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Effect of Sodium Butyrate on the Expression of Genes Transduced by Retroviral Vectors

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Abstract We have studied the effects of sodium butyrate (NaBu) on the expression of genes transduced by retroviral vectors and stably expressed in two salivary gland-derived cell lines, A5-DAP and A5-BAG, established earlier. These cell lines were obtained by infecting A5 cells with the retroviral vectors DAP and BAG, respectively, and by selecting neomycin-resistant transduced cells. A5-DAP cells express human placental alkaline phosphatase (PLAP) and A5-BAG cells bacterial β-galactosidase, both under the control of the viral long terminal repeat (LTR) enhancer-promoter. NaBu in the concentration of 2–8 mM inhibited the growth of A5-DAP cells, and induced the expression of heat-stable PLAP. These effects of NaBu were dose-dependent. Induction of PLAP in clones of A5-DAP cells that express different basal levels of the enzyme was not correlated with the relative inducibilty by NaBu. Exposure to 4 mM NaBu for 48 h increased the PLAP mRNA level by 31%. A5-DAP cells released, in a time-dependent manner, PLAP into the culture medium. Cells treated with NaBu released more PLAP than untreated cells in proportion to their elevated level of the enzyme. The parent A5 cells also express a low level of tissue non-specific type alkaline phosphatase, which was also induced by NaBu. NaBu inhibited the growth of A5-BAG cells also, and increased the β-galactosidase level. These data indicate the genes transduced by retroviral vectors can be induced by NaBu, which most likely interacts with the viral LTR. J. Cell. Biochem. 69:201–210, 1998.

Key words: sodium butyrate; alkaline phosphatase; A5 cells; A5-DAP cells; A5-BAG cells; β-galactosidase; retroviral vectors

Extensively studied during the past two decades, sodium butyrate (NaBu) exerts a wide variety of effects on cultured cells. In general, it inhibits cell growth, induces cell differentiation, modifies the expression of a number of genes and the phenotypes of transformed cells, alters the morphologic characteristics of different cell types [Tallman et al., 1977; Kruh, 1982; Azuma et al., 1986; Byrd and Alho, 1987; Byrd et al., 1987; Nakagawa et al., 1988; Naranjo et al., 1990; Buckley et al., 1996; Ishiguro and Sartorelli, 1996; Lallemand et al., 1996; Li et al., 1996], and induces apoptosis of some normal and neoplastic cells [Sadaie and Hager, 1994; Lee et al., 1996; Singh et al., 1997; Kurita-Ochiai et al., 1997; McBain et al., 1997]. In addition, NaBu induces the expression of integrated viral genes, selectively induces transcription of promoters adjacent to viral LTR [reviewed by Kruh, 1982; Yeivin et al., 1992], increases viral production by producing cell lines [Pagès et al., 1995], reactivates silenced virally transduced genes [Zhang et al., 1996; Chen et al., 1997;. Kashanchi et al., 1997], enhances the expression of recombinant plasmids in mammalian cells, and increases stable transformation efficiency [Gorman and Howard, 1983]. The precise mechanism by which NaBu modifies gene expression is not known. Available data indicate that NaBu, which is a reversible inhibitor of histone deacetylases, induces changes in chromatin composition and structure following an increase in histone acetylation [reviewed by Kruh, 1982; Buckley et al., 1996]. Posttranslational modifications of histones in chromatin is an important mechanism in the regulation of gene expression [Steger and Workman, 1996]. The levels of histone acetylation, which are the result of a dynamic equilibrium between the actions of histone deacetylase(s) and histone acetylase(s), may modify

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nucleosome association with regions of DNA involved in transcriptional control and thus influence the binding of transcriptional factors involved in the regulation of a set of cellular genes [Pagès et al., 1995, van Lint et al., 1996b]. The role of histone deacetylase in gene regulation has been further confirmed using specific inhibitors, trichostatin A and trapoxin, of the enzyme [Yoshida et al., 1995].

Previously, we have described the transduction of A5 cells, a rat submandibular gland-derived cell line, by the retroviral vectors BAG [Barka and van der Noen, 1996] and DAP [Barka and van der Noen, 1997]. These vectors code for *Escherichia coli* β-galactosidase (β-gal) [Cepko et al., 1984] and human placental alkaline phosphatase (PLAP) [Fields-Berry et al., 1992], respectively, under the control of Moloney (Mo) MuLV long terminal repeat (LTR) enhancer-promoter. We have established two cell lines, designated as A5-BAG and A5-DAP, which stably express β-gal and PLAP, respectively. Transduction of these genes into acinar cells of the submandibular gland in vivo and long-term expression of the transgenes were also observed following retrograde ductal injections of these vectors into rats in which division of acinar cells was induced by the administration of the β-adrenergic agonist isoproterenol (IPR).

