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Enhancement of Human Guanylate Cyclase Activity by Chemical Carcinogens (39794)

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Introduction. Guanylate cyclase (EC 4.6.1.2) catalyzes the conversion of guanosine triphosphate to guanosine 3′,5′-monophosphate (cyclic GMP). The enzyme is found in almost all mammalian cells and its product, cyclic GMP, is thought to be involved in cell growth. Cyclic GMP has been reported to increase DNA synthesis (1–3), increase protein synthesis (4, 5), and increase growth of fibroblasts (2) and thymocytes (6). In addition, cyclic GMP may be involved in malignant transformation (1), and the nucleotide has been reported to be increased in rat hepatomas (7) and in human adenocarcinomas (8). Guanylate cyclase itself is increased in the developing (fetal) rat heart (9) and liver (7).

The present study was devised to test the effect of chemical carcinogens on guanylate cyclase from a number of human tissues. Streptozotocin, an antibiotic isolated from *Streptomyces archomogenes,* is a specific pancreatic β-cell toxin utilized as a therapeutic agent in the treatment of metastatic insulin-producing tumors (10–12) and to induce experimental diabetes mellitus (13). Chemically, it is the 2-deoxy-D-glucose derivative of the carcinogen, methylnitrosourea (Fig. 1). Streptozotocin causes renal, liver, and pancreatic tumors in rats (14–17).

Recently, we demonstrated that streptozotocin and methylnitrosourea caused an *in vitro* enhancement of guanylate cyclase activity in a number of rat tissues (18). The present investigation demonstrates that these agents also result in enhancement of guanylate cyclase activity in a number of human tissues. In addition, hydrazine, a carcinogen occurring in tobacco (19, 20) and producing tumors in animals (21, 22), was also shown to enhance guanylate cyclase activity.

Materials and methods. Normal human tissues were obtained from autopsies performed at the Jackson Memorial Hospital and Miami Veterans Administration Hospital 1–12 hr after death. Tumor tissue was obtained from the surgical specimen at operation. All specimens were immediately placed in cold 0.03 *M* Tris-HCl, pH 7.6, and utilized within 30 min. Streptozotocin was a gift of the Upjohn Company (Kalamazoo, Mich.), and was dissolved immediately before use in a citric acid buffer adjusted to pH 4.5 with 1% NaOH. Methylnitrosourea was purchased from International Chemical and Nuclear Pharmaceutical Inc. (Plainview, N. Y.) and dissolved in this same buffer. Hydrazine was purchased from Eastman Kodak Chemicals (Rochester, N. Y.). Alumina oxide, neutral activity I, for column chromatography was obtained from E. Merck (Darmstadt, Germany). The [α-^{32}P]GTP was purchased from New England Nuclear Corp. (Boston, Mass.).

Guanylate cyclase activity was measured as previously described (9, 18) using a modification of the method of White and Zenser (23). The various tissues were homogenized in cold 0.03 *M* Tris-HCl, pH 7.6, and centrifuged at 37,000*g* in a Sorval refrigerated centrifuge at 4° for 15 min. After centrifugation, the supernatant solutions of the respective individual normal and tumor tissues were divided into 100-μl aliquots to be used for the control and carcinogen-containing enzyme incubations. The supernatants were then assayed at 37° for 10 min for guanylate cyclase activity, using a reaction mixture consisting of 20 m*M* Tris-HCl, pH 7.6; 5 mM $MnCl_2$; 2.67 m*M* cyclic GMP (used to minimize destruction of [^{32}P]GTP); a GTP

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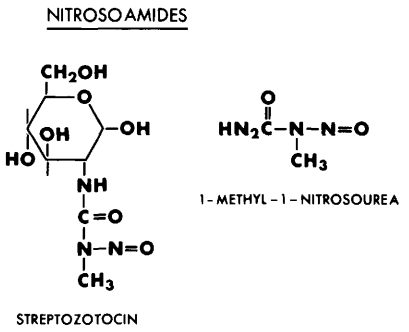

FIG. 1. Structure of streptozotocin and methylnitrosourea.

