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Butyrate-stimulated H_2S Production in Colon Cancer Cells

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Abstract

Butyrate is a short-chain fatty acid that arrests growth of various types of cells. H_2S can be endogenously produced by cystathionine γ-lyase (CSE) or cystathionine β-synthase (CBS) or both in colonic tissues. In this study, we observed endogenous H_2S production in a colon cancer cell line (WiDr) and colonic tissues through the activity of both CSE and CBS. After 24 h of incubation of WiDr cells, butyrate increased cell production of H_2S and upregulated CBS and CSE expressions. Both butyrate and NaHS (a H_2S donor) decreased cell viability in a dose-dependent manner. Blockade of CBS, but not CSE, decreased butyrate-stimulated H_2S production and reversed butyrate-inhibited cell viability. In addition, NaHS treatment stimulated the phosphorylation of extracellular signal-regulated kinase (ERK) and p38 mitogen–activated protein kinase (MAPK), but not c-Jun N-terminal kinase (JNK). Inhibition of the phosphorylation of either p38 MAPK or ERK did not abolish NaHS-induced cell death. Butyrate treatment increased the phosphorylation of ERK, not p38 MAPK and JNK, but inhibition of ERK and p38 MAPK phosphorylation did not inhibit butyrate-reduced cell viability. In conclusion, butyrate regulates endogenous H_2S production by stimulating CBS expression in colon cancer cells, but butyrate and H_2S inhibit cancer cell growth through different mechanisms. *Antioxid. Redox Signal.* 12, 1101–1109.

Introduction

Hydrogen sulfide (H_2S) in the lumen of the large intestine can be produced through fermentation of sulfur-containing amino acids and intestinal sulfomucin metabolism, mainly by commensal sulfate-reducing bacteria (14, 24). H_2S concentration reported in the lumen of human large intestine is as high as 3.4 mM (12, 14). Clinical studies have shown the involvement of H_2S in the development of colorectal cancer and ulcerative colitis due to its capacity to reduce butyrate β-oxidation in colonocytes (32, 34). Butyrate is present in the mammalian colon at high millimolar concentrations as a by-product of anaerobic bacterial fermentation of dietary fiber, and butyrate also serves as the principal energy source for colonic epithelial cells, in which it is metabolized by mitochondrial β-oxidation (16, 21, 30). Butyrate has been shown to induce growth inhibition and terminal differentiation of a variety of human colon cancer cell lines (3, 31). *In vivo* studies have linked butyrate levels with a decreased incidence of colon cancer. Butyrate instilled into the colonic lumen reduced tumor development in a chemical model of colon cancer (6).

The physiological importance of H_2S surfaced in the mid-1990s. We now know that H_2S, in addition to other endogenous gases, including nitric oxide and carbon monoxide, is one of gasotransmitters (40, 41). Two pyridoxal-5′-phosphate–dependent enzymes, cystathionine β-synthase (CBS) (EC4.2.1.22) and cystathionine γ-lyase (CSE) (EC 4.4.1.1), are responsible for the majority of endogenous production of H_2S in mammalian tissues that use cysteine or homocysteine as the main substrate (1, 18, 38, 50). Emerging evidence indicates that endogenous H_2S also can be produced and released by colonic tissue (8, 26, 49). In the intestinal system, H_2S relaxes ileal smooth muscle and increases colonic secretion (9, 13, 27). Despite clinical links between H_2S and colorectal disorder, as well as the known anticancer effect of butyrate, few studies have examined the interaction of butyrate and endogenous H_2S on intestinal cell growth.

The present study investigated the expressions of CBS and CSE and endogenous production of H_2S in colonic tissues and WiDr cells (a colon cancer cell line). The effects of butyrate on CBS and CSE expression and H_2S production in WiDr cells also were studied. Finally, the role of endogenous H_2S in the

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butyrate-reduced viability of WiDr cells and the underlying mechanisms were explored.

Materials and Methods

Cell culture

Colonic epithelial cell line WiDr cells derived from a human colon carcinoma (American Type Culture Collection, Manassas, VA) were cultured with RPMI 1640 medium (Sigma, Oakville, Ontario, Canada) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. The experiments were performed when the cells reached 70 to 80% confluence between passages 11 and 18. In all studies, cells were first incubated in the serum-free medium for 12 h and maintained in a quiescent state (G_0 phase), and then 10% serum was added together with different agents.

Cell-viability assays

Cell viabilities were assessed based on the conversion of trazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to purple formazan crystals by living cells. In brief, equal numbers of cells were plated onto each well of 96-well plates for 24 h. After different treatments, 20 μl (5 mg/ml) MTT was added to each well. The cells were then cultured at 37°C for 4 h, and the absorbance of formazan products at 570 nm was measured in a FLUOstar OPTIMA microplate spectrophotometer (BMG LABTEch, Offenburg, Germany). The cells incubated with control medium were considered 100% viable.

