CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE LEVELS IN NORMAL AND TRANSFORMED CELLS OF HIGHER PLANTS

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SUMMARY

Cyclic adenosine 3':5'-monophosphate (cAMP) concentrations were determined for various normal and transformed (crown-gall) plant tissues grown in sterile culture. No significant differences in cAMP concentrations were found between normal and transformed cells of Vinca rosea, Helianthus annuus, and Nicotiana tabacum, unlike the suppressed synthesis observed in transformed cells of mammalian systems. cAMP concentrations of these tissues in culture averaged 135 nanomolar. No correlation was found between cAMP concentrations and tissue culture generation times.

INTRODUCTION

Recently a number of reports have indicated that cyclic adenosine 3':5'-monophosphate has a regulatory function during growth of mammalian cells in vitro (1-3). cAMP levels are higher in slowly growing cells than in the more rapidly dividing mitotic cells (2, 4), and the addition of N^6-2^1-O-dibutyryl-cAMP (db-cAMP) (5) or prostaglandin PGE_1 (6) which activate adenyl cyclase (7), decreases the growth rate. Also, fibroblasts transformed by either viral

or chemical agents regain the morphological and physiological characteristics of non-transformed cells when exposed to either db-cAMP (8) or db-cAMP plus theophylline (9). cAMP plays a central role as a "mediator-messenger" for a number of hormones and cellular processes in animals (10). An accumulating body of evidence indicates that cAMP is also present in plant cells (11-18); further, this nucleotide may be involved in processes mediated by phytohormones such as gibberellins (19-24) and auxins (24-26). A plant cell division promoting factor, cytokinesin I, has also been reported which inhibits cAMP-phosphodiesterase activity in vitro (27). Cultured crown gall tumor cells contain a cytokinesin not found in normal cells (28) yet there is no information available concerning cAMP levels and their possible relation to growth control in these cells. Therefore, the purpose of this study was to determine the concentration of cAMP in normal and tumor plant cells in culture preliminary to evaluating the potential role of this nucleotide in plant cell transformation.

MATERIALS AND METHODS

Tissue cultures. The original clones of normal and crown gall tumor tissue cultures were obtained from Dr. A. C. Braun, Rockefeller University, New York, Dr. R. Manasse, Boyce Thompson Institute, Yonkers, New York, Dr. J. E. DeVay, University of California, Davis, and from our own collection.

Sterile callus cultures of normal and crown gall tumor tissues of Vinca rosea, Helianthus annuus, and Nicotiana tabacum cv. Havana were grown on agar media referred to under Table I.

Extraction and assay of cyclic AMP. The extraction procedure for cAMP was similar to that described by Steiner et al. (29). Tissue aliquots were quickly frozen by pressing them into thin layers between blocks of solid CO_2. The frozen tissue preparations, checked previously for bacterial contamination in medium 523 (30), were then ground in a mortar with ice cold 6% (w/v) tri-

chloroacetic acid (TCA) at 40 mg fresh tissue weight per ml TCA. TCA was removed by repeated extractions with anhydrous diethyl ether, and the extracts were clarified by centrifugation at 10,000 x g for 20 min at 4°C. The supernatant was heated at 80° for 2 min, centrifuged to remove any particulate matter, and then lyophilized. The lyophilized extract was resuspended in 0.05 M sodium acetate buffer, pH 6.2 and cAMP concentrations were determined by the assay of Steiner et al. (29).

RESULTS AND DISCUSSION

The results of these analyses are shown in Table 1. In contrast to studies with mammalian cells, no consistent differences is seen between cAMP concentrations in normal and tumorous cell lines. Likewise, there is no correlation between cAMP concentrations and the growth rate of the cell lines. Four types of assays have been used previously to detect cAMP in plant extracts: a) exposure of tissues to radioactive adenine followed by chromatographic analysis of the extracted labeled nucleotides (14, 15, 17), b) conversion of extracted adenine nucleotides by phosphodiesterase to AMP and then to ATP (11, 18); c) competition between extracted cAMP and exogenous radioactive cAMP for sites on a cAMP-binding protein (12); and d) stimulation of protein kinase activity (13, 18). Quantitative estimations of cAMP concentrations in higher plants have been made from the latter three assays and ranged from less than 1 to more than 900 nM (11, 12, 13, 18). We have employed the highly sensitive and specific radioimmunoassay of Steiner et al. (29) and found that the cAMP concentration in normal and tumor callus tissues from three plant species was consistently about 100 nM. In addition, preliminary experiments have shown that cAMP concentrations in leaf, stem, and root tissues of H. annuus are similar to those found in callus cultures in contradistinction to the report of 900 nM cAMP in Chinese cabbage leaves (18).

