Regulation by butyrate of the cAMP response to cholera toxin and forskolin in pituitary GH1 cells

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(Received June 19, 1990) — EJB 89 0752

In pituitary GH1 cells, a rat growth hormone-producing cell line, butyrate elicited a dose-dependent increase in chola toxin receptors as measured by an increased binding of ¹²⁵I-labeled chola toxin to the intact cells. Butyrate did not alter the affinity of chola toxin binding, the dissociation constant being 0.4 nM for both control and butyrate-treated cells. Despite the increased binding, the cAMP response to chola toxin was strongly reduced after exposure to butyrate. This reduction was dose-dependent and with butyrate 1–5 mM, intracellular and extracellular (medium) cAMP levels were decreased by more than 70% in cells incubated for 24 h with 1 nM chola toxin. Forskolin (30 µM) elicited a cAMP response similar to that found with the toxin, and a similar inhibition of cAMP was also found after incubation of GH1 cells with butyrate. Butyrate also affected basal cAMP levels which were reduced by 40–60% in cells cultured for 24–48 h with the fatty acid. In order to study whether butyrate influenced cAMP synthesis and/or cAMP degradation, adenylyl cyclase and phosphodiesterase activities were determined in control cells and in cells incubated for 24 h with chola toxin or forskolin. Butyrate had a dual effect since, besides activating phosphodiesterase by more than twofold, it also inhibited the cyclase by 40–50% in all groups. The in vitro response of adenylyl cyclase to stimulatory (NaF) and inhibitory (carbachol and adenosine) effectors was also examined. The absolute activity of the cyclase was always 40–50% lower in the cells incubated with butyrate, but the percentage change of activity obtained in butyrate-treated and untreated cells was unaltered. In addition, ADP-ribosylation of the guanine nucleotide stimulatory component of the cyclase (Gs) was not affected in the cells incubated with butyrate. These results suggest that the catalytic (C) subunit of adenylyl cyclase and/or its interaction with the regulatory components might be altered in butyrate-treated GH1 cells.

The inhibition of the cAMP response in GH1 cells was accompanied by an inhibition of a biological action of the nucleotide, namely growth hormone (somatotropin) production which is primarily controlled by thyroid hormones in these cells. Forskolin alone did not affect the somatotropin levels but potentiated the growth hormone response to triiodothyronine. Butyrate produced a dose-dependent inhibition of this response, which was totally abolished at concentrations of butyrate higher than 1 mM.

Butyrate exerts a variety of effects on growth, differentiation and metabolism in different types of cultured cells [1]. In neuroblastoma cells it has been noted that some of the butyrate effects could be mediated by an increase in intracellular cAMP levels which might reflect an augmented adenylyl cyclase activity [2]. In HeLa cells butyrate induces a severalfold increase of β-adrenergic receptor number and enhances agonist-stimulated adenylyl cyclase activity [3–5], by facilitating the ability of the regulatory components to couple to receptors [5]. Although the molecular mechanisms by which the receptors and G proteins interact to stimulate the catalytic (C) subunit has not yet been elucidated, the interaction among the different components of the cyclase could be affected by changes in the structure of the cell membrane.

Incubation with fatty acids is known to alter membrane structure and fluidity [6, 7], and butyrate specifically alters glycolipid membrane composition in cells by inducing an increase in its ganglioside content [8, 9]. As a consequence, butyrate induces an increase in chola toxin receptors in several cell types [10], since the toxin binds with high affinity to ganglioside GM1 in the cell membrane [11]. These results prompted us to investigate the effects of butyrate on basal cyclic AMP levels and on the response to chola toxin and forskolin in cultured growth-hormone-producing cells. Chola toxin stimulates adenylyl cyclase through ADP-ribosylation of the guanine nucleotide stimulatory component (Gs) of the enzyme [12], whereas the diterpene forskolin appears to activate the cyclase by a direct interaction with its catalytic C subunit [13, 14]. We reasoned that since butyrate increases chola toxin binding, it could enhance the response of the cyclase to the toxin but not to forskolin. However, our results indicate that in pituitary GH1 cells, treatment with butyrate effectively blocked the response to both compounds, even though it increased chola toxin binding to the cells.

