Stimulation of Synthesis and Secretion of Chorionic Gonadotropin Subunits by Eutopic and Ectopic Hormone-producing Human Cell Lines

Raymond W. Ruddon, Carmen Anderson, and Kimberly S. Meade-Cobun

ABSTRACT

The effects of dibutyryl cyclic adenosine 3'3':5'-monophosphate (AMP) and sodium butyrate on the synthesis and secretion of human chorionic gonadotropin (HCG) by trophoblastic and nontrophoblastic human cell lines were studied by radioimmunoassay and pulse-chase labeling techniques. Dibutyryl cyclic AMP stimulated synthesis and secretion of HCG-α and HCG-β subunits by the trophoblastic cell lines JAR and BeWo, whereas butyrate had no effect or decreased secretion. On the other hand, a number of nontrophoblastic cell lines (including the breast carcinoma lines ZR-75-31, BT-20, and MCF-7; the bronchogenic carcinoma line ChaGo; and the cervical carcinoma line HeLa S3) were induced to synthesize and secrete increased amounts of HCG subunits by butyrate, but dibutyryl cyclic AMP had less or no stimulatory effect. The nontrophoblastic brain tumor line CBT was an exception to this general rule in that HCG-β production was stimulated by dibutyryl cyclic AMP but not by butyrate. In all cases, the drug-induced increase in HCG subunit secretion was directly proportional to the elevation of HCG subunit synthesis. These data suggest that the differential effects of dibutyryl cyclic AMP and butyrate on trophoblastic and nontrophoblastic cells reflect differences in the transcription or translation of HCG subunit genes induced by these agents in the two cell types.

INTRODUCTION

One or both of the subunits of the placental glycoprotein hormone HCG are synthesized and secreted by a number of human malignant neoplastic cells in vivo and in vitro (1, 14, 15, 20, 21). The "eutopic" production of HCG subunits by trophoblastic malignant cells and the "ectopic" production by nontrophoblastic cells have been reported to be differentially affected by pharmacological agents such as dbcAMP and sodium butyrate. For example, dbcAMP induces the eutopic secretion of HCG subunits by BeWo, JAR, and JEG-3 choriocarcinoma cell lines, whereas exposure of these cells to sodium butyrate has no effect or inhibits secretion (1, 2, 4, 8, 9). On the other hand, butyrate induces the ectopic secretion of one or both HCG subunits in HeLa (3, 6, 11, 15), ChaGo bronchogenic carcinoma (4, 19), EICo breast carcinoma (8), CaSki cervical carcinoma (8), DoT cervical carcinoma (8), and line 163 ovarian carcinoma cells (10), but dbcAMP produces little or no stimulation of HCG secretion by ectopic HCG-producing cell lines (8). These data have led to speculation that the control of HCG secretion differs between trophoblastic and nontrophoblastic cell types (4, 8).

These previous studies did not determine whether HCG subunit synthesis is also stimulated by agents that induce HCG secretion, nor did they suggest what biochemical mechanism might explain the differences between eutopic and ectopic production of HCG subunits. We have found that the intracellular precursor forms of HCG subunits are similar in eutopic and ectopic HCG-producing cell lines (16, 18). There is an apparent difference, however, between eutopic and ectopic α subunit-producing lines in the kinetics of secretion. The rate of secretion of the α subunit by ChaGo bronchogenic carcinoma cells is somewhat slower than is the rate of secretion by JAR choriocarcinoma cells as observed by pulse-chase labeling techniques (18).

If indeed the control of synthesis and/or secretion of HCG subunits is different between trophoblastic and nontrophoblastic human cancer cells, important theoretical and practical questions would arise. For example, are there differences between the 2 cell types in the transcription or translation of HCG subunit genes or in the processing of the subunits? If so, do these differences reflect changes that occur in gene expression during the malignant transformation of cells? Could these differences be exploited to improve the clinical usefulness of HCG subunits as markers for human cancer? It is clear that much more needs to be learned about the processes controlling the synthesis and secretion of HCG subunits. In this paper, we address the questions of whether there is induction of both HCG subunit synthesis and secretion in human cell lines by dbcAMP and sodium butyrate and whether these agents can be used as probes to detect differences between eutopic and ectopic HCG production.

