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Nicotine Decreases Guanylate Cyclase Activity

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INTRODUCTION

The cyclic nucleotide, guanosine 3',5'-monophosphate (cyclic GMP) appears to serve a critical role in normal cell growth, DNA synthesis, and carcinogenesis (GOLDBERG et al. 1977). Tobacco smoke and hydrazine, a carcinogen which occurs in tobacco and tobacco smoke, have been shown to stimulate guanylate cyclase [E.C. 4.6.1.2], the enzyme that catalyzes the conversion of guanosine triphosphate to guanosine 3',5'-monophosphate (cyclic GMP), (ARNOLD et al. 1977, VESELY and LEVEY 1977a,b). Nicotine, the major alkaloid in tobacco, has been reported to be absorbed by all tissues and sixty-seven percent of nicotine in cigarette-smoke is retained in the lungs of smokers (LARSON et al. 1961). Since nicotine has some tumor promoting activity (BOCK and TSO 1975) we examined the effect of nicotine on guanylate cyclase activity in a variety of tissues.

METHODS

Tissues used in these experiments were obtained from male Sprague-Dawley rats, weighing 150-200 grams that had been maintained ad libitum on Purina Laboratory chow. Alumina oxide, neutral activity I for column chromatography, was obtained from E. Merck, (Darmstadt, Germany). The [^{32}P] GTP was from International Chemical and Nuclear Corporation, (Irvine, Calif.). Nicotine was obtained from Aldrich Chemical Company, Inc., (Milwaukee, Wis.) and dissolved in distilled water to prepare the concentration specified in the text.

Guanylate cyclase activity was measured as previously described (VESELY and LEVEY 1977a,b,c). 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 minutes. The nicotine was added to the supernatant enzyme prior to addition to the reaction mixture. The final concentrations of nicotine noted in the text were calculated by their concentration in the final incubation mixture. The supernatants plus nicotine were assayed at 37° for 10 minutes for guanylate cyclase activity, using a reaction mixture consisting of 20mM Tris HCl, pH 7.6; 5 mM $MnCl_2$; 2.67 mM cyclic GMP (5 mM creatine phosphate, 11.25 U creatine phosphokinase); 100 µg bovine serum albumin; 20 mM caffeine; [α-^{32}P]-GTP, approximately 5 x 10^5 cpm; and the enzyme

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preparation having 0.2 to 0.6 mg protein. Nicotine did not alter the pH of the reaction mixture (pH 7.6). 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 three minutes. After cooling in an ice bath, the [^{32}P] -cyclic GMP formed is isolated by sequential chromatography on Dowex-50-H^+ and alumina using the modification of KRISHNA and KRISHNAN (1975). The reaction mixtures were diluted with 0.5 ml of distilled water and transferred to a Dowex-50-H^+ column (10 x 75mM). The columns were then eluted with another 0.5 ml distilled H_2O and the eluates (1 ml) were discarded. The second 1 ml of water fraction eluted from the Dowex-50-H^+ column was allowed to directly pass through a column of dry neutral alumina (10 x 75 mM). The alumina columns were then eluted with 2 ml of 0.03 M Tris-HCl buffer, pH 7.6. The above three mls of elutant from the alumina column were collected directly into scintillation vials containing 15 mls of BRAY'S solution (1960). The eluates were then counted in a Packard Tri-Carb Liquid Scintillation spectrometer. The overall recovery of cyclic GMP after the two-stage chromatographic procedure was 90 to 95%. 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 LiCl as solvent and Chromar sheets (Mallinckrodt, St. Louis, Mo.) developed with absolute alcohol and concentrated NH_4OH (5:2, v/v). Protein was determined by the method of LOWRY et al. (1951).

RESULTS

Nicotine markedly decreased guanylate cyclase activity in all tissues tested (Table 1). The resultant decreases in cyclic GMP accumulation secondary to guanylate cyclase inhibition were highly significant. In terms of maximal inhibition with nicotine in vitro stomach > lung > pancreas > liver > heart > colon > spleen. The dose response relationship for nicotine is shown in Table 2. Maximal inhibition with nicotine was found at a concentration at which hydrazine has a maximal stimulatory activity (VESELY and LEVEY 1977a).

