on the energy source provided to the cells.

We first showed that butyrate increased *MUC3* and *MUC5B* expression, whereas acetate and propionate had no effect on these genes. Our results extend previous reports on the specific role of butyrate on the modulation of mucin synthesis and release (5, 16, 39). Interestingly, this specific effect of butyrate on mucins has also been described for other cellular functions such as differentiation and programmed cell death (7). The hypotheses that can be raised to explain butyrate specificity include a more efficient uptake of this SCFA by colonic epithelial cells, a specific role linked to the molecule of butyrate itself, or to its metabolism. SCFA uptake can follow different pathways, i.e., a nonionic passive diffusion, via a nonelectrogenic $SCFA^-/HCO_3^-$ antiport or via a $SCFA^-/H^+$ cotransport through monocarboxylates transporters (37). All of these pathways are common to acetate, propionate, and butyrate. In our study, the proportion of acetate and butyrate uptake by HT29-Cl.16E cells was similar, whereas butyrate but not acetate did modulate *MUC* gene expression. This implies that the difference in the effects on *MUC* gene expression caused by butyrate vs. acetate was not due to a difference in their uptake by the cells. The other two hypotheses are more likely: on one hand, the molecule of butyrate itself could directly act on gene expression, because butyrate responsive elements have been identified on various promoters of other genes than mucin genes (7). On the other hand, butyrate metabolism could be responsible for its effects, because it is well established that butyrate is the main energy source of colonocytes, representing 70% of their oxidative energy, whereas propionate and acetate are rather transferred to the portal circulation (35) and metabolized in liver and muscles.

We then demonstrated that butyrate effect on *MUC* gene expression was distinct according to the *MUC* gene studied, because in standard culture conditions, butyrate enhanced *MUC3* and *MUC5B* expression, but had no effect on *MUC2* expression and tended to decrease *MUC5AC*. Our results are in line with the findings of Velcich et al. (46, 47) who demonstrated that butyrate exposure stimulated *MUC3* but not *MUC2* gene expression in the parental undifferentiated HT29 cell line (2). Regulation pathways of *MUC* gene expression have recently been examined, but are still a matter of debate. First, the chromosomal localization of *MUC* genes could help in understanding their different modulations; *MUC3* is located on the chromosome 7q22, whereas *MUC2*, *MUC5AC*, and *MUC5B* belong to a cluster located on the chromosome 11p15.5, probably originating from an ancestral gene and possibly sharing pathways of regulation (15). Second, our demonstration that TSA reproduces the effects of butyrate only on the *MUC3* gene suggests that HDAC inhibition can be involved in the action of butyrate on *MUC3* expression but not on *MUC2*, *MUC5AC*, and *MUC5B* gene expressions. Other signaling pathways have been reported to be involved in *MUC2* gene regulation, such as protein kinase C pathway, the EGF-receptor/Ras/Raf/ERK cascade, or regulation by transcription factors of the Sp family (1, 25, 31). *MUC5AC* and *MUC5B* promoters also possess binding sites for Sp1 and NF-κB. Butyrate has also been shown in other

Table 3. *Influence of the extracellular pH on 2 mM butyrate uptake by HT29-C116E cells*

	Amount of SCFA	
	Given in 0.5 ml	Uptake in 24 h
pH 7.4	1.03±0.10	0.56±0.03
pH 6.4	1.09±0.09	0.47±0.10

Values are means ± SE in micromoles of 3 separate experiments.

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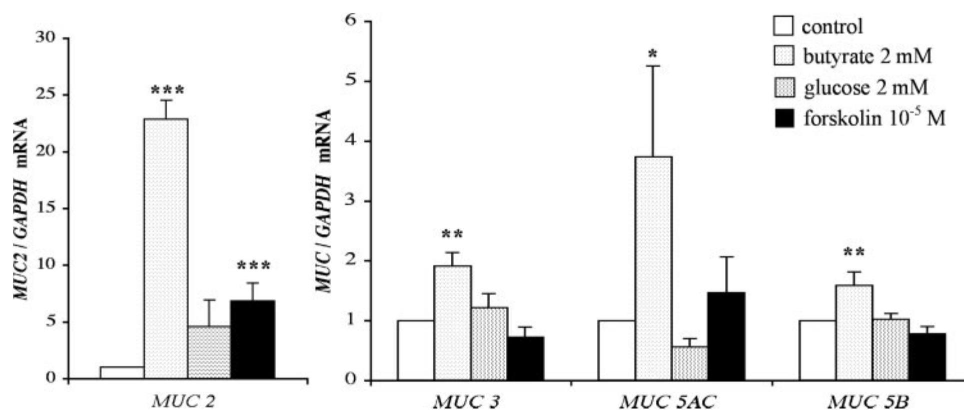

Fig. 5. Butyrate effects on *MUC* gene expression (after 24 h of incubation) in glucose-deprived medium. Graphs represent means ± SE of 4 separate experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.0001$ relative to control cells.

cell types and cellular functions to modulate NF-κB activation (38) or Sp1 binding (30). Further experiments are needed to test whether the butyrate effect on *MUC* gene expression can trigger one of these pathways in our in vitro model.

