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PROPERTIES OF GUANYLATE CYCLASE IN ADULT RAT LIVER AND SEVERAL MORRIS HEPATOMAS

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Summary

Guanylate cyclase (GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2) activity was examined in preparations from normal rat liver and a series of Morris hepatomas. Homogenate guanylate cyclase activities were 3.2, 1.6 and 1.2 nmol cyclic GMP formed per min/g tissue in normal liver and in two fast-growing hepatomas, 3924A and 9618A2, respectively. Enzymatic activity was found in all particulate fractions and in the supernatant fraction of all tissues examined. Liver had a particulate:soluble guanylate cyclase ratio of 0.24. Five hepatomas displayed particulate:soluble enzymatic ratios greater than one.

Particulate and soluble guanylate cyclase from hepatoma 3924A and hepatoma 9618A2 were examined in greater detail and compared to normal liver. Triton X-100 stimulated particulate guanylate cyclase 11-, 5-, and 2-fold in preparations from liver and hepatomas 3924A and 9618A2, respectively. Liver and hepatoma particulate and soluble guanylate cyclase preferred Mn^{2+} as the sole cation. While liver preparations could utilize Mg^{2+} when Mn^{2+} was present in low concentrations, the tumor enzymes did not utilize Mg^{2+} in the presence of Mn^{2+}. Calcium inhibited all particulate and stimulated all soluble preparations. ATP inhibited all preparations of guanylate cyclase. Sodium azide stimulated the particulate and soluble enzyme from liver 5- and 6-fold, respectively. Sodium azide did not alter guanylate cyclase activity from tumor preparations.

Introduction

Guanylate cyclase activity (GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2) has been found to be soluble and associated with membrane fractions in most mammalian tissue homogenates [1—6]. The soluble and particulate enzymes

differ with respect to activation and inhibition by Triton X-100, ATP, Ca^{2+}, *p*-chloromercuriphenylsulfonic acid, maleate, and sodium azide, and have different molecular sizes upon gel-filtration [3,5—7]. The basal activities and subcellular distribution of both forms of guanylate cyclase vary from tissue to tissue within the same animal [1—6]. Ratios of particulate to soluble guanylate cyclase activity were altered in liver during regeneration and development [4]. These studies describe an altered subcellular distribution of guanylate cyclase activity in various Morris hepatomas compared to normal liver. The properties of guanylate cyclase in these transplantable tumors were also different. Some of these studies were reported in abstract form [8].

Materials and Methods

Buffalo and ACI rats were inoculated subcutaneously with hepatoma minces in Washington and shipped to Charlottesville for study. Non-inoculated Buffalo and ACI littermates were also shipped to Charlottesville and were used as controls for the normal liver tissue studies. All animals were fed ad libitum, and were sacrificed by ether anesthesia or by decapitation when tumors were 2—5 cm in diameter. Tissues were quickly removed and placed in a cold solution of 0.25 M sucrose, 5 mM Tris · HCl buffer at pH 7.5, 1 mM dithiothreitol, and 1 mM EDTA. Liver and tumors cleaned of necrotic tissue were weighed and homogenized in 10 vols. of the same buffer in a glass homogenizer with a motor-driven Teflon pestle. Homogenates were filtered through two layers of gauze and centrifuged to obtain the various particulate fractions indicated. The 0—750 × *g*, 750—10 000 × *g* and 10 000—105 000 × *g* fractions were prepared with sequential centrifugation of preparations for 10, 20, and 90 min, respectively. All procedures were carried out at 4° C. All particulate fractions were resuspended in fresh sucrose/Tris buffer.

Assays for guanylate cyclase activity were performed at 37° C for 10 or 15 min with 50 mM Tris · HCl (pH 7.6), 10 mM theophylline, 0.1 mM dithiothreitol, 0.1 mM EDTA, 15 mM creatine phosphate, 20 μg creatine phosphokinase (120 units per mg), 1 mM GTP, 4 mM $MnCl_2$ (unless otherwise noted) and 10—20 μl of appropriately diluted enzyme in a final volume of 100 μl. Reactions were terminated by adding 1 ml of 50 mM sodium acetate buffer (pH 4.0) and immersion in ice. Reaction mixtures were heated at 90° C for 3 min and centrifuged; 50—150-μl aliquots of the supernatant fractions were used for radioimmunoassay of cyclic GMP formed [3—11]. With these conditions the amount of cyclic GMP formed was linear with enzyme concentration (5—30 μg of protein per 100 μl reaction mixture) and time (5—30 min) of incubation. Under these assay conditions with 10 mM theophylline less than 5—10% of added [^{3}H]cyclic GMP was degraded [3,7,11].

