[CANCER RESEARCH 44, 2924–2928, July 1984]

Effects of Dietary Compounds on α-Hydroxylation of *N*-Nitrosopyrrolidine and *N'*-Nitrosonornicotine in Rat Target Tissues[1]

Fung-Lung Chung,[2] Amy Juchatz, Jean Vitarius, and Stephen S. Hecht

Division of Chemical Carcinogenesis, Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595

ABSTRACT

Male F344 rats were pretreated with various dietary compounds, and the effects of pretreatment on the *in vitro* α-hydroxylation of *N*-nitrosopyrrolidine or *N'*-nitrosonornicotine were determined in assays with liver microsomes or cultured esophagus, respectively. Dietary compounds included phenols, cinnamic acids, coumarins, indoles, and isothiocyanates. Pretreatments were carried out either by administering the compound by gavage 2 hr prior to sacrifice (acute protocol) or by adding the compound to the diet for 2 weeks (chronic protocol). Acute pretreatment with benzyl isothiocyanate, allyl isothiocyanate, phenethyl isothiocyanate, phenyl isocyanate, and benzyl thiocyanate but not sodium thiocyanate inhibited formation of α-hydroxylation products of both nitrosamines. When the chronic pretreatment protocol was used, only phenyl isothiocyanate and sodium thiocyanate inhibited formation of α-hydroxylation products of both nitrosamines. Pretreatments with butylated hydroxyanisole, *p*-methoxyphenol, or *N*-acetylcysteine had little, if any, effect on the α-hydroxylation of *N*-nitrosopyrrolidine or *N'*-nitrosonornicotine. Chronic pretreatment with *p*-hydroxycinnamic acid, 4-hydroxy-3-methoxycinnamic acid, coumarin, umbelliferone, limetine, indole, indole-3-carbinol, indole-3-acetonitrile, and L-tryptophan induced activity for the α-hydroxylation of *N*-nitrosopyrrolidine. The results of this study indicate that isothiocyanates are possible candidates for further study as potential inhibitors of carcinogenesis by *N*-nitrosopyrrolidine and *N'*-nitrosonornicotine.

INTRODUCTION

Exposure to *N*-nitrosamines may be associated with human cancer (23). One way of preventing the carcinogenic effects of nitrosamines is to avoid exposure to these agents. However, elimination of nitrosamines from the human environment may not be possible. Furthermore, considerable evidence has indicated that nitrosamines can be formed *in vivo* by endogenous nitrosation (26, 28). Therefore, it is important, as an alternate approach, to discover inhibitors of nitrosamine carcinogenesis, especially those which occur in the normal human diet.

Wattenberg (37) has demonstrated inhibitory activities of some dietary-related compounds against the tumorigenic activities of certain polynuclear aromatic hydrocarbons such as 7,12-dimethylbenz(*a*)anthracene and benzo (*a*)pyrene. These compounds, which include phenols, cinnamic acids, coumarins, isothiocyanates, and indoles, are widely distributed in fruits and vegetables. These results may be related, in part, to the epidemiological findings which implicate fruit and vegetable consumption in the reduction of the incidence of certain human cancers (2, 3).

However, only scattered information is available regarding the inhibition of nitrosamine carcinogenesis by dietary compounds (24, 36). One potentially practical approach to identifying dietary compounds which may inhibit nitrosamine carcinogenesis is to assess their effects on the metabolic activation of nitrosamines in target tissues. Using this approach as an initial screening method for potential inhibitors, we have studied the effects of some dietary-related compounds and their structural analogues on the *in vitro* metabolism of 2 structurally related environmental nitrosamines, NPYR[3] and NNN (Chart 1; Refs. 16 and 32). The *in vitro* metabolic assays were carried out in target tissues using rat liver microsomes for NPYR and cultured rat esophagus for NNN.

