0021-972X/87/6405-1002$02.00/0
Journal of Clinical Endocrinology and Metabolism
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Vol. 64, No. 5
Printed in U.S.A.

8-Bromo-Adenosine 3′,5′-Monophosphate Regulates Expression of Chorionic Gonadotropin and Fibronectin in Human Cytotrophoblasts*

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ABSTRACT. Addition of 8-bromo-cAMP to primary cultures of human placental cytotrophoblasts results in significant alterations in the synthesis of secreted proteins, as detected by labeling with pulses of [^{35}S]methionine. Using immunoprecipitation techniques, we demonstrated that exposure to 8-bromo-cAMP prevented the *de novo* synthesis and secretion of the extracellular matrix component fibronectin, but enhanced the production of hCG subunits. The effects of the cyclic nucleotide on synthesis and secretion of these proteins were evident within 24 h. 8-Bromo-cAMP increased the cellular content of mRNA encoding the hCG α- and β-subunits and prevented the increase in fibronectin mRNA, as determined by blot hybridization analysis using specific cDNA probes. These findings demonstrate that cyclic nucleotides regulate the synthesis of several specific proteins in cultured human trophoblast by regulating levels of the mRNAs encoding the proteins. The actions of cyclic nucleotides in this regard may be essential for the normal expression of trophoblast endocrine function. (*J Clin Endocrinol Metab* **64:** 1002, 1987)

THE SYNCYTIOTROPHOBLAST of the human placenta, which elaborates a number of steroidal and protein hormones, is derived from mononuclear cytotrophoblasts which form a syncytium by cell fusion (1–3). Cytotrophoblasts do not synthesize large quantities of hormones as does the syncytiotrophoblast (3). For example, immunocytochemical staining for placental hormones such as hCG and human placental lactogen reveals that the proteins are localized to the syncytiotrophoblast (3). Recently, we found that the endocrine activity of cytotrophoblasts in culture could be markedly stimulated by 8-bromo-cAMP (4); the cAMP analog increased both hCG and progesterone secretion over a 48-h period. We concluded that a cAMP-mediated process triggers the functional differentiation of cytotrophoblasts to the mature trophoblast form. To further characterize the cAMP-activated differentiation of cytotrophoblasts, we examined the effects of 8-bromo-cAMP on specific protein synthesis and secretion using pulse labeling with [^{35}S]methionine with subsequent analysis of newly synthesized proteins by immunoprecipitation, electrophoresis, and fluorography. In addition, the effect of the cyclic nucleotide on levels of the mRNAs encoding fibronectin and the α- and β-subunits of hCG was determined. Our findings reveal that 8-bromo-cAMP promotes alterations in the synthesis and secretion of specific proteins, including fibronectin and the subunits of hCG by regulating mRNA expression.

Received November 4, 1986.
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* This work was supported by NIH Grants HD-06274 and HD-00715.
† Supported by a Rockefeller Foundation Biotechnology Career Fellowship.
‡ Supported by National Research Service Award HD-06881.
§ Supported by a grant from the Mellon Foundation.

Materials and Methods

Primary culture of cytotrophoblasts

Term placentae (n = 18) were obtained immediately after spontaneous vaginal delivery or uncomplicated cesarean section, and cytotrophoblasts were isolated as previously described by Kliman *et al.* (3). This procedure yields nearly pure cultures of cytotrophoblasts. Cytotrophoblasts were diluted to a concentration of 1 × 10^{6} cells/mL with Dulbecco's Modified Eagle's Medium (DMEM) containing 25 mM glucose, 25 mM HEPES, 50 μg/mL gentamycin, and 20% (vol/vol) heat-inactivated fetal calf serum, plated into 35-mm Nunclon (Nunc, Roskilde, Denmark) culture dishes (1 × 10^{6} cells in 2 mL medium), and incubated in humidified 5% CO_2-95% air at 37 C. Media were changed daily. 8-Bromo-cAMP (1.5 mM; Sigma Chemical Co.,

St. Louis, MO) was added to some cultures at the time of plating. This dose of 8-bromo-cAMP was previously found to produce a consistent stimulation of hCG secretion by cytotrophoblasts (4).