GH1 cells produce and release growth hormone (somatotropin) to the medium, and cAMP has been described to influence somatotropin production by pituitary cells [15]. Therefore, we studied the effect of butyrate on basal
somatotropin production and on the response to forskolin. Since thyroid hormones are the main regulators of growth hormone gene expression in these cells [16, 17], we also analyzed the influence of the fatty acid on the combined response to forskolin and 3,5,3'-triiodo-l-thyronine. The data obtained demonstrated that butyrate inhibited the effect of forskolin on somatotropin production concomitantly with the inhibition of the cAMP response to the diterpene.

**EXPERIMENTAL PROCEDURES**

**Materials**

Sodium butyrate, cholera toxin, carbachol and adenosine were obtained from Sigma. Forskolin was obtained from Calbiochem. \(^{125}\)I was from Amersham, \(^{[32P]}\)NAD, \(^{[2-}\)\(^{32P}\)ATP and \(^{[8-3H]}\)cAMP were from New England Nuclear. All other reagents were obtained from Sigma or Merck and were of the maximal purity available. Culture media and sera were from Gibco, and culture flasks and plates were purchased from Nunc.

**Cell culture**

Pituitary GH1 cells were grown in monolayers as previously described [16–19] but using RPMI instead of Ham’s F10 medium. For the experiments the cells were inoculated at an initial density of 20,000 cells/cm\(^2\) with RPMI medium containing 10% horse serum and 2.5% fetal calf serum; 24 h before the beginning of the experiments, the cells were shifted to a medium containing 10% serum treated with resin AG1X8 (Bio-Rad) and charcoal [17].

**Cholera toxin binding**

Cholera toxin was iodinated with chloramine T [20] to a specific activity of approximately 125 \(\mu\)Ci/\(\mu\)g and purified in cellulose CF-11 columns. The cells were grown in 10-\(cm^2\) plates and the binding assays were carried out in the cell monolayers in 1 ml serum-free medium containing \(^{125}\)I-labeled cholera toxin. Non-specific binding, determined in the presence of an excess cholera toxin (100 nM), was subtracted from the total binding to determine the amount of toxin specifically associated to the cells. The cultures were incubated for 45 min at 20–22°C, the medium was separated, and the cells washed three times with 4 ml ice-cold saline/phosphate buffer. The cells were then lysed in 0.4 M NaOH and transferred to tubes for determination of radioactivity. The radioactivity of the medium was also determined and considered to be the unbound ‘free’ toxin. An aliquot of the cell lysate was used for protein determination by the method of Lowry et al. [21]. All binding data were normalized to 100 \(\mu\)g cell protein and represent the mean of triplicate cultures which did not vary more than 5–10%.

**cAMP determination**

The cells were grown in 2-\(cm^2\) multiwell plates and after different treatments medium was removed and cells were thoroughly rinsed with ice-cold saline. The cAMP was extracted from the cells with 5% perchloric acid and the acid extracts were neutralized with KHCO\(_3\). The culture medium received the same treatment and was used to determine extracellular cAMP accumulation. Intracellular and medium cAMP content was assayed by radioimmunoassay in acetylated samples [22] as previously described [18] using the immunoreagents obtained from New England Nuclear.

**Adenyl cyclase assay**

Confluent GH1 cells grown in 75-\(cm^2\) flasks were detached from the flasks as described [23] and homogenized with a Dounce homogenizer in 1 ml of a buffer containing 27% (by mass) sucrose, 1 mM EDTA and 20 mM Na/Hepes pH 7.8. The cellular extracts contained approximately 1 mg protein/ml. Incubations were carried out at 32°C for 10 min as previously described [23] using 2–5 \(\times\) 10\(^6\) cpm/assay of \(^{[32P]}\)ATP. When present, 10 mM NaF, 10 \(\mu\)M carbachol, or 10 \(\mu\)M adenosine were added during the incubations. The reaction was stopped and the \(^{[32P]}\)cAMP formed was assayed by a modification [24] of the method of Salomon et al. [23].