For the determination of in situ levels of cyclic GMP, rats were anesthetized with ether and liver or hepatomas were quickly removed and homogenized in 6% trichloroacetic acid. Samples were extracted, purified, and assayed as described previously [10,12].

Radioimmunoassay was accomplished with newly synthesized [^{125}I]tyrosine methyl ester succinyl cyclic GMP that was purified by Sephadex G-10 column chromatography and cellulose thin layer chromatography [10,13]. The lower

limit of sensitivity of the cyclic GMP assay was 0.01—0.05 pmol [10,13]. Protein was determined by the method of Lowry et al. [14].

GTP, creatine phosphate, creatine phosphokinase, sodium azide and Triton X-100 were purchased from Sigma Chemical Company. Sephadex G-10 was obtained from Pharmacia Fine Chemicals. Other reagents were obtained as previously described [3—11].

Results

Guanylate cyclase activity was distributed in both particulate and soluble fractions from normal rat liver and five Morris hepatomas of varying rates of growth (Table I). Normal liver had a ratio of particulate guanylate cyclase to soluble guanylate cyclase of 0.24. Slow-growing, intermediate-growing and fast-growing hepatomas had particulate:soluble guanylate ratios greater than one.

Homogenate guanylate cyclase activities were 3.25, 1.64 and 1.21 nmol cyclic GMP formed per min/g tissue in normal liver and two fast-growing hepatomas, 3924A and 9618A2, respectively (Table II). Guanylate cyclase activity was associated with all crude particulate and soluble fractions in all tissues examined. Highest specific activities were observed in liver cytosol (43 pmol cyclic GMP per min/mg protein) and in the various particulate and soluble fractions of the two hepatomas (range 6—31 pmol cyclic GMP per min/mg protein).

Pretreatment of enzyme with 1% Triton X-100 increased particulate guanylate cyclase 11-, 5-, and 2-fold from liver, hepatoma 3924A and hepatoma 9618A2, respectively (Table III). Triton X-100 increased soluble guanylate cyclase activity from liver and hepatoma 3924A 50—60% and had no effect with soluble guanylate cyclase from hepatoma 9618A2. Therefore, significant amounts of latent activity in particulate fractions were apparent only after solubilization and activation with Triton X-100. As with other tissues, the apparent distribution of activity in subcellular fractions was altered with detergent [3,6].

Guanylate cyclase in all soluble and particulate fractions examined preferred Mn^{2+} as the sole cation (Table IV). Mg^{2+} was 5—20% as effective as Mn^{2+}. However, with normal liver, Mg^{2+} was quite effective in the presence of a low concentration of Mn^{2+} (0.1 mM) as reported previously [6,11]. This was not the case with the hepatoma fractions studied; Mg^{2+} was not effective as either sole cation or with low concentrations of Mn^{2+} (Table IV).

At less than optimal concentrations of Mn^{2+} (0.4 mM MnCl and 1 mM GTP) calcium chloride inhibited particulate guanylate cyclase from liver and both hepatomas. 80—90% inhibition was observed with 10 mM $CaCl_2$ (not shown). However, soluble fractions from liver and hepatomas were stimulated by 1 to 5 mM Ca^{2+}, and up to 3-fold stimulation of soluble guanylate cyclase was observed. At 4 mM Mn^{2+} the inhibitory and stimulatory effects of Ca^{2+} were diminished. Thus, the effects of Ca^{2+} on soluble and particulate guanylate cyclase from hepatomas were qualitatively similar to those with liver preparations [11].