MATERIALS AND METHODS

The source, characterization, and growth conditions of the cell lines used in this study have been reported previously (12, 15). Cells were grown as monolayer cultures in 60- or 100-mm-diameter Petri dishes. Fresh control medium or medium containing 10 mM sodium butyrate or 1 mM dbcAMP was added to cultures in the mid-log stage of growth. These drug concentrations were shown by previous dose-response studies with some of the cell lines to stimulate subunit secretion without being toxic to the cells (13, 15). However, both dbcAMP and butyrate slowed cell division at the concentrations used in

Received April 7, 1980; accepted August 26, 1980.

1 Research supported by the National Cancer Institute under Contract N01-CO-75380 with Litton Bionetics, Inc.
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: HCG, human chorionic gonadotropin; dbcAMP, dibutyryl cyclic adenosine 3'3':5'-monophosphate; RIA, radioimmunoassay; PBS, phosphate-buffered saline (0.01 M sodium phosphate, 0.14 M NaCl [pH 7.2]); SDS, sodium dodecyl sulfate.

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these experiments (13, 15). The BeWo cell line appeared to be more sensitive to the cytotoxic effects of butyrate than to that of the other cell lines studied, in that concentrations of butyrate (>10 mM) produced detachment of cells from the culture plate. This cytotoxic effect could explain, at least in part, the inhibitory effects of butyrate on HCG subunit synthesis and secretion by BeWo cells. Fresh medium with or without drugs was added at 0 time and 24 hr later. Forty-eight hr after the initial addition of control or drug-containing medium, the medium was collected and analyzed by RIA with antisera specific for the α and β subunits of HCG as described previously (15). The cells were washed with PBS, scraped from the plate with a rubber policeman into PBS, and lysed by sonication for 20 sec. Aliquots of the cell lysates were taken for protein assay and RIA of HCG-α and HCG-β subunits. The RIA for HCG-α was specific for free α subunit; cross-reactivity with HCG-β at B/B0 = 50% [the ratio of labeled standard antigen bound by antiserum in the presence of antigen-containing sample to labeled standard antigen bound by antiserum in the absence of antigen-containing sample (nonspecific binding subtracted from both values)] was < 0.3% and with complete HCG was < 3%. The HCG-β antiserum did not completely distinguish between the β subunit and complete HCG, but its cross-reactivity with the α subunit was < 0.6%. The limit of sensitivity for both the HCG-α and HCG-β assays was 0.5 ng/ml. All assays were run in duplicate, and dilution curves of the medium and cell lysates paralleled those of the standard ligands (15). RIA data were normalized for cellular protein. None of the serum-containing growth media used for the various cell lines reacted in the RIA’s.

Replicate plates from the same passage of each cell line were treated as described above and, 48 hr after initiation of drug treatment, were washed with methionine-free medium, incubated for 20 to 30 min in methionine-free medium, and pulsed for 1 hr with 100 μCi of [35S]methionine per ml (500 to 900 Ci/mmol; New England Nuclear, Boston, Mass.). Some plates were then washed 3 times in complete medium after the 1-hr pulse and ‘‘chased’’ for 4 hr in complete medium without [35S]methionine. Both the medium and the cells were collected for immunoprecipitation. Cells were washed and lysed in PBS containing 1.0% Triton X-100, 0.5% sodium deoxycholate, and 0.1% SDS (lysis buffer) by shearing with a 21-gauge needle. Culture medium was brought to the same concentration of lysis buffer by addition of a concentrated solution. Cell lysates and media were stored at −70° until analyzed and were clarified at 100,000 x g for 1 hr at 4°prior to immunoprecipitation. There were no significant differences in the total trichloroacetic acid-precipitable radioactivity before immunoprecipitation between the samples from control, butyrate-treated, or dbcAMP-treated samples, indicating that the effects of the drug treatments on total protein synthesis were minimal.

