Effects of Adenosine Cyclic Nucleotides on the Synthesis of Human Chorionic Gonadotropin in Transformed Human Placental Cells

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ABSTRACT

The synthesis of human chorionic gonadotropin (HCG) and its subunits was studied in simian virus 40 (SV40) tsA mutant-transformed human first-trimester and term placental cells at 33° (the temperature at which the cells have the transformed phenotype) and at 40° (the temperature at which the tsA transformants regain their nontransformed phenotype). 8-Bromo cyclic adenosine 3′:5′-monophosphate (SBrcAMP) and dibutyryl cyclic adenosine 3′:5′-monophosphate (Bt2cAMP) greatly induced the synthesis of human chorionic gonadotropin α (HCGα) with little or no stimulation of the synthesis of HCG in transformed placental cells grown at 33° or 40°. The ratio of HCGα to HCG in these cells, therefore, increased in the presence of either nucleotide. 8BrCAMP and Bt2cAMP also greatly induced the synthesis of HCGα in nontransformed secondary placental cells (at 6th to 20th passage), although the synthesis of HCG was not detectable in these cells under the experimental conditions used. The synthesis of HCG as well as HCGα was stimulated in choriocarcinoma cells by 8BrCAMP and Bt2cAMP. The ratio of HCGα to HCG in uninduced choriocarcinoma cells increased during growth in culture. 8BrCAMP stimulated the synthesis of HCG preferentially in these cells, thus decreasing the ratio of HCGα to HCG. Our data indicate that adenosine cyclic nucleotides have different effects on the production of HCG but not on HCGα in SV40 tsA-transformed placental cells and choriocarcinoma cells.

INTRODUCTION

Human tumors and tumor-derived cell lines are capable of producing "ectopic" proteins, which are not normally associated with the tissue of origin (4, 17, 21, 24, 32). Ectopic production of HCG and its subunits has been demonstrated in vivo and in vitro in many nontrophoblastic tumors (10, 18, 25, 26). HCG is a glycoprotein trophic hormone composed of 2 nonidentical subunits, α and β (20). It has been shown that the synthesis of HCG and HCGα is affected differentially in trophoblastic and nontrophoblastic tumor cells by the adenosine cyclic nucleotides (9, 12), sodium butyrate (8, 12), and 5-bromo-2′-deoxyuridine (9).

We have established stable cloned human placental cell lines by the transformation of normal placental cells with SV40 tsA mutants that are temperature-sensitive (ts) in the gene required for maintenance of transformation (5-7). The expression of differentiated functions in these placental cells could be controlled by changing the growth temperature. The tsA-transformed cells behave like transformed cells at the permissive temperature (33°) and like nontransformed differentiated cells at the restrictive temperature (40°). The availability of the tsA-transformed placental cells has prompted us to compare the effect of adenosine cyclic nucleotides, 8BrCAMP and Bt2cAMP, on the synthesis of HCG and HCGα by transformed placental cells and choriocarcinoma cells.

MATERIALS AND METHODS

Cells and Culture Conditions. The SV40 tsA mutant-transformed term placental cell lines (TPA30-1 and TPA30-6), the SV40 tsA mutant-transformed first-trimester placental cell lines (SPA255-26 and SPA255-27), and nontransformed secondary term placental (TP) cells at the 6th to the 20th passage have been described (5, 6). Nontransformed first-trimester placental (SP) cells were obtained by collagenase (0.1%; Worthington Biochemical Corp., Freehold, N. J.) digestion of human first-trimester placentas. Primary SP cells as well as secondary SP cells (at the 6th to 20th passage) were used in this study. Both transformed and nontransformed cells were grown in a modified minimal essential medium supplemented with 10% fetal bovine serum, streptomycin (100 μg/ml), and penicillin (100 units/ml). These transformed placental cells shed SV40; therefore, anti-SV40 serum (1%) was routinely added to the culture media to inhibit plaque formation. JEG-3 choriocarcinoma cells (14) were grown in Ham’s Medium F-12 supplemented with 10% fetal bovine serum, streptomycin, and penicillin. Media, fetal bovine serum, and anti-SV40 serum were obtained from Flow Laboratories, Rockville, Md.