ATP (0.2 to 5 mM) inhibited all guanylate cyclase activities examined (not shown). However, soluble guanylate cyclase from hepatoma 3924A and 9618A2 was more sensitive to ATP inhibition than the particulate activity. Sol-

TABLE I

PARTICULATE AND SOLUBLE GUANYLATE CYCLASE ACTIVITY IN NORMAL RAT LIVER AND MORRIS HEPTOMAS

Guanylate cyclase was determined in 105 000 × *g* supernatant and particulate fractions of homogenates as indicated in Materials and Methods. Values are mean activities ± S.E. of 2—8 preparations.

	Gyclic GMP in situ (pmol/mg protein)	Cyclic GMP formed				Ratio * particulate/ soluble
		Particulate		Soluble		
		nmol per min/g tissue	pmol per min/mg protein	nmol per min/g tissue	pmol per min/mg protein	
Normal liver	0.10 ± 0.01	0.60 ± 0.19	6.0 ± 0.8	2.51 ± 0.11	41.6 ± 3.6	0.24
Slow-growing hepatomas						
No. 20	0.19	1.17	37.6	0.44	13.7	2.66
No. 21	0.52 ± 0.06	1.81 ± 0.30	61.1 ± 3.5	0.88 ± 0.17	18.9 ± 0.8	2.06
Intermediate-growing hepatomas						
No. 9633F	0.39 ± 0.06	1.31 ± 0.71	21.6 ± 1.8	0.46 ± 0.10	9.1 ± 0.7	2.85
Fast-growing hepatomas						
No. 3924A	21.33 ± 1.66	1.30 ± 0.26	49.1 ± 3.6	0.40 ± 0.10	25.6 ± 1.9	3.25
No. 9618A2	0.99 ± 1.07	0.74 ± 0.11	29.7 ± 1.6	0.60 ± 0.13	22.6 ± 1.2	1.23

* Ratios were determined from columns containing nmol per min/g.

TABLE II

SUBCELLULAR DISTRIBUTION OF GUANYLATE CYCLASE ACTIVITY

Homogenates and the indicated fractions obtained after centrifugation were assayed for guanylate cyclase as described in Materials and Methods. Values are reported as mean activities ± S.E. of 3—4 preparations. Values in parentheses are percent of homogenate activity in each subcellular fraction.

	Cyclic GMP formed									
	Homogenate		(0—750 × *g*)		(750—10 000 × *g*)		(10 000—105 000 × *g*)		(105 000 × *g* supernatant)	
	nmol per min/g tissue	pmol per min/mg protein	nmol per min/g tissue	pmol per min/mg protein	nmol per min/g tissue	pmol per min/mg protein	nmol per min/g tissue	pmol per min/mg protein	nmol per min/g tissue	pmol per min/mg protein
Normal liver	3.25 ± 0.5 (100%)	21.6 ± 2.9	0.21 ± 0.07 (6.5)	5.7 ± 0.1	0.07 ± 0.02 (2.0)	1.1 ± 0.1	0.21 ± 0.05 (9.5)	11.8 ± 0.9	2.81 ± 0.30 (86.4)	43.3 ± 2.6
Hepatoma 3924A	1.64 ± 0.2 (100%)	15.4 ± 0.9	0.54 ± 0.08 (32.9)	30.8 ± 1.0	0.33 ± 0.01 (20.1)	6.8 ± 0.4	0.19 ± 0.02 (11.5)	6.6 ± 0.3	0.60 ± 0.07 (36.5)	23.0 ± 2.1
Hepatoma 9618A2	1.21 ± 0.08 (100%)	8.0 ± 0.4	0.27 ± 0.05 (22.3)	13.4 ± 0.8	0.24 ± 0.03 (19.8)	18.6 ± 0.5	0.23 ± 0.02 (19.0)	17.6 ± 0.9	0.47 ± 0.04 (38.8)	18.7 ± 1.0

TABLE III
EFFECT OF TRITON X-100 ON GUANYLATE CYCLASE ACTIVITY

105 00 × *g* soluble and particulate fractions were preincubated with 1% Triton X-100 at 4°C for 60 min prior to assay. Activities are means ± S.E. of 2—3 experiments.

