The Effects of Sodium Butyrate on Transcription Are Mediated through Activation of a Protein Phosphatase*

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In this study we have investigated the molecular mechanism by which sodium butyrate modulates gene expression when added to cultured cells. As a model system we used hepatoma tissue culture cells in which sodium butyrate treatment increases histone H1° mRNA level and decreases c-myc mRNA level. Because we observed that stimulation of histone H1° gene expression could take place in the absence of protein neosynthesis, we hypothesized that sodium butyrate induced a post-translational modification of a factor involved in the transcription process. Using different types of well-known kinase and phosphatase inhibitors, we studied the implication of kinase or phosphatase activity in this pathway. Interestingly, cell treatment with potent serine-threonine-phosphatase inhibitors, calyculin A or okadaic acid, prevented the regulation of both histone H1° and c-myc gene expressions by sodium butyrate. On the other hand, the tyrosine phosphatase inhibitor, vanadate, or the protein kinase C inhibitor, staurosporine, did not significantly modify sodium butyrate effects. Using protein phosphatase 1 and 2A for in vitro assays, we found a 45% increase of phosphatase activity after cell treatment by sodium butyrate, possibly due to a protein phosphatase 1-type protein phosphatase. These data strongly suggest that signaling pathway(s) triggered by sodium butyrate to modulate gene expression involve(s) a serine-threonine-phosphatase activity.

Interest in sodium butyrate studies was enhanced in 1977 by the observation of Riggs et al. that treatment of cultured cells with butyrate leads to the development of histone hyperacetylation (1). This effect results from the inhibition of the enzyme histone deacetylase and is reversed upon the removal of sodium butyrate (2, 3). Reversible histone acetylation is now supposed to play an important role in the regulation of chromatin structure and its transcriptional activity (4). Numerous studies have further shown that sodium butyrate exhibits a wide variety of other biological effects by mechanisms that are still not resolved. Its main biological properties concern cell growth, cell morphology, and gene expression (see Ref. 5 for review). Butyrate reduces the growth of many cell types (6–8); it can induce differentiation as observed with Friend erythroleukemia cells and HeLa cells (9, 10) or leads to a growth arrest at the G1 phase of the cell cycle as in hepatoma tissue culture (HTC) cells used in this study (11, 12). It has been established that butyrate suppresses, completely or partially, most of the transformation characteristics of several cell lines (13). For example, incubation of the human pancreatic adenocarcinoma cell line causes a dramatic decrease in cell proliferation, an increase in alkaline phosphatase activity, and an induction of secretory differentiation (14), whereas the murine rhabdomyosarcoma cell line is sensitive to sodium butyrate induction of creatine kinase, an enzyme characteristic of muscle (15). Apoptosis can also be induced by sodium butyrate treatment as seen in colon and colorectal tumor cell lines (16, 17).

The modulation of gene expression is one of the major effects of sodium butyrate. It is able to activate integrated viral DNA, and the treatment of cells latently infected with Epstein-Barr virus or HIV virus stimulates the synthesis and the expression of viral DNA (18, 19). In HeLa cells, butyrate activates "dormant" HIV by acting on the long terminal repeat-regulating element in a dose-dependent manner (20) and in chronically infected lymphoid and monocyte cells by acting on the TATA box (21).

The expression of many genes, including some oncogenes, is altered by sodium butyrate. The expression of c-myc mRNA is inhibited in 3T3 cells and in rectal carcinoma cells (22, 23), and in a previous study carried out with HTC cells we demonstrated that this effect did not result from the arrest of cell growth at the G1 phase of the cell cycle (24). Sodium butyrate treatment also diminishes retinoblastoma and p53 gene expressions as observed in a colon tumor cell line for both these genes (25) and for the p53 gene in Swiss 3T3 cells (22) and in a rectal carcinoma cell line (23). On the contrary, c-fos and c-jun gene expressions are induced by sodium butyrate as seen in Swiss 3T3 and HTC cell lines (12, 22, 24).