Measurement of H_2S production

The H_2S production rate was measured as described previously (44, 47). In brief, after different treatments, the cells were collected and homogenized in 50 m*M* ice-cold potassium phosphate buffer (pH 6.8). The flasks containing the reaction mixture (100 m*M* potassium phosphate buffer, 10 m*M* L-cysteine, 2 m*M* pyridoxal 5-phosphate, and 10% (wt/vol) cell homogenates) and center wells containing 0.5 ml 1% zinc acetate and a piece of filter paper (2×2.5 cm) were flushed with N_2 and incubated at 37°C for 90 min. The reaction was stopped by adding 0.5 ml of 50% trichloroacetic acid, and the flasks were incubated at 37°C for another 60 minute. The contents of the center wells were transferred to test tubes, each containing 3.5 ml of water. Then 0.5 ml of 20 m*M* *N,N*-dimethyl-*p*-phenylenediamine sulfate in 7.2 *M* HCl and 0.5 ml 30 m*M* $FeCl_3$ in 1.2 *M* HCl was added. The absorbance of the resulting solution at 670 nm was measured 20 min later with a FLUOstar OPTIMA microplate spectrophotometer.

Western blotting analysis

Cultured cells and mouse colonic tissues were harvested and lysed. Equal amounts of proteins were boiled and separated with SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane, as described previously (43). The primary antibody dilutions were 1:1,000 for CBS and CSE, phosphorylated or total extracellular signal–regulated kinase (ERK), p38 mitogen–activated protein kinase (MAPK), or c-Jun N-terminal kinase (JNK), and 1:10,000 for β-actin. Horseradish peroxidase–conjugated secondary antibody was used at 1:5,000. The immunoreactions were visualized with ECL and exposed to x-ray film (Kodak Scientific Imaging film, Kodak, Rochester, NY).

Short interfering RNA (siRNA) transfection

Predesigned CSE-targeted siRNA (CSE-siRNA) was purchased from Ambion (Austin, TX), and the targeted sequence (5′-CTATGTATTCTGCAACAAA-3′) was localized at a position 617 bases downstream of the start codon of CSE (GenBank Accession No. NM001902). Negative siRNA (Neg-siRNA), a 21-nucleotide RNA duplex with no known sequence homology with all the genes, was also from Ambion. Transfection of WiDr cells by siRNA was achieved by using the siPORTTM lipid transfection agent from Ambion (43). In brief, the cells were plated overnight to form 60 to 70% confluent monolayers. CSE-siRNA and the transfection reagent complex were added to the cells in serum-free medium for 4 h. Fresh normal growth medium was then added, and the cells were incubated for another 44 h. As control, Neg-siRNA was used to transfect WiDr cells.

Materials

Male C57BL/129SvEv mice (10 to 12 weeks) were used with an approved protocol by the Animal Care Committee of Lakehead University, Canada. After mice were anesthetized, colonic tissues were dissected and cleaned for protein extraction.

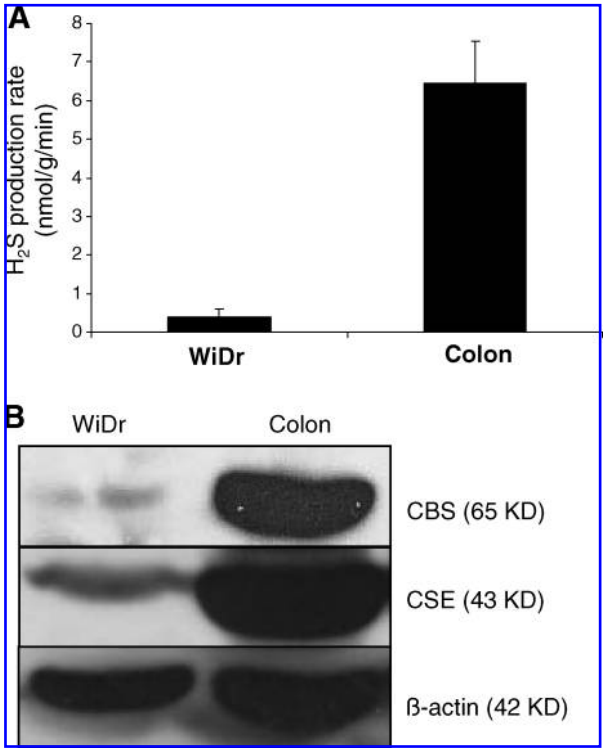

FIG. 1. Production of H_2S as well as expression of CBS and CSE in WiDr cells and colonic tissues. (**A**) H_2S production from WiDr cells and mouse colonic tissues. (**B**) Representative Western-blotting analysis on the protein expressions of CSE and CBS in WiDr cells and colonic tissues. Experiments in (A) and (B) were repeated 3 times.

The anti-CBS antibody and anti-CSE antibody were purchased from Novus Biologicals (Littleton, CO). The anti-MAPK antibodies and different MAPK inhibitors were obtained from New England Biolabs (Camarillo, CA). Horseradish peroxidase–conjugated goat anti-rabbit IgG antibody and goat anti-mouse IgG antibody were from Sigma. All other chemicals were from Sigma or New England Biolabs.

Statistical analysis

Data are presented as mean ± SEM, and the data represent at least three independent experiments. Statistical comparisons were made by using Student's *t* test, and the significance level was set at $p < 0.05$.

