ADENOSINE 3', 5'-CYCLIC MONOPHOSPHATE PHOSPHODIESTERASE ACTIVITIES IN THE X-IRRADIATION INDUCED RAT SMALL BOWEL ADENOCARCINOMA[1]

A.J. Lawson, D.D. Wall, J.W. Osborne, and R.H. Stevens

Radiation Research Laboratory
14 Medical Laboratories
University of Iowa
Iowa City, Iowa 52242

Received June 10, 1977

SUMMARY The adenosine 3', 5'-cyclic monophosphate phosphodiesterase (PDE) activities were evaluated in X-irradiation induced Holtzman rat small bowel adenocarcinoma and age-matched normal small intestine. Within normal small intestine, PDE activity was optimal at pH 7.4, and highly dependent upon the addition of Mg^{2+} or Mn^{2+}. Analyses of the rat small bowel adenocarcinoma revealed significantly elevated PDE activities above the normal small bowel which were found to be relatively constant throughout the length of the ileum and jejunum. These findings suggest that the diminished intracellular adenosine 3', 5'-cyclic monophosphate levels observed in this lesion (1) may be the consequence of elevated PDE activities.

INTRODUCTION

Many studies have conclusively demonstrated that adenosine 3', 5'- cyclic monophosphate (cAMP) acts as a central regulator at the molecular level of such diverse cell activities as mitosis, metabolism (enzyme activation, transcription, translation), and responses to extracellular signals (2). The intracellular levels of this cyclic nucleotide are dependent upon the intricate balance among the biosynthetic activity of the inner membrane bound enzyme adenylate cyclase [E.C.4.6.1.1] which synthesizes cAMP, its degradation to 5'-adenosine monophosphate (5'-AMP) by the hydrolytic enzyme cAMP-dependent phosphodiesterase (PDE) [E.C.3.1.4.17c], as well as utilization by cAMP-dependent protein kinases. Recently, we reported that the X-irradiation induced rat small bowel adenocarcinoma contained significantly reduced intracellular quantities of cAMP compared to the corresponding normal intestinal tissue (1). Similar diminished cAMP stores have been reported in a spontaneously occurring

[1] This investigation was supported by grant Ca 18327 awarded by the National Cancer Institute, DHEW.

ISSN 0006-291X

human colon adenocarcinoma when compared to the noninvolved adjacent tissue (3). These decreased cyclic nucleotide levels in the human colonic neoplasm were not attributed to the abnormal hydrolysis by the cAMP-dependent PDE because: (i) the enzyme inhibitor theophylline failed to elevate the tumor cell cyclic nucleotide concentrations, and (ii) no significant differences in cAMP PDE activities were observed although the high K_M enzyme activity was found to be slightly lower in the cancer tissue (3). We wish to report in this communication that unlike the human malignancy, the X-irradiation induced rat small bowel adenocarcinoma has significantly higher cAMP phosphodiesterase activities than normal tissue from comparable sites.

MATERIALS AND METHODS

Small bowel adenocarcinomas were induced in 200-250 g adult male Holtzman rats (Holtzman Co., Madison, Wisc.) by local X-irradiation of the hypoxic, temporarily exteriorized ileum and jejunum (4). The animals were lightly anesthetized with diethyl ether and the entire small bowel from the pyloric sphincter to the ileo-cecal valve was removed from the animals with either normal or adenocarcinomatous small bowel. The tissue (0.25 - 5.0 g) was quickly excised, rinsed free of fecal matter, trimmed of fat and connective tissue, washed with ice cold 0.05M Tris-HCl buffer (pH 7.4) and homogenized in buffer at 4°C utilizing a Polytron (Brinkman Instruments Inc., Westbury, N.Y.). The crude homogenate was centrifuged 500 xg for 5 min, and the decanted supernatant immediately assayed for cAMP-dependent PDE activities (5). The assay mixture contained 50 mM Tris-HCl (pH 7.4), 5 mM $MgSO_4$, 0.4 mM cAMP with 0.18 uCi of [^{3}H] cAMP (20 Ci/mM, Schwarz/Mann, Orangeburg, N.Y.) and tissue homogenate containing 20 - 60 ug DNA in a final volume of 900 ul. The assay mixture was kept at 4°C in an ice bath and upon completion of the mixture by addition of the tissue homogenate, the reaction was initiated by placing the mixture immediately in a rotating water bath at 37.0 $\pm$ 0.1°C for the prescribed time. The reaction was quenched by immediately returning the assay mixture to the ice bath and adding 1 ml of 2% $ZnSO_4$ followed by 1 ml of 1.9% $Ba(OH)_2$ (5). The assay was centrifuged 2000 xg for 10 min at 4°C and 500 ul of the supernatant containing unhydrolyzed cAMP counted for radioactivity in a Bray's scintillation cocktail with a Beckman Model 3155 liquid scintillation counter.