Detection of radioactively labeled, HCG-specific polypeptides in cell lysates and media was performed by immunoprecipitation with rabbit antisera directed against complete HCG, HCG-α, the COOH-terminal 15 amino acids of HCG-β, or nonimmune serum and by SDS-polyacrylamide gel electrophoresis followed by fluorography as described previously (16, 18).

RESULTS

Human cell lines known to produce HCG subunits eutopically or ectopically (15) were exposed to dbcAMP and sodium butyrate at concentrations known to stimulate secretion of subunits from a variety of cell types (Table 1). Both cells and growth media were examined by RIA to determine effects of the drugs on synthesis and secretion. As expected, the 2 choriocarcinoma lines, JAR and BeWo, contained and secreted both subunits, although BeWo contained more of the free α subunit relative to the β subunit than did JAR. Treatment with dbcAMP stimulated both synthesis and secretion of the α and β subunits by these cells; however, exposure to butyrate had little effect on α or β subunit synthesis and inhibited their secretion. Pulse-chase experiments corroborated the RIA data (Figs. 1 and 2). In the case of both JAR and BeWo cells, treatment with dbcAMP induced the synthesis of the M.W. 18,000 and 15,000 intracellular precursors of the α subunit and the M.W. 24,000 and 18,000 precursors of the β subunit that were reported previously to be present in α and β subunit-producing cells, respectively (16, 18). The dbcAMP-induced stimulation of secretion of the α and β subunits by these cells was also evident by analysis of the 4-hr chase medium. It should be noted that the bands representing the β subunits appear fainter than do the α subunit bands on some of these gels because there is one methionine in the β subunit and there are 3 methionines in the α subunit. The anti-COOH-terminal β subunit antiserum immunoprecipitated 2 bands from the medium of both choriocarcinoma lines (Figs. 1 and 2, Lane 11). The higher-molecular-weight form migrated identically to the placental β subunit and contained sialic acid. The lower-molecular-weight form appeared to contain less sialic acid as determined by neuraminidase digestion (17) and migrated somewhat faster than the free α subunit secreted by these cells. Dean et al. (5) have recently reported a similar observation for JEG-3 choriocarcinoma cells, and they suggest that the lower-molecular-weight band is the α subunit that is immunoprecipitated as part of complete HCG and perhaps has less carbohydrate than does the secreted free α subunit. This point remains to be clarified.

A number of breast carcinoma lines previously shown to secrete the α subunit ectopically (15) were also incubated in the presence of the inducing agents. As indicated in Table 1, butyrate induced the synthesis and secretion of the α subunit by the breast carcinoma lines ZR-75-31, BT-20, and MCF-7. A small amount of β subunit secretion was also noted in butyrate-treated BT-20 cultures. In the case of ZR-75-31 and BT-20, butyrate increased α subunit synthesis and secretion about 100-fold. Treatment of ZR-75-31 cells with dbcAMP also increased α subunit synthesis and secretion but only about 10-fold. Again, the pulse-chase data supported the RIA data and showed that both synthesis and secretion of the α subunit were stimulated in these cells (Figs. 3 to 5). The intracellular precursor forms of the α subunit and the secreted form that migrated like the placental α subunit were observed with these cells as for other α subunit producers (16, 18).

