CYCLIC AMP-INDUCED DIFFERENTIATED NEUROBLASTOMA CELLS: CHANGES IN TOTAL NUCLEIC ACID AND PROTEIN CONTENTS

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SUMMARY: The total DNA contents of neuroblastoma cells"differentiated"by dibutyryl cyclic AMP, prostaglandin E_1 and 4-(-3-butoxy-4-methoxybenzyl)-2-imidazolidinone treatment was about 50 percent that of control cells, indicating that cells were accumulated in the G_1-phase of the cell cycle. Sodium butyrate-treated cells were also accumulated in the G_1-phase; however, the expression of"differentiated"phenotype did not occur indicating that inhibition of cell division is not sufficient for morphological differentiation. A marked increase in RNA and protein contents of cyclic AMP-induced "differentiated" cells is consistent with an increase in the size of soma and nucleus.

INTRODUCTION

Cyclic AMP may be involved in the morphological"differentiation"of mouse neuroblastoma cells in culture. This is shown by the fact that dibutyryl cyclic AMP, prostaglandin E_1 (stimulator of adenylate cyclase) and 4-(-3-butoxy-4-methoxybenzyl)-2-imidazolidinone (inhibitor of cyclic AMP phosphodiesterase), cause morphological"differentiation"of neuroblastoma cells (1 - 3). The expression of the differentiated phenotype requires the assembly of microtubules and microfilaments and the synthesis of new protein, but not the synthesis of RNA (4). Cyclic AMP-induced"differentiated"cells lose tumorgenecity (5) and have elevated levels of cyclic AMP (unpublished observation), cyclic AMP phosphodiesterase (6), tyrosine hydroxylase (7), acetylcholinesterase (8), choline acetyltransferase (9); however, they do not show any change in the catechol-o-methyltransferase level (10). A marked increase in the size of soma and nucleus is seen during cyclic AMP-induced"differentiation"of neuroblastoma cells (1 - 3); therefore, changes

in the content of nucleic acid and protein are investigated. This paper shows that the total DNA contents of cyclic AMP-induced"differentiated"cells markedly decrease but total RNA and protein contents significantly increased. The pronounced reduction in the DNA content per cell is interpreted as evidence that most of the cells accumulate in the G_1-phase of the cell cycle.

MATERIAL AND METHODS

The procedures for culturing and maintenance of mouse neuroblastoma cells were described previously (8). A cholinergic clone NBE^- containing high choline acetyltransferase (ChA) but no tyrosine hydroxylase (11) was used in this study. N^6-O½-dibutyryl adenosine 3',5' cyclic monophosphate and 4-(-3-butoxy-methoxybenzyl)-2-imidazolidinone caused irreversible morphological differentiation in this clone provided the drug was allowed to remain in the medium for at least 3 days (11). This clone was relatively insensitive to prostaglandin E_1 in causing morphological differentiation. Cells (0.5 x 10^6) were plated in large Falcon plastic flasks (75 cm^2) and dibutyryl cyclic AMP (0.5 mM), sodium butyrate (0.5 mM), prostaglandin (PG) E_1 (10 ug/ml) or 4-(-3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO20-1724, 200 ug/ml) was added to flask 24 hours after plating. Medium was changed at day 2 and drug continued. A cell suspension was prepared 3 days after treatment using 0.25 percent viokase solution. An aliquot was used for determining cell number in the Coulter counter and the remaining sample was used for nucleic acid and protein assay. Nucleic acids were extracted by the method of Schneider (12). The DNA content was determined by the diphenylamine method of Burton (13), RNA content was determined by the Orcinol method of Cerrioti (14), protein content was determined by the method of Lowry et. al. (15). The data were expressed as pg/DNA/cell, pg RNA/cell and pg protein/cell.

RESULTS AND DISCUSSION

Table I shows that the differentiated cells induced by dibutyryl cyclic

TABLE I

Total DNA, RNA and protein contents in cyclic AMP-induced differentiated mouse neuroblastoma cells in culture

Treatment	DNA (pg/cell)	RNA (pg/cell)	Protein (pg/cell)
Control	13.3 ± 1.5*	15.3 ± 1.0	500 ± 29
DBcAMP	6.6 ± 0.6	33.6 ± 2.5	1580 ± 122
PGE_1	6.0 ± 1.6	24.4 ± 1.9	870 ± 47
R020-1724	6.7 ± 1.2	33 ± 1.8	1016 ± 54
Na butyrate	5.3 ± 1.0	31.2 ± 3.9	1479 ± 111

*Standard deviation

Cells (0.5×10^6) were plated in large Falcon plastic Flask (75 cm^2) and dibutyryl cyclic AMP (DBcAMP, 0.5 mM), prostaglandin (PG) E_1 (10 ug/ml), 4-(-3-butoxy-4-methoxybenzyl)-2-imidazolidinone (R020-1724, 200 ug/ml), and sodium butyrate (Na butyrate, 0.5 mM) were added separately 24 hours later. The total nucleic acid and protein contents were assayed 3 days after treatment. Each value represents an average of 4 to 6 samples.

AMP, PGE_1 and R020-1724 have DNA contents about 50 percent of control cells. This is interpreted to mean that the differentiated cells are in G_1-phase of the cell cycle. In asynchronous cell population, S-phase (late), G_2-phase and mitotic cells (before division) have about twice the amounts of DNA of those which are found after cell division (G_1 and early S-phase). Therefore, if "differentiated" cells accumulate in the G_1-phase of the cell cycle, the amount of DNA will be less than that of control cells distributed randomly throughout the cycle. Butyric acid, a degradative product of dibutyryl cyclic AMP in solution, inhibit cell division without causing morphological differentiation (1); however, the DNA content of butyric acid (sodium salt) treated cells is also about half of control cells, indicating that these cells are also blocked in the G_1-phase of the cell cycle. The total RNA and protein

contents of cyclic AMP-induced differentiated cells are about two-fold higher than in the controls (Table I). This is consistent with the fact that the size of nucleus and soma increases during differentiation of neuroblastoma cells. An adrenergic clone, $NBA_2(1)$ which contains an extremely low level of tyrosine hydroxylase but lacks ChA also shows changes in nucleic acid and protein contents similar to those observed in the cholinergic clone. The data presented here show that cyclic AMP-induced"differentiated"cells accumulate in the G_1-phase of the cell cycle. Sodium butyrate also block cells in the G_1-phase, but does not allow the expression of differentiated phenotype. This indicates that the inhibition of cell division is not sufficient for morphological differentiation as suggested elsewhere (16, 17, 18). Previous studies (1 - 3, 19, 20) also support the above suggestion.

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