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Urinary Excretion of Cyclic Guanosine 3′:5′-Monophosphate and Cyclic Adenosine 3′:5′-Monophosphate in Rats Bearing Transplantable Liver and Kidney Tumors[1]

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SUMMARY

Cyclic guanosine 3′:5′-monophosphate (cyclic GMP) and cyclic adenosine 3′:5′-monophosphate were measured in the urine of normal rats and those bearing transplantable liver and kidney tumors. The level of cyclic GMP ranged from 1.4 to 1.6 μmoles/g urinary creatinine in several strains of rats without tumors. Rats bearing Morris hepatomas 20, 21, 9618A and 9633F and kidney tumor MK2 had urine levels of cyclic GMP from 1.3 to 3.6 μmoles/g creatinine. Rats bearing the fast growing hepatomas 9618A2 and 3924A and Morris kidney tumor MK3 had urinary values of 5.6, 41.9, and 32.7 μmoles cyclic GMP per g creatinine, respectively. Urine levels of cyclic adenosine 3′:5′-monophosphate ranged from 10.1 to 19.7 μmoles/g creatinine in all normal and tumor-bearing rats and were not significantly different in any of the groups examined.

INTRODUCTION

Cyclic AMP[4] and cyclic GMP are quite ubiquitous in nature and have been found in most tissues and extracellular fluids. In spite of their general distribution and alteration with a variety of hormones, neurohormones, and drugs, levels of these nucleotides in extracellular fluids, particularly urine and plasma, have been useful in diagnosing some disorders and studying their pathophysiology (1, 2, 5, 8, 10, 11, 14, 16, 17, 20, 23). Urine levels of cyclic AMP and/or cyclic GMP are altered with some benign or neoplastic tumors, such as those associated with hyperparathyroidism (10, 14, 17, 20), ectopic parathyroid hormone-secreting tumors (10, 14), tumors associated with the syndrome of inappropriate antidiuretic hormone secretion (14), carcinoid tumors, pheochromocytoma (F. Murad, unpublished observations), and Cushing's disease (23). Hormonal derangement has been the explanation for altered cyclic nucleotide metabolism in all of these disorders.

Studies with cell cultures and several *in vivo* tumors have suggested that changes in cyclic AMP and cyclic GMP metabolism are associated with altered rates of cell growth and differentiation (12, 13, 15, 18, 21, 22). Levels of both cyclic nucleotides are increased in various *in vivo* hepatomas (21). This laboratory has also recently reported that urinary cyclic GMP is increased in rats bearing Morris hepatoma 3924A (15). The excretion of cyclic GMP in these animals decreased with radiotherapy, chemotherapy, or excision of tumor and correlated well with tumor size and growth. Thus, in this tumor model and perhaps in others, urinary cyclic GMP should prove to be a useful index of tumor size and its response to various agents. In this report we examined cyclic GMP and cyclic AMP excretion in rats with other transplantable liver and renal tumors to determine if this phenomenon was generally seen or specifically associated with some varieties of tumor. We found that urinary cyclic GMP, but not cyclic AMP, increased progressively with the growth of several but not all transplantable liver and kidney tumors studied.

MATERIALS AND METHODS

Buffalo and ACI rats were inoculated with tumor minces in Washington and shipped to Charlottesville for study. Noninoculated Buffalo and ACI littermates were also shipped to Charlottesville and were used as control animals. Throughout the study rats were provided free acess to laboratory chow and water. Individual rats were placed in metabolic cages for an 8-hr collection of urine samples (9 a.m. to 5 p.m.). Urine was collected at room temperature and, at the end of each collection, was stored at −20° until assayed (15–17).

Cyclic AMP and cyclic GMP levels were determined by previously described protein binding (9) and radioimmunoassay methods (19). Urinary levels of creatinine were determined with an automated picric acid method (15, 16). The values for urinary cyclic nucleotides reported are mean values ± S.E. and are expressed as μmoles cyclic nucleotide per g urinary creatinine (15, 17). Normalizing cyclic nucleotide excretion rates for urinary creatinine corrected values for small losses of urine in cages and containers.

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[4] The abbreviations used are: cyclic AMP, cyclic adenosine 3′:5′-monophosphate; cyclic GMP, cyclic guanosine 3′:5′-monophosphate.

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RESULTS

The excretion rates of cyclic AMP and cyclic GMP in 3 strains of non-tumor-bearing rats were approximately 12 and 1.5 μmoles cyclic nucleotide per g urinary creatinine, respectively. Throughout the course of study, the urinary levels of cyclic AMP did not change significantly, although with some tumors a tendency for small increases was observed (Table 1). This was seen in rats with either liver or kidney tumors that were as large as 6 cm in diameter.

In rats bearing slow growing hepatomas 20, 21, and 9618A, an intermediate growing hepatoma 9633F, or an intermediate growing kidney sarcoma (MK2), no changes in urinary cyclic GMP levels were observed. However, in rats bearing fast growing hepatomas 9618A2 and 3924A or an intermediate growing kidney adenoma (MK3), urinary cyclic GMP levels increased progressively with tumor growth. When tumors reached a diameter of 4 to 6 cm, urinary cyclic GMP increased 4- to 30-fold (Table 2). The observations with urinary cyclic AMP and cyclic GMP in rats with hepatomas 3924A are similar to our earlier report (15).