The standard diphenylamine deoxyribose reaction was utilized for the determination of DNA in the test samples (6). The enzyme activities were expressed as nanomoles cAMP hydrolyzed per ug DNA per minute $\pm$ S.E.M. Levels of significance were determined by Student's t-test (7).

RESULTS AND DISCUSSION

The DNA content of the sample was selected to represent the total number of cells from which the enzymes were being obtained on the rationale that intracellular DNA content is relatively constant in mononucleated cells whether derived from malignant or normal intestinal tissue (8). Furthermore, very little extracellular DNA would be expected in the rat intestinal tissues

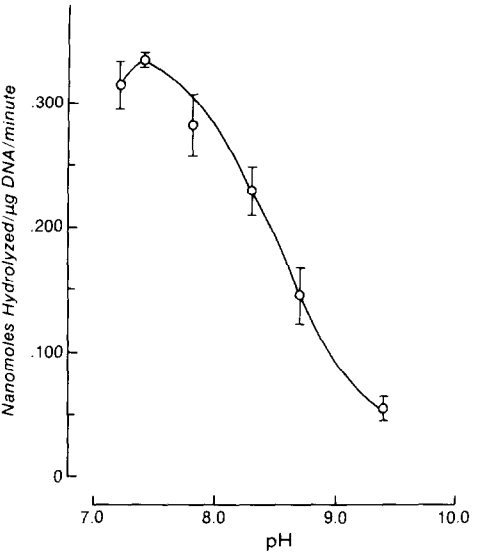

Figure 1. Influence of pH on the hydrolytic activity of the cAMP-dependent PDE in normal rat small intestine. The reaction mixture contained: Tris-HCl; 5 mM $MgSO_4$; 4 mM cAMP; and 20-60 µg DNA of tissue homogenate obtained 30-40 cm from the pyloric sphincter. Mean ± S.E.M. (n=4).

of interest. This is in contrast to the substantial quantities of extracellular protein (fecal material, mucous secretions, etc.) always present in these tissues even when animals have been fasted for several days.

Optimal conditions for evaluating the small intestine cAMP PDE activities was accomplished by analyses of pH and divalent cationic dependencies. Fig. 1 shows that the pH optimum for cAMP PDE as assayed was 7.4, and the hydrolytic capacity decreased rapidly as the conditions were made alkaline. Although some hydrolysis of cAMP to 5'-AMP was evident in the absence of added ions (i.e., TRIS-HCl), the PDE activities were strongly dependent on the addition of the divalent cations, Mg^{2+} or Mn^{2+}, with either SO_4^{2-} or Cl^- as the gegenions (Table 1). However, Ca^{2+} and Co^{2+} were ineffective and the addition of Zn^{2+} at both 0.5 and 5 mM completely abolished the phosphodiesterase activities.

Progress curves of the cAMP-dependent PDE activities in the crude cellular homogenates of the normal small bowel and the adenocarcinoma demostrated rectangular

Table 1

ION EFFECTS ON cAMP PHOSPHODIESTERASE ACTIVITIES

Ion	Molarity	Nanomoles Hydrolyzed/ µg DNA/minute
Tris·HCl	5×10^{-2}	0.184 ± 0.036 (4)[1]
$MgSO_4$	5×10^{-4}	0.372 ± 0.016 (5)
	5×10^{-3}	0.407 ± 0.017 (5)
$MgCl_2$	5×10^{-4}	0.385 ± 0.015 (5)
	5×10^{-3}	0.471 ± 0.024 (4)
$MnCl_2$	5×10^{-4}	0.242 ± 0.020 (5)
	5×10^{-3}	0.459 ± 0.017 (4)
$CaCl_2$	5×10^{-4}	0.196 ± 0.015 (5)
	5×10^{-3}	0.107 ± 0.013 (5)
$CoCl_2$	5×10^{-4}	0.209 ± 0.018 (5)
	5×10^{-3}	0.127 ± 0.010 (4)
$ZnSO_4$	5×10^{-4}	0 (5)
	5×10^{-3}	0 (5)

Reaction mixture contained 50 mM Tris-HCl, 0.4 mM cAMP, 20-60 µg DNA tissue homogenate obtained 30-40 cm from the pyloric sphincter; and the added ion as indicated.

[1] **mean ± S.E.M. (n)**

hyperbolic plots (Fig. 2). It is evident that significantly greater hydrolysis of cAMP by these PDE enzymes occurs in the X-ray induced adenocarcinoma. These elevated levels of enzyme activities are consistent with the almost two-fold decrease in intracellular cAMP concentration which was observed in this neoplasm (1). In contrast, DeRubertis and co-workers reported no differences in cAMP-dependent PDE activities in the human colon adenocarcinoma when compared with the noninvolved tissue. However, they too noted a diminished cAMP content in colonic tumor tissue (3). The differences in the results may be attributed to species differences, tissue type (i.e., small vs. large bowel), extent of autolysis subsequent to autopsy and enzyme activities determination, or some other undefined parameter.