Nontransformed secondary placental cells or the transformed placental cells in 25-cm flasks were initially changed at 33°. After 2 to 3 days at 33°, medium was changed, and nucleotide was added to the cultures. For temperature shift experiments, some of the cultures were shifted to 40° after...
media change. Primary SP cells were grown at 37°, and nucleotide was added 4 days after plating. Choriocarcinoma cells were grown at 37°, and nucleotide was added 2 to 3 days after subculturing.

Preparation of Cell Extracts. Cultures in 150-sq cm flasks were grown in the absence and presence of 8BrCAMP (1 mM) for 3 days, and medium was changed every day. The cells were harvested by removing the medium, washing the cells twice with 0.9% NaCl solution, and scraping the cells off the plastic surface with a rubber policeman. The cells were collected by centrifugation and frozen. The frozen pellet was resuspended in phosphate-buffered saline, and the cells were ruptured by sonication (Model DF-101 magnetostrictive oscillator; 250 watts, 10 cal; Raytheon Co., Manchester, N. H.) for 2 min at maximum power. The sonicates were centrifuged at 10,000 x g for 15 min, and the supernatant solutions were used for radioimmunoassays of HCG and HCGα. Protein was determined by the method of Lowry et al. (19).

Radioimmunoassays. HCG and HCGα in the culture media and cell extracts were determined by double-antibody radioimmunoassays (25). The antisera used were: anti-HCGα (SB6) for HCG and HCGβ (31), and anti-HCGα (CA3) for HCGα (8). The anti-HCGα serum measures HCGα, and the anti-HCGβ serum measures both HCGβ and HCG. The transformed placental cells synthesize native HCG as well as the free α and β subunits of HCG (6). Since anti-HCGβ serum was used, the amount of HCG indicated in this study represents the sum of HCG and HCGβ. Purified preparations of HCG (CR119; 11,600 IU/mg; ventral prostate assay; Second International Standard HCG) and of HCGα (CR117) were used as standards, and they were radioiodinated and used as tracers in the respective assays. Native HCG (CR119) has 1 to 3% cross-reactivity in the HCG assay; HCGα has 0.02 to 0.05% cross-reactivity in the HCG assay. Complete medium not exposed to cells had no detectable HCG or HCGα. Antisera, standard HCG, and HCGα were kindly provided by Drs. K. Catt, G. Hodgen, and H. Chen, NIH, Bethesda, Md.

RESULTS

Effect of Adenosine Cyclic Nucleotide on the Synthesis of HCGα and HCG by Nontransformed Primary, Secondary, and tsA-transformed Human Placental Cells. Bt2cAMP has been shown to stimulate the secretion of HCG in cultures of normal placenta (11, 12) and choriocarcinoma (12, 13, 27) cells. HCG secretion in cultured primary SP cells was also stimulated by 8BrCAMP (Table 1). However, HCG secretion by primary SP cells decreased rapidly in culture. In the absence of inducer, the levels of HCG secreted by primary SP cells from Day 4 to Day 7 and from Day 7 to Day 10 were 0.6 and 0.16%, respectively, of that secreted by these cells from Day 1 to Day 4. Therefore, primary placental cells rapidly lost their HCG-synthesizing capability in vitro. HCGα secretion by primary SP cells also diminished in culture, although to a lesser extent than that of HCG. The ratio of HCGα to HCG in primary SP cells increased with growth in culture (Table 1). Since HCG secretion by primary SP cells could not be maintained in culture, it is possible that the observed increase in HCG secretion by 8BrCAMP (as compared with the control uninduced cultures) resulted from more competent cells functioning in the presence of adenosine cyclic nucleotide. None of the 8BrCAMP-treated cultures secreted as much HCG as did those cultures at early stages of growth (from Day 1 to Day 4). On the other hand, HCGα secretion was greatly induced by 8BrCAMP. In the presence of 8BrCAMP, the levels of HCGα secreted by primary SP cells from Day 4 to Day 7 and from Day 7 to Day 10 were 4- to 5-fold higher than those secreted by the original cultures from Day 1 to Day 4. The ratio of HCGα to HCG in primary SP cells was therefore greatly increased by 8BrCAMP.

