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Two Components of Laetrile (Mandelonitrile and Cyanide) Stimulate Guanylate Cyclase Activity (40544)

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Recently, mandelonitrile β-glucuronide, the compound originally patented as Laetrile (1), has been synthesized from rat liver uridine diphosphate-glucuonosyl transferase (EC 2.4.1.17) and found to be mutagenic with *Salmonella typhimurium* strains TA 98 and TA 100 (2). Significant structural differences exist between mandelonitrile-β-glucuronide and amygdalin, the mandelonitrile-β-D-gentiobioside, reported (3–5) to be the major component of Mexican preparations marketed as laetrile. Analysis of recent samples of commercial laetrile indicate that mandelonitrile-β-glucuronide was not present; rather, the samples appeared to contain amygdalin and neoamygdalin, an isomer of amygdalin (2–5).

The present investigation examined the effect of amygdalin, neoamygdalin–amygdalin mixtures, mandelonitrile, and cyanide on guanylate cyclase activity. Recently several laboratories have demonstrated that chemical carcinogens can stimulate the activity of guanylate cyclase (EC 4.6.1.2), the enzyme catalyzing the conversion of guanosine triphosphate to guanosine 3′,5′-monophosphate (cyclic GMP). Hydrazine (6, 7), nitrosamines and nitrosamides (8, 9), sodium azide (10), and butadiene diepoxide (11) have been shown to stimulate guanylate cyclase activity. These findings are of potentially great importance because cyclic GMP may play a major role in normal and abnormal cell growth (12–16). Cyanide (CN^-) was tested in addition to amygdalin, neoamygdalin–amygdalin mixture, and mandelonitrile, as both mandelonitrile-β-glucuronide and the parent aglycone, racemic mandelonitrile, release CN^- under conditions of the mutagenic incubation studies (2). Oral feeding of both amygdalin and laetrile have recently been shown to release high levels of cyanide *in vivo* (17). The data in the present report indicate that guanylate cyclase activity is stimulated by mandelonitrile and cyanide but not by amygdalin or the neoamygdalin–amygdalin mixture.

Materials and methods. Tissues used in these experiments were obtained from male Sprague–Dawley rats, weighing 150–200 g, that had been maintained *ad libitum* on Purina Laboratory Chow. Mandelonitrile, amygdalin, and neoamygdalin–amygdalin mixture used in these experiments were isolated from commercial laetrile samples by Drs. Benson and Sheinin's laboratories (2). Potassium cyanide was obtained from Fisher Scientific Company (Fair Lawn, N.J.). All of the above were dissolved in triple-distilled water to obtain the various concentrations noted in the text. Alumina, neutral activity I for column chromatography, was obtained from E. Merck (Darmstadt, Germany). Analytical Grade Cation Exchange Resin (Dowex) AG-50WX8, 200–400 mesh was obtained from Bio-Rad Laboratories (Richmond, Calif.). The [^{32}P]GTP was from International Chemical and Nuclear Corporation, Irvine, California.

Guanylate cyclase activity was measured as previously described (6, 7, 18). The various tissues were homogenized in cold 0.03 *M* Tris–HCl, pH 7.6, and centrifuged at 37,000*g* in a refrigerated centrifuge at 4° for 15 min. The supernatants, to which amygdalin, neoamygdalin–amygdalin mixture, mandelonitrile, and cyanide had been added to achieve the final concentrations noted in Tables I and II was then assayed at 37° for 10 min. The reaction mixture consisted of 20 m*M* Tris–HCl, pH 7.6; 4 m*M* $MnCl_2$; 2.67 m*M* cyclic GMP (used to minimize destruction of [^{32}P]GTP); a GTP regenerating system (5 m*M* creatine phosphate and 11.25 units of creatine phosphokinase, EC 2.7.3.2); 100 μg

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TABLE I. THE EFFECT OF AMYGDALIN, NEOAMYGDALIN (AS A MIXTURE OF NEOAMYGDALIN AND AMYGDALIN), MANDELONITRILE, AND CYANIDE ON GUANYLATE CYCLASE ACTIVITY IN VARIOUS TISSUES.

	Cyclic 3′,5′-GMP (pmole accumulated/mg protein/10-min incubation)[a]				
Tissue	Control	Amygdalin (1 m*M*)	Neoamygdalin (1 m*M*)	Mandelonitrile (1 m*M*)	Cyanide (1 m*M*)
Liver	285 ± 6	290 ± 6[b]	293 ± 8[b]	438 ± 6[c]	356 ± 6[c]
Lung	1860 ± 8	1848 ± 12[b]	1852 ± 12[b]	3656 ± 12[c]	3046 ± 8[c]
Stomach	476 ± 6	484 ± 6[b]	472 ± 8[b]	948 ± 8[c]	896 ± 8[c]
Colon	592 ± 6	588 ± 6[b]	596 ± 4[b]	1106 ± 8[c]	998 ± 6[c]
Kidney	280 ± 12	276 ± 8[b]	286 ± 8[b]	404 ± 8[c]	378 ± 6[c]

[a] Each value is the mean ± SEM of nine determinations; all experiments were done in triplicate.
[b] Not significant.
[c] Significant at $P < 0.001$ for all tissues with mandelonitrile and potassium cyanide compared to control with Student's t test for unpaired values.