Radiolabeling of secreted and cellular proteins

Two hours before the time of pulsing, the medium was removed and replaced with 0.75 mL serum- and methionine-free DMEM. Two hours later, [^{35}S]methionine (100 μCi; New England Nuclear, Boston, MA) was added in a final volume of 1 mL. After 2 h of incubation, the media were collected, and cells were rinsed twice with serum-free DMEM containing methionine and harvested with a rubber policeman. The cells were pelleted by centrifugation and resuspended in phosphate-buffered saline containing 1.0% cholate and 0.1% sodium dodecyl sulfate (SDS). The resuspended cells were subjected to two freeze-thaw cycles, and unsolubilized material was sedimented by centrifugation at 13,000 × *g* for 10 min using a Brinkmann microfuge (Westbury, NY). The supernatant was removed for determination of trichloroacetic acid (TCA)-precipitable radioactivity and analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Equal volumes of culture medium (25 μL) and equal amounts of TCA-precipitable radioactivity from control and 8-bromo-cAMP-treated cells were loaded on the gels. The amount of cellular protein loaded in each lane ranged from 8–30 μg in these experiments. Mol wt markers were run on each gel (myosin, 200K; β-galactosidase, 116K; phosphorylase b, 97.4K; BSA, 66K; ovalbumin, 45K; carbonic anhydrase, 29K; trypsinogen, 24K; soybean trypsin inhibitor, 20.1K; α-lactalbumin, 14.2K). Selected calibration points are indicated on the figures.

Immunoisolation of fibronectin and hCG subunits

Fibronectin and hCG subunits were immunoisolated from equal volumes (20 μL) of medium from control and 8-bromo-cAMP-treated cells. Fibronectin was detected in medium using a polyclonal antibody raised in rabbits against human plasma fibronectin (Calbiochem, San Diego, CA). The immunoisolation procedure employed protein A-Sepharose CL-4B to precipitate the immunoglobulin G-bound fibronectin (5). To demonstrate specificity, some immunoisolations were performed in the presence of 200 μg unlabeled human plasma fibronectin (Biomedical Technologies, Inc., Cambridge, MA). The fibronectin immunoisolates were solubilized in final sample buffer containing 0.12 M Tris, 10% glycerol (vol/vol), 2% SDS (wt/vol), and 5% 2-mercaptoethanol (vol/vol) with heating at 90 C for 10 min and subjected to SDS-PAGE using 5% polyacrylamide gels in the presence of 2-mercaptoethanol, as described by Laemmli (6).

hCG subunits were immunoisolated using procedures described by Ivarie and Jones (7). Five microliters of a polyclonal antiserum raised in rabbits against purified α-subunit were employed. This antiserum was kindly provided by Dr. Steven Birken of Columbia University. Immunoisolation in the presence of excess hCG (50 IU hCG CR119 obtained from the National Pituitary Agency) was performed to verify specificity of the immunoisolation. The hCG immunoisolates were solubilized as described above and subjected to SDS-PAGE on 10% polyacrylamide gels in the presence of 2-mercaptoethanol.

The protocols used to immunoisolate fibronectin and the subunits of hCG were designed for quantitative recovery of labeled proteins from the aliquots of incubation medium. Thus, reexposure of the aliquots to antisera did not precipitate additional labeled proteins.

The polyacrylamide gels were impregnated with 1 M salicylate-1% glycerol, dried, and exposed to Kodak X-Omat x-ray film (Eastman Kodak, Rochester, NY) at −70 C. Some gels were stained with Coomassie blue before processing. The intensity of bands on the fluorograms was quantitated by densitometry using a soft-laser scanner.

RNA isolation, electrophoresis, and blot hybridization

In some experiments, cells were harvested for RNA isolation after exposure to 1.5 mM 8-bromo-cAMP or control medium for 24 or 48 h. RNA was isolated from cells by the guanidine isothiocyanate-cesium chloride method (8). Equal amounts of RNA from each treatment group were denatured and electrophoresed in formaldehyde-3-(N-morpholino)propane sulfonic acid agarose gels and transferred to nitrocellulose paper using standard procedures (8). The mobility of fragments from a *Hind* III digest of lambda DNA was used to estimate mRNA size. This lane was excised from the gel before transfer and stained with ethidium bromide, and migration of the DNA fragments was recorded for later comparison with autoradiograms.

Hybridizations with nick-translated cDNA probe were conducted according to the method of Berent *et al.* (9). Briefly, dried nitrocellulose filters were first moistened with 6 × SSC (1 × SSC = 0.15 M NaCl and 0.015 M sodium citrate), then placed in heat-sealable plastic bags with 5–8 mL prehybridization solution (50% formamide, 5 × SSC, 0.1% Ficoll, polyvinylpyrollidone, BSA, SDS, and 250 μg/mL denatured salmon sperm DNA) and incubated at 42 C for 4 h. This solution was replaced with fresh solution to which was added nick-translated cDNA (5–10 × 10^6 cpm/mL). Hybridizations were continued for 18–22 h. After hybridization, the filters were washed twice for 30 min at room temperature in 2 × SSC and 0.1% SDS, followed by two 45-min washes at 65 C in 1 × SSC and 0.1% SDS. After the washes, the filters were briefly blotted, wrapped in plastic, and placed with x-ray film (Kodak X-Omat) for autoradiography at −20 C for 2–96 h.