Results

Expressions of CBS and CSE as well as endogenous H_2S production in WiDr cells

A large amount of H_2S was produced by WiDr cell lysates (0.37 ± 0.07 nmol/g/min) and mouse colonic tissue homogenates (6.4 ± 0.8 nmol/g/min) (Fig. 1A). To elucidate the enzymatic basis of H_2S production in WiDr cells, we examined the expressions of CBS and CSE. The expressions of CBS and CSE proteins were observed in WiDr cells and colonic tissues (Fig. 1B). The expression level of CSE was higher than that of CBS in WiDr cells, and the expression levels of both CBS and CSE in colonic tissues were higher than those in cells (Fig. 1B).

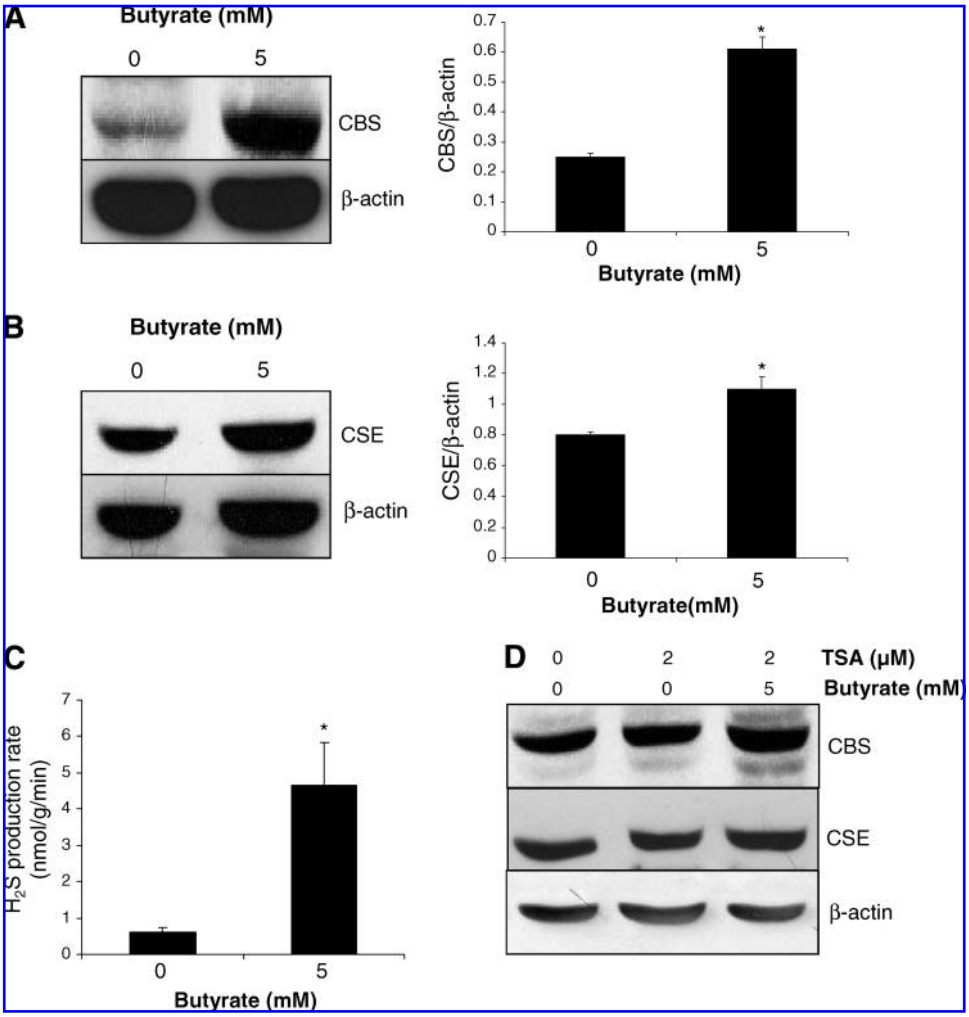

FIG. 2. Increased CBS and CSE expression and endogenous H_2S production by butyrate in WiDr cells. (**A**) CBS protein expression was increased in butyrate-treated cells. After incubation with 5 m*M* butyrate for 24 h, the cells were lysed, and 30 μg of protein was subjected to Western-blotting analysis by using anti-CBS antibody. Results are representative of at least three individual experiments. (**B**) CSE protein expression was increased in butyrate-treated cells. After incubated with 5 m*M* butyrate for 24 h, the cells were lysed, and 30 μg of protein was subjected to Western-blotting analysis with anti-CSE antibody. Results are representative of three individual experiments. The graphs in (A) and (B) represent the optical density of the bands of CBS and CSE normalized with the expression of β-actin. $^*p < 0.05$. (**C**) The endogenous H_2S production was higher in butyrate-treated cells. After the cells were incubated with 5 m*M* butyrate for 24 h, H_2S production rate was measured. Experiments were repeated at least 3 times. $^*p < 0.05$ *vs.* untreated control. (**D**) The expressions of CBS and CSE were not changed by TSA. After incubation with 2 μ*M* TSA for 24 h, the cells were lysed, and 30 μg of protein was subjected to Western-blotting analysis by using anti-CBS antibody or anti-CSE antibody. Results are representative of at least 3 individual experiments.