TABLE II. DOSE–RESPONSE RELATIONSHIPS OF AMYGDALIN, NEOAMYGDALIN (AS A MIXTURE OF NEOAMYGDALIN AND AMYGDALIN), MANDELONITRILE, AND CYANIDE ON HEPATIC GUANYLATE CYCLASE ACTIVITY

	Cyclic GMP (pmole accumulated/mg protein/10-min incubation)[a]			
Concentration (m*M*)	Amygdalin	Neoamygdalin	Mandelonitrile	Cyanide
0 (control)	285 ± 6	284 ± 6	282 ± 6	285 ± 6
3	290 ± 6[b]	282 ± 6[b]	499 ± 6[c]	365 ± 6[c]
1	280 ± 6[b]	294 ± 6[b]	479 ± 6[c]	345 ± 6[c]
0.1	278 ± 6[b]	278 ± 6[b]	438 ± 8[c]	340 ± 6[c]
0.01	276 ± 6[b]	282 ± 6[b]	344 ± 8[c]	295 ± 8[b]
0.001	282 ± 6[b]	274 ± 6[b]	317 ± 6[b]	288 ± 6[b]

[a] Each value is the mean ± SEM of nine determinations with all experiments being done in triplicate.
[b] Not significant.
[c] Significant at $P < 0.001$ with mandelonitrile and potassium cyanide compared to control with Student's t test for unpaired values.

of bovine serum albumin; 20 m*M* caffeine; and 1.2 m*M* [α-^{32}P]GTP, approximately 5 × 10^5 cpm. The enzyme preparation contained 0.1 to 0.4 mg of protein. The reaction was terminated by adding 10 μl of 0.1 *M* EDTA, pH 7.6, containing about 30,000 cpm of cyclic [^{3}H]GMP (to estimate recovery in the subsequent steps). The mixture was boiled for 3 min and cooled in an ice bath; the cyclic [^{32}P]GMP formed was isolated by sequential chromatography on Dowex-50-H^+ and alumina according to the modification of Krishna and Krishnan (19). The reaction mixtures were diluted with 0.5 ml of triple-distilled water and transferred to Dowex-50-H^+ columns (10 × 75 mm). The columns were then eluted with another 0.5 ml of distilled H_2O and the eluates (1 ml) were discarded. The second 1-ml water fraction eluted from the Dowex-50-H^+ columns was allowed to pass directly through a column of dry neutral alumina (10 × 75 mm) into scintillation vials containing 15 ml of Bray's solution. The alumina columns were then eluted into these vials with 2 ml of 0.03 *M* Tris–HCl buffer, pH 7.6, and the eluates were 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%. Blank ^{32}P counting rates averaged 40 to 50 cpm. In this assay system, cyclic GMP was linear with time for 20 min and with added protein from 50 to 400 μg.

Results and discussion. Mandelonitrile and cyanide stimulated guanylate cyclase in a wide range of tissues including liver, lung, stomach, colon, and kidney (Table I). Amygdalin and neoamygdalin had no effect in these same tissues (Table I). Table II shows the concentration–response relationships for these compounds in liver. Mandelonitrile stimulated hepatic guanylate cyclase activity over a concentration range of 0.001 to 3 m*M*. Cyanide stimulation was somewhat smaller but was observed over the concentration range of 0.1 to 3 m*M*. Concentrations in

excess of 3 m*M* did not produce any additional increase in guanylate cyclase activity.

It is of interest that mandelonitrile and cyanide stimulate guanylate cyclase at concentrations as low as any of the known nitrosamide and nitrosamine carcinogens which stimulate guanylate cyclase activity (6–11). Mandelonitrile rather than mandelonitrile-β-glucuronide was utilized in these experiments because analysis of recent samples of laetrile revealed that no mandelonitrile-β-glucuronide was present (2–5). These laetrile samples consisted mostly of amygdalin, the mandelonitrile-β-gentiobioside, which during metabolism forms mandelonitrile (3–5). Mandelonitrile, the breakdown product, had enhancing effects on guanylate cyclase activity while the parent compound, amygdalin, had no effect on guanylate cyclase activity. The guanylate cyclase–cyclic GMP system has been implicated in normal and abnormal growth (12–16) and a number of chemical carcinogens have been shown to enhance guanylate cyclase activity (6–11). Because of the possible relationship of the guanylate cyclase–cyclic GMP system and carcinogenesis, the data of the present investigation demonstrating that mandelonitrile enhances guanylate cyclase activity suggests that the possible carcinogenicity of laetrile used in man should be investigated. High levels of cyanide have recently been found to be released from laetrile and amygdalin *in vivo* (17). This fact taken in conjunction with cyanide enhancement of guanylate cyclase in the present investigation suggests that the effect of cyanide released from laetrile in man also needs further study.

Summary. The effect of four components of laetrile (mandelonitrile, amygdalin, cyanide, and a neoamygdalin–amygdalin mixture) on guanylate cyclase activity in a variety of tissues was examined. Mandelonitrile and cyanide, which can be released from mandelonitrile, stimulated the activity of rat guanylate cyclase (EC 4.6.1.2), an enzyme thought to be important in normal and abnormal cell growth. Amygdalin and neoamygdalin had no effect in these same tissues. The activation of guanylate cyclase by mandelonitrile was observed over the concentration range 0.001 to 3 m*M*, while cyanide stimulated the activity of guanylate cyclase in concentrations of 0.1 to 3 m*M*. Since many nitrosamine and nitrosamide carcinogens also stimulate guanylate cyclase activity, the present investigation suggests that possible carcinogenicity of laetrile used in man needs further study.

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