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Letter to the Editor

Cyclic Nucleotide Metabolism in a Hereditary Renal Rat Tumor*

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THE ROLE of cyclic nucleotides in the regulation of cell growth and their involvement in abnormal proliferative states has been the subject of numerous investigations [1–8]. Most of the work has been concentrated on cyclic AMP. Less information is available on the possible role of cyclic GMP. Goldberg *et al.* [9] have proposed that cAMP and cGMP may regulate cell proliferation by acting in opposition to each other. However, this view has been disputed and there are considerable controversies [10, 11].

In order to obtain more information on the possible role of cyclic nucleotide metabolism in tumor development, we have measured the levels of cAMP and cGMP as well as the activities of the enzymes involved in their synthesis and degradation in normal tissue as well as in tissue showing neoplastic growth. In the study we have used an inbred strain of rats with renal tumors (Tw). These rats develop multiple bilateral tumors of varying size. The tumors are inherited in a pattern consistent with a single autosomal dominant lethal gene. Occasionally, the tumors show metastatic spread [12, 13].

One year old rats were used in the present study. The tumor was removed from the kidney immediately after the animal was sacrificed. The tissue was minced with scissors and homogenized in a Potter–Elvehjem teflon glass homogenizer (1600 rev/min, 15 strokes) in 3 vol of ice cold TSE buffer (50 mM Tris–HCl, pH 7.4, 0.25 M sucrose, 2 mM EDTA).

cAMP and cGMP were determined with the radioimmuno assay kits of the Radiochemical Centre, Amersham, England. Adenylate cyclase was measured according to Jüppner and Hesch [14]. Guanylate cyclase was determined by modification of the method described by Nesbitt *et al.* [10]. The reaction mixture contained in a vol of 0.01 ml, 50 mM Tris–HCl, pH 7.8, 2.5 mM $MnCL_2$, 0.5 mM GTP, 1 mM theophylline, 30 μg of creatine kinase, 5 mM creatine phosphate and 50 mg of the crude enzyme homogenate. All incubations were carried out for 15 min at 37°C, and the reaction was terminated by boiling for 3 min. After centrifugation all samples were stored at −20°C until assayed for cAMP and cGMP by the radioimmuno assay method. The values obtained were corrected for the presence of cAMP and cGMP in the homogenates, and the results expressed as pmole of cyclic nucleotide formed per min/mg of protein. The assay mixture for measuring cyclic nucleotide phosphodiesterase activity, in a final volume of 100 μl, consisted of 40 mM Tris–HCl, buffer, pH 8.0, 5 mM $MgSO_4$, 50 μM $CaCl_2$, 1 μM cAMP or cGMP and 50 μl of homogenate. After incubation for 5 min at 37°C the reaction was stopped by boiling for 1 min. The cAMP and cGMP concentrations were measured before and after incubation by the radioimmunoassay method. The results are expressed as pmole of cyclic nucleotide transformed per min/mg of protein. Protein was determined by the method of Lowry *et al.* [15].

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Table 1 shows that the concentration of cAMP in the kidney tumor and in the normal kidney was the same. Likewise, no significant changes in the activities of cAMP cyclase and cAMP phosphodiesterase were observed in tumor tissue as compared to kidney without tumor.

From the results in Table 2 it can be seen that a marked decrease in tissue concentration of cGMP was found in the kidney tumors as compared to tumor-free kidneys. Thus, the concentration of cGMP in the tumor tissue was only half that found in normal kidneys. The change in cGMP concentration was associated with a decrease in cGMP cyclase activity. The activity of the enzyme in tumor tissue was reduced by 50% as compared to kidney without tumor. A small increase in cGMP phosphodiesterase activity was also observed in the kidney tumors. In the tissue surrounding the tumors, it was found that the concentration of cGMP as well as the activities of cGMP cyclase and phosphodiesterase were the same as in kidney without tumors.

Since the Tw-strain originates from Wistar rats, the concentrations of the cyclic nucleotides and the activities of the enzymes regulating their levels were also measured in kidneys from Wistar rats. It appears from Table 1 that the tissue concentration of cAMP as well as the levels of the cyclase and phosphodiesterase was the same in the kidneys of Wistar rats as in the Tw-strain. Table 2 shows that the concentration of cGMP and of cGMP cyclase and cGMP phosphodiesterase in the kidney of the Wistar rats was equal to that found in the kidneys of rats from the Tw-strain without any tumors.

The results obtained in the present study with extracts of rat kidneys agree well with previous work on transformed rat kidney fibroblasts [10]. In this study it was found that the level of cGMP as well as the activity of cGMP cyclase was markedly reduced compared to the levels of normal kidney cells. The present data do not support the view of Goldberg *et al.* [9] that cAMP and cGMP may regulate cell proliferation by acting in opposition to each other.

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Table 1. cAMP metabolism in kidney tumors and normal kidneys

Rat strain	Tumor	cAMP concentration	Enzyme activity: cAMP cyclase	Enzyme activity: cAMP phosphodiesterase
		(pmole/mg protein)	(pmole/min/mg protein)	(pmole/min/mg protein)
Tw	+	1.4±0.2*	4.1±0.2	124±18
	−	1.5±0.1	4.8±0.3	112± 5
Wistar†	−	1.5±0.2	5.1±0.5	116± 9

*Standard deviation of the mean. Each number represents the mean of 7 experiments with different animals.
†Wistar DK-Moll.

Table 2. cGMP metabolism in kidney tumors and normal kidneys

Rat strain	Tumor	cGMP concentration	Enzyme activity: cGMP cyclase	Enzyme activity: cGMP phosphodiesterase
		(pmole/mg protein)	(pmole/min/mg protein)	(pmole/min/mg protein)
Tw	+	0.050±0.005‡	4.1±0.5	124±17
	−	0.099±0.01	8.9±0.7	89± 8
Wistar§	−	0.105±0.01	7.3±0.3	116± 9

‡Standard deviation of the mean. Each number represents the mean of 7 experiments with different animals.
§Wistar DK-Moll.

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