Modulation of the Receptor-Coupled Adenylate Cyclase System in HeLa Cells by Sodium Butyrate

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ABSTRACT: Exposure of HeLa cells to 5 mM sodium butyrate, but not 0.6 mM, resulted in a more efficient coupling between their β-adrenergic receptors and the guanine nucleotide binding stimulatory (N_s) component of adenylate cyclase. Both concentrations of the fatty acid, however, caused an increase in receptor number. β receptors from control and butyrate-treated cells had the same affinity for isoproterenol. Modulation of this affinity by GTP was greatly enhanced, however, in cells treated with 5 mM butyrate compared to untreated and 0.6 mM butyrate treated cells. The concentration of isoproterenol required to half-maximally stimulate adenylate cyclase (K_{act}) was reduced in cells treated with 5 mM butyrate. In addition, the K_{act} for GTP in the presence, but not the absence, of isoproterenol was reduced. The effect of butyrate on the coupling between β receptors and N_s was analyzed in detail by monitoring the activation of N_s by guanine 5'-*O*-(3-thiotriphosphate) (GTPγS) in a two-step assay. In the absence of isoproterenol, N_s from control and 5 mM butyrate treated cells was activated to the same extent with the same time course and K_{act} for GTPγS. In the presence of isoproterenol, N_s from 5 mM butyrate treated cells was activated more rapidly and extensively than N_s from control cells. The K_{act} for both GTPγS and isoproterenol also was reduced. The rate of agonist-mediated activation of N_s was strongly dependent on temperature, which accentuated the differences between 5 mM butyrate treated and control cells. At 4 °C, the difference in rate was 8.8-fold. Although cells treated with 0.6 mM butyrate contained more β receptors than control cells, N_s from the former exhibited the same slow rate of activation with isoproterenol as N_s from the latter cells. Thus, the increased extent of activation of N_s from cells treated with 5 mM butyrate cannot be due to an increase in β receptors. From these results, it is proposed that untreated HeLa cells contain a population of N_s that can form a ternary complex with the receptor and agonist; dissociation of this complex, however, is poorly modulated by guanine nucleotides.

Over the last decade, great progress has been made in identifying the components of the β-adrenergic-responsive adenylate cyclase system. There are at least three separate membrane components: a specific β-adrenergic receptor, a stimulatory regulatory component (N_s)[1] that binds guanine nucleotides, and a catalytic component that converts ATP to cAMP (Rodbell, 1980; Ross & Gilman, 1980; Lefkowitz et al., 1983; Schramm & Selinger, 1984). Optimal stimulation by the β agonist requires efficient coupling not only between receptor and N_s but also between N_s and the catalytic component. Although β receptors (Cerione et al., 1983; Homcy et al., 1983; Cubero & Malborn, 1984) and N_s (Northrup et al., 1980; Hanski et al., 1981; Codina et al., 1984) have been purified, the mechanism by which receptor and N_s interact to stimulate enzyme activity, however, remains unknown.

One approach to investigating the coupling process is to have a system in which the efficiency of coupling can be modulated. HeLa cells have a substantial number of β receptors and an adenylate cyclase that is reponsive to nonhormonal effectors such as guanine nucleotides, NaF, forskolin, and cholera toxin but is poorly stimulated by catecholamines (Tallman et al., 1977, 1978; Henneberry et al., 1977; Lin et al., 1979; Kassis et al., 1984). When the cells are cultured in the presence of 5 mM sodium butyrate, they acquire 3-5-fold more β receptors and become highly responsive to β agonists. In a recent study (Kassis et al., 1984), it was demonstrated that, in addition to inducing a quantitative change in β receptors, 5 mM butyrate induced a qualitative change in N_s. Thus, the increased efficiency of the agonist-stimulated adenylate cyclase was due to an enhanced ability of N_s to interact with the receptors. The present studies were undertaken in order to analyze in detail the nature of the interaction between the receptor and N_s before and after butyrate treatment and explore the consequences of the observed change in N_s activity.

Experimental Procedures

Materials. (-)-Isoproterenol hydrochloride, ATP, GTP, Gpp(NH)p, phosphocreatine, creatine phosphokinase, (±)-propranolol hydrochloride, and sodium butyrate were purchased from Sigma. [α-^{32}P]ATP (25-30 Ci/mmol) was obtained from ICN, Irvine, CA, and New England Nuclear. (-)-([^{125}I]Iodocyano)pindolol (2200 Ci/mmol) was from New England Nuclear. GTPγS was from Boehringer Mannheim.

Cells and Membrane Preparation. HeLa cells, strain 30002 (Tallman et al., 1978), were grown as monolayers in Dulbecco's modified Eagle's medium containing 0.45% glucose, 10% fetal calf serum, and 50 μg/mL gentamicin (Kassis et al., 1984). Cultures for experiments were seeded at a density of (15-25) × 10^4 cells/cm^2 and fed daily. Where indicated, cells were incubated in medium containing sodium butyrate for 20-24 h. The cells were washed twice with ice-cold Dulbecco's phosphate-buffered saline minus Ca^{2+} and Mg^{2+},

[1] Abbreviations: N_s, stimulatory guanine nucleotide binding component of adenylate cyclase; ISO, (-)-isoproterenol; Gpp(NH)p, 5'-guanylyl imidodiphosphate; GTPγS, guanosine 5'-*O*-(3-thiotriphosphate); ICP, (iodocyano)pindolol; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; Hepes, 4-(2-hydroxymethyl)-1-piperazineethanesulfonic acid.