Zeatin and kinetin, two adenine derivatives having phytohormone properties, did not show any cAMP-like activity in the radioimmunoassay between concentra-

TABLE 1 Levels of cAMP and Generation Time in Normal and Crown Gall Tumor Tissues

Plant species	Tissue type	Transformed by bacterial strain	Phytohormones and cytokinins required	Growth medium	Original source of tissue	Generation time (days)	cAMP (nM)
Vinca rosea	normal	none	yes	a	g	26	163 ± 17.8
"	normal	none	yes	b	h	7.7	125 ± 23.1
"	habituated	none	no	b	i	7.5	141 ± 19.7
"	tumor	BP	no	c	h	19	147 ± 20.3
"	tumor	A6	no	c	h	29	124 ± 25.1
"	tumor	B6	no	b	i	5.4	95 ± 12.2
Helianthus annuus	normal	none	yes	d	j	7.8	131 ± 21.3
"	tumor	6121	no	d	j	37	159 ± 18.8
"	tumor	6121	no	d	j	13	128 ± 20.2
Nicotiana tabacum (var. Havana)	normal	none	yes	e	g	6.1	142
"	tumor	B6	no	f	g	6.7	131

cAMP concentrations, (nM) determined in triplicate by the method of Steiner et al. (29), represent average values with their standard error of the mean of 4 experiments. Only one experiment was done for N. tabacum. Reagents for the assay were purchased as a kit from Schwarz Mann Bioresearch Corp., Orangeburg, N.Y. Tissue fluid volumes were calculated from fresh weight determinations corrected for dry weight after incubation at 120° for 24 hr in vacuo. Generation times were determined from dry weight measurements taken during a 25-day time period. a, 2 times White's basal medium (32) except 6 mM $MgSO_4$, 0.058 mM $MnSO_4$, 27.8 mM $FeSO_4$, 37 mM Na_2 EDTA, 2.48 mM $Ca(NO_3)_2$ 4 H_2O, 0.274 mM NaH_2PO_4, 0.0188 mM $ZnSO_4$, 1.14 mg/L 2,4 dichlorophenoxyacetic acid, 4% sucrose; b, Braun and Wood (33); c, White (32); d, Hildebrandt et al. (34) except 2.5 mg/L nicotinamide; 2.5 mg/L myoinositol; 0.5 mg/L indoleacetic acid, 20% v/v coconut milk; e, Murashige and Skoog (35) except 1 mg/L gibberellic acid, 3 mg/L indoleacetic acid, 3 mg/L kinetin; f, Murashige and Skoog (35) without auxins and cytokinins; g, Dr. C. I. Kado, University of California, Davis, Ca.: h, Dr. A. C. Braun, The Rockefeller University, New York, N.Y.; i, Dr. R. Manasse, Boyce Thompson Institute, Yonkers, N.Y.; j, Dr. J. E. DeVay, University of California, Davis, Ca.

tions of 2×10^{-8} M and 2×10^{-7} M. Similarly neither compound, at concentrations between 10^{-7} M and 10^{-4} M, competed in the assay with 10^{-9} M cAMP. Furthermore, neither zeatin, at concentrations between 10^{-7} M and 10^{-3} M nor kinetin

between 10^{-7} M to 10^{-2} M, exhibited cAMP-like activity in the cAMP-dependent protein kinase assay (31).

No difference in cAMP levels were found in V. rosea tumor cells induced by A. tumefaciens B6 when grown in a minimal salts medium (32) or a phytohormone supplemented medium (33). Thus, different growth media used to achieve optimal growth of the tissues were not a factor in these studies.

In summary, the results of this study show that cAMP concentrations are similar in tumor and normal cells irrespective of their apparent growth rate. Since these data were obtained from cells growing asynchronously, small transitory changes in cAMP concentration during the cell cycle could remain undetected. The details of this possibility await the provision of further experimentation.

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