Butyrate increases CBS and CSE expression and endogenous H_2S production in WiDr cells

The changes of CBS and CSE protein expressions in WiDr cells after incubation with 5 m*M* butyrate for 24 h are shown in Fig. 2A and B. Butyrate significantly increased CBS and CSE protein expression. The cells treated with butyrate for 24 h had a higher H_2S production rate (4.75 ± 1.28 nmol/g/min) than that of untreated control cells (0.37 ± 0.07 nmol/g/min; $p < 0.05$) (Fig. 2C).

Butyrate is known as an inhibitor of histone deacetylase (30). To examine whether upregulation of CSE gene expression by butyrate is a result of inhibition of histone deacetylase, we treated the cells with trichostatin A (TSA), a specific histone deacetylase inhibitor. TSA at 2 μM had little effect on CBS and CSE protein expression (Fig. 2D). Furthermore, butyrate-stimulated CBS and CSE expression was not changed by TSA (Fig. 2D).

Butyrate and H_2S decrease WiDr cell viability

As shown in Fig. 3A, butyrate at 1 to 10 m*M* significantly decreased cell viability in a dose-dependent manner, and the cell viability with 5 m*M* butyrate treatment was only $68.7 \pm 2.6\%$ of that in the absence of butyrate ($p < 0.05$). We further tested the effect of exogenously applied H_2S on WiDr cell viability. Similar to butyrate, NaHS treatment (50 to 200 μM) for 24 h decreased cell viability, and the cell viability with 100 μM NaHS treatment was $60.6 \pm 9.8\%$ of that in the absence of NaHS (Fig. 3B; $p < 0.05$).

Knockdown of CSE expression does not block butyrate-inhibited cell viability

To address whether the antiproliferative effect of butyrate was mediated by increased CSE expression and H_2S production, we used the RNA-interference approach to knock down endogenous CSE expression. As shown in Fig. 4A, CSE-siRNA at 100 n*M* significantly inhibited CSE protein expression by 55%, whereas Neg-siRNA transfection had no effect on CSE expression. However, neither CSE-siRNA nor Neg-siRNA transfection reversed butyrate-stimulated H_2S production (data not shown) or butyrate-reduced cell viability ($p > 0.05$) (Fig. 4B). Blockade of CSE activity by DL-propargylglycine (PPG) in WiDr cells also did not reverse butyrate-induced H_2S production or butyrate-inhibited cell viability (data not shown).

CBS contributes to butyrate-stimulated H_2S production and butyrate-inhibited cell viability

We further tested the hypothesis that CBS activity may contribute to butyrate-stimulated H_2S production in WiDr cells. After the cells were incubated with CBS inhibitors amino-oxyacetate (AOA, 50 μM) or hydroxylamine (HA, 1 m*M*), butyrate-stimulated H_2S production was completely decreased to the basal level ($p < 0.05$) (Fig. 4C). Blockade of CBS activity by AOA also partially but significantly inhibited butyrate-induced cell death (Fig. 4D).

Altered MAPK phosphorylation by H_2S and butyrate

The MAPK pathway is an important signal mechanism for cell-growth regulation (46). Whether the MAPK pathway was involved in H_2S- or butyrate-reduced cell viability in WiDr cells was examined by using anti-phospho-MAPK antibodies. The same blots were later stripped and reprobed with an antibody that recognized both phosphorylated and unphosphorylated forms of MAPKs. Western blotting results in Fig. 5A showed that treatment with NaHS (100 μM) resulted in strong phosphorylation of ERK and p38 MAPK, but not of JNK. ERK activation appeared during the first 15 min of H_2S treatment, followed by dephosphorylation. After 30 min, ERK phosphorylation was again slowly increased and peaked at 8 h. A similar stimulatory effect of NaHS was observed on p38 MAPK. NaHS maximally activated p38 MAPK within 15 min of its application. The total amount of MAPK protein remained unchanged with NaHS stimulation. The effects of butyrate on MAPK activation also were explored. As shown in Fig. 5B, butyrate stimulated ERK phosphorylation at the first 15 min of treatment and peaked at 30 min. The p38 MAPK