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scraped in the same buffer, and collected by centrifugation (500*g* for 10 min at 4 °C). The cell pellets were suspended in a minimal volume of lysis buffer (Kassis & Fishman, 1982) and kept on ice for 15 min. The cells then were disrupted, and membranes were prepared as described previously (Kassis & Fishman, 1982).

Adenylate Cyclase Assay. Membranes (10–30 μg of protein) were assayed for adenylate cyclase activity as described earlier (Kassis & Fishman, 1982). Unless otherwise indicated, concentrations were as follows: ATP, 0.1 mM; guanine nucleotides, 50 μM; ISO, 10 μM; NaF, 10 mM. Labeled cAMP was isolated from the reaction mixtures by the method of Salomon et al. (1974).

Activation of N_s. N_s was activated by GTPγS in HeLa membranes by a modification of the two-step method described by Citri & Schramm (1982). In the first step, HeLa membranes (50 μg of protein) were incubated with 10 μM GTPγS and 1 μM propranolol or 10 μM ISO in a volume of 90 μL, which contained 2.5 μmol of Tris-HCl (pH 7.6), 0.5 μmol of $MgCl_2$, 50 nmol of ATP, 100 nmol of cAMP, 100 nmol of theophylline, 6 units of creatine kinase, 0.4 μmol of phosphocreatine, 100 nmol of dithiothreitol, and 100 μg of bovine serum albumin. Times and temperatures are indicated in the figure legends as are variations in ISO and GTPγS concentrations. Since preliminary experiments indicated that substantial activation of N_s occurred on ice, the reaction tubes were preincubated at the desired temperature for 2 min, at which time GTPγS was added. The second step measured the catalytic activity of adenylate cyclase resulting from N_s activated in the first step. Incubations were for 10 min at 30 °C and were started by adding 10 μL of a solution containing 10 mM GTP, 100 μM propranolol, 50 mM $MgCl_2$, and 1 μCi of [α-^{32}P]ATP.

Other Methods. Binding of ICP to membranes was determined essentially as described by Maguire et al. (1976). Membranes were incubated at 30 °C for 45 min with ICP and filtered under vacuum on glass-fiber filters. Nonspecific binding was determined in the presence of 10 μM (±)-propranolol and accounted for 5–30% of the total binding. Protein was determined with bovine serum albumin as the standared by the method of Lowry et al. (1951). Intracellular cAMP was extracted from the cells with 0.1 M HCl (Fishman, 1980) and determined by radioimmune assay (Rebois & Fishman, 1983).

Presentation of the Data. Values represent the mean ± standard deviation of triplicate determinations from representative experiments. Each experiment was repeated at least once, and comparable results were obtained. Because of variations in the absolute values among experiments, values from different experiments were not averaged. Where standard deviations are not indicated, they were less than 5% of the mean.

RESULTS

Effect of Butyrate Treatment on β Receptors and Reponsiveness of HeLa Cells to β Agonists. In agreement with previous studies (Kassis et al., 1984), treatment of HeLa cells with 0.6 and 5 mM butyrate resulted in a 2.3- and a 4.2-fold increase in β-receptors, respectively. HeLa cells exposed to 5 mM butyrate for 20 h exhibited an increased responsiveness to ISO compared to untreated cells (Figure 1). The agonist stimulated cAMP production of 30–50-fold whereas less than a 5–8-fold stimulation was observed in control cells. Moreover, when the dose reponse to ISO was examined in control and 5 mM butyrate treated cells, the concentration required for half-maximal stimulation (K_{act}) was shifted 3-fold lower in the latter cells (Figure 1). Values were 1050 ± 140 vs. 340 ± 85 nM (n = 3), respectively. Cells exposed to 0.6 mM butyrate exhibited the same low response to ISO, and the same K_{act} as that observed in control cells (data not shown) even though the numer of β receptors increased.

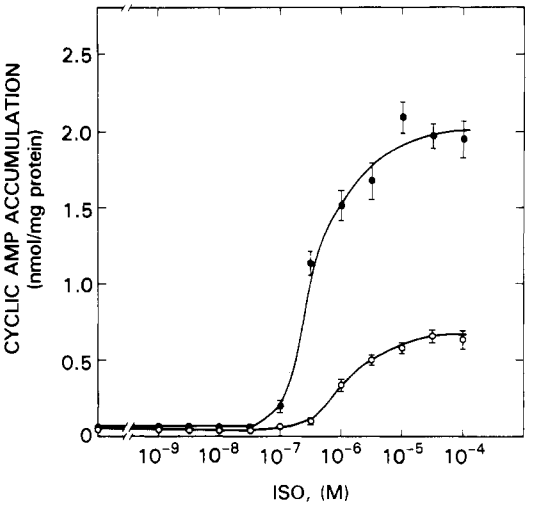

FIGURE 1: Effect of prior treatment of HeLa cells with butyrate on dose response to ISO. Control (○) and 5 mM butyrate treated (●) HeLa cells were incubated in serum-free medium buffered with 25 mM Hepes and containing 1 mM (isobutylmethyl)xanthine at 37 °C for 10 min in the presence of the indicated concentrations of ISO. The cells then were analyzed for intracellular cAMP content and protein as described under Experimental Procedures. Each value represents the mean ± SD of triplicate determinations from a representative experiment.