Tissue fraction	Cyclic GMP formed (pmol per min/mg)		Ratio +triton/—triton
	—Triton X-100	+Triton X-100	
Normal liver (particulate)	4.7 ± 0.4	53.1 ± 2.8	11.3
Normal liver (soluble)	40.0 ± 0.8	64.7 ± 2.9	1.6
Hepatoma 3924A (particulate)	46.2 ± 2.7	231.6 ± 17.1	5.1
Hepatoma 3934A (soluble)	19.4 ± 1.7	31.6 ± 1.6	1.5
Hepatoma 9618A2 (particulate)	30.5 ± 1.2	59.9 ± 3.1	2.0
Hepatoma 9618A2 (soluble)	26.1 ± 1.0	24.7 ± 1.1	0.9

TABLE IV

EFFECT OF CATIONS ON GUANYLATE CYCLASE ACTIVITY

105 000 × *g* soluble and particulate fractions were assayed for guanylate cyclase activity with the concentrations of Mn^{2+} and/or Mg^{2+} indicated. GTP was 1 mM in all incubations. Values reported are means ± S.E. of 2—3 preparations.

Tissue fraction	Cation concentration	Cyclic GMP formed pmol per min/mg protein
Liver (particulate)	0.1 mM Mn^{2+}	0.8 ± 0.1
	4.0 mM Mn^{2+}	5.4 ± 0.3
	4.0 mM Mg^{2+}	0.7 ± 0.1
	4 mM Mg^{2+} + 0.1 mM Mn^{2+}	3.4 ± 0.2
	4 mM Mg^{2+} + 4 mM Mn^{2+}	5.5 ± 0.3
Liver (soluble)	0.1 mM Mn^{2+}	2.1 ± 0.2
	4.0 mM Mn^{2+}	36.1 ± 1.0
	4.0 mM Mg^{2+}	2.2 ± 0.2
	4 mM Mg^{2+} + 0.1 mM Mn^{2+}	19.6 ± 0.8
	4 mM Mg^{2+} + 4 mM Mn^{2+}	35.7 ± 1.1
Hepatoma 3924A (particulate)	0.1 mM Mn^{2+}	7.7 ± 0.6
	4.0 mM Mn^{2+}	50.6 ± 2.1
	4.0 Mm Mg^{2+}	6.1 ± 0.4
	4 mM Mg^{2+} + 0.1 mM Mn^{2+}	7.0 ± 0.4
	4 mM Mg^{2+} + 4 mM Mn^{2+}	52.0 ± 1.7
Hepatoma 3924A (soluble)	0.1 mM Mn^{2+}	2.6 ± 0.2
	4.0 Mm Mn^{2+}	17.6 ± 1.1
	4.0 mM Mg^{2+}	2.2 ± 0.2
	4 mM Mg^{2+} + 0.1 mM Mn^{2+}	2.5 ± 0.3
	4 mM Mg^{2+} + 4 mM Mn^{2+}	16.7 ± 0.9
Hepatoma 9618A2 (particulate)	0.1 mM Mn^{2+}	3.6 ± 0.4
	4.0 mM Mn^{2+}	29.1 ± 1.2
	4.0 mM Mg^{2+}	3.8 ± 0.4
	4 mM Mg^{2+} + 0.1 mM Mn^{2+}	3.9 ± 0.5
	4 mM Mg^{2+} + 4 mM Mn^{2+}	31.6 ± 1.1
Hepatoma 9618A2 (soluble)	0.1 mM Mn^{2+}	3.9 ± 0.3
	4.0 mM Mn^{2+}	20.6 ± 1.1
	4.0 mM Mg^{2+}	4.1 ± 0.3
	4 mM Mg^{2+} + 0.1 mM Mn^{2+}	3.9 ± 0.3
	4 mM Mg^{2+} + 4 mM Mn^{2+}	21.1 ± 0.9

TABLE V

EFFECT OF SODIUM AZIDE ON GUANYLATE CYCLASE ACTIVITY

105 000 × *g* soluble and particulate fractions were preincubated with 1 mM NaN_3 for 10 min at 37°C prior to assay as described in Materials and Methods. Values are means ± S.E. of 3—5 experiments.