Among the multiple effects of sodium butyrate on gene expression, histone H1° gene induction has been well studied (see Ref. 26 for review). H1° is a histone H1 variant, preferentially found in nondenizing cells (27). The accumulation of histone H1° is observed in most cell types treated by sodium butyrate (28–30), and whereas the relation between histone H1° proliferation and differentiation is not yet clearly established, this process seems to be more related to terminal differentiation than to the decrease of proliferation itself (31, 32). Run-on experiments on sodium butyrate-treated cells have demonstrated that the modulation of the histone H1° mRNA amount results from a transcriptional control of gene expression (29). Studies of the Xenopus laevis promoter have shown that three DNA boxes are required for histone H1° gene induction by sodium butyrate (33), suggesting that this induced expression

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We dedicate this work to the memory of Professor Jacques Kruh.

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§ The abbreviations used are: HTC, hepatoma tissue culture; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; PP, protein phosphatase.
Butyrate Effects Are Mediated by a Protein Phosphatase

The molecular mechanism(s) by which sodium butyrate exerts its various effects is not known. To identify a molecular intermediate of the sodium butyrate signaling pathway on gene expression, histone H1° and c-myc genes have been chosen as models. In this study, we have observed that sodium butyrate increases the level of histone H1° mRNA and decreases that of c-myc by a mechanism involving a serine-threonine-phosphatase.

MATERIALS AND METHODS

Chemicals

Calyculin A, staurosporine, sodium orthovanadate, and okadac acid were obtained from LC laboratories (Woburn, MA), Me₂SO and sodium butyrate were from Merck (Darmstadt, Germany), actinomycin D was from Boehringer Mannheim (Meylan, France), and cycloheximide was from Sigma.

Cell Culture

HTC cells, an established cell line derived from Morris hepatoma (34), were grown at 37 °C in suspension in Swim’s S-77 medium (Life Technologies, Inc.) supplemented with 10% newborn calf serum as described by Hershko and Tomkins (35). For butyrate treatment, the cells were transferred into a fresh medium (6 × 10⁶ cells/ml) and supplemented with sodium butyrate at a final concentration of 5 mM for various periods of time. As mentioned under “Results and Discussion,” cells were also preincubated for 15 min before the addition of sodium butyrate, at the indicated concentrations of the kinase/phosphatase inhibitors: 50 nM calyculin A; 50 nM staurosporine; 5 μM sodium orthovanadate; 25 μM or 1 μM okadaic acid; or 45 μM Me₂SO. Cell viability was assessed by trypan blue dye exclusion.

PCR Amplification and Subcloning of the Rat Histone H1° Coding Region

Genomic DNA was prepared from liver of 24 h fasted Sprague-Dawley rats. The PCR reactions were performed in a total volume of 100 μl using 100 ng of genomic DNA in 250 μl each of the dNTPs, 16.6 mM SO₄(NH₄)₂, 67 mM Tris-HCl, pH 8.8, 6.7 mM MgCl₂, 1 mM β-mercapto-ethanol, 0.67 μM EDTA, pH 8, 10% Me₂SO, and 0.5 units of Thermus aquaticus DNA polymerase (Perkin-Elmer, Foster City, CA). The primers were chosen from the mouse histone H1° cDNA sequence (36) as follows: the 5′ primer corresponded to the initiation codon region (ATG-GACCCGAGAATCCTACCTC) and the 3′ primer was the complementary sequence of the termination codon region (TCACTTCTTGGTGTCCCCT). The primers were added to the PCR mixture at a final concentration of 0.5 μM. The cycling conditions were 94 °C for 1 min initially, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 40 s, extension at 72 °C for 50 s, and a final extension of 72 °C for 5 min. The PCR product was cloned in a pUC vector (Pharmacia LKB, Upsala, Sweden), and 10 independent clones were sequenced.

