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PRELIMINARY COMMUNICATIONS

NONCOMPETITIVE INHIBITION OF SOLUBLE GUANYLATE CYCLASE BY 2'-DEOXYGUANOSINE-3'-MONOPHOSPHATE

Cheranjiv L. Kapoor* and Gopal Krishna

Section on Drug Tissue Interaction, Laboratory
of Chemical Pharmacology, National Heart, Lung,
and Blood Institute, National Institutes of Health,
Bethesda, MD 20205, U.S.A.

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Recent studies by Sahyoun et al. (1) have shown that fat cell membrane adenylate cyclase is specifically inhibited by 2'-deoxy-3'-AMP. In this communication we report that analogously guinea pig pancreas guanylate cyclase [GTP pyrophosphate-lyase (cyclizing) EC 4.6.1.2] is specifically inhibited by 2'-deoxy-3'-GMP.

[α-^{32}P]GTP (sp. act., 30 Ci/mmole) and [^{3}H]cGMP (sp. act., 4.5 to 10 Ci/mmole) were obtained from the New England Nuclear Corp., Boston, MA. Dowex 50-H^+ resin (Bio-Rad, AG 50.W x 4 H^+ form, 200-400 mesh) was obtained from Bio-Rad Laboratories, Richmond, CA. Neutral alumina (activity grade 1) was obtained from ICN Nutritional Biochemicals, Cleveland, OH. 3-Isobutyl-1-methylxanthine (IBMX) was obtained from the Aldrich Chemical Co., Milwaukee, WI. [α-^{32}P]GTP and unlabeled GTP were purified by ion exchange chromatography on a Dowex 50-H^+ column (2). Deoxynucleotides were obtained from the Sigma Chemical Co., St. Louis, MO. All other unlabeled nucleotides, creatine phosphate and creatine kinase were obtained from the Boehringer-Mannheim Co., New York, NY. Other reagents were of analytical grade.

Guinea pigs (male NIH Hartley strain, 300-350 g weight) were fasted overnight and decapitated. The soluble and particulate fractions of guinea pig pancreas were prepared by homogenization of tissue in 10 vol. of ice-cold 0.3 M sucrose containing 25 mM Tris HCl buffer (pH 7.4) in a glass homogenizer with a Teflon pestle. All subsequent procedures for preparation of the enzyme were carried out at 4°. The homogenate was centrifuged at 109,000 *g* for 1 hr. The supernatant fraction was removed and saved. The pellet was homogenized with an amount of the sucrose-Tris buffer equal to the original volume of the homogenate and recentrifuged at 109,000 *g* for 1 hr. The washing procedure was repeated once and the pellet was resuspended in the buffer. The resuspended washed pellet was incubated for 1 hr at 4° in the buffer containing 1% Triton X-100 (3). The Triton-treated enzyme was then centrifuged at 59,000 *g* for 1 hr; the resulting supernatant fraction represented the Triton-dispersed enzyme.

Guanylate cyclase activity was assayed as described by Krishna and Krishnan (2). The incubation mixture (100 µl) contained 50 mM Tris HCl (pH 7.4), 30 mM sucrose, 0.5 mM IBMX, 1 mM [^{3}H]cGMP (36,000 d.p.m.), various concentrations of [α-^{32}P]GTP and $MnCl_2$, 10 mM creatine phosphate, 20 µg of creatine phosphokinase (140-165 units/mg of protein) and inhibitors to be tested. The assay was initiated by the addition of enzyme (40-50 µg protein), incubated for 15 min at 37°, and terminated by the addition of 100 µl of 100 mM Na_2 EDTA. All of the reaction mixture was sequentially chromatographed first on Dowex 50-H^+ and then on neutral alumina (2). Recovery of 70-80 percent of added [^{3}H]cGMP was routinely obtained. The blank values were usually between 0.001 and 0.003 percent of added GTP. Protein was estimated by the method of Lowry et al. using bovine albumin as a standard (4).

*Present address: Division of Endocrinology, Department of Medicine, University of North Carolina, Chapel Hill, NC 27514.

Figure 1a shows the inhibition of soluble guanylate cyclase from guinea pig pancreas by various concentrations of 2'-deoxy-3'-GMP when assayed at varying concentrations of substrate Mn:GTP. The inhibition of the enzyme by 2'-deoxy-3'-GMP was noncompetitive as shown by the double reciprocal plot (Fig. 1b) and the Dixon Plot. The K_i value was about 50 μM.

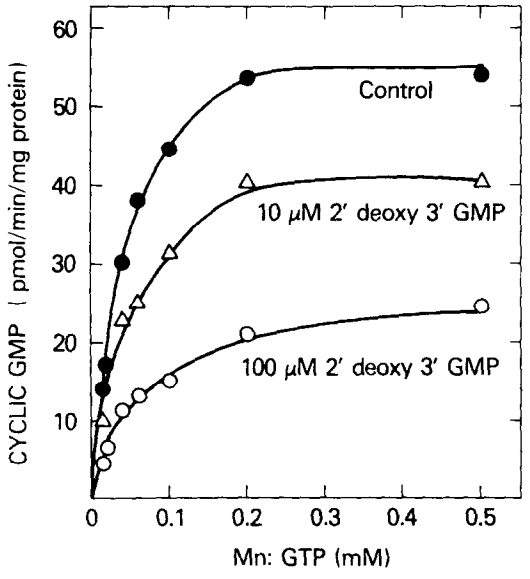

Fig. 1a. Inhibitory effect of 2'-deoxy-3'-GMP on the pancreatic guanylate cyclase. Guanylate cyclase was assayed in the 109,000 *g* supernatant fraction of guinea pig pancreas with various concentrations of Mn:GTP (sp. act. of GTP 20 d.p.m./pmole) with 2 mM excess Mn^{2+} concentration as described in the text. 2'-Deoxy-3'-GMP was added at 10 μM and 100 μM concentrations. The incubations were started by the addition of 50 μg protein of the supernatant fraction. The guanylate cyclase activity values are the means of four determinations.