Tissue fraction	Cyclic GMP formed (pmol per min/mg)		Ratio NaN_3/—NaN_3
	—NaN_3	+NaN_3	
Normal liver (particulate)	5.1 ± 0.4	27.0 ± 0.2	5.3
Normal liver (soluble)	42.0 ± 0.9	280.7 ± 9.4	6.7
Hepatoma 3924A (particulate)	51.1 ± 2.8	54.1 ± 3.4	1.0
Hepatoma 3924A (soluble)	22.6 ± 1.7	27.0 ± 1.9	1.2
Hepatoma 9618A2 (particulate)	33.4 ± 1.4	37.6 ± 1.7	1.1
Hepatoma 9618A2 (soluble)	23.2 ± 1.3	25.4 ± 1.3	1.1

uble guanylate cyclase from liver [11] and other tissues [3,5] is also more sensitive to ATP inhibition. With hepatomas, seventy to ninety percent inhibition was observed with 5 mM ATP.

Sodium azide, a potent nucleophile and metabolic inhibitor, markedly increased guanylate cyclase activity from soluble and particulate fractions of normal liver (5—7-fold, Table V) as reported previously [7,15]. However, soluble and particulate activities from hepatoma 3924A or 9618A2 were not altered with 1 mM NaN_3.

Discussion

Cyclic GMP has been postulated to act in an opposing direction to cyclic AMP in mammalian tissues [16—18], and the cyclic GMP:cyclic AMP ratio has been reported to be increased in liver neoplasia [19,20], in transformed cells [21,22], and in proliferating epidermal tissues [23]. The levels of both of these nucleotides are influenced by hormones and numerous pharmacological agents. Regulation of the in vivo levels of the nucleotides may occur through alterations of synthesis, hydrolysis, and cellular excretion. Studies with the proliferating cell systems of lymphocytes [16], epidermal tissues [23], fibroblasts [16,21,22], and plants [24] support the concept that the two cyclic nucleotides promote opposing regulatory influences that may involve cellular growth. Some reports, however, have not supported this concept [25], and it is obivous that regulatory effects of these nucleotides on growth, if they indeed exist, must be quite complex.

Further, the complexity of the regulation of cyclic GMP levels in tissues is apparent from these hepatoma studies. In situ levels of cyclic GMP are 0.1 and 21.3 pmol/mg protein in liver and hepatoma 3924A, respectively (Table I). Experiments with tissue slices indicate that hepatoma 3924A can maintain basal levels of cyclic GMP that are 5—20 times higher than normal liver (unpublished observations). Yet the activities of guanylate cyclase are 22 and 15 pmol cyclic GMP formed per min/mg protein in homogenates of liver and hepatoma 3924A, respectively. Although the total basal activity of guanylate cyclase is less in the

hepatomas examined, the apparent properties and subcellular distribution of the enzyme are somewhat different than liver. In all hepatomas examined guanylate cyclase is predominantly in particulate fractions. This has also been observed in regenerating liver, fetal liver [4], and two renal tumors [26]. Thus, the localization of enzyme in particulate fractions seems to correlate best with the elevated levels of cyclic GMP observed in hepatomas (Table I and ref. 19) and renal tumors [26]. The greater susceptibility of the soluble enzyme to ATP inhibition also suggests that the particulate enzyme could be more important in regulating cyclic GMP synthesis and levels. Enzyme from hepatoma is less able to utilize Mg^{2+} in the presence of low concentrations of Mn^{2+}. The stimulatory effects of Triton X-100 were less with preparations from hepatomas. We have reported previously that NaN_3 increases cyclic GMP synthesis in preparations from liver and some other tissues and its effect on cyclic GMP accumulation is not due to altered cyclic GMP or GTP hydrolysis [7]. Guanylate cyclase from hepatomas is not stimulated by sodium azide. In other experiments (not shown) this appears to be due to the absence of a protein activator factor in hepatomas required for the azide response [15]. Analogous to the studies reported here with liver and hepatomas, sodium azide also increases soluble and particulate guanylate cyclase activity from rat kidney but not from renal tumors MK2 and MK3 [7,26]. The effects of Ca^{2+} and ATP with guanylate cyclase from hepatomas are qualitatively similar to normal liver.