RNA Isolation and Analysis

Total RNAs from 3 × 10⁶ HTC cells have been prepared using the RNA now reagent (Biogentex Inc., Seabrook, NJ). For Northern blot analysis, 30 μg of RNA was fractionated on 0.8% agarose gels containing 2.2 M formaldehyde and transferred onto Genescreen Plus nylon membranes. Blots were hybridized with either the 585-base pair rat histone H1° coding sequence described above, or with a human c-myc probe corresponding essentially to the third exon of the c-myc gene (kindly provided by Y. Le Garrec). DNA probes were labeled with the random megaprime system (Amersham Corp.). After hybridization, the filters were washed at 42 °C in 0.2 X SSC, 0.1% SDS. The filters were then exposed for autoradiography to XAR5 Kodak films for 2 days at −70 °C with an intensifying screen. Dehybridizations were performed at 68 °C in 0.1% SDS.

Apoptosis Testing

The percentage of HTC cells undergoing apoptosis was determined using the Apoptosis Detection kit (R & D Systems, Europe LTD) based on the use of annexin V and propidium iodide. Following culture, the cells were stained for subsequent analysis on a Epics Elite Cell Cytometer (Coultronics). 5,000 cells were counted.

Enzyme Activity Determination

Assays of Metabolic Enzymes—Enzyme catalytic activities were measured under routine conditions on a Hitachi 717 automated analyzer. They were performed with reagent kits from Boehringer Mannheim (Meylan, France) for alanine aminotransferase (EC 2.6.1.2), aspartate aminotransferase (EC 2.6.1.1), γ-glutamyltransferase (EC 2.3.2.2), and alkaline phosphatase (EC 3.1.3.1) or from bioMérieux (Marcy-l’Etoile, France) for 5′-nucleotidase (EC 3.1.3.5) and acid phosphatase (EC 3.1.3.2) according to the manufacturer’s instructions. Cell-free extracts were prepared as follows: 10⁷ centrifuged HTC cells were washed three times and then lysed by vortexing in 0.5 ml of a buffer containing 0.25% sodium deoxycholate, 10 mM Tris, pH 8. After 10 min of centrifugation at 15,000 rpm, enzymatic activity determination was performed on the supernatant. When stated in the text, cells were treated by either sodium butyrate or the inhibitors as described above.

Assays of Protein Phosphatases 1 and 2A—Protein phosphatase 1 (PP1) and 2A (PP2A) activities were determined using the protein phosphatase assay system (Life Technologies, Inc.) according to the manufacturer’s protocol. Phosphorylase phosphatase activity was measured as the release of trichloroacetic acid soluble phosphate from [32P]phosphorylase a. One unit corresponds to a release of 1.0 nmol phosphate/min at 30 °C. Nonradioactive assays were performed using the serine-threonine phosphatase assay system (Promega, Madison, WI) according to the manufacturer’s protocol. This system, designed for the convenient dosage of protein phosphatase 2A, is based on a molybdate:malachite green:phosphate complex formed with free phosphate released from a phosphopeptide substrate.

RESULTS AND DISCUSSION

In this study histone H1° and c-myc genes have been used as models to investigate the signaling pathway used by sodium butyrate to modulate gene expression. We have chosen these two genes because sodium butyrate has been described to exert an opposite effect on their respective mRNA amounts. The decrease of c-myc gene expression upon sodium butyrate treatment in the HTC cell line has been previously described (24), whereas the behavior of histone H1° mRNA amount in this cell line remained to be determined. Numerous studies have shown that the level of histone H1° mRNA generally increases after cell exposure to sodium butyrate (28, 29, 37), but two cell lines have been described to display other features. The human pro-myelocytic HL-60 cell line failed to induce any accumulation of histone H1° mRNA in presence of the inducer (38), whereas the human promyelocytic cell line HepG2 expressed a high level of histone H1° mRNA, which could not be further increased by the inducer (39). Consequently, we studied histone H1° expression in HTC cell line prior to and after addition of sodium butyrate. Fig. 1 shows a Northern blot analysis of histone H1° mRNA as a function of the time of treatment of cells with 5 mM sodium butyrate. We used as a probe the rat histone H1° coding sequence fragment obtained by PCR as described under “Material and Methods.” The autoradiograph displays a 2.2-kilobase
sodium butyrate and 10^{-2} M butyrate (B4); sodium butyrate and 5 \mu g/ml actinomycin D (B4 + Act); or sodium butyrate and 10^{-4} M cycloheximide (B4 + Cyc). RNAs were analyzed by Northern blot as described under "Materials and Methods" and hybridized with a histone H1° probe. 6, 320-nm UV revelation of the membrane after transfer of ethidium bromide-stained mRNA.