Secondary TP or SP cells at the 6th to 20th passages did not synthesize detectable amounts of HCG in culture, indicating that they were dedifferentiated further in vitro. These nontransformed cells, however, synthesized measurable amounts of HCGα in vitro. HCGα synthesis in secondary TP and SP cells was greatly induced by the adenosine cyclic nucleotides, 8BrCAMP and Bt2cAMP. HCG, however, was not detectable in these cells, even in the presence of 8BrCAMP or Bt2cAMP. Data for secondary TP cells are shown in Chart 1.

Transformation of placental cells by SV40 tsA mutants not only permits the propagation and cloning of these cells but also prevents the placental cells from dediffereniatating. As a consequence, the tsA-transformed placental cells synthesized HCG as well as HCGα in culture. The synthesis of HCGα in these transformed cells was induced by 8BrCAMP or Bt2cAMP (Chart 1). The synthesis of HCG in the transformed placental cells, however, was only slightly enhanced by 8BrCAMP or Bt2cAMP (Chart 1). Furthermore, it appeared that the induction was not dependent upon the concentration of the nucleotide added. The ratio of HCGα to HCG in these cells, therefore,
increased greatly in the presence of 8BrCAMP or Bt2CAMP (Table 2). When the ratio of HCGα to HCG was normalized to the value for the respective control noninduced cultures, a ratio as high as 70 was attained in the presence of 8BrCAMP in TPA30-6 cells.

Effect of Adenosine Cyclic Nucleotides on the Synthesis of HCGα and HCG by JEG-3 Choriocarcinoma Cells. The synthesis of HCGα as well as HCG was induced in JEG-3 choriocarcinoma cells by 8BrCAMP and Bt2CAMP (Chart 1). The ratio of HCGα to HCG in these cells (the ratio was normalized to that for the control noninduced cultures) was nearly unity in the presence of 8BrCAMP and was increased to approximately 2 in the presence of Bt2CAMP (Table 2). The synthesis of HCGα was also induced by 8BrCAMP and Bt2CAMP in the nontransformed and tsA-transformed placental cells. The increase in the ratio of HCGα to HCG by adenosine cyclic nucleotides in nontransformed primary SP cells and the tsA-transformed placental cells suggests that the regulation of HCG, not its α subunit, in primary and tsA-transformed placental cells may differ from the regulation in choriocarcinoma cells.

Since butyric acid cleaved from Bt2CAMP affects the synthesis of HCG and HCGα in both transformed placental cells and choriocarcinoma cells (8, 12), 8BrCAMP alone was used in the following experiments.

The synthesis of HCG was decreased and the synthesis of HCGα was increased during growth of JEG-3 choriocarcinoma cells in culture (Chart 2). The ratio of HCGα to HCG in these cells, therefore, increased with growth (Chart 2, inset). In the presence of 8BrCAMP, however, the levels of HCGα and HCG were maintained at nearly a constant ratio regardless of the growth of the cells. Accordingly, growth of JEG-3 cells in the presence of 8BrCAMP prevented these cells from synthesizing excess amounts of HCGα.

Intracellular and extracellular hormones were affected in a...
Control SBrcAMP SBrcAMP

Day 3.

immediately.

and allowed to incubate further, the level of HCGα dropped choriocarcinoma cells (Chart 2). As the ratio decreased in induced cells were washed with medium containing no inducer 26 first-trimester placental cells are shown in Chart 7. When induced level of HCGα in the transformed first-trimester or term placental cells grown either at 33° or 40°. Data for SPA255-26 first-trimester placental cells are shown in Chart 7. When induced cells were washed with medium containing no inducer and allowed to incubate further, the level of HCGα dropped immediately.