It remains to be established whether or not the altered properties of guanylate cyclase in hepatomas are in part or solely responsible for the increased levels of cyclic GMP observed in these tumors. Equally important would be the examination of cyclic GMP hydrolysis by phosphodiesterase in these preparations.

This study does not address the question of cellular heterogeneity and its alteration in hepatomas. This may account for the altered cellular distribution of guanylate cyclase and its properties in hepatomas. It seems unlikely, however, that this could explain the loss of the NaN_3 activation of guanylate cyclase in tumors.

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References

1 Hardman, J.G. and Sutherland, E.W. (1969) J. Biol. Chem. 244, 6363—6370
2 Ishikawa, E.M., Ishikawa, S., Davis, J.W. and Sutherland, E.W. (1969) J. Biol. Chem. 244, 6371—6376
3 Kimura, H. and Murad, F. (1974) J. Biol. Chem. 249, 6910—6916
4 Kimura, H. and Murad, F. (1975) Proc. Natl. Acad. Sci. U.S. 72, 1965—1969
5 Kimura, H. and Murad, F. (1975) Metabolism 24, 439—445
6 Kimura, H. and Murad, F. (1975) Life Sciences 17, 837—844
7 Kimura, H. and Murad, F. (1975) J. Biol. Chem. 250, 8016—8022
8 Criss, W.E., Murad, F. and Kimura, H. (1976) Clin. Res. 24, 51a

9 Kimura, H. and Murad, F. (1974) J. Biol. Chem. 249, 329—331
10 Kimura, H., Thomas, E. and Murad, F. (1974) Biochim. Biophys. Acta 343, 519—528
11 Kimura, H. and Murad, F. (1975) J. Biol. Chem. 250, 4810—4817
12 Murad, F. and Kimura, H. (1974) Biochim. Biophys. Acta 343, 275—286
13 Steiner, A.L., Parker, C.W. and Kipnis, D.M. (1972) J. Biol. Chem. 247, 1106—1113
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
15 Mittal, C.K., Kimura, H. and Murad, F. (1975) J. Cyclic Nucleotide Res. 1, 261—269
16 Goldberg, N.D., Haddox, M.K., Estensen, R., White, J.G., Lopez, C. and Hadden, J.W. (1974) in Cyclic AMP, Cell Growth and the Immune Response, by (Braun, W., Lichtenstein, L. and Parker, C., eds.), pp. 247—262, Springer-Verlag, New York
17 Goldberg, N.D., Haddox, M.K., Hartle, D.K. and Hadden, J.W. (1973) in Pharmacology and the Future of Man, 5th International Congresss of Pharmacology Vol. 5, pp. 146—169, Karger, Basel
18 Goldberg, N.D., Haddox, M.K., Nicol, S.E., Glass, D.B., Sanford, C.H., Kuehl, Jr., F.A. and Estensen, R. (1975) Adv. Cyclic Nucleotide Res. 5, 307—338
19 Thomas, E.W., Murad, F., Looney, W.B. and Morris, H.P. (1973) Biochim. Biophys. Acta 297, 564—567
20 Goldberg, M.L., Nurke, G.C. and Morris, H.P. (1975) Biochem. Biophys. Res. Commun. 62, 320—327
21 Moens, W., Vokaer, A. and Kram, R. (1975) Proc. Natl. Acad. Sci. U.S. 72, 1063—1067
22 Rudland, P.S., Seeley, M. and Seifert, W. (1974) Nature 251, 417—419
23 Voorhees, J.J., Stawiski, M., Duell, E.A., Haddox, M.K. and Goldberg, N.D. (1973) Life Sci. 13, 639—653
24 Haddox, M.K., Stephenson, J.H. and Goldberg, N.D. (1974) Fed. Proc. 33, 522
25 Chlapowski, F.J., Kelly, L.A. and Butcher, R.W. (1975) Adv. in Cyclic Nucleotide Res. 6, 245—338
26 Criss, W.E., Murad, F. and Kimura, H. (1976) J. Cyclic Nucleotide Res. 2, 11—19