Two other ectopic α subunit-producing lines, ChaGo and HeLa, were also stimulated to synthesize and secrete more of the α subunit after 48 hr of exposure to butyrate (Table 1). In the case of HeLa, the induction of α subunit synthesis and secretion by butyrate was 50- and 20-fold, respectively. Treatment of HeLa cells with dbcAMP produced a 3- to 4-fold increase in α subunit synthesis and secretion. Butyrate induced α subunit synthesis and secretion about 3-fold in ChaGo cells. Pulse-chase data for ChaGo is shown in Fig. 6. The pulse-
Stimulation of HCG Synthesis and Secretion

Table 1
Induction of HCG subunit production in human cell lines by dbcAMP or sodium butyrate

Cells were treated with 1 mM dbcAMP or 10 mM sodium butyrate for 48 hr prior to harvesting. The concentration of HCG subunits was determined in cell lysates and media by RIA as described in "Materials and Methods." Values are expressed as ng/mg cellular protein for both the cell lysates and media. The medium values represent the amount secreted during the second 24-hr period of drug treatment.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tissue of origin</th>
<th>Fraction</th>
<th>Control</th>
<th>dbcAMP</th>
<th>Sodium butyrate</th>
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<tr>
<td>JAR</td>
<td>Choriocarcinoma</td>
<td>Cells</td>
<td>26</td>
<td>23</td>
<td>24</td>
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<td></td>
<td></td>
<td>Medium</td>
<td>110</td>
<td>41</td>
<td>93</td>
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<td>20</td>
<td>1.5</td>
<td>15</td>
</tr>
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<td></td>
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<td>1.3</td>
<td>59</td>
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<td>Cells</td>
<td>3</td>
<td>ND</td>
<td>347</td>
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<tr>
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<td></td>
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<td>ND</td>
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<tr>
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<td>ND</td>
<td>143</td>
</tr>
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<td></td>
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<td>12</td>
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<td>1714</td>
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<td>Breast carcinoma</td>
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<td>ND</td>
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<td>ND</td>
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<td>Cells</td>
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<td>Cervical carcinoma</td>
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Table 1 notes:
- ND, not detectable; <0.1 ng/mg.
- _, values not determined in this experiment. Previous experiments showed the HCG-β values to be the same or less than values for the control, untreated cultures; for example, in another experiment, the intracellular values were 71 ng HCG-β per mg cellular protein for the control and 45 ng/mg for the butyrate-treated cultures.

The data also indicate that the intracellular precursor forms whose synthesis is stimulated by dbcAMP or butyrate are identical by SDS-polyacrylamide gel electrophoresis to those identified previously in α or β subunit-producing cells (16, 18).

DISCUSSION

The results of these experiments show that the agents dbcAMP and sodium butyrate can induce HCG subunit synthesis and secretion in a variety of human cancer cell types. A number of previous reports indicated that one or the other of these agents could stimulate HCG secretion by human cells (1-4, 6, 8-11, 15, 19). Our experiments show that the increased secretion can be accounted for by increased synthesis of the subunits. For example, based on the intracellular and secreted levels of the subunits (Table 1), in JAR cells, dbcAMP produces 1.6- and 1.5-fold increases in α subunit synthesis and secretion, respectively, as well as 3.3- and 3.4-fold increases in β subunit synthesis and secretion, respectively. Sodium butyrate induces α subunit synthesis 115-fold and secretion 85-fold in ZR-75-31 breast carcinoma cells. In ChaGo cells, butyrate increases α subunit synthesis 2.9-fold and secretion 2.5-fold, and in HeLa cells, 54- and 20-fold increases in α subunit synthesis and secretion, respectively, are seen after butyrate treatment. In CBT cells, dbcAMP induces β subunit synthesis 1.8-fold and secretion 1.8-fold. Of course, one could argue that the increased intracellular content of the subunits noted by RIA after treatment with inducing agents is due to decreased degradation rather than to increased synthesis. However, the pulse-chase experiments clearly show that there is increased incorporation of [35S]methionine during a 1-hr pulse period. Moreover, we have shown previously that butyrate-induced stimulation of α and β subunit secretion by HeLa cells is blocked completely by treatment of the cells with actinomycin D or cycloheximide (15).
beled amino acid into the precursor forms is consistent with the idea that de novo synthesis is stimulated by the inducing drugs, and the fact that the observed increased secretion is directly proportional to augmented synthesis indicates that the drugs are not merely acting on some posttranslational processing step leading to increased subunit secretion.