Both nitrosamines are believed to be metabolically activated by α-hydroxylation which gives reactive species that can damage DNA (14). Alternatively, these reactive intermediates can react with H_2O, forming stable metabolites. 4-Hydroxybutyraldehyde from NPYR can be assayed by HPLC as its 2,4-dinitrophenylhydrazone derivative (1). 4-Hydroxy-1-(3-pyridyl)-1-butanone (keto alcohol) and its oxidized metabolite, 4-oxo-4-(3-pyridyl)butyric acid (keto acid), formed by 2'-hydroxylation of NNN, and 4-hydroxy-4-(3-pyridyl)butyric acid (hydroxy acid), formed by 5'-hydroxylation of NNN, can be analyzed by HPLC (15). Quantitative assays for these metabolites and derivatives were used as indicators of the amounts of reactive precursors formed by these α-hydroxylation pathways.

MATERIALS AND METHODS

Chemicals. NPYR was obtained from Aldrich Chemical Co., Milwaukee, WI. The 2,4-dinitrophenylhydrazone of 4-hydroxybutyraldehyde was prepared as described previously (13). NADPH, NADP, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from Sigma Chemical Co., St. Louis, MO. 2,4-Dinitrophenylhydrazine was obtained from Eastman Kodak Co., Rochester, NY. Sucrose (special enzyme grade) was from Schwarz/Mann, Orangeburg, NY. [2'-^{14}C]NNN (51.7 mCi/mmol or 18.4 mCi/mmol) was purchased from New England Nuclear, Boston, MA. Unlabeled NNN reference metabolites were synthesized as described previously (13). Butylated hydroxyanisole was purchased from Sigma Chemical Co., St. Louis, MO. *p*-Methoxyphenol, *o*-hydroxycinnamic acid, *p*-hydroxycinnamic acid, 4-hydroxy-3-methoxycinnamic acid, coumarin, umbelliferone, limetine, indole, indole-3-carbinol, indole-3-acetonitrile, L-tryptophan, *N*-acetylcysteine, allyl isothiocyanate, phenyl isothiocyanate, sodium thiocyanate, phenyl isocyanate, benzyl isocyanide, and benzyl thiocyanate were all purchased from Aldrich Chemical Co. Benzyl isothiocyanate was obtained from Fluka, Hauppauge, NY, and phenethyl isothiocyanate was purchased from Eastman Kodak Co.

[1] This study was supported by National Cancer Institute Grants CA-32272 and CA-32519. This is Paper 68 in the series, "A Study of Chemical Carcinogenesis."

[2] To whom requests for reprints should be addressed.

Received December 1, 1983; accepted March 22, 1984.

[3] The abbreviations used are: NPYR, *N*-nitrosopyrrolidine; NNN, *N'*-nitrosonornicotine; HPLC, high-pressure liquid chromatography.

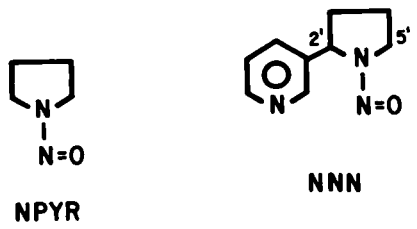

Chart 1. Structures of NPYR and NNN.

Animal Treatments. Male F344 rats (200 to 300 g) were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. Rats were housed in groups of 2 or 3 in solid-bottomed polycarbonate cages with hardwood bedding. Animals were kept at 20 ± 2° (S.D.) and 50 ± 10% relative humidity with a 12-hr light-dark cycle. Purina lab chow and water were administered *ad libitum*. For the acute studies, rats were treated by gavage with chemical (1 mmol/kg of body weight) in corn oil and sacrificed after 2 hr. The control groups received only corn oil, and 1 control group (3 rats) was used for every 3 treated groups. For most of the chronic studies, rats were fed *ad libitum* with NIH-07 diet containing the appropriate chemical (0.03 mmol/g of diet). For benzyl isothiocyanate, allyl isothiocyanate, phenethyl isothiocyanate, and phenyl isothiocyanate, the doses were reduced to 0.003 mmol/g of diet due to toxicity. The appropriate chemical and NIH-07 diet were thoroughly mixed with a mechanical mixer and stored at 4° before use. The control rats were fed NIH-07 diet, and 1 control group (4 rats) was used for every 8 treated groups. After 2 weeks of feeding, the animals were sacrificed by decapitation on Day 15.

