MODIFICATION OF PHOSPHOLIPID METABOLISM IN DIBUTYRYL-cAMP-MEDIATED MORPHOLOGICAL CONVERSION OF CHO CELLS

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SUMMARY: The cellular uptake and incorporation of [1-^{14}C]palmitic acid and ^{32}P into lipids of Chinese hamster ovary cells, clone K_1 (CHO-K_1) have been investigated under conditions where the cells are converted from the compact, epithelial-like shape to the elongated fibroblast-like morphology by $N^6,O^{2'}$-dibutyryl adenosine 3':5'-phosphate. The primary alteration in lipid metabolism accompanying the morphological conversion to the fibroblast form was an increased incorporation of lipid precursors into all phospholipid classes and a decreased incorporation into the "neutral" lipid fraction. These results reflect the cells' need for phospholipid precursors when the membrane expands to form the fibroblast shape. When the fibroblast-shaped cells were allowed to revert to the epithelial shape, lipid metabolism was similar to that found in untreated cells.

INTRODUCTION

Treatment of Chinese hamster ovary cells, clone K_1 (CHO-K_1) with $N^6,O^{2'}$-dibutyryl adenosine 3':5'-phosphate (Bt_2cAMP) converts the compact epithelial-like cells to a fibroblast-like shape (1-3). The morphological conversion of these cells is accompanied by biochemical changes that are opposite those found in fibroblasts transformed by viruses or carcinogenic agents (3). Treated cells exhibit restoration of contact inhibition of growth, a decrease in agglutinability with wheat germ agglutinin or concanavalin A, and an increase in collagen synthesis (2).

Because some of the changes are associated with modification of the surface membrane properties, we determined how lipid metabolism was affected by the morphological conversion. We found that the morphological conversion of CHO-K_1 cells to the fibroblast shape induced by Bt_2cAMP is accompanied by an elevated incorporation of [1-^{14}C]palmitic acid and ^{32}P into cellular phospholipids. This increased incorporation of precursors into phospholipids of the

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fibroblast-like cells correlates with the increase in membranes formed during the conversion.

MATERIALS AND METHODS

The sodium salts of Bt_2cAMP and adenosine 3′:5′-phosphate (cAMP) were obtained from P-L Biochemicals. We purchased [1-^{14}C]palmitic acid (54.3 mCi/mmol) and ^{32}P (disodium phosphate) from New England Nuclear.

In the [1-^{14}C]palmitic acid study, CHO-K_1 cells (4) were grown in plastic culture dishes (15 × 60 mm diam, Falcon Plastics) containing 5 ml of F12 medium (5) supplemented with 10%, dialyzed, heat-inactivated (56°C, 30 min) fetal calf serum (Pacific Biological Co.). In the ^{32}P experiments, the cells were cultured in flasks (75 cm^2 surface area, Falcon Plastics) containing 10 ml of medium. All cultures were initiated at a density of 1 to 2 × 10^5 cells per dish or flask, allowed to develop for 48 to 72 hr to approximately 2 to 3 × 10^6 cells per culture, and then replenished with fresh prewarmed medium with or without 1 mM Bt_2cAMP just before adding the labeled precursors.

Cell numbers on duplicate cultures were determined using a Coulter counter. All incubations were at 37°C in a 100% humidified atmosphere containing 5% CO_2. Morphology of cells was routinely examined by light microscopy.

Lipids were extracted by the method of Bligh and Dyer (6), except the methanol contained 2% glacial acetic acid. Phospholipids were separated by thin-layer chromatography on Silica Gel HR in chloroform-methanol-acetic acid-water (50:25:8:4, v/v) and "neutral" lipids were analyzed on Silica Gel G layers developed in hexane-diethyl ether-acetic acid (80:20:1, v/v) (7). The distribution of radioactivity on the chromatogram was determined (8).