DISCUSSION

Several laboratories have described a correlation of increased cyclic GMP levels in cell cultures and *in vivo* tumors with increased growth rate (12, 13, 15, 18, 21, 22). It was also shown that Morris hepatoma 3924A contained very high levels of cyclic GMP, about 100- to 200-fold higher

Table 1
Level of cyclic AMP in urine of tumor-bearing rats

Tumor and rat strain	No. of animals	μmoles cyclic AMP/g creatinine		
		Control or no tumor detectable	Tumor, 1-2 cm in diameter	Tumor, 4-6 cm in diameter
Non-tumor-bearing rats				
Sprague-Dawley	6	10.1 ± 1.3[a]		
Buffalo	10	10.7 ± 1.4		
ACI	12	13.1 ± 1.6		
Hepatoma-bearing rats				
20 (Buffalo)	3	12.6 ± 1.6	12.9 ± 1.4	16.7 ± 1.8
21 (Buffalo)	3	11.7 ± 1.2	13.6 ± 1.4	12.8 ± 1.6
9618A (Buffalo)	2	14.9 ± 2.0	13.9 ± 1.7	14.9 ± 1.9
9633F (Buffalo)	2	16.3 ± 1.8	15.6 ± 1.9	17.6 ± 2.1
3924A (ACI)	12	13.6 ± 1.7	12.7 ± 1.9	14.8 ± 2.1
9618A2 (Buffalo)	13	15.4 ± 1.4	16.9 ± 1.6	11.7 ± 1.4
Kidney tumor-bearing rats				
MK2 (Buffalo)	9	12.9 ± 1.7	14.7 ± 1.9	19.7 ± 2.1
MK3 (Buffalo)	11	16.7 ± 1.9	15.9 ± 2.2	19.1 ± 2.3

[a] Mean ± S.E.

Table 2
Level of cyclic GMP in urine of tumor-bearing rats

Tumor and rat strain	No. of animals	μmoles cyclic GMP/g creatinine				
		Control or no tumor detectable	Tumor, 1-2 cm in diameter	Tumor, 2-3 cm in diameter	Tumor, 3-4 cm in diameter	Tumor, 4-6 cm in diameter
Non-tumor-bearing rats						
Sprague-Dawley	6	1.5 ± 0.07[a]				
Buffalo	10	1.6 ± 0.08				
ACI	12	1.4 ± 0.06				
Hepatoma-bearing rats						
20 (Buffalo)	3	1.6 ± 0.06	1.6 ± 0.06	2.6 ± 0.09	3.2 ± 0.10	3.6 ± 0.10
21 (Buffalo)	3	1.4 ± 0.07	1.4 ± 0.07	3.3 ± 0.09	2.8 ± 0.09	2.9 ± 0.11
9618A (Buffalo)	2	1.3 ± 0.07	1.6 ± 0.06	1.8 ± 0.09	1.8 ± 0.08	1.9 ± 0.10
9633F (Buffalo)	2	1.4 ± 0.05	1.8 ± 0.06	1.6 ± 0.08	1.8 ± 0.07	1.5 ± 0.08
3924A (ACI)	12	1.3 ± 0.09	3.9 ± 0.09[b]	10.4 ± 0.20[c]	22.4 ± 0.82[c]	41.9 ± 2.81[c]
9618A2 (Buffalo)	13	1.4 ± 0.09	1.9 ± 0.09	2.6 ± 0.10	3.3 ± 0.12	5.6 ± 0.23[b]
Kidney tumor-bearing rats						
MK2 (Buffalo)	9	1.2 ± 0.05	1.3 ± 0.05	1.8 ± 0.07	2.2 ± 0.07	2.9 ± 0.09
MK3 (Buffalo)	11	1.3 ± 0.07	2.9 ± 0.11[b]	9.7 ± 0.46[c]	17.1 ± 0.81[c]	32.7 ± 1.62[c]

[a] Mean ± S.E.
[b] $p < 0.05$.
[c] $p < 0.01$.

than normal liver or slow growing hepatomas (21). The properties and subcellular localization of guanylate cyclase in this tumor are different from normal liver (12).[5] This may be offered as 1 explanation for the increased levels of cyclic GMP. Rats bearing Morris hepatoma 3924A also have increased urine levels of cyclic GMP, while urinary cyclic AMP is unaltered. Urinary cyclic GMP increased as much as 20-fold with tumor growth, and urinary cyclic GMP correlated well with tumor size and alterations with irradiation, chemotherapy, and surgical removal (15). While the elevated levels of cyclic GMP in urine may represent release from the tumor and renal clearance (3, 4), other mechanisms have not been excluded.

The present study was conducted to determine if urinary cyclic GMP was increased in animals bearing other tumors. Of the 6 hepatoma lines and 2 renal tumors examined, 3 tumors were associated with increased urine levels of cyclic GMP: hepatoma 3924A, hepatoma 9618A2, and renal tumor MK3. Urinary cyclic AMP was unaltered with all tumors examined.

Since hepatomas 3924A (21), 9633F and 9618A2 and renal tumors MK2 and MK3 all have elevated tissue levels of cyclic GMP compared to normal liver or normal kidney (6, 7, 21), a simple explanation for the increased urinary cyclic GMP in animals with some but not all of these tumors cannot be offered at present. Current studies are directed at this question. In any event, these studies indicate that urinary cyclic GMP may be useful in evaluating tumor size in some animal tumors. These studies also suggest the need for clinical studies to determine the possible usefulness of urinary cyclic GMP to diagnose or evaluate therapeutic responses in patients with various tumors.

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