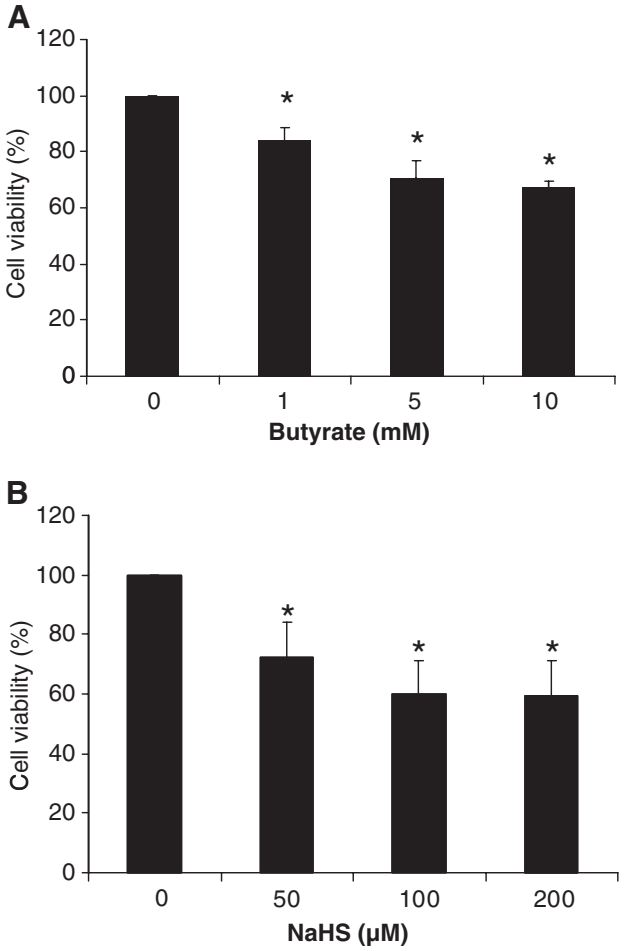

FIG. 3. Reduced cell viability with butyrate and NaHS. (**A**) Butyrate decreased WiDr cell viability. After the cells were incubated with different concentrations of butyrate (1–10 m*M*) for 24 h, cell viability was analyzed with MTT assay. Experiments were repeated 4 times. $^*p < 0.05$ *vs.* untreated control. (**B**) NaHS decreased cell viability. After the cells were incubated with different concentrations of NaHS (50–200 μM) for 24 h, cell viability was analyzed with the MTT assay. Experiments were repeated 4 times. $^*p < 0.05$ *vs.* untreated control.