Although the entire ileum and jejunum were exposed to the X-irradiation,

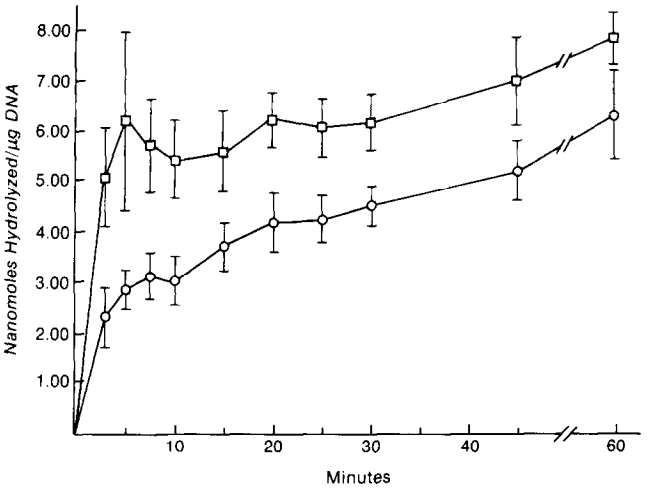

Figure 2. Comparison of cAMP-dependent PDE activities from 8 normal rat small bowels (O—O) and 4 x-irradiation induced adenocarcinomas (□—□) of the small bowel plotted as the mean ± S.E.M.

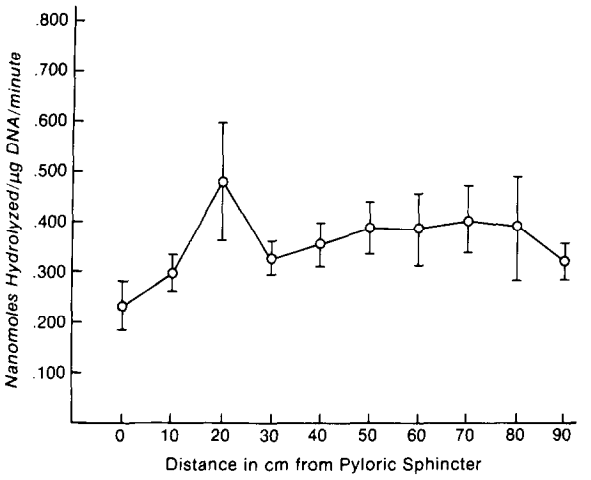

Figure 3. Activity of cAMP-dependent PDE throughout the length of the rat small intestine. Data represent the mean ± S.E.M. (n=11) of 4 cm segments obtained at the indicated distance from the pyloric sphincter in normal rat small bowel.

tumors developed at various loci throughout this length. Consequently, it was important to establish whether an activity gradient of cAMP-dependent PDE existed in the small intestine. The length of the rat small bowel was normalized to 100 cm for comparison of PDE activity among various animals. We observed that

the cAMP-dependent PDE activities were relatively constant throughout the entire length of the ileum and jejunum (Fig. 3). Therefore, the enzyme activity differences cannot be attributed to the location of the segment being analyzed, and that no preferred site for neoplastic transformation exists.

In summary, it has been observed that the X-irradiation induced rat small bowel adenocarcinoma has a significantly greater cAMP PDE activity than normal small intestinal tissue. Whether this alteration is attributable to a change in a single isoenzyme or whether the activities of multiple enzymes are all increased, remains to be elucidated with future kinetic studies of purified phosphodiesterases.

REFERENCES

1. Stevens, R.H., Smith, D.D., Osborne, J.W., and Oberley, L.W., (1976) J. Natl. Cancer Inst. 57, 43-45.
2. Robison, A.G., Butcher, R.W., and Sutherland, E.W. (1971) Cyclic AMP, Academic Press, New York.
3. DeRubertis, F.R., Chatoyin, R., and Field, J.B. (1976) J. of Clin. Invest. 57, 641-649.
4. Coop, K.L., Sharp, J.G., and Osborne, J.W., (1974) Cancer Res. 34, 1487-1494.
5. Schonhofer, P.S., Skidmore, I.F., Bourne, H.R., and Krishna, G., (1972) Pharmacology 7, 65-77.
6. Burton, K., (1956) Biochem. J. 62, 315-323.
7. Daniels, W.W. (1974) Biostatistics, pp. 123-130, John Wiley and Sons, Inc., New York.
8. Bertalanffy, L. (1960) Principles and Theory of Growth. In: Fundamental Aspects of Normal and Malignant Growth, pp. 157-159, Elsevier Publishing Co., New York.