Stimulation by N\textsuperscript{6},O\textsuperscript{2}-Dibutyryl Cyclic Adenosine 3':5'-Monophosphate of Ectopic Production of the Free β Subunit of Chorionic Gonadotropin by a Human Brain Tumor Cell Line

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ABSTRACT

Previous studies have favored a basic difference in the regulation of specialized protein production by cells derived from the usual tissue of origin (eutopic) and cancer cells derived from a tissue not normally producing the protein (ectopic). Thus N\textsuperscript{6},O\textsuperscript{2}-dibutyryl cyclic adenosine 3':5'-monophosphate was believed to stimulate only eutopic (but not ectopic) chorionic gonadotropin production, and butyrate to stimulate only ectopic (but not eutopic). However, in CBT, a human brain tumor cell line, we find that N\textsuperscript{6},O\textsuperscript{2}-dibutyryl cyclic adenosine 3':5'-monophosphate, but not butyrate, stimulated ectopic production of the β subunit of chorionic gonadotropin. We conclude that neither butyrate nor cyclic adenosine 3':5'-monophosphate derivatives reliably discriminate ectopic from eutopic regulation.

INTRODUCTION

Regulation of protein production in eukaryotic cells is poorly understood. We do not know, for example, why normal placental trophoblast produces large amounts of the glycoprotein CG\textsuperscript{2} ("eutopic" production), whereas production of this protein by normal cells of other tissues ("ectopic" production) is barely or not at all detectable (2, 20). It has long been recognized that eutopic production of CG continues when trophoblastic cells become malignant (choriocarcinoma). Only in the past decade, however, have we become aware that, when certain nonplacental cells become malignant, they too may acquire the capacity for ectopic production of CG, or one (or both) of its α and β subunits (36). In fact, such ectopic production may occur at rates higher than those for eutopic production by cells of placental origin (26).

Recently, it has been suggested that there may be a consistent difference in the cellular regulation of ectopic versus eutopic protein production (12, 21, 23). Thus, dibutyryl cAMP stimulated eutopic production of CG or its subunits, or both, by 4 cell lines derived from choriocarcinomas (1, 13, 39); but butyrate had little or no effect (12, 21). On the other hand, ectopic production of these proteins was enhanced by butyrate, but little or not at all by the cAMP derivative, in a variety of nontrophoblastic cell lines. These include several HeLa lines (11, 18, 21, 27, 37), 2 lines derived from other carcinomas of the cervix (21), a line derived from a bronchogenic carcinoma (12, 13, 27, 37), and a line derived from an ovarian cystadenocarcinoma (23). If this difference between butyrate and dibutyryl cAMP were consistent, it could lead to advances in our understanding, particularly since recent work has suggested a specific regulatory mechanism for butyrate at the level of transcription (9, 31). Our experience with the CBT cell line reported below, however, suggests that butyrate and dibutyryl cAMP stimulation do not invariably distinguish ectopic from eutopic protein production.

MATERIALS AND METHODS

CBT is an established cell line derived from a human brain tumor (29). The patient, a 49-year-old woman, had no history of gestational neoplasm. The primary tumor had the appearance of glioblastoma multiforme, and the CBT cells produced a fibrosarcoma in athymic ("nude") mice. Neither the primary tumor nor the established CBT cell line, nor the tumor produced by CBT cells in athymic mice resembled choriocarcinoma morphologically.

Sodium butyrate was obtained from ICN Pharmaceutical Co. (Plainview, N. Y.). Dibutyryl cAMP was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Cells were grown at 37° in 25-sq cm flasks in 3 ml of Roswell Park Memorial Institute Medium 1640 containing 20% fetal calf serum, penicillin (30 μg/ml), streptomycin (50 μg/ml), gentamicin (50 μg/ml), and sodium butyrate, or dibutyryl cAMP, or control buffer. On each day, we counted cell number by hemocytometer, determined cell protein by the method of Lowry et al. (28), and measured CG-β by homologous double-antibody radioimmunoassay (42) in each of 3 flasks.