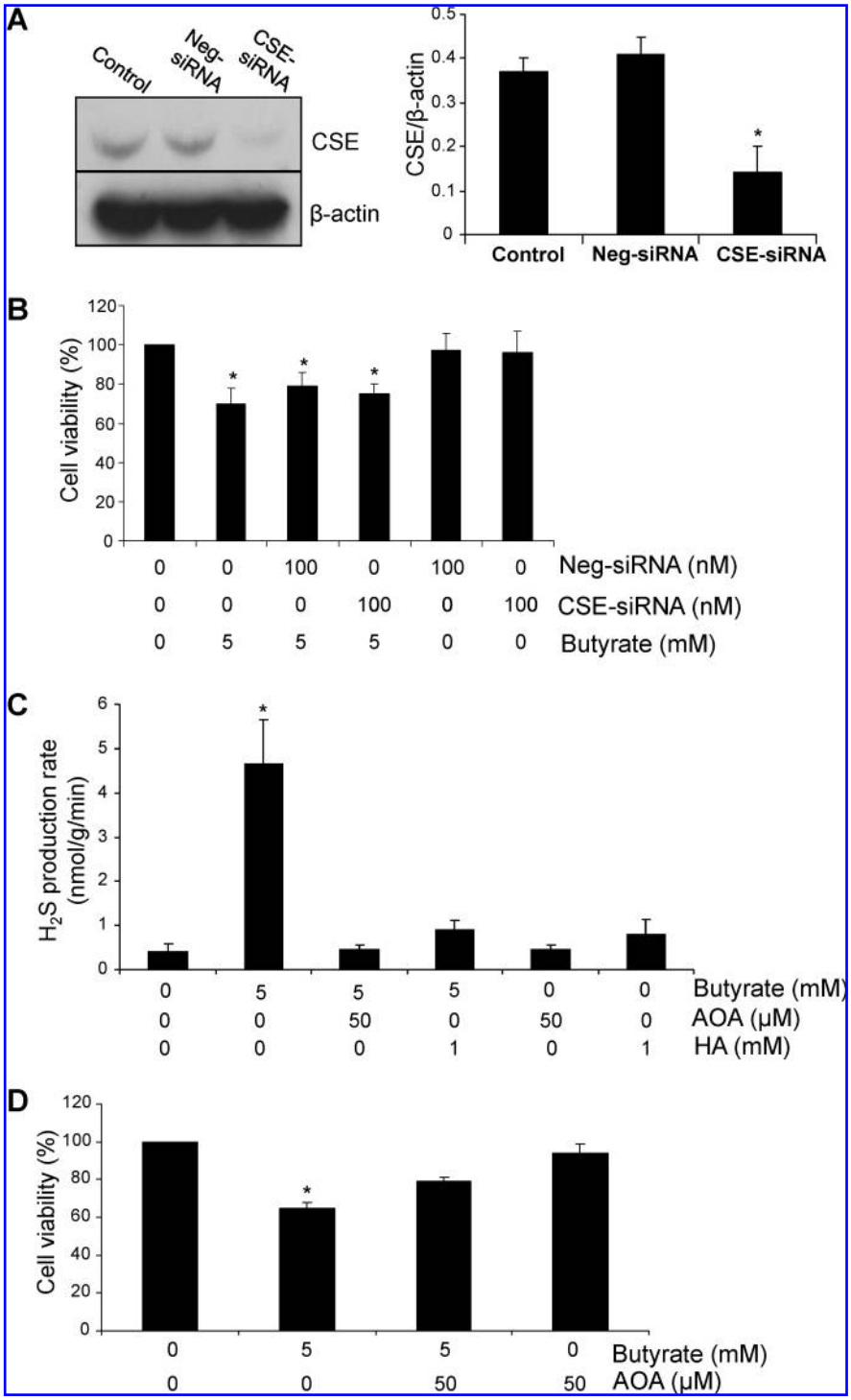

FIG. 4. Inhibition of CBS reversed butyrate-stimulated H_2S production. (**A**) CSE-siRNA transfection significantly decreased CSE protein expression in WiDr cells. After transfection with CSE-siRNA or Neg-siRNA at 100 n*M* for 48 h, the cells were collected and subjected to Western-blotting analysis. Representative Western-blotting results from three individual experiments are shown on the top, and a summary of relative abundance levels of CSE protein is shown on the bottom. $^*p < 0.05$ *vs.* Neg-siRNA–treated cells or untreated control cells. (**B**) Inhibition of CSE did not reverse butyrate-decreased cell viability. After transfection with CSE-siRNA or Neg-siRNA at 100 n*M* for 24 h, the cells were incubated with 5 m*M* butyrate for another 24 h. Cell viability was analyzed with the MTT assay. Experiments were repeated 3 times. $^*p < 0.05$ *vs.* untreated control. (**C**) Blockage of CBS activity reversed butyrate-stimulated H_2S production. Experiments were repeated at least 3 times. $^*p < 0.05$ *vs.* all other groups. (**D**) Blockage of CBS activity reversed butyrate-decreased cell viability. After the cells were incubated with 5 m*M* butyrate for 24 h in the presence or absence of 50 μM AOA, cell viability was analyzed with the MTT assay. Experiments were repeated 3 times. $^*p < 0.05$ *vs.* all other groups.

and JNK phosphorylation as well as the total MAPKs remained unchanged with butyrate treatment (not shown).

To determine whether decreased viability of WiDr cells by NaHS or butyrate was modulated by ERK or p38 MAPK activation, we treated WiDr cells with U0126 (a selective inhibitor of the MEK/ERK signaling pathway) and SB203580 (a p38 MAPK inhibitor). As shown in Fig. 6A, U0126 at 1 μM had no effect on H_2S-decreased cell viability ($p > 0.05$), whereas SB203580 (1 μM) further worsened NaHS-decreased cell viability ($p < 0.05$). Neither U0126 (1 μM) nor SB203580 (1 μM) had an effect on butyrate-decreased cell viability (Fig. 6B).

Discussion

Gasotransmitters are a family of endogenous gas molecules involved in regulation of physiologic and pathologic functions of mammalian cells (11, 29, 41). H_2S, a novel gasotransmitter, has been extensively studied in recent years (11, 22). Abnormal metabolism and functions of H_2S have been

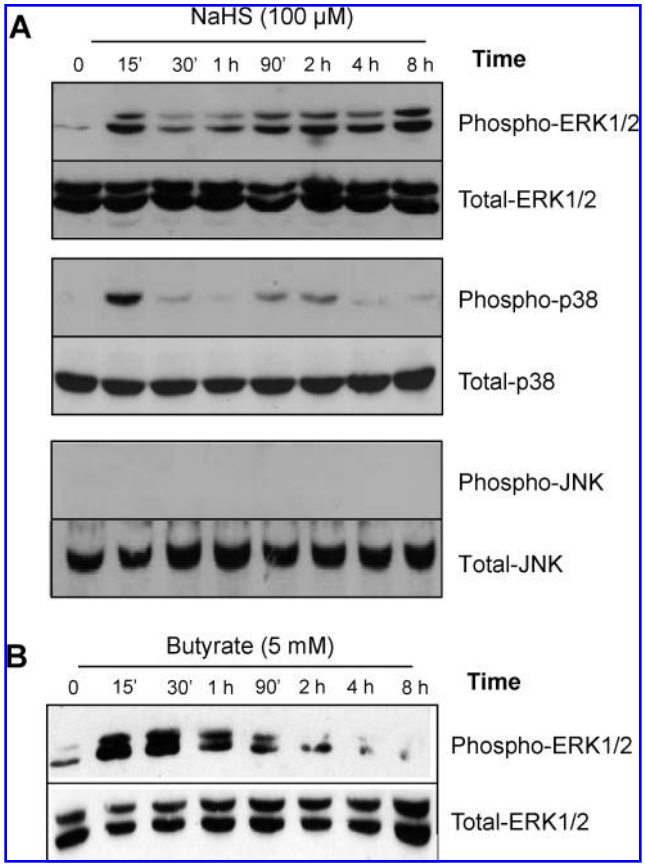

FIG. 5. Activation of MAPKs by NaHS and butyrate. (**A, B**) Time course of the activation of MAPKs induced by NaHS and butyrate. After incubation with 100 μM NaHS or 5 mM butyrate for the indicated times, the cells were collected and subjected to Western-blotting analysis by using different MAPK antibodies. Results are representative of three individual experiments.