**ADP-ribosylation**

A detailed description of the ADP-ribosylation assay has been given elsewhere [23]. The assays were carried out with 10 \(\mu\)l appropriately diluted homogenates to give a concentration of 1 mg/ml and \(^{[32P]}\)NAD (2–5 \(\times\) 10\(^6\) cpm) with or without 600 \(\mu\)g cholera toxin/ml previously activated for 30 min at 32°C with dithiothreitol and sodium dodecyl sulfate in the conditions previously described [23]. After incubation for 30 min at 32°C, the reaction was stopped by addition of 1 ml ice-cold 20% trichloroacetic acid. The trichloroacetic-acid-insoluble material was washed with ethyl ether [23] and the samples were analyzed for incorporation of \(^{[32P]}\)ADP-ribosylated units of GH1 cells by resuspending in 20 \(\mu\)l Laemmli’s sample buffer [26] at room temperature and loading onto 11% SDS/polyacrylamide slab gels. Electrophoresis was performed according to Laemmli, and the gels were dried and autoradiographed to visualize bound \(^{32P}\).

**Phosphodiesterase assay**

Cyclic nucleotide phosphodiesterase was measured using methods described elsewhere [27, 28]. The assay mixture, in a final volume of 100 \(\mu\)l, consisted of 20 mM Tris/HCl, 1 mM \(\text{Cl}_2\)Mg, 1 mM EGTA, 60 units 5'-nucleotidase, 100 \(\mu\)g soybean trypsin inhibitor, 100 \(\mu\)M cAMP, approximately 60000 cpm \(^{[3H]}\)cAMP, and an appropriate dilution of the cell homogenates. The mixture was incubated for 20 min at 30°C in the presence or absence of 0.2 mM CaCl\(_2\). The reaction was stopped by addition 1 ml of activated AG1X8 resin (Bio-Rad) suspended 1:3 in distilled water. After centrifugation at 1000 \(\times\) g for 15 min, \(^3\)H was measured in a 0.4-ml aliquot of the supernatant. The hydrolyzed cAMP was calculated by subtracting blank values obtained with boiled extracts, and applying the adenosine retention power of the resin as a correction factor. Degradation of the substrate was always less than 20% of the initial concentration.

**Quantification of somatotropin production**

To study the growth hormone (somatotropin) response, the cells were grown in 2-\(cm^2\) multiwell plates with 1 ml medium. After a 24-h incubation in the presence or absence of 1 mM butyrate, the cells received 5 \(\mu\)M 3,5,3’-triiodo-l-thyronine and/or 30 \(\mu\)M forskolin. After 24 h, the medium was saved and the cells were washed with saline and used for quantification of cell protein [21]. In GH1 cells the accumu-
indicated for 24 h. Binding was determined by incubating the cells with 0.12 nM $^{125}$I-labeled cholera toxin ($[^{125}\text{I}]\text{ChT}$) as described under Experimental Procedures. In this and in the following figures each data point represents the mean ± SD of triplicate cultures which normally did not vary more than 10–15%.

ulation of somatotropin in the medium reflects the rate of hormone intracellularly and somatotropin is stable in the culture medium [16, 17]. Somatotropin was determined by means of a specific radioimmunoassay using the immunoreagents provided by the NIAMDD, and all samples were assayed at least at two different dilutions.

RESULTS

Effect of butyrate on cholera toxin binding to GH1 cells

Fig. 1 shows the effect of different concentrations of butyrate on binding of $^{125}$I-labeled cholera toxin. Butyrate produced a dose-dependent increase of cell-associated radioactivity. A half-maximal effect was found at approximately 1.6 mM, and the maximal increase (1.9-fold over control levels) was found at 5 mM. This effect was due to an increase in specific binding with no change in non-specific binding (less than 4% of the total), or in toxin degradation (less than 7%). Binding studies were also performed in untreated cells and in cells treated with 1 mM butyrate in the presence of increasing concentrations of $^{125}$I-labeled cholera toxin. The inset in Fig. 2 shows that butyrate increased binding at all concentrations examined. When the binding data were analyzed by the method of Scatchard, linear plots were found (Fig. 2). There was no significant difference in the slopes but the maximal binding capacity increased by 33% in the butyrate-treated cells. Therefore, butyrate did not alter the affinity of cholera toxin binding ($K_d = 0.4$ nM in both groups) but increased the number of binding sites.