RESULTS

The cellular uptake of [1-^{14}C]palmitic acid in CHO-K_1 cells grown in the presence of Bt_2cAMP (1 mM) for 24 hr was considerably higher (∿39%) than untreated cells (Table I). Significantly more of the label was found in phospholipids and much less in the "neutral" lipid fraction of the treated cells. Bt_2cAMP did not affect the distribution of ^{14}C among the phospholipid classes. The major labeled lipids in both the control and treated cultures were phosphatidylcholine (67%), phosphatidylethanolamine (15%), sphingomyelin (11%), and phosphatidylserine plus phosphatidylinositol (6.6%). Bt_2cAMP decreased the incorporation of [1-^{14}C]palmitic acid into the "neutral" lipid fraction, e.g., triacylglycerols (55% of controls), alkyldiacylglycerols (62% of controls), and the cholesterol ester-wax fraction (94% of controls). cAMP, which causes no morphological change, caused a slight increase in the cellular uptake of [1-^{14}C]palmitic acid in 24-hr cultures, but it did not alter its incorporation into "neutral" lipids (Table I).

TABLE I

EFFECT OF Bt_2cAMP AND cAMP ON THE DISTRIBUTION OF [1-^{14}C]PALMITIC ACID IN LIPIDS OF CHO-K_1 CELLS

Additions	^{14}C-Activity relative to controls		
	Cellular uptake into total lipids	Phospholipids	"Neutral" lipids
Bt_2cAMP (1 mM)	139	159	84
cAMP (1 mM)	115	122	98

Cultures were labeled for 24 hr with [1-^{14}C]palmitic acid in the presence or absence of the additions. The values in this table represent the average of duplicate samples and are typical of results obtained in other incubations carried out under identical conditions. The relative values are based on the following dpm per 10^6 cells for the controls: total lipids (383,000-443,000), phospholipids (278,000-324,000), and "neutral" lipids (105,000-119,000). Control values equal 100 in the table.

Cultures incubated with 1 mM Bt_2cAMP for 12 hr were pulse labeled with ^{32}P for 40 min in the presence or absence of Bt_2cAMP in the labeling medium. The data presented in Table II indicate that fibroblast-like cells had a 1.4 times higher cellular uptake of ^{32}P than the epithelial-like cells, with or without Bt_2cAMP present in the media. The quantity of ^{32}P incorporated into phospholipids of fibroblast-like cells under these conditions was approximately twice that of epithelial-like controls. Cells treated with 1 mM cAMP showed no alterations in their morphology or the cellular uptake and metabolism of ^{32}P.

Morphologically converted fibroblast-like cells that were allowed to revert to the epithelial-like morphology by removal of Bt_2cAMP, followed by incubatior in fresh medium for 12 hr, exhibited reversal of the cellular uptake and incorporation of ^{32}P into lipids to levels found in untreated cultures. The data in Table III indicate that ^{32}P uptake by the morphologically "reverted" epithelial-like cells was similar to the untreated epithelial-like controls. Incorporation of ^{32}P into phospholipids of the "reverted" epithelial-like cells was 13% higher than the controls. On the other hand, both cellular uptake and the incorporation of ^{32}P into lipids were lower in the "reverted"

TABLE II

^{32}P INCORPORATION INTO PHOSPHOLIPIDS OF THE FIBROBLAST- AND EPITHELIAL-LIKE FORMS OF CHO-K_1 CELLS

Cell shape	Bt_2cAMP (1 mM)	Total cellular uptake	Total phospholipids	
			dpm/10^6 cells	Percent incorporated
Fibroblast-like	+	274,000 ± 19,100	1,833 ± 48	0.67
	-	277,000 ± 900	1,908 ± 57	0.69
Epithelial-like	+	202,000 ± 660	928 ± 15	0.46
	-	197,000 ± 10,000	910 ± 76	0.46

Fibroblast-like cells were induced by incubating cultures for 12 hr in the presence of Bt_2cAMP (1 mM). Values in the table represent averages from duplicate incubations ± the range of the deviation from the mean. Fibroblast-like cells (2.74×10^6/flask) and epithelial-like cells (3.03×10^6/flask) were allowed to incorporate ^{32}P (10.9 µCi/ml) in 5 ml medium for 40 min in the presence (+) or absence (-) of Bt_2cAMP.