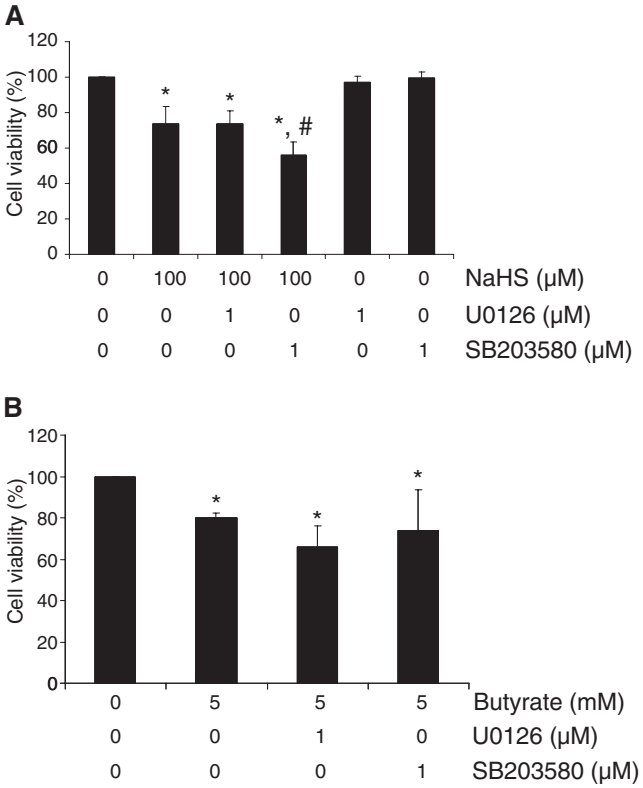

FIG. 6. Effects of MAPK inhibitors on NaHS- or butyrate-reduced cell viability. (**A**) SB203580 and U0126 did not inhibit NaHS-induced cell death. WiDr cells were treated with or without MEK/ERK inhibitor U0126 (1 μM), or p38 MAPK inhibitor SB203580 (1 μM), or NaHS (100 μM) for 24 h. Experiments were repeated 3 times. $^{*}p < 0.05$ *vs.* untreated control; $^{\#}p < 0.05$ *vs.* butyrate-treated cells. (**B**) U0126 and SB203580 had no effect on butyrate-reduced cell viability. WiDr cells were treated with or without U0126 (1 μM), or SB203580 (1 μM), or butyrate (5 mM) for 24 h. Experiments were repeated 3 times. $^{*}p < 0.05$ *vs.* untreated control.

linked to many diseases (25, 28, 33, 45). Endogenously produced or exogenously applied H_2S regulates cell growth or death in a multitude of settings, and unbalanced cell proliferation and apoptosis due to the altered metabolism and functions of H_2S under different pathologic conditions have been documented (10, 46–48).

Endogenous H_2S is generated from L-cysteine and homocysteine metabolism, catalyzed by CBS or CSE or both (1, 38). As a pyridoxal phosphate–dependent enzyme, CSE is expressed in a range of mammalian cells and tissues, and it seems to be the main H_2S-forming enzyme in the cardiovascular system, pancreas, and liver (19, 47, 50). Deficiency of CSE results in disorders such as hypertension, atherosclerosis, and homocystinuria because of decreased H_2S levels and increased homocysteine levels (17, 28, 45). In some tissues, CBS and CSE are both needed for generation of H_2S, whereas in others, one enzyme suffices (38). CBS-derived H_2S is produced mostly in neural tissues (22). Recently, another enzyme, 3-mercaptopyruvate sulfurtransferase, in combination with cysteine aminotransferase, was reported to produce H_2S from cysteine in brains and vascular endothelium (36, 37). In the present study, we first found that a large amount of H_2S could be produced and released by WiDr cells and colonic tissues (Fig. 1A). We further observed marked CBS and CSE protein expression in WiDr cells as well as in colonic tissues with Western blotting (Fig. 1B). The expression levels of CSE protein are higher than those of CBS (Fig. 1B). Others also reported that both CSE and CBS are highly expressed in colon, but the CSE level was more abundant than CBS in the intestinal system (8, 26).

H_2S is known to be produced inside the intestine by bacteria as a poison that inhibits cellular respiration at the level of cytochrome oxidase. Our present study, as well as other reports, demonstrated that H_2S is also endogenously produced in colonic tissues and WiDr cells, and that exogenously applied H_2S at physiologically relevant concentrations reduces cell viability (Fig. 3B). Treatment with NaHS also significantly reduces HT-29 cell (one cancer colon cell) proliferation (24). These findings are perhaps surprising, given the large quantities of H_2S present in the intestine (12, 14). Inside the colon, the mucosa may serve as a particularly effective barrier to the diffusion of H_2S across to the muscle layers (12, 34). The colonic mucosa is endowed with an efficient H_2S-detoxifying mechanism, oxidizing more than 300 μM H_2S daily in the rat colon (21). These observations may have physiologic and pathologic relevance in conditions in which H_2S production is

increased or in which barrier or detoxification mechanisms are impaired (32, 34).