Table I: Effect of GTP on Affinity of HeLa β Receptors for ISO: Pseudo Hill Coefficients from Competition Binding Curves[a]

butyrate treatment (mM)	pseudo Hill coeff (*nH*)			
	expt I		expt II	
	−GTP	+GTP	−GTP	+GTP
0	0.743	0.528 0.910	0.892	0.708 0.927
0.6	*b*	*b*	0.856	0.608 0.855
5.0	0.655	1.08	0.635	0.996

[a] Hill plots were made from the agonist competition curves described in Figure 2 (experiment II) and from a separate experiment (experiment 1), and pseudo Hill coefficients (*nH*) were extrapolated by linear regression analysis. The correlation coefficients were >0.994 for all of the plots. In the presence of GTP, the plots of data with membrane from control and 0.6 mM butyrate treated cells were biphasic, and both values are given (see text). [b] Not determined.

Effect of Butyrate Treatment on Affinity of β Receptors for Agonist. The affinity for the agonist ISO was determined by its ability to compete with the labeled antagonist ICP for binding to the β receptors (Figure 2). The concentration of ISO required for half-maximal inhibition (IC_{50}) was similar for membranes from HeLa cells treated with 0, 0.6, and 5 mM butyrate being 107, 107, and 92 nM, respectively. When binding was assayed in the presence of 0.1 mM GTP, the affinity for ISO of the β receptors from control and low butyrate treated cells was shifted only 5.9-fold to the right (Figure 2A,B). In contrast, GTP shifted the agonist affinity 19.4-fold after 5 mM butyrate treatment (Figure 2C).

As the competition curves for membranes from control and low butyrate treated cells remained shallow in the presence of GTP, the data were analyzed by Hill plots. In the absence of GTP, linear plots with "pseudo" Hill coefficients (*nH*) of less than unity were obtained with all three membrane preparations (Table I). In the presence of GTP, a linear plot with

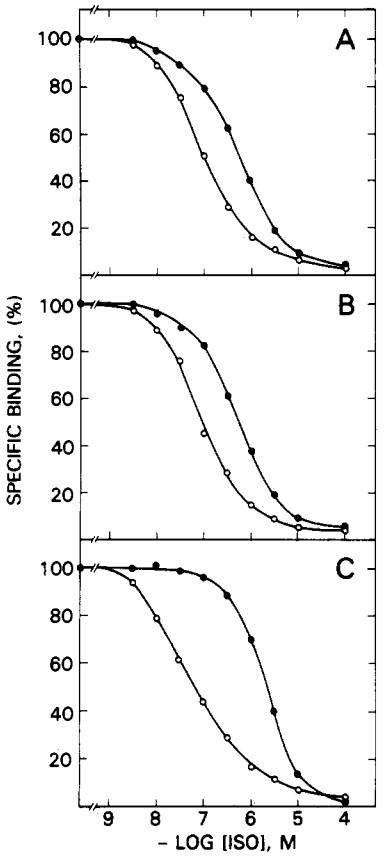

FIGURE 2: Affinity of β receptors for ISO in membranes from HeLa cells treated with (A) 0, (B) 0.6, and (C) 5 mM butyrate. Membranes were incubated with 70 pM [^{125}]ICP in the presence of increasing concentrations of ISO with (●) and without (○) 0.1 mM GTP. Values have been corrected for nonspecific binding and are presented as the percent of specific binding measured in the absence of ISO.

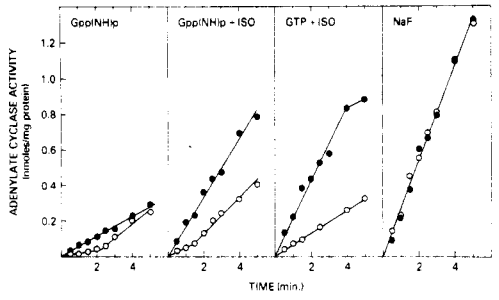

FIGURE 3: Time course of adenylate cyclase activity in membranes from control (○) and 5 mM butyrate treated (●) HeLa cells. Membranes were incubated at 30 °C for the indicated times with either 50 μM Gpp(NH)p, 50 μM Gpp(NH)p plus 10 μM ISO, 50 μM GTP plus 10 μM ISO, or 10 mM NaF, and the amount of accumulated cAMP was determined as described under Experimental Procedures.

an *nH* of unity was obtained with membranes from 5 mM butyrate treated cells. In contrast, the Hill plots of the agonist competition curves for membranes from control and 0.6 mM butyrate treated cells were biphasic with a break in the plot occurring around the IC_{50}. At low concentrations of ISO, the *nH* values were significantly below unity, and at higher concentrations, they approached unity (Table I).