Plasmid preparation, isolation, and nick-translation

A plasmid containing a 440-basepair cDNA insert for the αhCG mRNA that contains the complete 5′ noncoding sequence and all but the last amino acids of the coding region (10) was generously provided by Dr. Irving Boime of Washington University (St. Louis, MO). A 579-basepair cDNA which codes for human hCGβ (11) was generously provided by Dr. John Fiddes, California Biotechnology (Mountain View, CA). A plasmid containing a 1300-basepair cDNA insert for the human fibronectin mRNA that corresponds to a portion of the 3′ coding sequence and the 3′ noncoding sequence (12) was provided by Dr. Mon-Li Chu, Thomas Jefferson School of

Medicine (Philadelphia, PA). A plasmid containing a 2100-basepair insert representing a full-length cDNA for the human γ-actin mRNA (13) was provided by Dr. P. Gunning, Stanford University School of Medicine (Palo Alto, CA).

The cDNA inserts were released by appropriate restriction enzyme digestion and isolated from preparative low melting point agarose gels for nick-translation. The cDNAs were labeled to specific activities of 1.5–3 × 10^8 cpm/μg with a nick-translation kit from Bethesda Research Laboratories (Gaithersburg, MD).

Analytical methods, reagents, and supplies

Protein was determined by the method of Bradford (14). hCG was measured with a specific RIA recognizing hCGβ (Corning Medical, Medford, MA). This assay was calibrated to the Second International Standard.

Ficoll, polyvinylpyrrolidone, BSA, and 8-bromo-cAMP were purchased from Sigma (St. Louis, MO); [^{32}P]dCTP (SA, >3000 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Nitrocellulose was purchased from Schleicher and Schuell (Keene, NH). All other reagents for mRNA analyses were purchased from Bethesda Research Laboratories.

Statistical analysis

Values presented are the mean ± SE of the indicated number of separate experiments. Each experiment was replicated on at least two occasions with different cell preparations.

Results

Effects of 8-bromo-cAMP on incorporation of [^{35}S]methionine into proteins secreted by cultured cytotrophoblasts

Examination of radiolabeled proteins in the media from cultures of control cytotrophoblasts by SDS-PAGE using 5% and 10% polyacrylamide gels in the presence of 2-mercaptoethanol consistently revealed the presence of a protein with an apparent mol wt of 220,000 (Fig. 1). The intensity of the 220,000 mol wt band in media of control cultures increased several-fold between the first and second days of culture, whereas the intensity of the other bands remained more or less constant. The incorporation of label into the 220,000 mol wt protein in media of cytotrophoblasts cultured in the presence of 8-bromo-cAMP for 48 h was far less than that in the control incubations. This reduction was most apparent after 48–60 h of continuous exposure to the cAMP analog when labeling of the 220,000 mol wt protein was 10% or less that of the controls. However, the cyclic nucleotide appreciably increased the labeling of secreted proteins migrating with mol wt in the range of 17,500–20,000 (Fig. 1).

The 220,000 mol wt protein in the medium was identified as fibronectin by immunoisolation with a specific antiserum (Fig. 2). The immunoisolated protein migrated with authentic fibronectin, and inclusion of unlabeled