A series of studies demonstrated that butyrate inhibits growth and induces differentiation and apoptosis of colonic tumor cells (3, 30, 31). Such an antiproliferative effect was confirmed in the present study (Fig. 3). However, the precise mechanism of growth suppression by butyrate in colon cancer cells has not been clarified. Frequently described is the effect on gene expression of butyrate. Many butyrate-responsive genes that are involved in the processes of apoptosis, proliferation, and differentiation have been identified (30). Butyrate also can influence cell proliferation through the release of growth factors or gastrointestinal peptides, such as gastrin, or modulation of mucosal blood flow (30). In this study, we found that butyrate increased CBS and CSE expression as well as H_2S production (Fig. 2). Considering the proapoptotic effect of H_2S derived from CSE on some types of cells, the butyrate-decreased cell viability seen in this study might be mediated by increased CSE expression (2, 11, 25). Conversely, our study does not support this hypothesis. RNAi inhibited endogenous CSE expression by 55% in WiDr cells (Fig. 4A), and this treatment did not reverse butyrate-stimulated H_2S production and did not alter the cell-killing effect of butyrate (Fig. 4B). Blockade of CSE activity by PPG in WiDr cells also did not reverse butyrate-induced H_2S production and butyrate-inhibited cell viability (data not shown). More interesting, we found that blockade of CBS activity by incubating the cells with AOA (50 μM) or HA (1 mM) significantly decreased butyrate-induced H_2S production (Fig. 4C), and AOA at 50 μM also partially but significantly reversed butyrate-inhibited cell viability (Fig. 4D), which suggests that increased H_2S production derived from CBS may partially contributed to butyrated-inhibited cell viability. The reason that butyrate upregulates both CSE and CBS expression but only stimulates CBS to increase H_2S production is currently unknown. Conversely, both H_2S and butyrate exert multiple effects in modulating inflammation, oxidative stress, defense barrier function, and butyrate metabolism in the intestinal system. Therefore, butyrate and H_2S may offer synergistic anticancer action (2, 12, 42).

The precise mechanisms underlying butyrate-increased CBS and CSE expression are not well understood. Butyrate has been shown to act as an inhibitor of histone deacetylase, resulting in a relative hyperacetylation of core histone proteins (14). Hyperacetylation of histones disrupts ionic interactions with the adjacent DNA backbone, creating less densely packed chromatin, or euchromatin, and allowing transcription factors to activate specific genes (15). Our study shows that TSA, a specific histone deacetylase inhibitor, had few effects on CBS and CSE expression. It is thus suggested that the upregulation of *CBS* and *CSE* genes by butyrate in colon cancer cells does not go though the histone hyperacetylation pathway (Fig. 2D). Butyrate has been shown to alter the binding of regulatory transacting proteins to specific DNA sequences that control the expression of the gene (7). Butyrate decreases the expression of cyclin D1 in epidermoid carcinoma cells and increases the expression of IGFBP-3 in breast cancer cells (39). Butyrate also regulates the expression of $p21^{waf1/cip1}$ in a variety of cancer cells such as osteosarcoma, colon carcinoma, glioma, and lung cancer cells (5, 20, 23).

To gain mechanistic insight into the roles of H_2S and butyrate on regulating WiDr cell growth, we investigated the activation of MAPKs in WiDr cells. MAPKs play important roles in the cellular response to gasotransmitters, growth factors, cytokines, or environmental stress (44). The three distinct groups of MAPKs are ERK1/2, p38 MAPK, and JNK. In the current study, we provided evidence that both H_2S and butyrate increased the activity of ERK. Suppression of ERK activity by U0126, however, had no effect on cell viability change mediated by NaHS or butyrate (Figs. 5 and 6). NaHS, but not butyrate, also increased the p38 MAPK activation. To our surprise, the suppression of p38 MAPK activation by SB253580 further strengthened, rather than inhibited NaHS-reduced cell viability (Fig. 6). These data suggested that NaHS-induced phosphorylation of p38 MAPK may not be responsible for NaHS-induced WiDr cell death. H_2S stimulates or represses activations of ERK or p38 MAPK or both in different cells, including human aorta smooth muscle cells, HEK-293 cells, human granulocytes, and pancreatic β cells (27, 43, 44, 46).

Similar to H_2S, different effects of butyrate on MAPK activation are reported (4, 35). Upregulation of GADD153 and induction of apoptosis by butyrate in CT-116 human colon adenocarcinoma cells involved ERK activation (35). However, exposure of DU145 cells (human prostate cancer cells) to butyrate stimulated p38 MAPK and JNK activation, but not ERK activation, during apoptosis (4). All these suggest that cell type-specific activations of MAPK by H_2S or butyrate are required for a variety of cell-fate determinations.

In summary, this study reported novel effects of butyrate on CBS and CSE expression as well as H_2S production in epithelial cancer cells. Butyrate and H_2S both can offer antiproliferative action, but through different mechanisms and pathways. These observations may have a wide impact on the study of colon cancer biology and the prevention of colon cancer and chronic intestinal epithelial disorders. The potential physiologic and pathologic implications of these synergistic effects between butyrate and H_2S merit further investigation.

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

AOA = amino-oxyacetate
CBS = cystathionine β-synthase
CSE = cystathionine γ-lyase
ERK = extracellular signal-regulated kinase
HA = hydroxylamine
H_2S = hydrogen sulfide
JNK = c-Jun N-terminal kinase
MAPK = mitogen-activated protein kinase
NaHS = sodium hydrogen sulfide
siRNA = short-interfering RNA
TSA = trichostatin A

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