Influence of butyrate on the cAMP response to cholera toxin and forskolin

Fig. 3 illustrates the effect of incubation with increasing concentrations of butyrate on the stimulation of cAMP levels by 1 nM cholera toxin. The toxin caused a sevenfold increase in intracellular cAMP, and butyrate at concentrations higher than 0.1 mM caused a dose-dependent reduction on the response to cholera toxin (Fig. 3A). With butyrate 1–5 mM, cAMP was almost as low as in untreated cells. The fatty acid also produced a similar decrease of uninduced cAMP levels which were reduced by more than 60% with butyrate > 1 mM.

The extracellular cAMP (Fig. 3B) paralleled the intracellular cAMP, and butyrate also produced a dose-dependent inhibition of cAMP accumulation into the medium. In agreement with our previous results in GH1 cells [18, 19], after 24 h, intracellular cAMP represented less than 10% of the cAMP released by the cells into the culture medium.

Fig. 4 shows the inhibitory effect of 1 mM butyrate on the response to 1 nM and 100 nM cholera toxin. In agreement with our previous results [18, 19], 0.1 nM cholera toxin elicited a greater cAMP response than that produced by the highest concentration. Basal cAMP decreased by 40% and the response to the toxin was inhibited by more than 60% in the cells incubated with butyrate. Although this inhibition was less marked than that obtained in Fig. 3, where the cells had been pre-incubated with butyrate for 24 h, these results show that pre-incubation with the fatty acid is not required in order to decrease the response to the toxin.

That the inhibitory effect of butyrate is not restricted to stimulation with cholera toxin is illustrated in Fig. 5, where the effect of butyrate on the cAMP response to forskolin was examined: 5 μM and 30 μM forskolin stimulated cAMP production to a similar extent as 1 nM cholera toxin; cAMP accumulation was also greatly diminished in the presence of 1 mM butyrate.

We next examined the reversibility of the effect of butyrate on basal and cholera-toxin-stimulated cAMP. Fig. 6 shows the time course of cAMP accumulation in the medium from cells which were pre-incubated for 24 h with or without 1 mM butyrate and were then washed free of the fatty acid. In untreated cells cAMP accumulated rapidly after incubation with the toxin and remained constant over 5–20 h. In butyrate-preincubated cells, cAMP levels were lower during the period examined; 2 h after removal of butyrate, cAMP accumulation in response to cholera toxin was 20-fold lower in the cells previously exposed to the fatty acid. The difference became gradually smaller, and 20 h after butyrate removal cAMP levels were 4-fold lower in untreated cells. In unstimulated cells, cAMP levels were also 30–50% lower in cells previously exposed to the fatty acid during the whole experimental period.
Adenylyl cyclase and phosphodiesterase activity

The decreased cAMP response in the cells incubated with butyrate may occur as a result of a decrease in cAMP synthesis, an increase in degradation, or by a combination of both effects. To differentiate between these possibilities, adenylyl cyclase and phosphodiesterase activity were determined in GH1 cells incubated with and without butyrate. Fig. 7 shows the influence of butyrate on adenylyl cyclase activity. Basal enzyme levels were decreased by approximately 40% in cells treated with 1 mM butyrate for 24 h. Forskolin (30 μM) and chelera toxin (1 nM) induced an 8–10-fold increase in adenylyl cyclase activity over basal levels, and butyrate also decreased activity by 50% under these conditions.