Kinetics of Adenylate Cyclase Activity. In order to further characterize the nature of the β receptor/N_s interaction, the time course of the adenylate cyclase reaction in the presence of various effectors was determined (Figure 3). When the enzyme was assayed in the presence of Gpp(NH)p, membranes from control HeLa cells exhibited a lag of 140 s during which the activation of the enzyme proceeded at a slower rate.

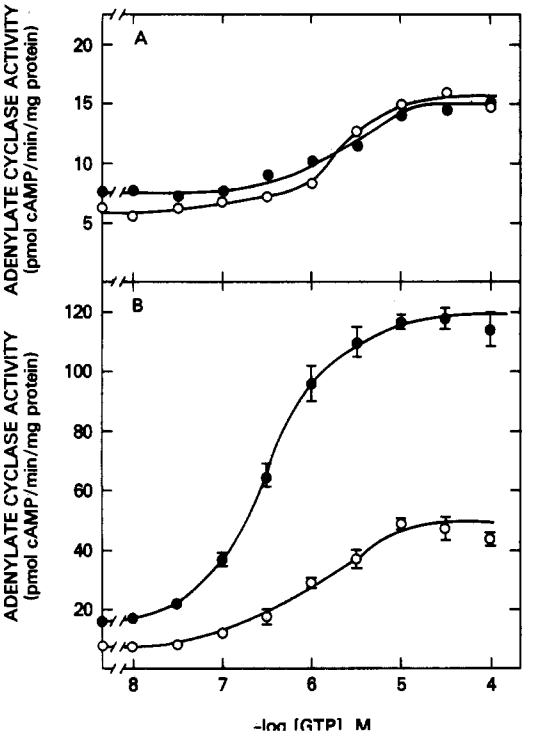

FIGURE 4: Effect of GTP concentration on adenylate cyclase activity in membranes from control (○) and 5 mM butyrate treated (●) HeLa cells. Membranes, incubated with increasing GTP concentrations in the absence (A) and presence (B) of 10 μM ISO, were assayed for adenylate cyclase activity.

Addition of ISO to the reaction mixture reduced the lag to 80 s but did not abolish it. No detectable lag was observed with membranes from cells treated with 5 mM butyrate either in the absence or in the presence of the agonist. When activity was measured in the presence of GTP and ISO, no lag period was detected with either membrane preparation. The rate of activation, however, was several fold faster after 5 mM butyrate treatment. In contrast, 5 mM butyrate treatment had no effect on the response of HeLa adenylate cyclase to nonhormonal effectors such as NaF.

The stimulation of adenylate cyclase by increasing concentrations of GTP in the absence and presence of ISO also was measured (Figure 4). In the absence of agonist, GTP stimulated the enzyme in membranes from control and 5 mM butyrate treated HeLa cells to the same extent and with the same K_{act} (2.0 vs. 1.9 μM). When 10 μM ISO was included in the assay, the K_{act} for GTP was shifted 5.4-fold to the left (to 0.35 μM) with the enzyme from the butyrate-treated cells but was only slightly reduced with the control enzyme (to 1.6 μM).

Kinetics of N_s Activation. In order to directly examine the interation of β receptors with N_s, a two-step assay was employed (Citri & Schramm, 1982). Membranes were incubated with GTPγS to activate N_s under different conditions; then, the membranes were assayed for adenylate cyclase activity in the presence of excess GTP and propranolol, which prevents further activation of N_s. As GTPγS is poorly hydrolyzed and its activation of N_s is not reversed by excess GTP (Citri & Schramm, 1982), the amount of adenylate cyclase activity measured in the second step will be an accurate reflection of the extent of N_s activation during the first step.

At 30 °C in the presence of propranolol (Figure 5), GTPγS activated N_s from control and 5 mM butyrate treated HeLa cells to the same extent and with the same K_{act} (180 vs. 150 nM). When ISO replaced propranolol in the activation step,

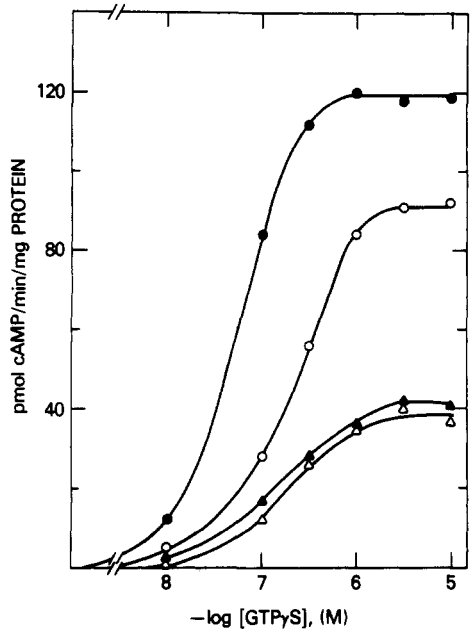

FIGURE 5: Effect of GTPγS concentration on activation of N_s. Membranes from control (○, △) and 5 mM butyrate trated (●, ▲) HeLa cells were incubated at 30 °C for 3 min with the indicated concentrations of GTPγS in the presence of 1 μM propranolol (△, ▲) or 10 μM ISO (○, ●). The sample then were assayed for adenylate cyclase activity to determine activated N_s as described under Experimental Procedures. Values have been corrected for activity obtained in the absence of GTPγS.