epithelial-like cells, 23% and 36% less, respectively, when compared to the fibroblast-like controls. The major portion of ^{32}P in the phospholipids was in phosphatidylcholine (47-75%), phosphatidylserine plus phosphatidylinositol (5-31%), and phosphatidylethanolamine (5-25%). Although variation in the distribution of ^{32}P existed between different batches of cell cultures, there was always an increased incorporation of ^{32}P in the total phospholipid fraction of the fibroblast-like cells. Increased incorporation of ^{32}P into the phosphatidylserine plus phosphatidylinositol fraction always was associated with a decrease in the ^{32}P incorporated into phosphatidylethanolamine.

DISCUSSION

Our results show that the morphologically converted fibroblast-like cells induced by Bt_2cAMP exhibit increased incorporation of labeled precursors into phospholipids. With palmitic acid, the increased incorporation was consistently preceded by an approximate 4-hr lag in cells undergoing the process, as well as in cells that had undergone complete morphological conversion. Since only cells in the G_1 phase of the cell cycle are responsive to morpho-

TABLE III

EFFECT OF REVERSAL OF MORPHOLOGICAL CONVERSION ON ^{32}P INCORPORATION INTO PHOSPHOLIPIDS OF CHO-K_1 CELLS

Cell shape	Total cellular uptake	Total phospholipids	
		dpm/10^6 cells	Percent incorporated
Epithelial-like[a]	223,000 ± 9,730	1,982 ± 144	0.89
Fibroblast-like[b]	285,000 ± 9,960	3,481 ± 207	1.22
Reverted epithelial-like[c]	220,000 ± 3,860	2,238 ± 60	1.02

[a]Untreated control (36 hr old); [b]cells from *a* were treated with Bt_2cAMP (1 mM) for 12 hr; [c]cells from *b* were in medium without Bt_2cAMP for 12 hr. Cells were labeled with ^{32}P (10.7 μCi/ml) in 5 ml medium for 40 min without Bt_2cAMP. Values in the table represent averages obtained from duplicate incubations ± the range of the deviation from the mean. See text for ^{32}P distribution in phospholipid classes.

logical effect of Bt_2cAMP (9), it may require at least 4 hr for a significant fraction of cells to exhibit the manifested changes observed in lipid metabolism.

The presence of 1 mM Bt_2cAMP in the medium during the labeling period had no effect on the cellular uptake or incorporation of lipid precursors in cells that were maintained in the epithelial-like and fibroblast-like forms. These findings indicate that the increased incorporation of the precursors into phospholipids of the fibroblast-like cells is not associated with the initial quiescence of cell movement and membrane ruffing caused by Bt_2cAMP at the cell surface. It is of interest that when the morphologically converted fibroblast like cells are allowed to revert to the epithelial-like morphology, the cells again exhibit a decreased uptake and incorporation of ^{32}P that is comparable to the untreated control levels. The ^{32}P experiment, together with the experiments on palmitate incorporation into lipids, including the observed lag period, is consistent with the notion that the temporal relationship is most pronounced only in fully converted fibroblast-like cells and not with either the initiation or the early process of morphological conversion. Our data

with cAMP and its essential lack of an effect on lipid metabolism in CHO-K_1 cells appear to be consistent with its relative inactivity in morphological conversion (10).

Porter et al. (11) estimated the average length of the control, untreated CHO cells to be 15 μm and that of "reverse transformed" cells to be 85 μm. Also, Hsie et al. (10) calculated a sizable volume increase in the morphologically converted fibroblast-like cells (1.9 μl/10^6 cells) compared to epithelial-like cells (1.6 μl/10^6 cells). Thus, the increased uptake of lipid precursors into phospholipids during and after morphological conversion of the CHO cells to the fibroblast shape would appear to be associated with the increase in cell size and membrane pool that accompanies morphological conversion.

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