NaF increased enzyme activity by more than twofold in homogenates from untreated or forskolin-treated cells, whereas it slightly decreased activity in the cells incubated with the toxin. Under all conditions, the activity of the enzyme was reduced by 30–40% in the homogenates from the cells incubated with butyrate. Carbachol and adenosine decreased adenylyl cyclase by 40–50% in all groups; the inhibitory effect of butyrate was again observed in the presence of these compounds. It is important to observe that, although the absolute activity of the cyclase was always lower in the cells incubated with butyrate, treatment of cells with the fatty acid interfered little with the degree to which the different effectors act, the percentage of the stimulation or inhibition being approximately the same in homogenates of control and butyrate-treated cells.

In order to ADP-ribosylate G subunits, GH1 cell extracts were incubated with [32P]NAD and activated chelera toxin (Fig. 8). When analyzed by polyacrylamide gel electrophoresis three main radioactive bands (120, 45 and 24 kDa) were observed. The 45-kDa polypeptide was specifically labeled by the toxin and therefore represents the α component of Gs. Densitometry of the autoradiogram did not demonstrate changes in the extent of labeling of Gs in the cell treated with butyrate. As expected [23], previous exposure of the cell cultures to the toxin resulted in an essentially complete disappearance of sites susceptible to subsequent in vitro ADP-ribosylation with [32P]ADP-ribose. Pre-incubation with the toxin also decreased incorporation into the 120-kDa acceptor which could represent nuclear poly(ADP-ribose) synthetase, in agreement with our previous data showing that incubation of GH1 cells with chelera toxin for 24 h decreases nuclear ADP-ribosylation [19]. The 24-kDa band, that represents the self ADP-ribosylated A subunit of the toxin [29], was not altered by any treatment.
Fig. 7. Influence of butyrate on basal and stimulated adenylyl cyclase activity. GH1 cells were incubated for 24 h with (stippled bars) or without (open bars) 1 mM butyrate. The cultures received no additions (control), 30 μM forskolin or 1 nM cholera toxin. Adenylyl cyclase was determined as described under Experimental Procedures and when indicated 10 mM NaF, 10 μM carbachol (Cchol) or 10 μM adenosine (Ado) were added during the assay to the cell homogenates.

Fig. 8. ADP-ribosylation of Gs by cholera toxin in intact and butyrate-treated GH1 cells. Cells were treated or not with 1 mM butyrate for 24 h in the presence or absence of 1 nM cholera toxin. The cells were homogenized and tested for ADP-ribosylation with activated cholera toxin and [32P]NAD. The [32P]ADP-ribosylated proteins were separated by SDS/PAGE and identified by autoradiography. The apparent molecular masses of standard proteins electrophoresed in parallel lanes are given. Lanes 1 and 2, control cells; lanes 3 and 4, butyrate-treated cells; lanes 5 and 6, cholera-toxin-treated cells; lanes 7 and 8, butyrate + cholera-toxin-treated cells.

Phosphodiesterase activity is shown in Fig. 9. Incubation with either forskolin or cholera toxin for 24 h did not alter cyclic nucleotide phosphodiesterase activity in GH1 cells. However, butyrate produced a more than twofold increase in activity in all experimental groups.

Influence of butyrate on the biological action of cAMP

We then examined whether or not the decrease in cAMP levels caused by butyrate was accompanied by an inhibition in the biological action developed by the nucleotide in GH1 cells. In Fig. 10 somatotropin production by GH1 cells incubated for 24 h with increasing concentrations of butyrate and then exposed for an additional 24 h period to 5 nM triiodothyronine and/or 30 μM forskolin is shown. In the absence of thyroid hormones, neither butyrate nor forskolin significantly influenced somatotropin levels. Triiodothyronine elicited an 11-fold stimulation of somatotropin production, and cAMP potentiated the effect of triiodothyronine, increasing somatotropin 20-fold over control levels. Butyrate did not affect the response to triiodothyronine at concentrations below 1 mM, whereas at 3 mM significantly decreased it. Butyrate produced a dose-dependent inhibition of forskolin-stimulated somatotropin production. The dose/re-
response of this inhibitory effect of the fatty acid was almost identical to that found for the inhibition of cAMP production (Fig. 3) and with concentrations 1 mM and higher the response to forskolin was totally blocked.