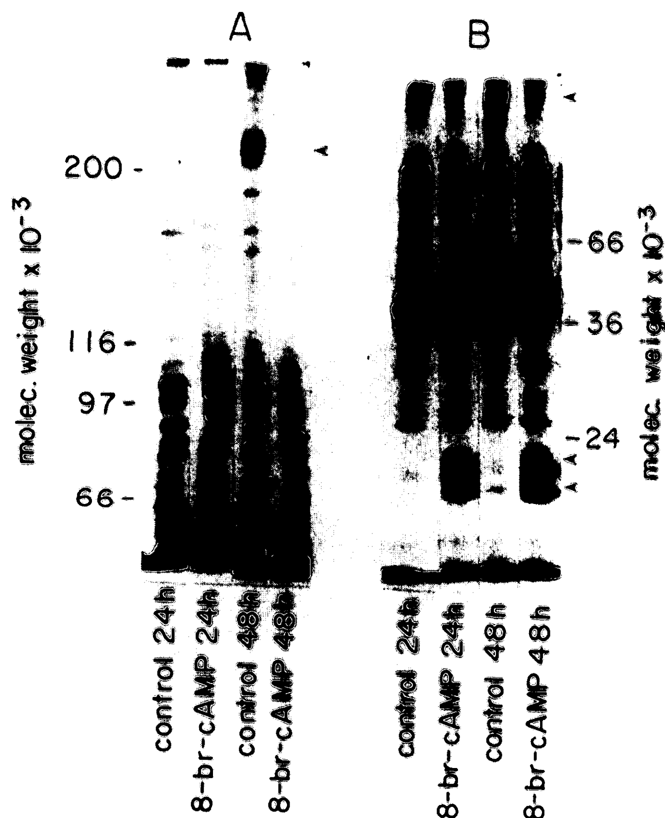

FIG. 1. Effects of 8-bromo-cAMP on synthesis of cytotrophoblast secretory proteins. The fluorograms are of 5% (A) and 10% (B) SDS-polyacrylamide gels, revealing labeled proteins secreted into the incubation medium by cytotrophoblasts cultured in the absence (control) and presence of 8-bromo-cAMP (1.5 mM). *Arrows* mark the location of a 220,000 mol wt protein, subsequently identified as fibronectin, and the 17,500–19,600 mol wt proteins where immunoisolatable hCG subunits migrate. Similar results were obtained with media from four other experiments.

human fibronectin in the immunoisolation reaction prevented the detection of the labeled 220,000 mol wt protein, establishing the specificity of the immunoisolation procedure. Immunoisolation of fibronectin from equal volumes of incubation medium collected from control and 8-bromo-cAMP-treated cultures confirmed the dramatic reduction in secretion of this protein by the cyclic nucleotide analog-treated cells.

At least some of the radiolabeled proteins migrating with apparent mol wt of 17,500–20,000 were identified as hCGα by immunoisolation with a specific antiserum (Fig. 3). These proteins were barely detectable in immunoisolates from control media, but were readily detectable in equal volumes of media from 8-bromo-cAMP-treated cytotrophoblasts. The isolation of several distinct labeled proteins representing α-subunits was expected on the basis of previous studies of hCG subunit synthesis by placental and choriocarcinoma cells (15). The higher mol wt band (~19,600) represents the free α-subunit,

FIG. 2. Immunoisolation of fibronectin from media of control and 8-bromo-cAMP-treated cells. Equal volumes of incubation media from control and 8-bromo-cAMP-treated cultures were subjected to immunoisolation with a specific antiserum to fibronectin. The labeled protein immunoisolated from control medium was not detected when immunoisolations were performed in the presence of excess unlabeled fibronectin (+ fibronectin) or when nonimmune serum was used. The *arrowhead* indicates the migration of purified fibronectin as detected by Coomassie blue staining. The results presented were reproduced on three occasions using media from two different cultures.

while the lower mol wt band (~17,500) represents α-subunit dissociated from hCG. A band migrating with an apparent mol wt around 28,000 was seen in overexposed fluorograms of immunoisolates from medium of 8-bromo-cAMP-treated cells. This presumably represents the β-subunit of hCG immunoisolated along with the α-subunit. The incorporation of smaller quantities of label into the β-subunit was expected because it contains only one methionine residue whereas the α-subunit contains three (16). The radiolabeled proteins migrating with mol wt of the hCG subunits were not detected when immunoisolations were performed in the presence of excess unlabeled hCG, confirming the specificity of the immunoprecipitation. The labeled protein at the top of the gel, which is prominent in the immunoprecipitates from control media and less intense in those from 8-bromo-cAMP-treated cultures, is probably fibronectin, which is known to bind to *Staphyloccus aureus* (17), and would be recovered along with hCG bound by the antibodies.

In confirmation of our previous findings (4), 8-bromo-cAMP stimulated hCG secretion, as measured using a RIA specific for the β-subunit, coinciding with the increase in secretion of labeled hCG subunits. After 48 h of culture, hCG was barely detectable in medium (<50 mIU/mg protein) from control cultures, whereas the hormone was always present in substantial quantities in medium of cells cultured for 24 or 48 h in the presence of 8-bromo-cAMP (>1000 mIU/mg protein).