The maximal expression is obtained after 6 h and remains high for longer times of treatment. This experiment reveals that like many other cell lines, HTC cells normally express a low amount of histone H1° mRNA and that this quantity can be increased when cells are cultured in presence of sodium butyrate.

In B16 melanoma cell line, Rousseau et al. had established that the histone H1° mRNA accumulation induced by sodium butyrate resulted from an activation of the transcription rate of the gene (29). To check whether the histone H1° mRNA accumulation observed in HTC cell line could also be related to an increase of the transcription rate, exponentially growing cells were treated for 4 h with 5 mM sodium butyrate (B4), sodium butyrate and 5 \mu g/ml actinomycin D (B4 + Act); or sodium butyrate and 10^{-4} M cycloheximide (B4 + Cyc). RNAs were analyzed by Northern blot as described under "Materials and Methods." The same filters have been hybridized with both probes successively: a, histone H1° probe; b, c-myc probe; c, 320-nm UV revelation of the membrane after transfer of ethidium bromide-stained mRNA.

The presence of an inhibitor of the translational apparatus, sodium butyrate failed to increase histone H1° mRNA amount, strongly suggesting that, as in B16 cells, this process would require an active transcriptional mechanism in HTC cells.

To investigate how sodium butyrate could act on histone H1° gene expression, we wondered whether protein neosynthesis was required for this induction. Histone H1° mRNAs from HTC cells simultaneously treated for 4 h with the inducer and cycloheximide (10^{-4} M) were analyzed by Northern blot. The presence of an inhibitor of the translational apparatus did not alter the increase of the histone H1° mRNA amount induced by sodium butyrate (Fig. 2, lane B4 + Cyc), indicating that the induction of histone H1° gene expression could take place in the absence of protein neosynthesis.

Taken together, these data led us to postulate that sodium butyrate could modulate gene expression by acting at the post-translational level of a factor involved in the transcriptional apparatus. Because numerous proteins implied in transcription control are now known to be regulated by phosphorylation/dephosphorylation cycles, we examined the role of kinase or phosphatase activity in this pathway.

In a first set of experiments, we investigated the modulation of histone H1° and c-myc gene expressions due to sodium butyrate treatment after 4 h of culture in presence of the protein kinase C inhibitor staurosporine or the potent serine-threonine-phosphatase inhibitor calyculin A. As such components could possibly induce cell lethality when added to the culture medium for several hours, HTC cell viability was assessed by trypan blue dye exclusion. After 4 h of culture, the percentage of cell death (<1%) was similar in control cells and in cells cultivated in the presence of either staurosporine (50 nm) or calyculin A (50 nm), showing that the use of these components did not seem to alter cell viability under these experimental conditions. When used in the experiments presented above, inhibitors were systematically added to the culture medium 15 min before the addition of sodium butyrate. Northern blot experiment using the histone H1° probe (Fig. 3a) showed that the expected increase of the H1° mRNA amount due to sodium butyrate induction (lane B4) was highly reduced when cells had been simultaneously cultivated with the inhibitor calyculin A (lane C). Hybridization of the same filter with the c-myc probe (Fig. 3b) also showed that in the presence of calyculin A (lane C), sodium butyrate failed to provoke the decrease of the c-myc mRNA level. On the contrary, the effect of sodium butyrate on the expression of these two genes was not significantly altered by staurosporine (Fig. 3, lane S), strongly suggesting that the protein kinase C was not implied. From these observations, it can be concluded that a protein phosphatase highly sensitive to the calyculin A inhibitor might be specifically involved in the sodium butyrate pathway to modulate gene expression.