DISCUSSION

In this study, we have examined basal cAMP and its response to cholera toxin and forskolin in pituitary GH1 cells incubated with butyrate. Our results show that, in control non-stimulated cells, basal cAMP levels were significantly decreased after exposure to butyrate. This contrasts with the reported elevation of cAMP levels in neuroblastoma cells incubated with butyrate [12] and indicates the specificity of the effect of the fatty acid in the different cell types.

Butyrate has been described as increasing cholera toxin binding in other cell types [10] due to an increase in ganglioside GM1 content, the reported toxin receptor [1]. The number of cholera toxin receptors found by us in untreated GH1 cells is at least twofold higher than that described for HeLa or C6 cells, and similar to that found in Friend cells [10]. We have provided evidence that butyrate causes an increase in cholera toxin receptors in GH1 cells, although this increase was less marked that that observed in HeLa or C6 cells [10]. This also occurred in Friend cells, thus suggesting that the quantitative effect of butyrate could depend on the initial concentration of receptors. On the other hand, the affinity of the toxin for control and butyrate-treated GH1 cells was identical, and appeared to be similar to that described in HeLa cells [10]. The increase in the number of binding sites without changes in affinity suggests that the concentration of GM1 ganglioside is increased in the membranes of GH1 cells exposed to the fatty acid.

After the initial high affinity binding of the B protomer subunit of the toxin to GM1, the A protomer component is internalized and exerts its effect by catalyzing the ADP-ribosylation of the stimulatory Gs component of the cyclase [12]. Therefore, it was expected that the increase in cholera toxin binding brought about by butyrate must be followed by an increased modification of Gs and by a subsequent elevation of adenyl cyclase activity and cAMP synthesis. However, our results clearly show that in butyrate-treated GH1 cells, cAMP levels are strongly decreased, rather than increased, in response to cholera toxin. The effect of butyrate was found to be dose-dependent and to be more accentuated when the cells were pre-incubated with butyrate before exposure to the toxin than when both compounds were added simultaneously to the cells. In addition, the reversal of the effect of the fatty acid was slow, suggesting that a direct ‘allostic’ effect of the butyrate molecule on some of the adenyl cyclase components is very unlikely.

To test whether the inhibition of cAMP formation could reflect a decreased presence of a functional Gs, we also tested the cAMP response to forskolin which activates the cyclase by a direct interaction with its catalytic subunit [13] and is capable of stimulating cAMP in cells lacking Gs [30]. The data obtained indicated that a clearly diminished response to forskolin was also found in butyrate-treated cells and that, therefore, a predominant effect of butyrate on Gs is unlikely. The presence of unaffected Gs in homogenates of butyrate-treated cells was also demonstrated by ADP-riboleylation, since [32P]ADP-ribose incorporation to the a subunit was not different from that found in control GH1 cells. In agreement with that found in other pituitary cells [23], treatment of intact cells with cholera toxin resulted in modification of essentially all Gs molecules as shown by the inhibition of in vitro [32P]ADP-ribose incorporation; this also occurred in the cells incubated with butyrate.

A decreased accumulation of cAMP could be caused by an increase in phosphodiesterase activity and/or by an inhibition of the adenyl cyclase. We found that butyrate had a dual effect in GH1 cells, inhibiting cAMP synthesis and enhancing cAMP degradation, which results in an additive effect and a marked inhibition of cAMP levels. Phosphodiesterase activity was not altered by cholera toxin or forskolin in GH1 cells, whereas it increased by more than twofold in the cells treated with the fatty acid. In contrast, butyrate significantly decreased basal levels of adenyl cyclase activity and, as expected, treatment with cholera toxin and forskolin strongly activated the cyclase. The enzyme from butyrate-treated cells retained its susceptibility to become activated by both compounds, but the absolute activity was decreased to half the values found in the cells which were not exposed to the fatty acid.