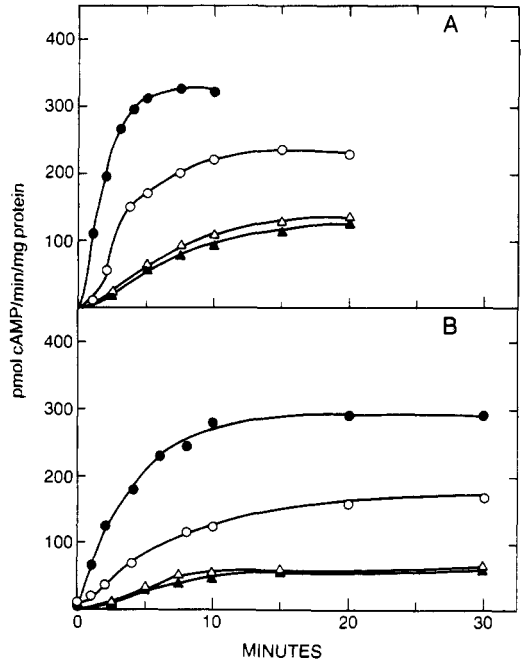

FIGURE 6: Time course of activation of N_s by GTPγS at 30 (A) and 18 °C (B). Details are the same as those in the legend to Figure 5 except the GTPγS concentration was 10 μM and the incubations times were varied as indicated.

the K_{act} for GTPγS was reduced to 53 nM for N_s from the latter cells but remained unchange (215 nM) for N_s from the control cells. In the presence of the agonist, maximal activation of N_s from control cells was significantly less than that of N_s from cells treated with 5 mM butyrate.

The rate of N_s activation was determined at different temperatures by incubating membranes with GTPγS in the presence of either 1 μM propranolol or 10 μM ISO (Figures 6 and 7). The rate of activation is defined as the slope of the

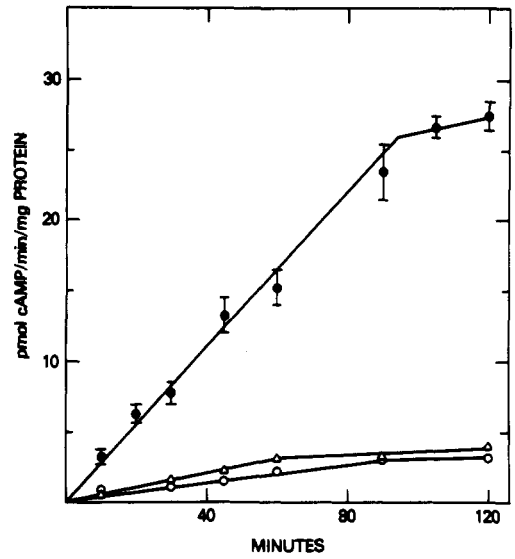

FIGURE 7: Time course of activation of N_s by GTPγS at 4 °C. Membranes from HeLa cells treated with 0 (○), 0.6 (△), and 5 (●) mM butyrate were incubated at 4 °C with 10 μM GTPγS and 10 μM ISO for the indicated times and assayed for activation of N_s.

linear portion of the curves and is expressed as units per minute of activation. One unit represents 1 pmol of cAMP produced per minute per milligram of protein in the adenylate cyclase assay (second step). When N_s was activated at 30 °C by GTPγS in the presence of propranolol (Figure 6a), the rate (18 vs. 16 units/min) and extent of activation were the same for N_s from control and 5 mM butyrate treated cells. In the presence of agonist, N_s from the former cells was activated at a slower rate than N_s from the latter cells (46 vs. 96 units/min) and to a lesser extent (72%). These differences were accentuated at lower temperatures where the rates of activation were slowed down. At 18 °C in the presence of antagonist, the rate of N_s activation in both types of membranes was the same (10 units/min) as was the extent of activation (Figure 6B). In the presence of agonist, the rates were 16 and 58 units/min before and after 5 mM butyrate treatment, respectively; and, the extent of activation of N_s from control cells was only 55% of that observed for N_s from cells exposed to 5 mM butyrate. An extreme case was obtained at 4 °C in the presence of agonist (Figure 7). While N_s from 5 mM butyrate treated cells was activated substantially (0.28 unit/min), very little activation of N_s from control cells occurred (0.034 unit/min). The 8.2-fold faster rate of activation was reflected in the 8.6-fold higher extent of activation by 2 h of incubation at 4 °C.