Calyculin A has been described as a potent and specific inhibitor of PP1 and PP2A (40, 41), both of which belong to the serine-threonine phosphatase group. To investigate further the mechanism by which sodium butyrate modulates gene expression, a second set of similar experiments was performed using two other protein phosphatase inhibitors, okadaic acid, and vanadate. Okadaic acid is another serine-threonine phosphatase 1 and 2A inhibitor (42), whereas vanadate is known to inhibit tyrosine phosphatase proteins (43). As before, the estimation of cell lethality, performed after 4 h of culture using the blue trypan exclusion test, did not show any difference between control cells and cells treated with either okadaic acid (1 \mu M) or vanadate (5 \mu M). Northern blot data presented in Fig. 4 show that in presence of 5 \mu M vanadate (lane V) sodium butyrate is still able to alter histone H1° and c-myc mRNA amount, strongly suggesting that no protein tyrosine phosphatase is involved in this pathway.

When 1 \mu M okadaic acid was added to the culture medium (Fig. 4, lane AO1), sodium butyrate was neither able to induce an increase of the histone H1° mRNA level nor able to provoke a decrease of the c-myc mRNA amount. This observation shows...
that the use of okadaic acid at a 1 μM concentration highly prevents the effect of sodium butyrate on both histone H1° and c-myc gene expressions. On the contrary, the use of 25 nM okadaic acid (Fig. 4, lane AO2) does not significantly affect sodium butyrate effects on these two genes, showing that when used at this lower concentration okadaic acid is not able to exert its antagonist effect under our experimental conditions. This dose-dependent response must still be analyzed carefully. Indeed, although PP2A has been described to be a more sensitive enzyme than PP1 to okadaic acid (44), comparison of these two data could not lead to the conclusion that PP1 was the main target of sodium butyrate. According to the intracellular concentrations of PP1 and PP2A and because some cells may be relatively impermeable to this inhibitor, it may be necessary to use concentrations of okadaic acid higher than those employed in vitro to inhibit these phosphatases in cultured cells (45). Therefore, the observed effect could be the consequence of the inhibition of a serine-threonine protein phosphatase, which could be here either PP1, PP2A, or an unknown phosphatase.

Because all these inhibitors are diluted in MeSO, total RNAs of HTC cells cultivated for 4 h in presence of 45 μM MeSO and 5 mM sodium butyrate have been loaded onto the gel (Fig. 4, lane DMSO). This MeSO concentration, which is the highest concentration of MeSO used, does not significantly modify sodium butyrate effects. This control shows that the significant effects observed above are not simply due to the presence of MeSO in the cell culture medium.

Taken together, these results strongly suggest again that a protein phosphatase is involved in the signaling pathway triggered by sodium butyrate to modulate histone H1° and c-myc gene expressions. Because this main observation is based on cell treatments with two potentially toxic drugs for 4 h, extended experiments have been performed to check whether cell integrity was still intact. As calyculin A and okadaic acid are commonly used for less than 1 h, their potential toxic effects following several hours of incubation are still unknown. Using trypan blue dye exclusion test, we found that the percentage of cell death was not altered by the additional drugs in the culture medium. Experiments have thus been set up to control that HTC cells were not engaged into apoptosis at first and that some of their intracellular functions were not altered. The percentage of cells undergoing apoptosis has been quantitatively determined by virtue of their ability to bind annexin V and exclude propidium iodide, which allows detection of cells in the early phases of apoptosis (46–48). The test has been performed in duplicate on control cells and on cells cultivated for 4 h in presence of sodium butyrate alone or supplemented with one of the two drugs. We have observed that the percentage of cells undergoing apoptosis and necrosis was not significantly different in control cells, in cells treated with sodium butyrate, and in cells treated simultaneously with sodium butyrate and either calyculin A (50 nM) or okadaic acid (1 μM). Intracellular functions of the cell have been estimated using activity measurement of enzymes implied in hepatic metabolic pathway. Alanine aminotransferase, aspartate aminotransferase, l-γ-glutamyltransferase, and 5′-nucleotidase activities have been measured from control cells and from cells treated for 4 h with sodium butyrate alone or in presence of either calyculin A (50 nM) or okadaic acid (1 μM). In all cases, no significant enzymatic activity differences were detectable in extracts from control cells and from cells treated with sodium butyrate alone or supplemented with the inhibitors. These control experiments show that HTC cell viability was not altered and that cellular metabolism was still preserved after 4 h of incubation in presence of calyculin A or okadaic acid.