Effect of 8BrcAMP on the Synthesis of HCGα and HCG by tsA-transformed Placental Cells Grown at the Permissive and the Restrictive Temperatures. In the absence of inducer, nontransformed secondary placental cells at the 6th to the 20th passage synthesized low levels of HCGα in culture. 8BrcAMP greatly increased the synthesis of HCGα in these secondary TP and SP cells at both 33° and 40° (Chart 3). HCG synthesis was not detected in these cells in the presence of 8BrcAMP at either temperature. The level of HCGα in nontransformed secondary TP cells in both the presence and the absence of 8BrcAMP was consistently higher than that in secondary SP cells. This may reflect the HCGα levels in vivo at these 2 stages of gestation. In the tsA-transformed first trimester and term placental cells, 8BrcAMP induced HCGα synthesis at 33° as well as at 40° (Charts 4 and 5). At 33°, HCG synthesis in the transformed placental cells was induced slightly by 8BrcAMP. At 40°, 8BrcAMP inhibited the synthesis of HCG after 2 or more days of exposure. The decline in HCG synthesis at 40° might have resulted from 8BrcAMP-induced cytotoxicity. On the other hand, in the same culture, HCGα synthesis was greatly induced by 8BrcAMP at 40°. The effects of 8BrcAMP were not due to effects on secretion because intracellular and extracellular hormones were affected in a similar fashion by 8BrcAMP (Table 3). Therefore, the observed induction was not due to effects on hormone secretion.

Table 3: Effect of 8BrcAMP on intracellular and extracellular accumulation of HCG and HCGα

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Control</th>
<th>SBrcAMP</th>
<th>Control</th>
<th>SBrcAMP</th>
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<tr>
<td>SPA255-26</td>
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<td></td>
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<tr>
<td>Control</td>
<td>1.4 ± 0.1</td>
<td>18.5 ± 1</td>
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<td>44 ± 1</td>
<td>828 ± 10</td>
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<td>JEG-3</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>0.09 ± 0.005</td>
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<td>0.2 ± 0.01</td>
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<td>3.4 ± 0.1</td>
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<tr>
<td>Control</td>
<td>21.7 ± 0.2</td>
<td>124 ± 2</td>
<td>13.5 ± 1</td>
<td>337 ± 6</td>
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<td>8BrcAMP</td>
<td>2,196 ± 70</td>
<td>18,828 ± 1,000</td>
<td>627 ± 30</td>
<td>12,070 ± 1,226</td>
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</tr>
</tbody>
</table>

a Mean ± S.E. 
b Numbers in parentheses, ratio of HCG (or HCGα) of SBrcAMP-treated cultures to that of the control cultures; thus, a ratio of 1.0 represents no stimulation.

Table 3: Effect of 8BrcAMP on intracellular and extracellular accumulation of HCG and HCGα

Cells in 150-sq cm flasks were treated with 8BrcAMP (1 mm) for 3 days with daily medium change. Hormone levels were determined in cells and media collected on Day 3.

Effect of 8BrcAMP on the synthesis of HCGα and HCG by tsA-transformed Placental Cells Grown at the Permissive and the Restrictive Temperatures. In the absence of inducer, nontransformed secondary placental cells at the 6th to the 20th passage synthesized low levels of HCGα in culture. 8BrcAMP greatly increased the synthesis of HCGα in these secondary TP and SP cells at both 33° and 40° (Chart 3). HCG synthesis was not detected in these cells in the presence of 8BrcAMP at either temperature. The level of HCGα in nontransformed secondary TP cells in both the presence and the absence of 8BrcAMP was consistently higher than that in secondary SP cells. This may reflect the HCGα levels in vivo at these 2 stages of gestation. In the tsA-transformed first trimester and term placental cells, 8BrcAMP induced HCGα synthesis at 33° as well as at 40° (Charts 4 and 5). At 33°, HCG synthesis in the transformed placental cells was induced slightly by 8BrcAMP. At 40°, 8BrcAMP inhibited the synthesis of HCG after 2 or more days of exposure. The decline in HCG synthesis at 40° might have resulted from 8BrcAMP-induced cytotoxicity. On the other hand, in the same culture, HCGα synthesis was greatly induced by 8BrcAMP at 40°. The effects of 8BrcAMP were not due to effects on secretion because intracellular and extracellular hormones were affected in a similar fashion by 8BrcAMP (Table 3). In the presence of 8BrcAMP, the ratio of HCGα to HCG (as compared with the control uninduced cultures) in the tsA-transformed placental cells increased (Chart 6), whereas the ratio decreased in choriocarcinoma cells (Chart 2).