In general, our data support the previous observations regarding the differences between trophoblastic and nontrophoblastic cells in their susceptibility to stimulation by butyrate and dbcAMP (1, 2, 4, 8). With few exceptions, the nontrophoblastic cells are induced to synthesize and secrete HCG subunits by butyrate more than they are by dbcAMP, whereas the reverse is true for trophoblastic cells. In some cases, dbcAMP also induces subunit production by nontrophoblastic cells (e.g., ZR-75-31 and HeLa) but usually to a much lesser extent than does butyrate. A small stimulation (<2-fold) of subunit production in some nontrophoblastic cell lines by dbcAMP was also observed by Hussa et al. (8). The clearest exception to the rule is the CBT cell line derived from a glioblastoma multiforme. HCG-β production is stimulated by dbcAMP but not by butyrate. This has also been observed by Rosen et al. (13) for the CBT line.

The biochemical explanation for the differential effects of butyrate and dbcAMP on HCG subunit production by trophoblastic and nontrophoblastic cell lines is not yet apparent. We have observed previously that the kinetics of a subunit secretion by the trophoblastic cell line JAR differs from that of the nontrophoblastic lines ChaGo and HeLa (17, 18). Very little of the mature, fully glycosylated α subunit accumulates in JAR cells during pulse-chase experiments; however, the mature α subunit appears in the chase medium by 1 hr after initiation of chase. In ChaGo cells, the mature α subunit is more clearly detectable intracellularly, and the mature α subunit does not accumulate in the chase medium in detectable amounts until about 2 hr after initiation of the chase (17). Thus, the control of secretion may be different in trophoblastic and nontrophoblastic cells. Hussa (7) has suggested that exocytosis is not the mechanism by which HCG subunits are secreted from trophoblastic cells. Our data indicate that the mature subunits do not accumulate in trophoblastic cells and, by implication, that they are not stored in secretory vesicles in these cells, suggesting that the concentration and storage steps of the classical pathway for protein secretion are abbreviated or omitted in this instance. If the mechanisms of secretion are different between trophoblastic and nontrophoblastic cells, this could perhaps partially explain the differences in response to dbcAMP and butyrate. However, for the cells utilized in this study, increased synthesis occurs in every case where increased secretion is seen. Presumably, then, the drugs have differential effects on gene transcription and/or translation in these types of cells. Nucleic acid hybridization studies utilizing complementary DNA probes reflecting the sequences of the HCG-α and HCG-β genes should help resolve these questions.

ACKNOWLEDGMENTS

The authors thank Helen Beck and Jo Ann Tichnell for their help in preparation of the manuscript.

REFERENCES

Fig. 1. Pulse-chase labeling of JAR cells: effects of dbcAMP on HCG-α and HCG-β production. Cells were grown to mid-log stage and then incubated with fresh control medium or medium containing 1 mM dbcAMP for 48 hr prior to labeling. Fresh medium with or without drug was added at 0 time and 24 hr later. Medium was then removed, and the cells were starved for 20 min in methionine-free medium, pulsed for 1 hr with 100 μCi [35S]methionine per ml, and chased for 4 hr as described in “Materials and Methods.” Lysates of cells pulsed for 1 hr and 4-hr chase media were immunoprecipitated with anti-α subunit, anti-COOH-terminal β subunit, or nonimmune rabbit sera (1/5000 dilution). Lanes 1 (molecular weight standards from top to bottom): ovalbumin, 46,000; carbonic anhydrase, 30,000; and cytochrome c, 12,300. Lanes 2 and 3 (control cells): 2, anti-α subunit; 3, anti-β subunit. Lanes 4 to 6 (dbcAMP-treated cells): 4, anti-α subunit; 5, anti-β subunit; 6, nonimmune serum. Lanes 7 to 9 (control medium): 7, anti-α subunit; 8, anti-β subunit; 9, nonimmune serum. Lanes 10 to 12 (dbcAMP-treated medium): 10, anti-α subunit; 11, anti-β subunit; 12, nonimmune serum. Arrows, migration of the M.W. 24,000, 18,000, and 15,000 intracellular precursor of the α and β subunits (left) and the secreted forms of the α and β subunits (right).