A similar situation was found when NaF was present in the assay. NaF was an effective stimulator of the cyclase in control cells and in cells incubated with forskolin, whereas after treating the cells with cholera toxin resulted in an inhibition of activity. This has been interpreted as the result of the dual effect of fluoride to activate Gs (stimulatory) and Gi (inhibitory) at the same time [23, 31]. Since, in the cells treated with cholera toxin, Gs is fully activated, the inhibitory effect of NaF becomes apparent. The effect of butyrate was equally visible in the absence and the presence of stimulatory regulation by NaF and, although under all conditions the response in terms of percentage of the respective control remained unaltered, a reduction of cyclizing activity was always observed.

To test whether activation of Gi could be responsible for the inhibitory action of butyrate on the cyclase, the effect of inhibitory hormones was also examined. Carbachol and adenosine are able to decrease cyclase activity in other pituitary cells [23, 32] and they also decreased activity by 40–50% when added to homogenates of GH1 cells. Although absolute activities were again decreased in butyrate-treated membranes as compared with control membranes in all groups, the relative inhibition of activity attainable by activation of the Gi pathway with either carbachol or adenosine remained unaltered from cells incubated with butyrate. This suggests that activation of Gi probably does not play a fundamental role in the inhibitory effect of the fatty acid.

Taken together, our results show that, whereas cyclase activity is decreased in butyrate-treated cells, when analyzed in terms of percentage of control, the effectiveness with which inhibitory or stimulatory effectors of the cyclase G components act is unaffected. This suggests that a decrease in the functional catalytic C subunit and/or a change in its interaction with the regulatory components could be responsible for the observed effect. An alteration in the coupling among the different components of the cyclase would not be unexpected in cells incubated with butyrate, since this compound appears to induce a variety of biochemical changes in the cell membrane [1] and incubation of cultured cells with fatty acids alters membrane lipid composition and, as a consequence, membrane fluidity [6, 7]. In this respect it is interesting to note that butyrate increases the responsiveness of HeLa cells to catecholamines [3–5]. In these cells butyrate induces large increases in β receptor number and qualitative changes in the
regulatory component that facilitate its ability to couple to receptors but do not alter its ability to respond to other non-hormonal effectors [5]. Therefore, these observations and the effect of butyrate in GH1 cells may be the consequence of a direct modification in the cyclase components or may be indirectly caused by changes in other membrane components which in turn modulate cyclase activity.

Pituitary GH1 cells and other related cell lines have been proven to be a very useful model for studying hormonal regulation of somatotropin production. In these cells triiodothyronine regulates somatotropin gene expression [17], and other hormones and factors modulate the response to thyroid hormones [33-36]. Among the physiological compounds that regulate somatotropin, growth-hormone-releasing hormone (GRH) acting through an elevation of intracellular cAMP appears to increase somatotropin synthesis in pituitary cells [15]. We examined the effect of cAMP on somatotropin production by GH1 cells. Incubation with forskolin instead of cholera toxin was chosen, since the toxin decreases thyroid hormone receptor levels, whereas the diterpene does not affect this receptor [18]. Our results show that cAMP by itself does not induce somatotropin production, but potentiates the effect of triiodothyronine. This is similar to that found with glucocorticoids, other important regulators of somatotropin gene expression [33, 34]. Butyrate has been described to influence somatotropin production by cultured pituitary cells [35, 36] and we found that with concentrations of butyrate higher than 1 mM the response to triiodothyronine is significantly decreased. Since butyrate inhibits the cAMP response to forskolin, the fatty acid should also inhibit the somatotropin response caused by forskolin. Our results indicate that the response to forskolin was in fact abolished in the presence of butyrate, thus showing that the decreased cAMP response is reflected in the inhibition of the biological function of the nucleotide in somatotropin-producing cells.

We thank Aida Villa for her expert technical assistance. This work has been supported by grants from the Directrion General de Investigacion Cientifica y Tecnica (PM88-0007) and Fondo de Investigaciones Sanitarias of the Seguridad Social of Spain, and a grant from the Comite Conjunto Hispano-Norteamericano para la Cooperacion Cientifica y Tecnologica (CCB 8409-013).

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