Another main finding of this study is that the effects of butyrate on *MUC* gene expression are dependent on the energy source available. Indeed, in conditions of glucose deprivation, the simulation of *MUC3* and *MUC5B* by butyrate was maintained, but *MUC5AC* and especially *MUC2* expressions were enhanced. In our model, these changes of *MUC* gene expression in glucose-deprived medium cannot be accounted for by cell death. This is the first report of an enhancement of butyrate effect on the modulation of *MUC* gene expression during glucose deprivation. Such an effect has, however, been reported for another gene involved in intestinal cell differentiation, i.e., alkaline phosphatase (24). In addition, butyrate uptake by the cells was increased in the absence of glucose. The association between increased butyrate uptake by the cells and the enhancement of its effects on *MUC* gene expression, support the hypothesis of the role of butyrate metabolism in the modulation of gene expression. Thus butyrate metabolism could then be involved, not only on mucin synthesis (16), but also at the transcriptional level. Indeed, Leschelle et al. (26) showed that butyrate acts not only on proliferation by itself but also by its metabolites generated in the mitochondria upstream of acetyl-CoA synthesis. The involvement of very early steps in butyrate metabolism in these regulations is confirmed by our results, because the high increase of *MUC2* expression in the absence of glucose is neither reproduced by glucose itself nor by another SCFA. Mariadason et al. (28) also showed that when colonocytes preferentially use glucose as an energy source, the provided butyrate remains available for genetic effects through the histone deacetylase pathway, whereas when cell metabolism turns into preferential butyrate oxidation, butyrate effects could involve its metabolites. Our results also show that 24 h of incubation with butyrate are required to obtain modulation of *MUC2* and *MUC5AC* gene expression. We did not characterize the metabolism of SCFA by HT29-Cl16E cells, but Leschelle et al. (26) demonstrated that HT29 cells oxidize into CO_2 only the 1.5% of butyrate provided at 10 mM in 90 min. A few more hours would then be required to obtain efficient amounts of butyrate metabolism products, which is in line with the hypothesis of the role of its metabolites. Moreover, the upregulation of *MUC2* and *MUC5AC* by butyrate occurring only when it is the only energy source for the cells also supports the hypothesis of the role of its metabolites. But our study does not allow us to conclude that butyrate per se does not have any effect on *MUC* gene expression; we showed that *MUC3* expression is enhanced by butyrate, probably through a HDAC pathway, which is thought to involve the butyrate molecule per se (29). We rather suggest that *MUC3* gene expression could be modulated by butyrate per se, whereas the *MUC2* and *MUC5AC* gene modulation could involve butyrate metabolites.

In conclusion, we demonstrated that butyrate specifically modified *MUC* gene expression in intestinal goblet cells. In glucose-rich medium, *MUC3* expression was enhanced but not *MUC2*. However, increasing butyrate consumption by the cells with a glucose-deprived medium led to a strike increase of *MUC2* gene expression. Butyrate metabolism could then be involved not only in its effects on mucin synthesis (16), but also as early as on the gene transcription. The distinct effects of butyrate on the membrane-associated

Table 4. *Influence of glucose deprivation on butyrate uptake by HT29-C116E cells*

	Amount of SCFA	
	Given in 0.5 ml	Uptake in 24 h
Glucose-rich	1.03±0.10	0.56±0.03
Glucose-free	0.90±0.03	0.72±0.10

Values are means ± SE in micromoles of 3 separate experiments.

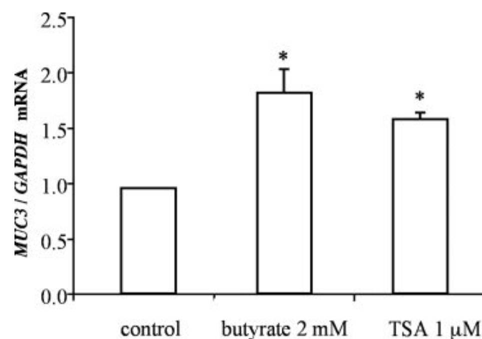

Fig. 6. Trichostatin A (TSA) reproduced the effects of butyrate on *MUC3* gene expression (after 24 h of incubation) in glucose-deprived medium. HT29-Cl.16E monolayers were submitted to a 24-h butyrate (2 mM) or TSA (1 μM) treatment and then to RT-PCR analysis for *MUC3*. Graphs represent values ± SE of 4 separate experiments. $*P < 0.05$ relative to control cells.

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MUC3 and on the secreted MUC2 could result in modifications of the properties of the mucus layer they compose and of its protective function.

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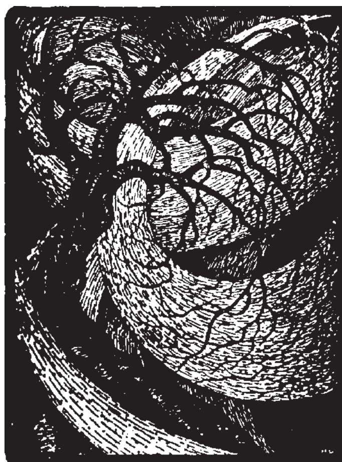

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