Fig. 2. Pulse-chase labeling of BeWo cells: effects of dbcAMP on HCG-α and HCG-β production. Experimental procedures were the same as those described in the legend to Fig. 1. Same sequence of antisera was used for all groups. Lanes 1 to 3 (control cells): 1, anti-α subunit; 2, anti-β subunit; 3, nonimmune serum. Lanes 4 to 6 (dbcAMP-treated cells); Lanes 7 to 9 (control medium); Lanes 10 to 12 (dbcAMP-treated medium); Lane 13 (molecular weight standards).

Fig. 3. Pulse-chase labeling of ZR-75-31 cells: effects of butyrate and dbcAMP on HCG-α production. Cells were incubated for 48 hr with or without 10 mM sodium butyrate or 1 mM dbcAMP. Experimental procedures were as described in the legend to Fig. 1. Same sequence of antisera was used for all groups. Lanes 1 and 2 (control cells): 1, anti-α subunit; 2, nonimmune serum. Lanes 3 and 4 (butyrate-treated cells); Lanes 5 and 6 (dbcAMP-treated cells); Lanes 7 and 8 (control medium); Lanes 9 and 10 (butyrate-treated medium); Lanes 11 and 12 (dbcAMP-treated medium); Lanes 13 (molecular weight standards from top to bottom): phosphorylase B, 92,500; bovine serum albumin, 66,000; ovalbumin, 46,000; carbonic anhydrase, 30,000; and cytochrome c, 12,300.

Fig. 4. Pulse-chase labeling of BT-20 cells: effects of butyrate on HCG-α production. Experimental procedures were as described in the legends to Figs. 1 and 3. Same sequence of antisera was used for all groups. Lanes 1 and 2 (control cells): 1, anti-α subunit; 2, nonimmune serum. Lanes 3 and 4 (butyrate-treated cells); Lanes 5 and 6 (control medium); Lanes 7 and 8 (butyrate-treated medium); Lane 9 (molecular weight standards).

Fig. 5. Pulse-chase labeling of MCF-7 cells: effects of butyrate on HCG-α production. Experimental procedures, sequence of antisera, and order of presentation were the same as for Fig. 4.

Fig. 6. Pulse-chase labeling of ChaGo cells: effects of butyrate and dbcAMP on HCG-α production. Experimental procedures were as described in the legends to Figs. 1 and 3. Same sequence of antisera was used throughout. Lanes 1 to 3 (control cells): 1, anti-α subunit; 2, anti-β subunit; 3, nonimmune serum. Lanes 4 to 6 (butyrate-treated cells); Lanes 7 to 9 (dbcAMP-treated cells); Lanes 10 to 12 (control medium). Lanes 13 to 15 (butyrate-treated medium).

Fig. 7. Pulse-chase labeling of CBT cells: effects of dbcAMP on HCG-β production. Experimental procedures were as described in the legend to Fig. 1. Lanes 1 to 3 (control cells): 1, anti-HCG; 2, anti-COOH terminal β subunit; 3, nonimmune serum. Lanes 4 and 5 (dbcAMP-treated cells): 4, anti-HCG; 5, anti-COOH terminal β subunit. Lanes 6 and 7 (control medium): 6, anti-HCG; 7, anti-COOH terminal β subunit. Lanes 8 to 10 (dbcAMP-treated medium): 8, anti-HCG; 9, anti-COOH terminal β subunit; 10, nonimmune serum. Lane 11 (molecular weight standards).
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