In the present paper, we describe the effects of NaBu on the growth of A5-BAG and A5-DAP cells, and on the expression of the transduced genes coding for β-gal and PLAP. NaBu, in a dose- dependent manner, inhibited the growth of both cell lines, and induced β-gal and PLAP, respectively. The induction of PLAP by NaBu was unrelated to basal levels of expression in clones of A5-DAP cells. These data are consistent with the view that NaBu induces transcription of the viral LTR enhancer-promoters irrespective of their site of chromosomal integration.

MATERIALS AND METHODS

Cell Lines and Culture

A5 cells, originally named RSMTx, were a gift of Dr. B.J. Baum. A5-BAG and A5-DAP cells were established in our laboratories [Barka and van der Noen, 1996, 1997]. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics. n-Butyric acid sodium salt (Sigma Chemical Co., St. Louis, MO) stock solution was prepared either in full medium (20 mM) or in water (1 M), and added to cultures grown in 35-mm plastic dishes. Triplicate dishes were used to assay growth by counting the cells in a hemocytometer after trypsinization and to determine alkaline phosphatase activity. DNA was measured by a spectrofluorometric method [Hinegardner, 1971] using deoxyadenosine as standard and assuming that 1 mg DNA = 0.385 mg of deoxyadenosine.

Assay of Alkaline Phosphatase Activity

Alkaline phosphatase (AP) activity was measured in triplicates using THERMOmax™ Microplate Reader (Molecular Devices Corp. Menlo Park, CA). The reaction mixture consisted of 50 µl alkaline buffer (2-amino-2-methyl-1-propanol, 1.5 mol/L, pH 10.3, Sigma Diagnostics, 221, Sigma Chemical Co.) supplemented with 1% Nonidet P-40, 50 µl cells suspension (10,000–20,000 cells in PBS) and 50 µl of substrate solution (p-nitrophenyl phosphate disodium salt, final concentration 5 mM). In preliminary experiments we have established that this concentration of Nonidet P-40 lyses the cells but has no effect on alkaline phosphatase activity. For kinetic assays, the plate was placed into the chamber of the plate reader with the temperature set for 37°C. After a 4-min lag period, the optical density at 405 nm minus nonspecific background at 650 nm of each well was determined at 1-min intervals over a period of 35 min. Blank wells contained H_2O. The maximum rate of reaction, V_{max} , calculated using the software (SOFTmax) of the plate reader, is given in mOD/min (millioptical density units per minute). This was converted into mOD/min/10^6 cells, or, by using appropriate p-nitrophenol standards, into nmol/min/10^6 cells. In all instances, the correlation coefficient of the kinetic plot was < 0.9.

Assay of β-Galactosidase Activity

β-Galactosidase activity of A5 and A5-BAG cells (50,000–100,000 cells) was measured by the method of Norton and Coffin [Norton and Coffin, 1985] with o-nitrophenyl β-D-galactoside as substrate. The method was modified by including 0.5% Triton X-100 into the buffer to lyse the cells.

Secretion of Alkaline Phosphatase by A5-DAP Cells

A5-DAP clone 3 cells, which reveal high basal level of AP activity, were plated in 35-mm dishes,

10^5 cells/dish, in full medium. To 3 dishes, NaBu was added to a final concentration of 4 mM. There were 3 control dishes. Forty-eight hours later, the dishes were rinsed two times with PBS, and 0.5 ml RPMI 1640 medium (without phenol red) (Life Technologies, Gaithersburg, MD) was added. After 30-min incubation at 37°C, the spent medium was withdrawn and replaced with 0.5 ml fresh medium. This was repeated again after 30-min incubation. At the end of the 90-min incubation period, the cells were trypsinized and counted. One control and one butyrate-treated dish, which were not incubated with the RPMI medium, were also trypsinized and counted. The spent medium samples were centrifuged for 4 min at 14,000 rpm, and AP activity was assayed using 50-µl samples in Thermomax plate reader. AP activity of cells was also measured as described above. The results are expressed as V_{max} = nmol/min/10^5 cells.

Quantitation of PLAP mRNA

The relative concentrations of PLAP mRNA were determined using a nonradioactive dot blot/ribonuclease protection assay described by Zhan et al. [1997], and modified as follows.

Preparation of digoxigenin (DIG)-labeled cRNA probes. Probes were prepared using Promega "Riboprobe® in vitro Transcription System" and "DIG RNA labeling mix" (unless indicated otherwise, all reagents were obtained from Boehringer Mannheim, Gmbh, Mannheim, Germany, and the manufacturer's protocols were used). The plasmid pSVT7, a gift of Dr. J Millán, which contains a 2.8-kb fragment of human placental alkaline phosphatase [Millán, 1986] was digested with EcoRI/KpnI. The resulting 2-kb fragment was subcloned into pGEM®-3Zf(+) vector. This plasmid was linearized with EcoRI to serve as template for RNA transcription by SP6 RNA polymerase to generate DIG-labeled antisense probe. For transcription by T7 RNA polymerase to generate the DIG-labeled sense probe, the plasmid was linearized with KpnI and treated with Klenow DNA polymerase to convert the 3' overhang to blunt end. The yield of DIG-labeled RNA was estimated using DIG-labeled Control RNA. The DIG-labeled RNAs were used for hybridization without further purification.