8BrcAMP must be present continuously to maintain an induced level of HCGα in the transformed first-trimester or term placental cells grown either at 33° or 40°. Data for SPA255-26 first-trimester placental cells are shown in Chart 7. When induced cells were washed with medium containing no inducer and allowed to incubate further, the level of HCGα dropped immediately.

DISCUSSION

The investigation of genetic regulation of HCG synthesis in human placenta has suffered from the lack of a reliable in vitro system. Neoplastic transformation of human organs usually results in the alteration of the control mechanism for the synthesis of certain proteins specific for that tissue. The difficulties inherent in establishing and maintaining placental explants (or primary cultures) have limited the usefulness of these cultures. In an attempt to circumvent some of these problems, or at least provide an alternative approach, we and others have taken a somewhat different approach. Differentiated cells have been transformed in tissue culture by temperature-sensitive mutants of RNA or DNA tumor viruses (3, 5, 6, 22). By a variety of criteria, such transformed cells behave like tumor cells at the permissive temperature but mimic the normal nontransformed tissue at the nonpermissive temperature. Conclusions from studies of gene regulation carried out in such cells at the

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HCG Synthesis in Placental Cells

Chart 4. Effect of 8Br-cAMP on the synthesis of HCG and HCGα in tsA-transformed first-trimester placental cells grown at 33° and 40°. Cultures were grown in the absence and in the presence of 8Br-cAMP (1 μM) and were incubated at 33° or 40°. Medium was changed every day. HCG and HCGα in the culture medium were estimated by radioimmunoassays. Each value represents the average of 2 determinations from each of 2 cultures (4 determinations total); bars, range. ○, control; ●, 8Br-cAMP.

Chart 5. Effect of 8Br-cAMP on the synthesis of HCG and HCGα in tsA-transformed term placental cells grown at 33° and 40°. Cultures were grown in the absence and in the presence of 8Br-cAMP (1 μM) and were incubated at 33° or 40°. Medium was changed every day. HCG and HCGα in the culture medium were estimated by radioimmunoassays. Each value represents the average of 2 determinations from each of 2 cultures (4 determinations total); bars, range. ○, control; ●, 8Br-cAMP.

nonpermissive temperature may be more nearly like normal cells.

We investigated the synthesis of HCG and HCGα in the tsA-transformed first-trimester and term placental cells (grown at 33° or 40°) and in choriocarcinoma cells. We thought that adenosine cyclic nucleotides, inducers of HCG and HCGα in choriocarcinoma cells, might have a different effect in SV40-transformed cells versus the choriocarcinoma cells. In the tsA-transformed placental cells at either 33° or 40°, 8Br-cAMP greatly induced the synthesis of HCGα with little or no stimulation of the synthesis of HCG. In choriocarcinoma cells,
8BrCAMP greatly induced the synthesis of HCG as well as HCGα. The difference between the 2 types of placental cells was more evident when the data were expressed as the ratios of HCGα to HCG. In the presence of 8BrCAMP, the ratio of HCGα to HCG increased (as compared with the control unin-
duced cultures) in the tsA-transformed placental cells, whereas the ratio decreased in choriocarcinoma cells.

8BrCAMP also greatly induced the synthesis of HCGα in primary SP cells and secondary TP and SP cells. In the presence of 8BrCAMP, the ratio of HCGα to HCG increased in primary SP cells, like that in tsA-transformed placental cells. It appears that the effect of 8BrCAMP on HCG and HCGα synthesis in tsA-transformed placental cells closely resembles that in nontransformed cells. Transformation of placental cells by SV40 therefore may not have interfered with the normal control mechanism for the synthesis of HCG. The differential responses to 8BrCAMP in tsA-transformed placental cells and choriocarci-
coma cells suggest that HCG synthesis in choriocarcinoma patients may be regulated abnormally.

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