DECREASED BASAL CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE LEVELS IN MORRIS HEPATOMA 5123 t.c.(h)

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SUMMARY

The basal and glucagon-stimulated cyclic adenosine 3',5'-monophosphate levels of Morris hepatoma 5123 t.c.(h) have been compared to those of rat liver. Basal levels of this nucleotide were found to be significantly lower in the hepatoma than were those of liver when compared on either a wet weight, dry weight or protein basis. These results are analogous to the findings of numerous studies on transformed cells in culture; they are, however, also unique in that this is the only known in vivo study reporting substantially reduced cyclic adenosine 3',5'-monophosphate levels in a solid, transplantable hepatoma. Administration of glucagon to tumor-bearing rats produced only a 2-fold increase in the hepatoma cyclic adenosine 3',5'-monophosphate levels compared to a 37-fold rise in liver, suggesting that this tumor's adenylate cyclase is less responsive to glucagon stimulation than is that of liver.

INTRODUCTION

A number of recent studies indicate that cAMP* is involved in regulating cell growth and proliferation. For example, it has been shown that transformed mammalian cells in culture have lower basal levels of cAMP than do corresponding normal cells (1-3); further, when cAMP levels in transformed cells are raised either by adding exogenous cAMP (or its dibutyryl derivative) or by the presence of phosphodiesterase inhibitors such as theophylline, the altered growth and proliferative characteristics revert to those of untransformed cells i.e. growth becomes regulated, the abnormally high proliferation rate decreases to normal and, contact inhibition and differentiation are restored (1-7).

*Abbreviation for cyclic adenosine 3',5'-monophosphate.

Since cancer cells in vivo resemble transformed cells in culture with respect to the altered properties mentioned above, the above findings suggest that transformation of cells to the malignant state is associated with reduced cellular cAMP levels. The applicability of this hypothesis to solid, transplantable tumors in vivo has been questioned recently (8-10) since these workers did not find any appreciable reduction in cAMP levels of the hepatomas studied.

To further investigate the validity of the above hypothesis to in vivo tumors, the cAMP levels, determined by the radioimmunoassay method (11), were compared for Morris hepatoma 5123 t.c.(h) and liver. In addition, the effect of glucagon on cAMP levels was studied to determine the relative in vivo responsiveness of this tumor's adenylate cyclase system to this hormone. The results indicate that the basal cAMP levels of this hepatoma are significantly lower than those of liver and that this tumor is substantially less responsive to glucagon than is liver.

MATERIALS

The animals used in this study were adult male Buffalo strain rats (200-350 g) obtained from Simonsen Laboratories, Gilroy, California. These animals were housed in air-conditioned, windowless rooms with regulated light cycles from 7:30 a.m. to 7:30 p.m.. Food and water were available ad libitum except for the glucagon experiments in which all food was withheld for 18 hours prior to the experiment.

The tumor, designated as Morris hepatoma 5123 t.c.(h), has been carried in our laboratory by serial subcutaneous inoculations of saline-hepatoma cell suspensions into the inguinal region of the animal. This tumor was derived from the hepatoma 5123 t.c. subline initially provided by Dr. H.P. Morris (12). Although the average

growth rate (5 weeks) of 5123 t.c.(h) has not been altered appreciably during the numerous generations in our laboratory, its cAMP levels differ markedly from the parent subline (10); for this reason the designation for our hepatoma has been modified.

The cAMP radioimmunoassay kits (Schwarz-Mann) were obtained from Picker Nuclear, Montreal, Canada. The glucagon (crystalline porcine) was generously supplied by Dr. R.J. Hosley of the Eli Lilly Co., Indianapolis, U.S.A.. The solution for subcutaneous injection consisted of 0.8 mg glucagon per ml of 0.154 M NaCl (pH 3).

METHODS

The rats were sacrificed either by decapitation or by ether anesthesia since it was found that similar cAMP levels were obtained using either method. Portions of liver or non-necrotic hepatoma tissue were rapidly removed and frozen in liquid nitrogen. Known weights of frozen tissue (10-25 mg) were homogenized in 1.5 ml of ice-cold TCA (6%) and centrifuged at 2,400 x g for 25 minutes at 4°C. The supernatant was extracted three times with 5 ml of diethyl ether (water saturated) and evaporated to dryness under nitrogen at 65°C. The resulting residue was suspended in an appropriate volume of sodium acetate buffer (0.05 M, pH 6.2) for assay; the volume of dilution was dependent upon the expected concentrations of cAMP in the residue. The cAMP content was determined by the radioimmunoassay method developed by Steiner et al (11).