Isolation of RNA and hybridization. Total RNA was extracted from untreated A5-DAP cells as well as from A5-DAP cells exposed to 4 mM NaBu for 48 h using a commercial kit (Tri Reagent, Sigma Chemical Co.). The RNA samples were diluted with a solution consisting of DEPC-treated H_2O:20 X SSC:formaldehyde, 5:3:2, and 1 µl aliquots containing 0.625–5.0 µg RNA were dotted on positively charged nylon membrane (Boehringer Mannheim). The membranes were cross-linked by UV light (UV STRATALINKER(tm) 1800, Stratagene, LaJolla, CA) and prehybridized for 3 h. This was followed by hybridization overnight in the same solution to which 200 ng/ml DIG-labeled, denatured cRNA probe was added. Prehybridization and hybridization were carried out at 68°C. The hybridization solution was prepared according to the protocol of Boehringer Mannheim (The Genius(tm) System User's Guide for Filter Hybridization, Version 2.0).

After hybridization the membranes were washed 2 x 5 min in 2 x SSC/0.1% SDS at room temperature and 2 x 15 min in 0.1 x SSC/0.1% SDS at 68°C. The membranes than were equilibrated in a buffer and treated with 1 g/ml RNase A (Sigma Chemical Co.) for 10 min at room temperature as described by Zhan et al. [1997].

The DIG-labeled RNA hybrids were visualized using anti-digoxigenin-alkaline phosphatase Fab fragments and 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium method. Incubation was 30 min at room temperature in the dark. After incubation, the membranes were rinsed in distilled water, and dried at room temperature.

The signals were quantified using Arcus II scanner (AGFA-Gevaert, NV) and MD ImageQuant software, Version 3.3 (Molecular Dynamics, Sunnyvale, CA). This quantification provides a valid measure of the relative levels of mRNA. In a series of preliminary experiments, we have validated the quantitation of DIG-labeled RNA by the technique as described above. First, serial dilutions of a dye were used to validate the quantifying procedure using the scanner and the software program. Second, by using DIG-labeled Control RNA, we have observed a linear relationship between the amount of RNA (in dots) and the intensity of staining in a wide range, 15 pg to 4 ng, of RNA amounts. Finally, by using dots of purified alkaline phosphatase (alkaline phosphatase from bovine intestinal mucosa, Sigma Chemical Co.) stained with the X-phosphate/NBT technique, we have observed a linear relationship between the intensity of staining of the dots and the length of incubation between 10–80 min.

RESULTS

Effect of NaBu on the Growth and Alkaline Phosphatase Activity of A5 and A5-DAP Cells

The A5 cell line was established from the submandibular glands of weaning rats by treatment of explanted tissue fragments with 3-methylcholanthrene [Brown et al., 1989]. The cell line is probably of ductal origin. A5-DAP cell line was established from A5 cells infected with the retroviral vector DAP and selecting the neomycin (G418) resistant cells. A5-DAP cells express heat-resistant, membrane-bound PLAP [Barka and van der Noen, 1997]. A5 cells also reveal low levels of alkaline phosphatase activity which is heat-sensitive, tissue non-specific type.

Exposure to 2 mM NaBu inhibited the growth of both A5 and A5-DAP cells (Fig. 1). At the same time, alkaline phosphatase was induced in both cell lines. Exposure of A5 cells to 2 mM NaBu for 48 or 72 h increased AP activity 10-fold or 18-fold, respectively. The induction of AP activity in A5-DAP cells was about 3-fold after 48-h and 2.5-fold after 72-h treatment with NaBu (Fig. 2).

In both untreated and NaBu-treated A5 cells, the AP was heat-sensitive; after exposure to 65°C for 20 min, 2.8 and 1.4%, respectively, of enzyme activity remained compared to those of cells kept at 0°C (100%). AP in A5 cells treated with NaBu was sensitive to L-homoarginine, less sensitive to L-leucine, and resistant to L-phenylalanine. This and the heat sensitivity indicate that the enzyme induced by butyrate in A5 cells was of tissue non-specific type. In contrast, AP in NaBu-treated A5-DAP cells was relatively heat-resistant; 60–83% of enzyme activity remained after exposure to 65°C for 20 min. It was resistant to L-homoarginine but sensitive to L-phenylalanine, indicating that the induced enzyme is of the placental type (Table I).

Inhibition of growth and induction of AP by NaBu were concentration-dependent. NaBu in concentrations of 0.5 and 1.0 mM had no effect on growth of A5 cells. Growth was inhibited by 2–8 mM NaBu by 36–64% (Fig. 3). Induction of AP was significant only with 2, 4, and 8 mM NaBu; 8 mM NaBu increased AP activity/cell 8-fold (Fig. 4).