To test the possibility that these differences in N_s activation simply reflect differences in receptor densities between control and 5 mM butyrate treated HeLa cells, two approaches were used. The first approach utilized membranes from HeLa cells treated with 0.6 mM butyrate. Although these membranes contain 2.5-fold more β receptors than control membranes, they exhibited the same slow rate of N_s activation in the presence of ISO as control membranes at 4 °C (Figure 7) and at 18 °C (data not shown).

The second approach was to activate HeLa N_s with a saturating concentration of GTPγS and different concentrations of agonist. Control membranes were incubated with a saturating concentration of ISO (10 μM) whereas membranes from 5 mM butyrate treated cells were incubated with a subsaturating concentration (0.7 μM). On the basis of the data in Figure 2C, a K_d for ISO of 1.02 μM in the presence of guanine nucleotides was calculated.[2] Thus, only 41% of

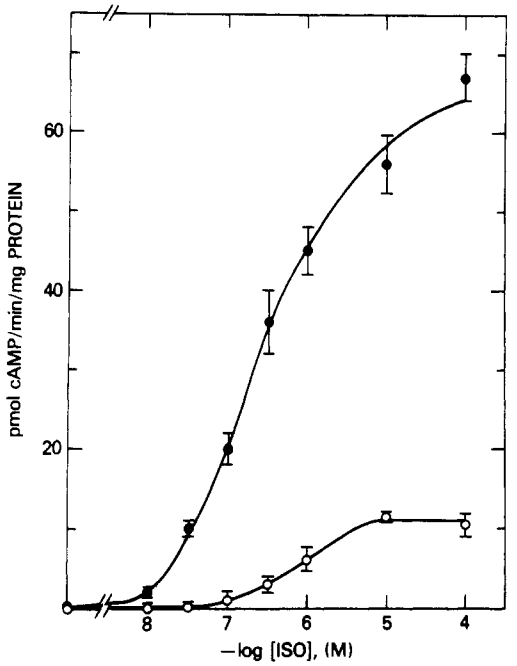

FIGURE 8: Effect of ISO concentration on activation of N_s. Membranes from control (○) and 5 mM butyrate treated (●) HeLa cells were incubated at 4 °C for 40 min with 10 μM GTPγS and the indicated concentrations of ISO. The samples then were assayed for activated N_s. Values represent the mean ± SD of triplicate determinations and have been corrected for activities obtained for samples incubated with GTPγS plus propanolol during the first step of the assay.

the receptors would be occupied at this concentration of agonist. The rate of activation of N_s at 18 and 4 °C was 2.8- and 7.9-fold faster, respectively, in membranes from 5 mM butyrate treated cells than in control membranes under these conditions. The K_d for ISO measured under competition binding conditions with GTP may not reflect the true affinity of the receptor for its agonist under conditions used for activating N_s. Therefore, the effect of agonist concentration on the rate of activation of N_s by GTPγS at 4 °C was determined (Figure 8). The membranes were incubated with GTPγS and agonist for 40 min, during which time activation of N_s was linear (Figure 7). The K_{act} for ISO was 0.23 μM for N_s from 5 mM butyrate treated cells compared to 1.03 for N_s from control cells; these values are similar to those required to stimulate cAMP production in intact cells (Figure 1). It is clear from the data in Figure 8 that, at saturating concentrations of ISO, N_s from the butyrate-treated cells was activated at a faster rate than can be accounted for by the differences in receptor number. At a suboptimum concentration (0.1 μM) that should occupy less than a third of the receptors in membranes from 5 mM butyrate-treated cells,[3] the activation of N_s was still greater than that observed in control membtanes at saturating agonist concentrations.

Although the rate of N_s activation is reduced at subsaturating concentrations of ISO, the extent of activation is not. Activation of N_s in membranes from 5 mM butyrate treated cells was determined at 18 °C with 0.1 and 10 μM ISO. The respective rates were 8.8 and 17.4 units/min whereas the maximum extents of activation were 200 ± 37 and 220 ± 22 pmol min^{-1} (mg of protein)$^{-1}$.

Discussion

In a previous study, it was demonstrated that treatment of HeLa cells with 5 mM butyrate caused quantitative changes in β receptors and qualitative changes in N_s, which improved its ability to interact with the receptors but not with the catalytic component of adenylate cyclase (Kassis et al., 1984). On the basis of the various experiments in the present study, the coupling efficiency between N_s and agonist-occupied β receptors is extremely high in HeLa cells treated with 5 mM butyrate compared to untreated cells as is the modulation by guanine nucleotides. Thus, the K_{act} for ISO was reduced 3-fold, and the K_{act} for GTP in the presence of ISO was reduced 5.4-fold after butyrate treatment. In the absence of ISO, there was little difference in the K_{act} for GTP between adenylate cyclase from control and butyrate-treated cells.

There was a substantial lag in the rate of activation of the control enzyme by Gpp(NH)p, which was reduced but not eliminated by the agonist. In contrast, there was no lag for Gpp(NH)p activation of the enzyme from 5 mM butyrate treated cells. Such lags in Gpp(NH)p activation have been observed in other adenylate cyclase systems and usually are reduced by addition of hormone (Londos et al., 1974; Salomon et al., 1975; Rodbell, 1975; Levitzki et al., 1976). It has been proposed that the lag represents the slow exchange of GDP bound to N_s for Gpp(NH)p, which is accelerated by hormone-occupied receptor (Cassel & Selinger, 1978).