regenerating system (5 m*M* creatine phosphate, 11.25 U of creatine phosphokinase); 100 μg of bovine serum albumin; 20 mM caffeine; [α-^{32}P]GTP, approximately 5 $\times$ 10^5 cpm; and the enzyme preparation having 0.2 to 0.6 mg of protein, in a final volume of 75 μl. The reaction was terminated by the addition of 10 μl of 0.1 *M* EDTA, pH 7.6, containing about 30,000 cpm of [^{3}H]cyclic GMP (to estimate recovery in the subsequent steps) and boiling for 3 min. After cooling in an ice bath, each reaction mixture was applied to 1 g of neutral alumina oxide columns and washed with 0.03 *M* Tris-HCl, pH 7.6. The first milliliter of elutant from the column was discarded since the majority of the cyclic GMP appeared in the second, third, and fourth milliliters of elutant. The above 3 ml of elutant from the column were collected directly into scintillation vials containing 15 ml of Bray's solution (24). The elutants were then counted in a Packard Tri-Carb liquid scintillation spectrometer. All of the ^{32}P-containing material was identifiable as cyclic GMP, as determined by thin-layer chromatography on cellulose (PEI, Brinkman) using 1 *M* formic acid, 1 *M* LiCl as solvent, and Chromar sheets (Mallinchrodt, St. Louis, Mo.) developed with absolute alcohol and concentrated NH_4OH (5:2, v/v) (23). In four experiments the product of the incubations was assayed using cyclic GMP radioimmunoassay (25) in order to confirm that the material was cyclic GMP. The experimental results were similar to those obtained with the guanylate cyclase assay.

Results. Streptozotocin and methylnitrosourea enhancement of guanylate cyclase activity in various human tissues. The structure of streptozotocin containing the carcinogen moiety, methylnitrosourea, is shown in Fig. 1. Both streptozotocin and methylnitrosourea enhanced guanylate cyclase activity in all human tissues tested (Table I). The resulting stimulation of cyclic GMP accumulation was two- to sixfold and significant ($P < 0.001$) in all tissues tested. In general, the enhancement of guanylate cyclase activity by methylnitrosourea was as great or greater than streptozotocin. The order of potency for stimulation by methylnitrosourea was colon > liver > breast > kidney > duodenum > lung > stomach > pancreas. These findings were similar to those with streptozotocin except that breast was the least sensitive to streptozotocin. The concentration-response relationship of the two agents was approximately the same in liver, being greatest at 20 m*M* and unresponsive at 1 m*M*. Concentrations in excess of 20 m*M* did not produce any additional increase above those observed at 20 m*M*. The similarity in dose-response relationships supports the concept that the nitrosourea moiety is responsible for the activation of guanylate cyclase.

Enhancement of guanylate cyclase activity by hydrazine. Hydrazine (H_2N-NH_2) also enhanced guanylate cyclase activity in all human tissues tested (Table I). The maximal stimulation produced by a concentration of 100 m*M* hydrazine was considerably in excess of that produced by streptozotocin and methylnitrosourea, being approximately 52-fold in pancreas, 35-fold in kidney, 21-fold in lung, 24-fold in breast, 9-fold in liver, 7-fold in duodenum, and 3-fold in stomach. Hydrazine produced a maximal stimulation of the enzyme in each tissue at 100 m*M* and approached nonstimulative levels at 10 m*M*.

Enhancement of particulate guanylate cyclase activity. The 37,000*g* supernatant preparation of guanylate cyclase from these human tissues contained about 95% of the total guanylate cyclase activity. Guanylate cyclase activity in the precipitate "particulate guanylate cyclase" had no significant enhancement with streptozotocin and only 1^1/$_2$- to 2^1/$_2$-fold increase with methylnitrosourea. Hydrazine, however, stimulated

particulate human guanylate cyclase from 38- to 80-fold.

Enhancement of guanylate cyclase activity in tumors. It was of interest to determine whether human tumors contained guanylate cyclase activity and whether or not the enzyme could be stimulated by streptozotocin, methylnitrosourea and hydrazine. As shown in Table II, the three tumors examined had guanylate cyclase activity which was stimulated significantly by the three agents.