Inhibition of growth of A5-DAP cells by NaBu in 2.0–8.0 mM concentrations was 42–73%. NaBu was inhibitory even at 0.5-mM concentration, but this inhibition was not statistically significant (Fig. 3). The AP activity of untreated A5-DAP cells was 10-fold higher than the activity of untreated A5 cells. NaBu induced AP at

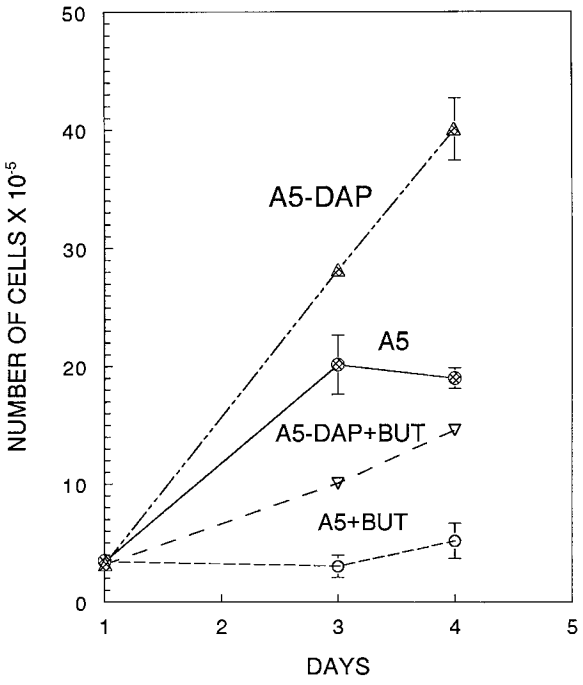

Fig. 1. The effect of sodium butyrate on the proliferation of A5 and A5-DAP cells. Cells were grown in complete medium supplemented with 2 mM sodium butyrate. The values represent means ± S.E. of triplicate dishes.

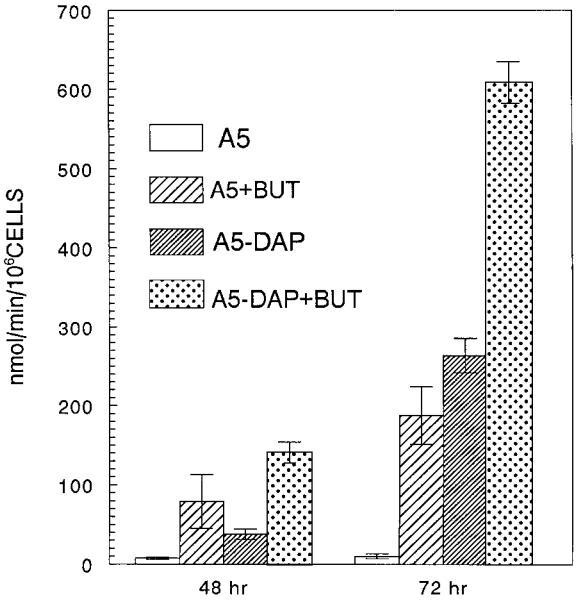

Fig. 2. The effect of sodium butyrate on the alkaline phosphatase activity of A5 and A5-DAP cells. Cells were grown in full medium supplemented with 2 mM sodium butyrate. After 48- or 72-h exposure, alkaline phosphatase activity was determined as described in Materials and Methods. Means ± S.E. of triplicate dishes, each assayed in triplicate, are shown.

TABLE I. Effect of Inhibitors on the Alkaline Phosphatase Activity of A5 and A5-DAP Cells Treated With Sodium Butyrate*

	L-homoarginine		L-leucine		L-phenylalanine	
	3 mM	5 mM	3 mM	5 mM	3 mM	5 mM
A5 + NaBu	44	39	89	77	91	89
A5-DAP + NaBu	100	100	93	94	77	65

*A5 cells (Clone 2) and A5-DAP cells were exposed to 2 mM NaBu for 72 h. AP activity was measured in triplicates, and the averages of inhibition as percent of control (100%) are shown.