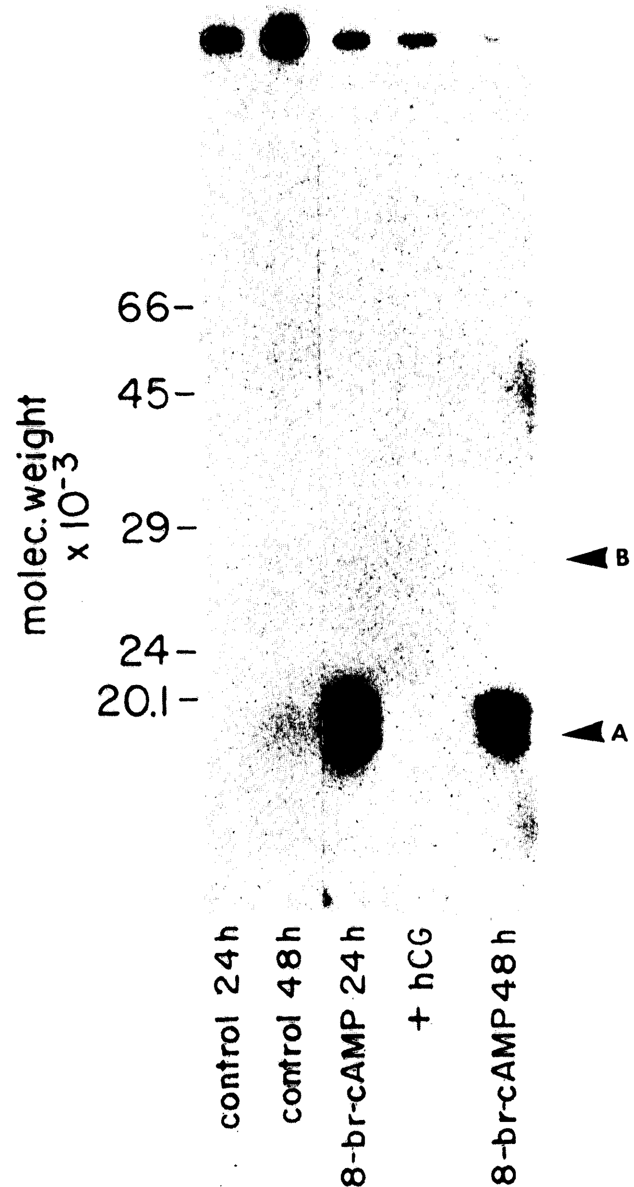

FIG. 3. Fluorogram of an immunoprecipitate of hCG subunits from media of control and 8-bromo-cAMP-treated cytotrophoblasts. Equal volumes of incubation media from control and 8-bromo-cAMP-treated cultures were immunoprecipitated with a specific antiserum to hCGα. No immunoprecipitation of the labeled subunits was found when excess unlabeled hCG was included (+ hCG). The labeled protein of high mol wt is probably fibronectin, which binds to *Staphyloccus aureus* and would thus be precipitated in the immunoisolation process. The A and B *arrows* indicate the migration of dissociated hCGα and hCGβ, respectively, as detected by Coomassie blue staining. Similar results were obtained in two other experiments.

Effects of 8-bromo-cAMP on incorporation of [^{35}S]methionine into cytotrophoblast cellular proteins

Exposure of cytotrophoblasts to 8-bromo-cAMP for 24–60 h resulted in some notable changes in protein synthesis, including a reduction in the labeling of a protein with an apparent mol wt of 220,000, which is presumably fibronectin (either intracellular or fibronectin bound to the cell surface) and an increase in labeling of proteins of 28,000 (2.3-fold at 48 h), 17,000 (4.8-fold at 48 h), and 12,000–14,000 (4.6-fold at 48 h) mol wt (Fig. 4). With respect to total label incorporation into cellular proteins, 8-bromo-cAMP treatment did not substantially alter the specific activity of total proteins after either 24 of 48 h of exposure [91 ± 5% of control specific activity at 24 h (n = 3) and 98 ± 16% at 48 h (n = 3)].

Effects of 8-bromo-cAMP on fibronectin and hCG mRNA levels

RNA blot hybridization analysis was conducted on nitrocellulose filters blotted with equal amounts of total cellular RNA from cytotrophoblasts cultured for 24 or 48 h in the presence or absence of 1.5 mM 8-bromo-cAMP. Nick-translated ^{32}P-labeled human fibronectin cDNA hybridized with a band of approximately 7.8 kilobases (Fig. 5), the expected size of the fibronectin mRNA (12). While fibronectin mRNA levels in untreated cells increased substantially during the 48 h of culture, mRNA levels in 8-bromo-cAMP-treated cells remained low. These results closely parallel the incorporation of [^{35}S]methionine into fibronectin in untreated and cAMP-stimulated cells.

In marked contrast to the results with fibronectin mRNA, 24 or 48 h of treatment with 8-bromo-cAMP