DISCUSSION

The role of guanylate cyclase and cyclic GMP in cell biology has been difficult to define. As a result of a decade of research attention has been focused on the role of cyclic GMP in normal and abnormal cell growth (KRAM and TOMKINS 1973, KIMURA and MURAD 1975, VESELY et al. 1976, DERUBERTIS et al. 1976a, GOLDBERG et al. 1977). Recently great interest has been evoked by the findings of several laboratories that chemical carcinogens influence the activity of guanylate cyclase by either increasing or decreasing its activity. Hydrazine (VESELY and LEVEY 1977a,b), nitrosamines and nitrosamides (DERUBERTIS and CRAVEN 1976b, VESELY et al. 1977c,d), tobacco smoke and nitrous oxide (ARNOLD et al. 1977), and butadiene diepoxide (VESELY and LEVEY 1977e) increase guanylate

TABLE 1 Effect of Nicotine on Guanylate Cyclase Activity in Various Tissues

Tissue	Cyclic GMP (pmoles accumulated/mg protein/10 minute incubation)[a] Control	Nicotine (100 mM)
Lung	1375 ± 12	117 ± 4[b]
Liver	275 ± 6	32 ± 4
Pancreas	201 ± 6	17 ± 6
Stomach	390 ± 6	15 ± 4
Colon	605 ± 8	80 ± 6
Spleen	831 ± 12	112 ± 8
Heart	163 ± 6	18 ± 4

[a]Each value is the mean ± S.E.M. of triplicate determinations in each of three separate experiments.

[b]$p < 0.001$ for all tissues with nicotine compared to control by Student's t test for unpaired values.

TABLE 2 Dose Response Relationship of Nicotine on Hepatic Guanylate Cyclase

Concentration (mM)	Cyclic GMP (pmoldes accumulated/mg protein/10 minute incubation)[a] Nicotine	Hydrazine
0	285 ± 6	285 ± 6
100	34 ± 4[b]	9000 ± 20
50	230 ± 6[b]	2000 ± 12
20	261 ± 6[NS]	--
10	284 ± 6[NS]	250 ± 6
1	286 ± 6[NS]	--

[a]Each value is the mean ± S.E.M. of triplicate determinations in each of three separate experiments.

[b]$p < 0.001$ with nicotine as compared to control by Student's test.

[NS] Not significant.

cyclase activity. In contrast, azo dyes, polycyclic aromatic hydrocarbons, aromatic amines, aflatoxins (VESELY and LEVEY 1977f) dimethylhydrazine, and hydrazine sulfate (VESELY et al. 1977b) decrease guanylate cyclase activity.

Nicotine, which has been reported to be absorbed in all tissues (LARSON et al. 1961), inhibited guanylate cyclase activity in every tissue tested including liver, lung, pancreas, stomach, colon, spleen, and heart. The relationship of the guanylate cyclase inhibition to the deleterious effects of nicotine such as

its reported tumor-promoting activity (BOCK and TSO 1975) is unclear. However, the connection may reside in the area of DNA synthesis. Cyclic GMP increases DNA synthesis (KRAM and TOMKINS 1973 , RUDLAND et al. 1974, WEINSTEIN et al. 1974) and this may account, at least in part, for the effects of cyclic GMP on normal and abnormal cell growth. Hydrazine increases DNA synthesis (BANKS et al. 1967) and increases guanylate cyclase activity. Conversely, nicotine has been shown to decrease DNA synthesis (VALADON and MUMMERY 1974, ROSZMAN et al. 1975). Thus, the observation that nicotine-induced a decrease in guanylate cyclase activity and cyclic GMP generation is consistent with, and may be the mechanism by which, nicotine decreased DNA synthesis.

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