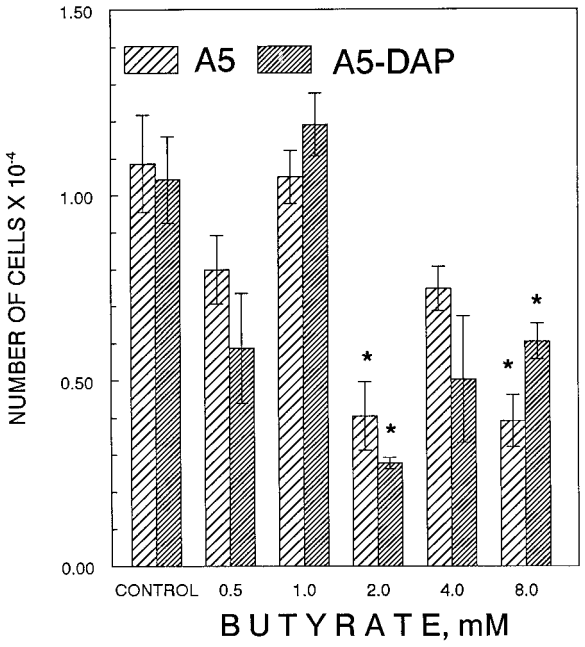

Fig. 3. The effect of different concentrations of sodium butyrate on the proliferation of A5 and A5-DAP cells. Cells were grown in full medium supplemented with the indicated concentrations of sodium butyrate for 48 h. Values are means ± S.E. of triplicate dishes. Asterisks, here and in all subsequent figures, indicate statistically significant difference, $P > 0.01$, between control and treated dishes.

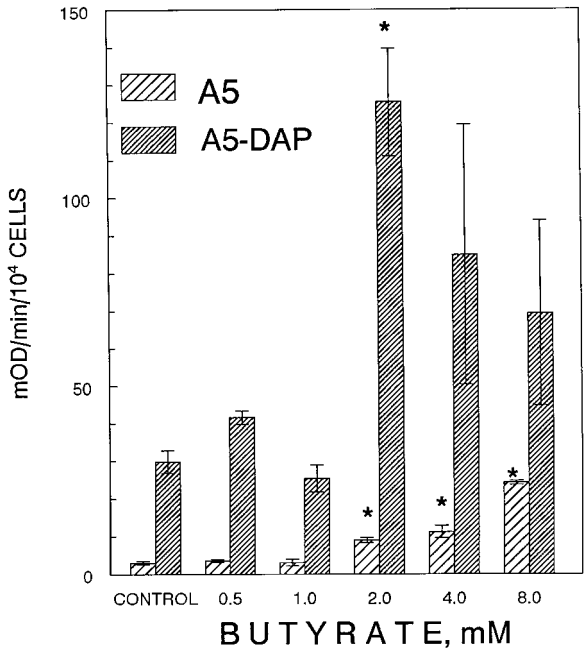

Fig. 4. The effect of different concentrations of sodium butyrate on the alkaline phosphatase activity of A5 and A5-DAP cells. Cells were exposed to the indicated concentrations of sodium butyrate for 48 h. Alkaline phosphatase activity was assayed as described in Materials and Methods. Means ± S.E. of triplicate dishes are shown.

all concentrations tested, but a significant increase was seen only with 2 mM of NaBu, which was 5.2-fold of control. In contrast to A5 cells, even 0.5 mM increased the enzyme activity 1.7-fold. However, the dose-dependency of induction was not obvious (Fig.4).

We have shown previously that clones of A5-DAP cells, even in early passages, display different levels of AP activity [Barka and van der Noen, 1997]. Based on those data, we have investigated whether the basal levels of AP activity of different clones correlate with the degree of inducibility of the enzyme by NaBu. To this end, we have selected five clones of A5-DAP cells displaying different levels of AP activity with V_{max} (nmol/min/10^6cells) ranging from 8 to 574. Exposure to 4 mM NaBu for 46 h increased AP activity 1.6–4.7-fold in the different clones (Fig. 5). There was no direct correlation between the degree of induction of AP activity (relative inducibility) and the basal expression level. Thus, clones with different levels of basal activity revealed comparable degrees of induction.

Effect of NaBu on the Level of PLAP mRNA in A5-DAP Cells

The levels of PLAP mRNA in A5-DAP cells and A5-DAP cells exposed to 4 mM NaBu for 48 h were determined using a DIG-labeled cRNA in a dot blot/ribonuclease protection assay. Treatment with NaBu caused a 31% increase in the relative con-

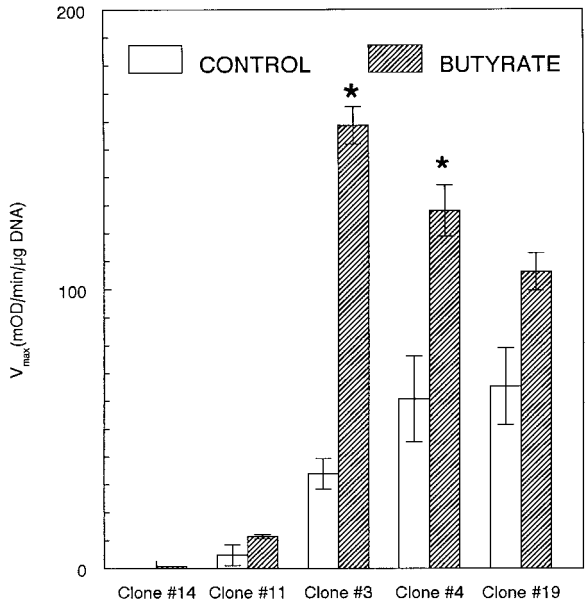

Fig. 5. The effect of sodium butyrate on the alkaline phosphatase activity of clones of A5-DAP cells. Five clones of A5-DAP cells displaying different levels of alkaline phosphatase activity (control) were treated with 4 mM sodium butyrate for 46 h. Alkaline phosphatase activity was determined as described in Materials and Methods. Means ± S.E. of triplicate dishes are shown.

centration of PLAP mRNA compared to that of untreated cells (Fig. 6). A comparable increase was seen using exposure to 2 mM NaBu for 48 h in a second experiment (data not shown). This finding indicates that NaBu acts at the transcriptional level in increasing PLAP in transduced cells.