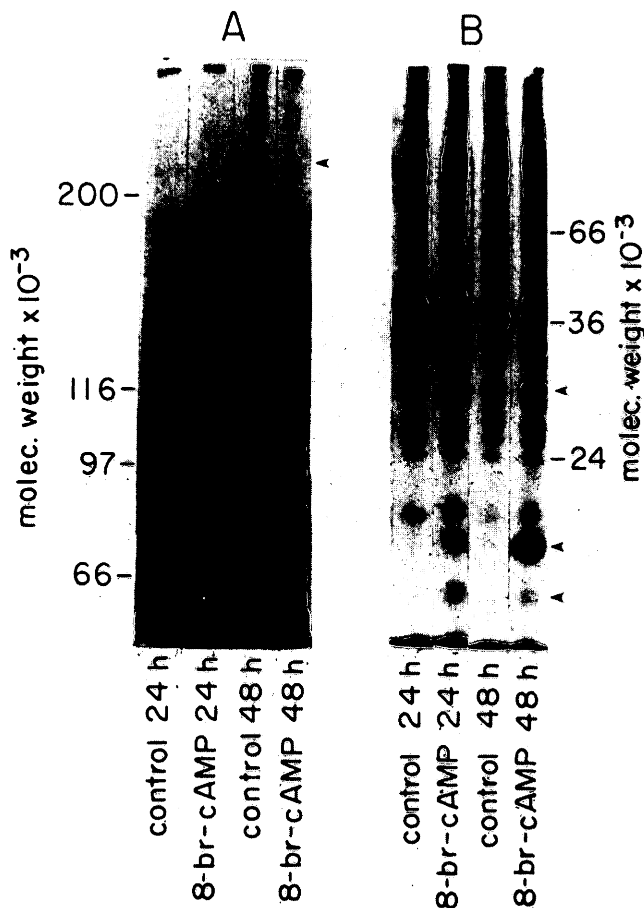

FIG. 4. Fluorograms of 5% (A) and 10% (B) SDS-polyacrylamide gels of labeled cellular proteins from control and 8-bromo-cAMP-treated cytotrophoblasts. Equal quantities of TCA-precipitable radioactivity from control and 8-bromo-cAMP-treated cells were analyzed. The *arrows* mark labeled proteins altered by 8-bromo-cAMP treatment. Similar results were obtained in four other experiments.

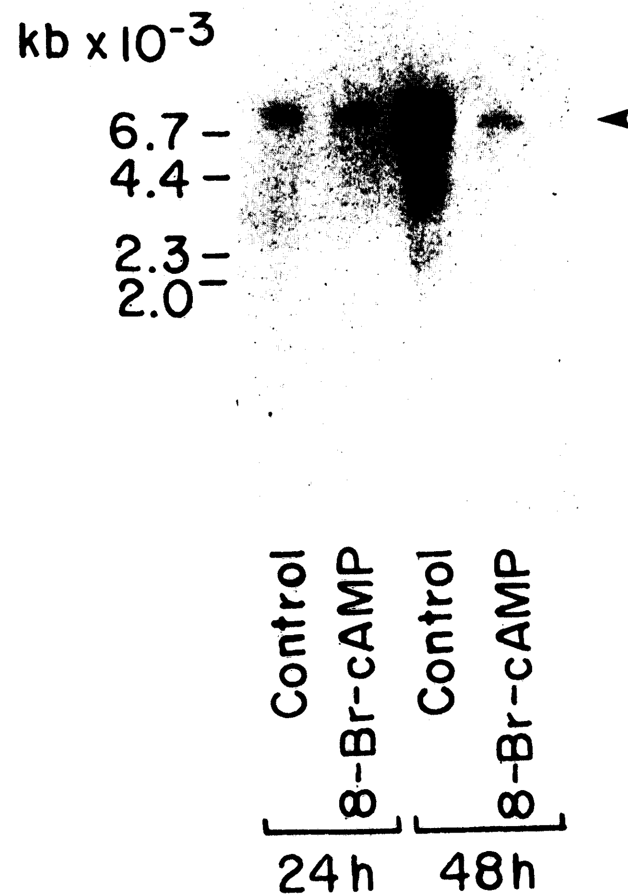

FIG. 5. Effects of 8-bromo-cAMP on fibronectin mRNA levels in cultured cytotrophoblast cells. Cytotrophoblast cultures were exposed to control medium or 1.5 mM 8-bromo-cAMP for 24 or 48 h, the cells were then harvested, and total cellular RNA was prepared. After electrophoresis and transfer of equal amounts (15 μg) of RNA from control and treated cells, the nitrocellulose filters were probed with a nick-translated ^{32}P-labeled cDNA for human fibronectin mRNA. The migration of fragments of *Hind* III-digested λ DNA appear to the *left*. The probe hydridized with a RNA band of appropriate size [~7.8 kilobases (Kb)] in both control and 8-bromo-cAMP-treated cells, but the signal became more intense in control cells during the 48 h of culture, whereas it remained low in cells exposed to 8-bromo-cAMP for 48 h. These findings were replicated in two separate experiments.

caused a dramatic increase in the cellular content of the 0.85-kilobase α hCG mRNA (18) (Fig. 6, lanes 1 and 2) and the 1.05-kilobase mRNA encoding the β subunit (Fig. 7). Thus, the changes in the hCG mRNA content of these cells directly paralleled the synthesis and secretion of hCG previously described.