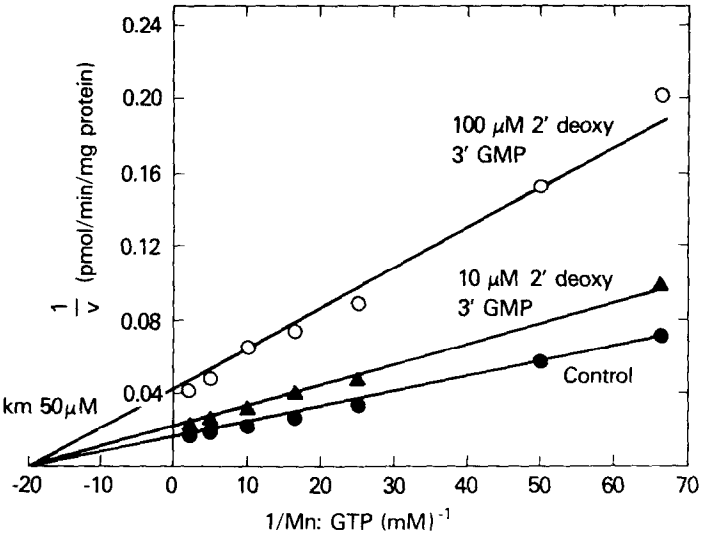

Fig. 1b. Lineweaver-Burk plot of data in Fig. 1a.

The effects of 2'-deoxy-mononucleotides on the soluble and Triton-dispersed particulate guanylate cyclase of guinea pig pancreas were compared (Table 1). 2'-Deoxy-3'-AMP and 2'-deoxy-3'-CMP at concentrations up to 500 μM were unable to inhibit either the soluble or particulate guanylate cyclase. However, at a concentration of 100 μM, 2'-deoxy-3'-GMP inhibited the soluble guanylate cyclase by more than 50 percent but did not affect the Triton-dispersed particulate enzyme (Table 1). Similar inhibition of soluble guanylate cyclase of lung and liver (50-70 percent) was also obtained by 100 μM 2'-deoxy-3'-GMP.

Table 1. Effects of deoxynucleotide-monophosphates on pancreatic guanylate cyclase activity*

Nucleotides	Concn (mM)	Percent of control activity+	
		Soluble	Triton-dispersed particulate
2'-Deoxy-3'-AMP	0.1	95.8	110.0
	0.5	100.0	97.4
	1.0	93.2	89.0
2'-Deoxy-3'-GMP	0.1	48.9	97.1
	0.5	27.6	104.0
	1.0	25.2	52.4
2'-Deoxy-3'-CMP	0.1	114.0	96.5
	0.5	106.0	95.6
	1.0	98.2	106.0

*Guanylate cyclase was assayed with 0.5 mM [α-^{32}P]GTP (sp. act. 21.3 d.p.m./pmole) and 2.5 mM $MnCl_2$, as described in the text. Various deoxynucleotides were present at the concentrations indicated and incubations were started by the addition of 50 μg protein of the supernatant fraction or 52 μg protein of the Triton-dispersed particulate fraction. The guanylate cyclase activity values are the means of duplicate determinations.

+Guanylate cyclase activity values are reported as percent of enzyme activity when incubated in the absence of various deoxynucleotides. They were 38.4 and 31.9 pmoles/min/mg of protein for soluble and Triton-dispersed particulate enzyme respectively.

The present study describes a specific noncompetitive inhibition of guanylate cyclase by micromolar concentrations of 2'-deoxy-3'-GMP. These findings are analogous to the inhibition of fat cell adenylate cyclase by 2'-deoxy-3'-AMP. Moreover, these results indicate a possible role of 2'-deoxy-3'-GMP in the modulation of cyclic GMP synthesis in pancreas and in other tissues (5-7).

REFERENCES

1. N. Sahyoun, D.J. Schmitges, M.I. Siegel and P. Cutrecasas, Life Sci. 19, 1961 (1976).
2. G. Krishna and N. Krishnan, J. Cyclic Nucleo. Res. 1, 293 (1975).
3. H. Kimura and F. Murad, J. biol. Chem. 249, 6910 (1974).
4. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. 193, 265 (1951).
5. C.L. Kapoor and G. Krishna, Biochim. biophys. Acta 544, 102 (1978).
6. D. Wallach and I. Pastan, Biochem. biophys. Res. Commun. 72, 859 (1976).
7. N.D. Goldberg and M.K. Haddox, A. Rev. Biochem. 46, 823 (1977).