Release of AP From Untreated and NaBu-Treated A5-DAP cells

Previously, we have established that the membrane-bound PLAP expressed by A5-DAP cells is released into the culture medium [Barka and van der Noen, 1997]. We have now investigated the release of AP from butyrate-treated cells, which express a higher level of enzyme activity. PLAP was released, or secreted, in a time-dependent fashion from both untreated and butyrate-treated cells. The release of PLAP was correlated with the level of AP activity of the cells. Thus, treatment with NaBu increased AP activity about 2-fold, and the butyrate-treated cells secreted, on a per cell basis, about twice as much AP as the untreated cells (Fig. 7). In this experiment, butyrate caused a 56% inhibition of growth.

Effect of NaBu on the Growth and β-Galactosidase Activity of A5 and A5-BAG Cells

The above experiments established that a transduced gene, specifically the one coding for PLAP transduced by the retroviral vector DAP and stably expressed in A5-DAP cells, can be induced by NaBu. To further the notion that NaBu can induce integrated viral genes, we have carried out experiments with another cell line, A5-BAG. This cell line, which was established by infecting A5 cells with the BAG retroviral vector, stably expresses *Escherichia coli* β-galactosidase as described previously [Barka and van der Noen, 1996]. The parent A5 cells also express low levels of β-gal activity, which is about 1/10 of that of A5-BAG cells.

Exposure to 2 mM NaBu inhibited the growth of A5-BAG cells (Fig. 8), and induced β-gal activity. After 48-h exposure the activity was 4-fold and

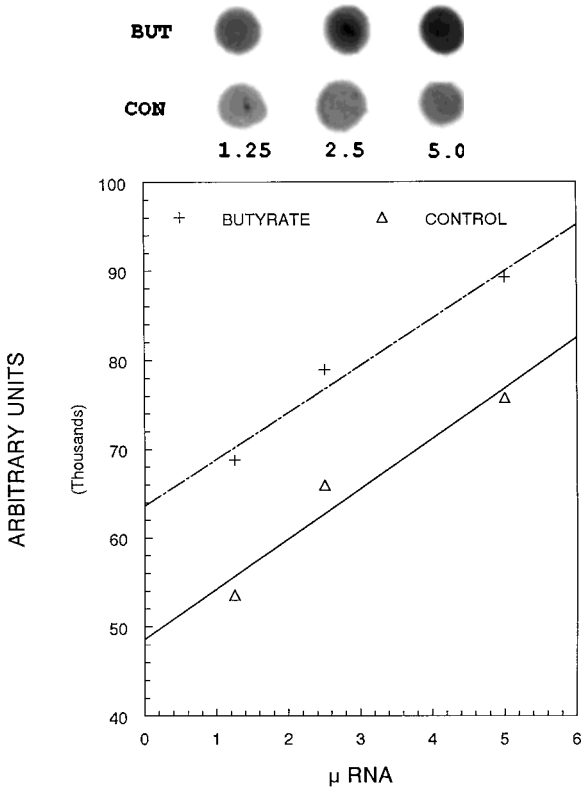

Fig. 6. The effect of sodium butyrate on the level of placental alkaline phosphatase mRNA. Total RNA was isolated from A5-DAP cells and from A5-DAP cells cultured in the presence of 4 mM sodium butyrate for 48 h. The relative concentrations of PLAP mRNA were determined using a DIG-labeled cRNA in a dot blot/ribonuclease protection assay, as described in Materials and Methods. **Top:** Dot blots. The numbers indicate total RNA in µg. CON = untreated culture; BUT = culture treated with butyrate. **Bottom:** Linear regression analysis of the intensity of the hybridization signal. There was a linear relationship between the amount of RNA and the intensity of the signal. The probabilities of linear regressions were: control: $r^2 = 0.825$; butyrate-treated: $r^2 = 0.9671$. The difference between the control and treated values was statistically significant, $P = 0.0024$ (paired *t*-test).