All these data strongly suggest that a protein phosphatase would act as an intermediate of sodium butyrate effects on H1° and c-myc gene expressions. Because sodium butyrate is able to enter into HTC cells (49), two hypotheses may be proposed: (i) sodium butyrate is a direct effector of the protein phosphatase; (ii) sodium butyrate modulates some factor(s) upstream the phosphatase, which then belongs to a signal transduction cascade. To test these hypotheses, in vitro protein phosphatase activities have been assayed on HTC cell extracts. Although our data suggested that the implied phosphatase could be either PP1 or PP2A, alkaline and acid phosphatase activities have also been measured because it has been previously described that sodium butyrate could induce alkaline phosphatase activities in a cell line derived from a human colon carcinoma metastasis (50). Both activities have been determined using classical methods, as described under “Materials and Methods.” We found that acid phosphatase activity levels were not significantly different in cell-free extracts, supplemented or not with 5 mM sodium butyrate, from control cells and from cells treated for 4 h with sodium butyrate (data not shown). Surprisingly, no alkaline phosphatase activities were found from HTC control cells and from cells treated by sodium butyrate. Because it was detectable in two other hepatoma cell lines (HepG2 and FAO), this indicates that alkaline phosphatase activity is probably too weak in HTC cells to be detected using classical enzymatic dosages. Anyway, we did not found any stimulation of acid and alkaline protein phosphatase activities in HTC cells following sodium butyrate treatment. PP1- and PP2A-type activities have been determined using the classical substrate, [32P]phosphorylase a, as described under “Materials and Methods.” Dosages performed on cell-free extracts from control cells and from cells treated for 4 h with sodium butyrate revealed that the presence of the compound leads to a 45% increase of the amount of 32P released (Table I). This effect was not found when sodium butyrate (5 mM) was directly added to cell-free extract of untreated cells. These observations, which are in agreement with the involvement of a protein phosphatase as an intermediate of sodium butyrate effects, also suggest that the activation of the implied phosphatase would not be simply due to a direct interaction between the protein and
sodium butyrate. The link between sodium butyrate and the protein phosphatase would then be made by one or several factors that remain to be identified.

Because the main data presented in this work were based on the simultaneous treatment of cultured cells with sodium butyrate and protein phosphatase inhibitors, additional experiments have been performed to control that the inhibitors were able to act in the presence of the compound. As expected, protein phosphatase activities were drastically diminished when cells were treated for 4 h either with 5 mM sodium butyrate and 50 nM calyculin A or with 5 mM sodium butyrate and 1 μM okadaic acid (Table I). When cells were treated with 5 mM sodium butyrate and 25 nM okadaic acid, we observed a partial diminution of protein phosphatase activities, which corresponded to about half the activity found with sodium butyrate alone. These controls show that protein phosphatase activities have been affected by the presence of the inhibitors in the culture medium. The drastic diminution, observed when 50 nM calyculin A or 1 μM okadaic acid were used, reveals that almost all PP1 and PP2A enzymatic activities were abolished when sodium butyrate was neither able to induce an increase of the histone H1* mRNA amount nor able to provoke a decrease of the c-myc mRNA level. On the contrary the partial decrease of the phosphatase activities, observed with the use of 25 nM okadaic acid, was not sufficient to affect sodium butyrate effects. Because it has been described to be less sensitive to okadaic acid (44), PP1 could then appear to be a better candidate than PP2A for mediating sodium butyrate effects on gene expression.