Glucagon was administered subcutaneously at a dose of 0.4 mg per 100 g body weight and the animals sacrificed 15 minutes after injection (8, 13).

Protein was determined using the method of Lowry et al (14).

RESULTS AND DISCUSSION

As shown in Table I, the basal cAMP levels of Morris hepatoma

5123 t.c.(h) are significantly lower than host liver ($p \ll 0.001$) and normal liver ($p < 0.001$) when compared on either wet weight, dry weight or protein basis. These findings are in agreement with the tissue culture studies, but are at variance with those recently reported for other hepatomas (8-10). It is of particular interest that the cAMP levels of the tumor used in the present study are substantially less than those reported by Thomas et al (10) for its parent subline, 5123 t.c.; this difference cannot be attributed to the use of different cAMP assay methods since the concentrations of this nucleotide in liver compare favourably with those reported by these workers. In view of these divergent cAMP levels in hepatomas, can the hypothesis that "malignant transformation of cells is associated with reduced cAMP levels" be considered to be valid for solid tumors in vivo? A deficiency of cAMP appears to be associated with at least some in vivo tumors, since it has been shown that the growth of a mouse lymphosarcoma (15), a rat carcinosarcoma (16), rat mammary tumors (17) and a human epidermoid carcinoma (18) can be suppressed by administered cAMP, dibutyryl cAMP or theophylline. However, additional studies of this type are required for other in vivo tumors in order to establish whether the elevation of cellular cAMP levels with these agents will suppress tumor growth in general, or whether only the tumors having abnormally low cAMP levels respond to treatment with cAMP or phosphodiesterase inhibitors. If tumors having cAMP levels that are as high or higher than those of the normal counterpart are also suppressed by these agents, this may indicate: (a) that these tumors have a deficiency of "available" cAMP which could be due, for example, to alterations in cAMP-binding to cellular components (19) or alternatively: (b) that these neoplasms have a corresponding increase in a cellular substance that is antagonistic to the actions of cAMP.

Table I. Basal and glucagon-stimulated cAMP levels in Morris hepatoma 5123 t.c.(h) and rat liver.

cAMP levels*	Basal			After glucagon	
Tissue	Normal liver	Host liver	Hepatoma	Host liver	Hepatoma
Wet weight	0.64** ± 0.07*** (11)	0.73 ± 0.03 (16)	0.27 ± 0.02 (18)	27.30 ± 3.23 (7)	0.58 ± 0.05 (12)
Dry weight	2.18 ± 0.24 (11)	2.61 ± 0.11 (16)	1.31 ± 0.10 (18)	97.73 ± 11.56 (7)	2.81 ± 0.24 (12)
Protein	3.79 ± 0.41 (11)	4.37 ± 0.18 (16)	2.37 ± 0.18 (18)	163.53 ± 19.35 (7)	5.09 ± 0.44 (12)

*Picomoles of cAMP per mg wet weight, dry weight or protein; all samples were run in duplicate.

**Standard error.

***Number of animals.

An example of such a substance is cyclic guanosine 3',5'-monophosphate, which is believed to promote cellular events that are antagonistic to those mediated by cAMP (20). In summary, the differences observed above for hepatoma cAMP levels do not invalidate the foregoing hypothesis, however they do suggest that malignant transformation *in vivo* may be associated with a net decrease in availability or efficacy of cAMP rather than a reduction of cAMP levels *per se*.

The results shown in Table I also indicate that the *in vivo* responsiveness of hepatoma 5123 t.c.(h) adenylate cyclase-cAMP

system to glucagon is much less than that of liver; these findings are in general agreement with other *in vivo* (8) and *in vitro* studies (21, 22). It would appear that the glucagon responsiveness of hepatomas depends on at least two factors: first, the tumor growth rate ie. the more rapid the growth the less responsive the tumor cAMP system is to glucagon (21); a second factor is the tumor transport capability for certain amino acids such as ∝-amino-isobutyrate ie. hepatomas with high aminoisobutyrate distribution ratios are poorly responsive to glucagon stimulation (8).

The relationship between tumor responsiveness to endogenous substances affecting the cAMP system (eg. glucagon, prostaglandins, etc.) and parameters such as growth rate, differentiation, amino acid transport and malignancy is, however, poorly understood at the present time.

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