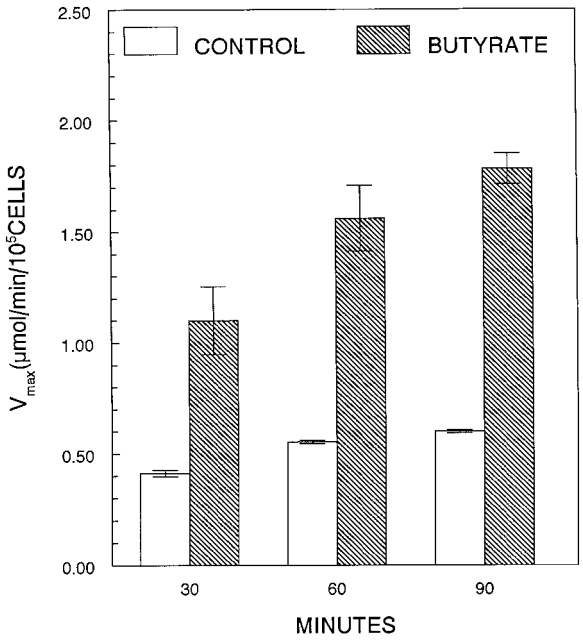

Fig. 7. Time-dependent release of alkaline phosphatase from A5-DAP cells and from A5-DAP cells that were cultured with 4 mM sodium butyrate for 48 h. The amount of alkaline phosphatase activity released during a 90-min incubation in a serum-free medium was determined as described in Materials and Methods. Means of triplicate assays ± S.E. are shown.

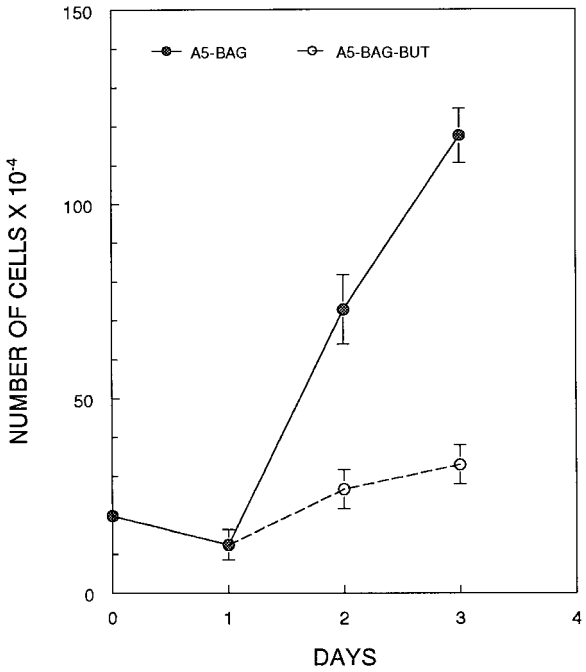

Fig. 8. The effect of sodium butyrate on the proliferation of A5-BAG cells. Cells were grown in complete medium supplemented with 2 mM sodium butyrate. The values represent means of triplicate dishes ± S.E.

after 72 h about 3-fold of control. In A5 cells, NaBu increased β-gal activity 2.5-fold (Fig. 9).

DISCUSSION

The two cell lines, A5-DAP and A5-BAG, used in the present study were established by infecting A5, a salivary gland-derived cell line, by the retroviral vectors DAP and BAG, respectively, and by selecting the neomycin resistant cells. A5-DAP cells express PLAP and A5-BAG cells β-gal under the control of the viral enhancer-promoter LTR. The salient findings of this study are that NaBu enhances the expression of these transgenes and that the induction of PLAP by NaBu is not correlated with the basal expression of the enzyme in clones of A5-DAP cells. Interaction of NaBu and trichostatin A with viral enhancers-promoters has been describe [Kruh, 1982; Hoeben et al., 1991; Laughlin et al., 1993; van Lint et al., 1996a; Dion et al., 1997], but the interaction of these inhibitors with the Moloney MuLV LTR [Pagès et al., 1995] has not been analyzed in details. In the case of the LTR of the Moloney murine sarcoma virus (MoMSV), sodium butyrate responsive element (BRE) was localized in the enhancer region and was found to be promoter dependent [Yeivin et al., 1992]. BREs have also been localized to two regions of the LTR of the human immunodeficiency virus type 1 (HIV-1) in HeLa cells. Induction of gene expression directed by HIV-1 LTR is mediated by the interaction of NaBu with cellular transcriptional factors that bind to the HIV LTR [Bohan et al., 1989].

Clones of A5-DAP cells revealed a great variation in the expression of PLAP [Barka and van der Noen, 1997]. The same phenomenon was observed with clones of A5-BAG cells with respect to the expression of β-gal (unpublished observation). These variations were seen even with first or second passage of cultures, and appear to be unrelated to silencing of transgenes, which may occur with long-term culture of some of the genes transduced by retroviral vectors. Clonal variation in β-gal expression in BAG-infected murine fibroblasts has been ascribed to de novo methylation of cytidine residues and could be reversed by treatment with azacytidine [Hoeben et al., 1991]. In addition to promoter-enhancer functions, higher order of regulatory effects exist that vary according to the actual positions of the site of integration of the gene. In analyzing the inducibility of PLAP by NaBu in 5 clones of A5-DAP cells, we have found no correlation between the basal and