To check this hypothesis, a second set of phosphatase activity assays has been performed using, instead of [32P]phosphorylase a, a phosphopeptide as substrate. This nonradioactive method (see “Materials and Methods” for references) preferentially allows PP2A activity measurement because the phosphopeptide is a poor substrate for the PP1 enzyme. Dosages performed on cell-free extracts from control cells and from cells treated for 4 h with sodium butyrate did not show the previous phosphatase activity stimulation observed when phosphorylase a was used as substrate. However, a slight increase in the phosphatase activities was detectable. These data reveal that the 45% increase of phosphatase activities previously obtained was probably not due to PP2A. Anyway, the implication of PP2A in sodium butyrate pathway cannot be totally ruled out, because we found, using the phosphopeptide as substrate, a little activation of phosphatase activities that could be attributed to PP1, to PP2A, or to an unknown phosphatase. Taken together, these results are in favor of a main implication of PP1 in the stimulation of protein phosphatase activities observed when sodium butyrate is added to cultured cells. Consequently, PP1 is a good candidate to be an important intermediate in the modulation of gene expression by sodium butyrate.

In this work, we have shown that sodium butyrate treatment causes an increase of histone H1* mRNA amount in HTC cells. Data obtained using actinomycin D and cycloheximide strongly suggest that this effect results from an active transcriptional mechanism that does not require protein neosynthesis. As a consequence, this enhancement of the transcription rate could result from chromatin structure alterations and/or be due to the modulation of a transcription factor activity by post-translational modifications. To check the second hypothesis, we performed experiments using different well known protein kinase and phosphatase inhibitors. Histone H1* and c-myc genes were chosen as models because sodium butyrate exerts opposite effects on their respective mRNA amounts. Using two different potent serine-threonine phosphatase inhibitors, calyculin A and okadaic acid, we drastically inhibited sodium butyrate effect on histone H1* and c-myc gene expressions. Data obtained from phosphatase activity assays and the fact that two inhibitors produce similar effects strengthen the hypothesis that the presence of sodium butyrate disturbs the phosphorylation/dephosphorylation balance of the cell. Based on these observations, we propose that a serine-threonine protein phosphatase, possibly a PP1-type, is involved in this cellular process.

This result is significant for several reasons. Although numerous studies have previously shown that sodium butyrate could modulate many gene expressions, the mechanism(s) implicated in this process was unknown. For the first time, our data suggest that a serine-threonine protein phosphatase could act as a molecular intermediate in the signaling pathway used by sodium butyrate to modulate histone H1* and c-myc gene expressions. This observation means that sodium butyrate would use at least one common way to induce or repress gene expression. This leads to the hypothesis that a serine-threonine protein phosphatase would act at the beginning or very early in this signaling pathway. The numerous effects of sodium butyrate could therefore be explained by the fact that such serine-threonine protein phosphatases have a lot of different substrates. Because it is now well established that the cell cycle is finely regulated by phosphorylation/dephosphorylation mechanisms, our data may have important implications for the understanding of the process through which sodium butyrate leads to the arrest of cell growth at the G1 phase.

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### Table I

|        | units/mg |
|--------|----------|
| T      | 3.6      |
| B4     | 5.3      |
| C      | 0.6      |
| OA1    | 0.7      |
| OA2    | 2.3      |

**Effects of sodium butyrate and inhibitors on PP1 and PP2A type protein phosphatase activities**

*In vitro* phosphorylase phosphatase activities were measured as the release of phosphate from [32P]phosphorylase a. Assays were performed on cell-free extracts from control HTC cells (T) or from cells treated for 4 h with 5 mM sodium butyrate (B4); sodium butyrate and 50 nM calyculin A (C); sodium butyrate and 1 μM okadaic acid (OA1); or sodium butyrate and 25 nM okadaic acid (OA2). One unit corresponds to a release of 1.0 nmol phosphate/min at 30 °C. Presented values are the mean of three independent experiments.
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