RESULTS

Neither free α subunit of CG, nor placental lactogen (41), nor pregnancy-specific β1-glycoprotein (22), another placental protein, were detected (<1.5 ng/ml) in these cultures. Material behaving immunochemically like CG-β, but differing from complete heterodimer CG, was detected in both media and control samples taken from the CBT cultures (Chart 1).

The secretion rate of CB-β by the CBT cells was increased.
3- to 7-fold by 1 mM dibutyryl cAMP but little, if at all, by 2 mM butyrate (Chart 2) or by 5 mM butyrate (data not shown). Radioimmunoassay of cell sonicates showed that the inability of butyrate to stimulate CG-β secretion was not due to a failure to secrete intracellular CG-β (Table 1). Replacement of the sodium butyrate by a less concentrated (0.2 mM) solution permitted almost normal cell multiplication but did not increase CG-β production, and addition of 1 mM theophylline to the medium did not enhance the stimulation afforded by dibutyryl cAMP alone (Table 1). CG-β production by the CBT cells was not stimulated by hexamethylene bisacetamide (Table 1), a known stimulator of procollagen production by these cells (29) as well as an inducer of hemoglobin production by Friend virus-transformed erythroleukemia cells (30). Dimethyl sulfoxide, another inducer of hemoglobin (17) and inducer of a nonhistone chromatin protein (24) in the erythroleukemia cells, also failed to enhance CG-β production by the CBT cells; in fact, CG-β production was decreased by this agent (Table 1).

**DISCUSSION**

Isolated or unbalanced production of CG-β has been reported both in vivo (3, 42) and in vitro (21, 35, 37, 40). Except for certain intracranial germ cell tumors, the production of which may be eutopic, we are unaware of in vivo CG-β production by brain tumors. One of 4 other brain tumor cell lines, also derived from a glioblastoma, produced CG-β (35); it might be worthwhile to screen a variety of brain tumors for such ectopic production.

Instances of isolated or unbalanced production of the α subunit have also been documented (16, 27, 34, 35, 37, 38). These data are consistent with the presence of separate mRNA's for each subunit. Indeed, recent experiments with isolation and translation of placental (4) and pituitary (19) mRNA, correlation of subunit production and chromosome loss from human-mouse hybrid cells (5), and, finally, isolation, clon-
ing, and sequencing of the complementary DNA for the α subunit (15) strongly support this model.

The failure of butyrate to enhance ectopic CG-β production by CBT cells differs from its stimulation of ectopic hormone production in most other (11-13, 18, 21, 23, 27, 37) but not all (21) cell lines examined. The finding that dibutylryl cAMP stimulated ectopic production of CG-β by a non-choriocarcinoma mesenchymal cell line is novel. These data suggest that neither butyrate nor dibutylryl cAMP should be considered a reliable "probe" in discriminating between eutopic and ectopic protein regulation.

Recently, small amounts of CG-like material have been found in testis (7), pituitary (10), other viscera (8, 43), urine (32), and serum (6) of persons not known to be pregnant or bearing a neoplasia. Material with CG-like properties has also been detected in occasional culture media of normal human fibroblasts (35); and another placental protein, pregnancy-specific βi-glycoprotein, has been found in virtually all fibroblast cultures examined (14, 33). Moreover, a number of proteins, believed earlier to be produced solely by pituitary and other noncerebral tissues, have now been demonstrated in normal brain (25). Thus, the distinction between eutopic and ectopic production is becoming increasingly blurred.

The CG in nonplacental sites differed from CG of placental origin not only in its very low concentration but also in its decreased affinity for concanavalin A (43). Although these may be due solely to regional differences in posttranslational processing, we cannot yet rule out differences in the regulation of transcription or translation, or even differences in the primary gene transcript. Study of cell lines such as CBT may yield further insight into these questions.

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