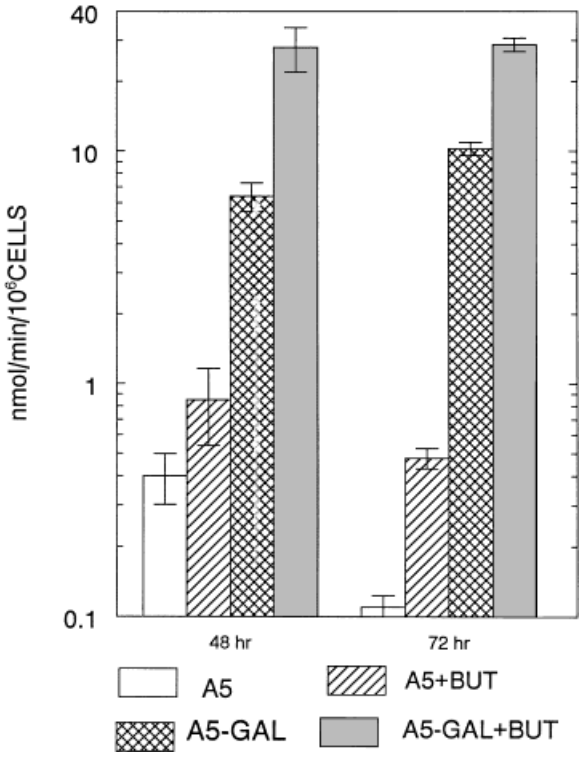

Fig. 9. The effect of sodium butyrate on the β-galactosidase activity of A5 and A5-BAG cells. Cells were grown in full medium supplemented with 2 mM sodium butyrate. After 48- or 72-h exposure, β-galactosidase activity was determined as described in Materials and Methods. Means ± S.E. of triplicate dishes, each assayed in triplicate, are shown. The difference between control and butyrate-treated values were statistically significant, $P < 0.01$.

induced levels of enzyme activity. This suggests additional control mechanisms in inducibility by butyrate acting on the viral LTR. This is in contrast to the finding that in AKV leukemia virus-infected cells the relative inducibility of integrated vectors by dexamethasone correlated inversely with the basal expression levels [Duch et al., 1993].

The parent A5 cells from which the A5-BAG and A5-DAP cells derived also express low level of AP activity. AP in A5 cells has not been studied, but its heat sensitivity and the pattern of inhibition by amino acids indicate that it is tissue non-specific type. This enzyme was also induced by NaBu, and the degree of inducibility exceeded that observed with A5-DAP cells. Induction of different types of AP by NaBu has been observed frequently with various cell types [Griffin et al., 1974; Morita et al., 1982; Tsao et al., 1982; Herz and Halwer, 1983; Jemmerson et al., 1985; Gum et al., 1987; Telfer and Green, 1993; Fleming et al., 1995, and references therein), but the BREs in AP genes have not been characterized.

NaBu increased the level of PLAP mRNA in A5-DAP cells. This increase, although statistically significant, was modest, 31%. Whether NaBu acts at the level of transcription or by increasing the half-life of PLAP mRNA was not investigated. Although NaBu is a pleiotropic agent [Kruh, 1982], its effects on gene expression are most likely related to its activity as a reversible inhibitor of histone deacetylase(s). This is supported by the close parallelism between the effect of NaBu and specific inhibitors of histone deacetylase trichostatin A and trapoxin on gene expression in different cell types. Increased acetylation of histone tails, which is determined by the dynamic equilibrium of the actions of histone acetyltransferases and histone deacetylases, is associated with transcriptional activity, and underacetylation with repression [Turner, 1993; Tsukiyama and Wu, 1997; Hartzog and Winston, 1997; Luger et al., 1997; Grunstein, 1997]. Since both acetyltransferases and deacetylases are transcriptional factors or form complexes with certain transcriptional factors, a mechanism exists for the regulation of selective gene expression [van Lint et al., 1996b; Bannister and Kouzarides, 1996; Ogryzko et al., 1996; Rundlett et al., 1996; Steger and Workman, 1996; Tsukiyama and Wu, 1997]. Acetylation of specific lysine residues may afford further control mechanism affecting selective gene expression [Turner, 1993; Rhodes, 1997].

A5-DAP cells express membrane-bound, heat-stable PLAP, which could be released by treatment with bromelain or phosphatidylinositol-specific phospholipase C. Furthermore, some of the enzyme is released by the cells into the incubating medium in a time-dependent manner. We have provided evidence also that the enzyme is secreted into the saliva by acinar cells that were transduced by the retrograde ductal injection of the DAP retroviral vector [Barka and van der Noen, 1997]. A5-DAP cells treated with NaBu had higher levels of AP activity, and released proportionally higher amounts of AP than the untreated cells. Whether NaBu, butyrate prodrugs [Chen and Breitman, 1994; Newmark and Young, 1995], or long-lasting analogues, could induce the expression of transgenes in vivo deserves to be studied since it may provide a means of increasing delivery of specific gene products.

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