Discussion. The results demonstrate that the carcinogens streptozotocin, methylnitrosourea, and hydrazine enhance the activity of the enzyme guanylate cyclase, catalyzing the production of cyclic GMP. Of relevance to the carcinogenicity of these three agents is the fact that cyclic GMP is involved in several processes related to cell growth (1–8), and has recently been implicated in cells undergoing malignant transformation (1). Kimura and Murad found elevated cyclic GMP levels in rat hepatomas (7) and DeRubertis *et al.* (8) reported increased concentrations of cyclic GMP in adenocarcinoma of the human colon. Thus, if cyclic GMP is a significant factor in malignant transformation, the ability of streptozotocin, methylnitrosourea, and hydrazine to induce tumors may be related to their capacity to enhance guanylate cyclase activity, which in turn would increase the production of cyclic GMP. Other structurally related carcinogens have also been shown to increase guanylate cyclase activity. In this regard, DeRubertis and Craven have reported that dibutylnitrosoamine, diethylnitrosomine, nitrosopiperidine, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine enhance guanylate cyclase activity in rat liver and kidney (26), and Kimura *et al.* have shown that sodium azide is a potent stimulator of liver guanylate cyclase activity (27).

We have also shown that the guanylate cyclase is present in tumors obtained from human lung, breast, and pancreas, and can be stimulated by the three carcinogens used in the present investigation. It is difficult to

TABLE I. STREPTOZOTOCIN, METHYLNITROSOUREA, AND HYDRAZINE ENHANCEMENT OF GUANYLATE CYCLASE ACTIVITY IN HUMAN TISSUES.

	Cyclic GMP (picomoles accumulated per milligram of protein per 10-min incubation)			
Tissue	Control (citric acid)	Streptozotocin 10 m*M*	Methylnitrosourea 10 m*M*	Hydrazine 100 m*M*
Liver	271 ± 4[a]	825 ± 6*	1486 ± 10*	2480 ± 10*
Lung	777 ± 6	2745 ± 3	3125 ± 6	16162 ± 12
Colon	688 ± 6	4344 ± 12	5188 ± 10	5987 ± 6
Stomach	1083 ± 14	2213 ± 14	2927 ± 29	3616 ± 18
Duodenum	445 ± 6	2405 ± 12	2003 ± 13	3222 ± 6
Kidney	100 ± 3	548 ± 6	488 ± 6	3476 ± 12
Pancreas	189 ± 3	425 ± 12	369 ± 11	9425 ± 10
Breast	167 ± 2	257 ± 6	857 ± 3	4002 ± 12

[a] Mean ± SE for six samples.
* $P < 0.001$ for all tissues with streptozotocin, methylnitrosourea, or hydrazine additions compared to control with Student's *t* test.

TABLE II. STREPTOZOTOCIN, METHYLNITROSOUREA, AND HYDRAZINE ENHANCEMENT OF GUANYLATE CYCLASE ACTIVITY IN HUMAN TUMORS.

	Cyclic GMP (picomoles accumulated per milligram of protein per 10-min incubation)			
Tissue	Control	Streptozotocin 10 m*M*	Methylnitrosourea 10 m*M*	Hydrazine 100 m*M*
Squamous cell CA[a] of lung	575 ± 6[b]	4491 ± 6*	4191 ± 6*	3982 ± 14*
Ductal CA of breast	25 ± 3	2354 ± 6	2454 ± 14	2054 ± 12
Pancreatic adenocarcinoma	409 ± 4	1298 ± 12	862 ± 12	1051 ± 6

[a] CA = carcinoma.
[b] Mean ± SE of three samples.
* $P < 0.001$ for all tissues with streptozotocin, methylnitrosourea, or hydrazine additions.

extrapolate beyond these observations to the induction process of tumors since guanylate cyclase activity in the fully developed tumor may not reflect the initial events of tumor induction. However, if a chemical carcinogen causes tumor growth in humans then continued exposure to the carcinogen might promote continued growth of the tumor.

Summary. The effect of three carcinogens, streptozotocin, methylnitrosourea, and hydrazine, on guanylate cyclase activity in a variety of human tissues obtained from autopsy specimens 1 to 12 hr after death was examined. Streptozotocin and methylnitrosourea produced two- to sixfold increases in guanylate cyclase activity in lung, liver, colon, kidney, stomach, duodenum, heart, and pancreas. Hydrazine, increased guanylate cyclase activity 21-fold in lung, 52-fold in pancreas, 35-fold in kidney, 24-fold in heart, and 9-fold in liver. The data in this report may help explain the tumor-inducing capacity of these three carcinogens.

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