The interaction between N_s and β receptors also was determined by competition binding experiments. In the absence of guanine nucleotides, the affinity of the β receptor for ISO was the same in membranes from control and 0.6 and 5 mM butyrate treated cells. In the presence of GTP, agonist affinity was reduced almost 20-fold in the latter, and the pseudo-Hill coefficient became unity. This degree of modulation is characteristic of highly coupled systems (Ross et al., 1977; De Lean et al., 1980). In contrast, the affinity of β receptors in membranes from control and low butyrate treated cells for the agonist was poorly modulated by GTP and reduced less than 6-fold. Even more unusual was the fact that the Hill plots became biphasic, which is suggestive of two populations of binding sites. It is believed that the high-affinity state of the receptor for the agonist represents a receptor/N_s complex (De Lean et al., 1980; Schramm & Selinger, 1984). In the presence of guanine nucleotides, the complex dissociates to form the low-affinity state of the receptor. If this theory is correct, then only a part of β receptor/N_s complexes in control membranes are dissociating in the presence of GTP to yield receptors with a low affinity for the agonist. The rest remain as complexes with a high affinity for the agonist.

By use of a two-step assay, the interaction between β receptors and N_s was examined directly. In the first step, N_s was activated by GTPγS, a poorly hydrolyzable analogue of GTP, and agonist. In the second step, the activity of adenylate cyclase was determined under conditions where further activation (or deactivation) of N_s is prevented (excess antagonist and GTP). When an antagonist is substituted in the first step, the activation of N_s without receptor participation can be determined. Under these latter conditions, the rate and extent of activation of N_s by GTPγS as well as the concentration dependence of the latter were identical in membranes from control and 5 mM butyrate treated cells. This confirms previous work that the amount of N_s and its ability to activate the catalytic component of adenylate cyclase remain unchanged after HeLa cells are exposed to 5 mM butyrate (Kassis et al., 1984).

[2] The K_d value for ISO was calculated from the equation $K_d = IC_{50}/(1 + [ICP]/K_{ICP})$, where K_{ICP} is the affinity for ICP (94 pM), [ICP] is the concentration of ICP (70 pM), and IC_{50} is the concentration of ISO required for 50% inhibition of ICP binding (1.78 μM from Figure 2C).

[3] Assuming that the K_{act} calculated from Figure 8 represents the true affinity for ISO under these conditions.

When an agonist was included in the activation step, N_s from cells treated with 5 mM butyrate was activated faster and to a greater extent than control N_s. Furthermore, the K_{act} of N_s for GTPγS was 4.1-fold lower after butyrate treatment, which is in agreement with the reduction of the K_{act} for GTP as measured in the adenylate cyclase assay. Similarly, the K_{act} for ISO was 4.5-fold lower. These differences in agonist-mediated activation of N_s from control and 5 mM butyrate treated cells cannot be due to differences in receptor number. N_s from cells treated with 0.6 mM butyrate was activated at a similar rate and to the same extent as control N_s even though the former cells contained 2.5-fold more β receptors than the latter cells. When the agonist concentrations were adjusted to have similar numbers of occupied receptors in membranes from control and 5 mM butyrate treated cells, the rates and extents of activation of N_s from the latter cells were still higher than those of N_s from control cells.

It is believed that the receptor acts as a catalyst to activate N_s and thus adenylate cyclase (Schramm, 1976; Tolkovsky & Levitzki, 1978; Citri & Schramm, 1980). When a poorly hydrolyzable analogue of GTP is used, eventually all of the N_s will be activated even when the number of receptors or concentration of agonist is limiting. This was confirmed by showing that N_s from cells treated with 5 mM butyrate was activated at a slower rate but eventually to the same extent under subsaturating and saturating concentrations of agonist. Thus, the difference in the extent of activation of N_s from control and 5 mM butyrate treated HeLa cells in the presence of agonist, but not antagonist, is an anomaly.

It has been proposed that, in the presence of agonist, an agonist/receptor/N_s ternary complex is formed (De Lean et al., 1980; Limbird et al., 1980; Korner et al., 1982; Schramm & Selinger, 1984). When GTP (or in this case GTPγS) is added, the complex dissociates and an activated N_s is produced, which in turn can activate C. To explain the anomalous behavior of the control N_s, I propose that although the ternary complex can form, not all of it dissociates in the presence of the guanine nucleotide. Thus, only a part of the N_s becomes activated in the presence of agonist. This explanation is consistent with the unusual competition binding data.