Finally, hybridization of RNA samples with nick-translated human actin cDNA revealed that 8-bromo-cAMP caused only a modest (<20%) decrease in actin mRNA levels in these experiments. Therefore, the effects of 8-bromo-cAMP on hCG or fibronectin mRNA levels were not due to any major alterations in overall cellular mRNA content.

Discussion

Cytotrophoblasts respond to 8-bromo-cAMP with a striking increase in their capacity to synthesize and secrete progesterone and hCG (Ref. 4 and present studies). The cAMP analog appears to effect this change at least in part by increasing the synthesis of the subunits of hCG, and it also increases the synthesis of a number of unidentified proteins. In addition, the synthesis and secretion of at least one protein, fibronectin, is suppressed. The fact that 8-bromo-cAMP did not significantly alter the specific activity of total cellular proteins and that incorporation of [^{35}S]methionine into some proteins was increased whereas it was decreased in others indicate that treatment with the cAMP analog did not substantially alter methionine pool size.

Fibronectin, a component of the extracellular matrix, is synthesized and secreted in substantial amounts by cultured cytotrophoblasts. The significance of the 8-bromo-cAMP-provoked reduction in fibronectin synthesis and secretion is not yet clear. However, fibronectin is thought to function in cell adhesion and migration (19). Exogenous fibronectin blocks myoblast fusion (20). It is of interest to note that cAMP analogs stimulate fibronectin production by certain cells [rabbit cornea (21)] and that enhanced production of this glycoprotein occurs with anchorage-independent cell growth (22). Fibronec-

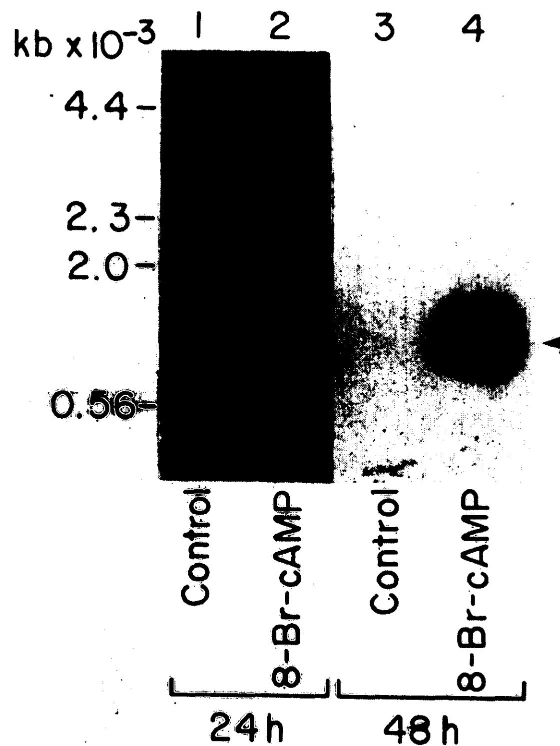

FIG. 6. Effects of 8-bromo-cAMP on hCGα mRNA levels in cultured cytotrophoblasts. Cytotrophoblast cultures were exposed to control medium or 1.5 mM 8-bromo-cAMP for 24 h (lanes 1 and 2) or 48 h (lanes 3 and 4), and the cells were then harvested for preparation of RNA. After electrophoresis and transfer of equal amounts (15 μg) of RNA from control and treated cells, the nitrocellulose filters were probed with a nick-translated ^{32}P-labeled cDNA for hCGα. The probe hybridized with a single RNA band of appropriate size [0.85 kilobase (Kb)] in both control and 8-bromo-cAMP-treated cells, but the signal was dramatically increased with 24 h of cyclic nucleotide treatment (lane 2). In a second experiment a marked increase in hybridizable hCG mRNA was found in cells exposed to 8-bromo-cAMP for 48 h (lane 4).