There are several possibilities to explain the inability of the ternary complex in control HeLa cells to dissociate and generate an activated N_s. Schramm and co-workers [see Schramm & Selinger (1984)] have proposed that during formation of the ternary complex both β receptor and N_s undergo conformational changes, the high-affinity state of the receptor for agonist is formed, and the guanine nucleotide binding site on N_s is exposed. Upon binding of the nucleotide, the complex dissociates to form activated N_s and the low-affinity state of the receptor. In control HeLa cells, N_s may not undergo this conformational change and thus not be able to bind GTP (or GTPγS) in the presence of agonist. The receptor will remain in its high-affinity state, and N_s will not be activated. Alternatively, N_s may bind the nucleotide but fail to undergo the approriate conformational change required for dissociation of the ternary complex. Under these conditions, N_s will be unable to productively interact with C.

One further unusual observation was the strong differential effect of temperature on agonist-mediated activation of N_s from control and 5 mM butyrate treated HeLa cells. Lower temperatures limited the activation of N_s from control cells much more than that of N_s from the butyrate-treated cells. Whereas the rates differed by 2-fold at 30 °C, the differences increased to 4-fold at 18 °C and over 8-fold at 4 °C. There was a corresponding differential reduction in the extent of activation of N_s. On the basis of these results, it would appear that the modulation of the receptor/N_s complex by guanine nucleotides in control HeLa cells is more sensitive to temperature than that in butyrate-treated cells.

From other studies [see Schramm & Selinger (1984)], membrane lipids and membrane fluidity appear to have an important role in the β-adrenergic-sensitive adenylate cyclase. In the turkey erythrocyte, stimulation of adenylate cycylase by isoproterenol, but not by NaF, is enhanced at low temperatures by treating the membranes with long-chain unsaturated fatty acids such as *cis*-vaccenic acid, which increase membrane fluidity (Orly & Schramm, 1976; Rimon et al., 1978, 1980). Treating the turkey erythrocyte membranes with butyrate, however, had no effect (Orly & Schramm, 1976). Although the mode of action of sodium butyrate is still unknown, it is important to point out that in HeLa cells butyrate acts as an inducer and its effects on β-receptor number and coupling to N_s require protein and RNA synthesis (Tallman et al., 1977; Henneberry et al., 1977). Thus, the effects of butyrate described here or in previous studies are not due to the direct incorporation of butyrate into the plasma membrane.

The ability to increase the coupling efficiency between receptors and N_s by treating HeLa cells with 5 mM butyrate may prove useful for elucidating the coupling process. From the present studies, its appears that N_s in control HeLa cells can interact with the β receptor. This interaction, however, does not lead to an efficient activation of N_s because modulation of the interaction by guanine nucleotides is impaired. In this regard, control HeLa cells are distinct from variants of S49 lymphoma cells (Bourne et al., 1981). These include UNC in which N_s can interact with the catalytic component but not with the receptor and Ha21 in which N_s can interact with the receptor but not with catalytic component. In Ha21 membranes, the affinity of the β receptors for agonist is modulated by guanine nucleotides (Bourne et al., 1981). Thus, HeLa cells may provide a unique model for investigating the mechanism of activation of adenylate cyclase by hormones.

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Registry No. GTP, 86-01-1; GTPγS, 37589-80-3; Gpp(NH)p, 34273-04-6; $CH_3(CH_2)_2CO_2H$, 107-92-6; adenylate cyclase, 9012-42-4; isoproterenol, 7683-59-2.

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Cloning, Expression, and Purification of Gene 3 Endonuclease from Bacteriophage T7†

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ABSTRACT: The structural gene for the single-stranded endonuclease coded for by gene 3 of bacteriophage T7 has been cloned in pGW7, a derivative of the plasmid pBR322, which contains the λ P_L promoter and the gene for the temperature-sensitive λ repressor, cI857. The complete gene 3 DNA sequence has been placed downstream of the P_L promoter, and the endonuclease is overproduced by temperature induction at mid-log phase of *Escherichia coli* carrying the recombinant plasmid pTP2. Despite the fact that cell growth rapidly declines due to toxic effects of the excess endonuclease, significant amounts of the enzyme can be isolated in nearly homogeneous form from the induced cells. An assay of nuclease activity has been devised using gel electrophoresis of the product DNA fragments from DNA substrates. These assays show the enzyme to have an absolute requirement for Mg(II) (10 mM), a broad pH optimum near pH 7, but significant activity from pH 3 to pH 9, and a 10-100-fold preference for single-stranded DNA (ssDNA). The enzyme is readily inactivated by ethylenediaminetetraacetic acid or high salt. The differential activity in favor of ssDNA can be exploited to map small single-stranded regions in double-stranded DNAs as shown by cleavage of the melted region of an open complex of T7 RNA polymerase and its promoter.

An endonuclease coded for by gene 3 of bacteriophage T7 has been implicated in the degradation of host DNA which occurs after T7 phage infection (Center et al., 1970), in DNA maturation (Paetkau et al., 1977), and in genetic recombination (Powling & Knippers, 1974; Kerr & Sadowski, 1975; Lee et al., 1976; Lee & Sadowski, 1981). The gene 3 enzyme can catalyze an endonucleolytic cleavage of both single- and double-stranded DNA (Center & Richardson, 1970a,b; Sadowski, 1971). The rate of hydrolysis of single-stranded DNA has been reported to be at least 100 times greater than that of duplex DNA (Center et al., 1970). The enzyme introduces

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