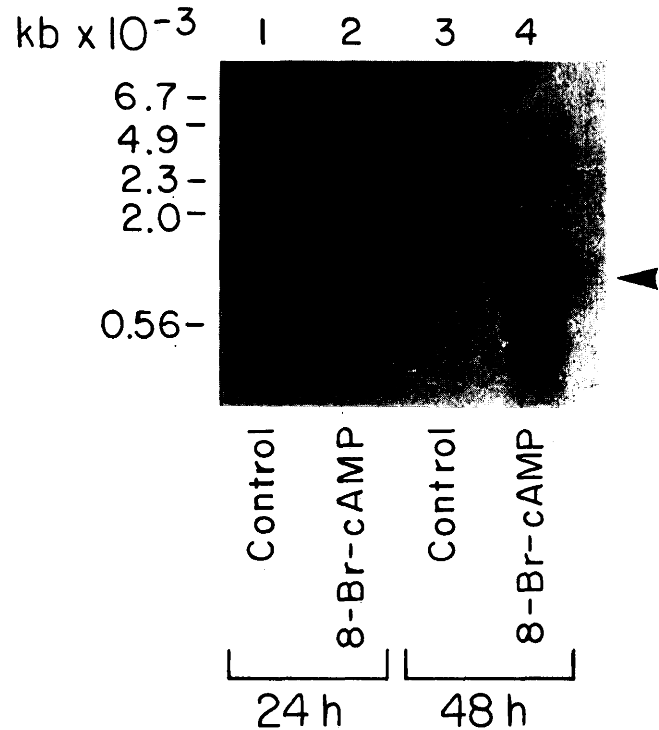

FIG. 7. Effects of 8-bromo-cAMP on hCGβ mRNA levels in cultured cytotrophoblasts. Cultures were exposed to control media (lanes 1 and 3) or 1.5 mM 8-bromo-cAMP for 24 (lane 2) or 48 h (lane 4). RNA was extracted, and equal amounts (10 μg) were electrophoresed and transferred to nitrocellulose paper. The filters were probed with a ^{32}P-labeled cDNA for hCGβ. The probe hybridized with a single RNA band of 1.05 kilobases (Kb) in samples from 8-bromo-cAMP-treated cells. Similar results were obtained in two other experiments.

tin also seems to play an important role in modulating cellular phenotype and metabolism, including adrenergic differentiation of neural crest cells (23), the morphology of cultured chondrocytes (24), and activation of genes involved in lipid synthesis in 3T3 adipocytes (25). In the latter system, fibronectin inhibits the expression of lipogenic enzymes. Hence, the reduction in elaboration of fibronectin caused by the cyclic nucleotide may facilitate the expression of genes directing endocrine activity of the trophoblast and the process of syncytium formation resulting from cell fusion. Immunohistochemical studies of human placenta suggest that actively replicating cytotrophoblasts elaborate fibronectin, whereas mature trophoblastic elements do not (26). These observations are consistent with the concept of an inverse relationship between fibronectin synthesis and differentiated function in the trophoblast.

cAMP affects synthesis of the proteins we studied by regulating levels of their mRNAs. The cellular content of mRNA encoding hCGα and hCGβ was markedly increased within 24 h of 8-bromo-cAMP treatment, whereas the rise in mRNA encoding fibronectin was prevented. The finding that hCGα and hCGβ mRNAs were increased is consistent with the recent report that cAMP analogs increase hCG subunit mRNAs in cultured choriocarcinoma cells (27). Moreover, cAMP analogs increase the expression of chloroamphenicol acetyltransferase encoded by a chimeric construct containing the 5′ flanking sequence of the α subunit gene transfected into a responsive recipient cell line (28). Thus, cAMP can activate the transcription of genes encoding placental peptides. Our findings clearly demonstrate that the cyclic nucleotide can also prevent or reduce the expression of specific mRNAs (*e.g.* fibronectin) while having a minimal effect on others (*e.g.* actin).

There is notable similarity between the effects of 8-bromo-cAMP on specific protein synthesis by cytotrophoblasts and the effects of tropic hormones on protein synthesis by ovarian granulosa cells. Immature granulosa cells secrete fibronectin, and hormonal (*e.g.* FSH) stimulation of these cells, which induces their differentiated functions, is associated with markedly reduced synthesis and secretion of this protein compared to control cells (29, 30). Moreover, we have also found that 8-bromo-cAMP stimulates the synthesis of adrenodoxin, a component of the cholesterol side-chain cleavage system, by cytotrophoblasts (31) just as in cultured granulosa cells (32, 33). These observations lead us to propose that the events that underlie the expression of endocrine activities of cytotrophoblasts and granulosa cells, two cell types that secrete both steroid and protein hormones required for establishment and maintenance of pregnancy, are similar if not identical.

Acknowledgments

We thank Dr. Irving Boime, Washington University (St. Louis, MO), for helpful discussions and Ms. Janet Brennar for her assistance in preparation of this manuscript.

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Important Announcement

Search for Editor-in-Chief of ENDOCRINOLOGY

After five years of devoted service to the Society, Dr. Nicholas S. Halmi will have completed his term as Editor-in-Chief of *Endocrinology* on December 31, 1987. The Society is indebted to Dr. Halmi and his Editors for an outstanding performance, which has maintained *Endocrinology* among the top scientific Journals.

The Publications Committee solicits your recommendations for individuals who might serve as Editor-in-Chief of *Endocrinology*. The office carries a budget for supporting a staff and an honorarium. Please send your recommendation, preferably with curricula vitae, to:

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