Metabolism. After the rats were sacrificed, the esophagus and liver were removed. Preparation of liver microsomes followed a procedure previously described (13). Microsomes were assayed for protein concentration by the method of Lowry *et al.* (22). The assay for NPYR metabolism was carried out as follows (1). A mixture of 40 μmol of NPYR, 0.6 μmol of NADPH, 0.15 ml of microsomal suspension, 10 units of glucose-6-phosphate dehydrogenase, 10 μmol of glucose 6-phosphate, and 10 μmol of $MgCl_2$ was brought to a total volume of 2.0 ml with 0.185 mmol Tris-HCl buffer (pH 7.5) and incubated at 37° for 60 min. This incubation mixture was then quenched with 2 ml of ethanol. After removal of protein by centrifugation, 3 ml of the supernatant was treated with 0.2 ml of 0.15 M 2,4-dinitrophenylhydrazine reagent. A 0.04-ml aliquot of this sample was neutralized and analyzed by HPLC.

Rat esophagus cultures were established by the published method (15). Appropriate doses of [2′-^{14}C]NNN were added to 5.0 ml of Williams Medium E with supplements. Three different concentrations of [2′-^{14}C]-NNN were used: (*a*) 4.96 μM (10^6 dpm; specific activity, 18.4 μCi/μmol); (*b*) 1.82 μM (10^6 dpm; specific activity, 51.7 μCi/μmol); and (*c*) 0.18 μM (10^5 dpm; specific activity, 51.7 μCi/μmol). All 3 concentrations gave satisfactory HPLC radiograms and similar results, but Concentration C was the most economical. For a typical incubation, the dishes were placed in a controlled atmosphere culture chamber atop a rocking apparatus in a 37° warm room. The chamber was rocked at a rate of 10 cpm, alternatively exposing the sections to the atmosphere (95% O_2/5% CO_2) and to the culture medium. The incubations were carried out for 24 hr.

Analysis of Metabolites. Details of the HPLC assay for NPYR α-hydroxylation by hepatic microsomes have been described (1). Levels of α-hydroxylation were determined from the height of the peak corresponding to 4-hydroxybutyraldehyde-2,4-dinitrophenylhydrazone. Results were expressed as nmol/mg of protein.

Aliquots of the esophageal culture medium were analyzed directly for NNN metabolites, using an improved HPLC procedure.[4] The radioactivity associated with the 3 major metabolites, keto alcohol, keto acid, and hydroxy acid, was measured, converted to nmol, and divided by the total number of nmol in the sample analyzed to give the normalized value. Each esophagus was recovered fromthe culture medium, lyophilized, and weighed (average dry weight, 22.5 mg). Results were expressed as nmol/mg of dry weight of esophagus.

An index of inhibition or induction was calculated by dividing the mean values of the metabolites in the experiments with treated rats by the mean values in the corresponding control experiments. The resulting index value was >1 for induction and <1 for inhibition of metabolite formation. The statistical significance of the index was calculated by the 2-sample *t* test.

RESULTS

In the acute studies, treatment of rats with the tested compounds at a concentration of 1 mmol/kg of body weight did not cause observable toxicity, as evidenced by no death among treated rats before sacrifice and the normal gross appearance of the livers. In the chronic studies, most of the compounds tested at 0.03 mmol/g of diet caused no apparent toxic effects. Treated rats had weight gains that were comparable to those of control rats during the 2-week feeding period. However, allyl isothiocyanate, phenethyl isothiocyanate, benzyl isothiocyanate, and phenyl isothiocyanate showed possible toxicities, as evidenced by considerable weight loss (5 to 50 g) in the treated rats. In view of this fact, the doses of these compounds were reduced to 0.003 mmol/g of diet. At this dose, no apparent toxicities were observed. On the average, each rat consumed about 15 g of diet/day. Therefore, the total doses of the isothiocyanates in the chronic studies were about 2.1 mmol/kg of body weight, whereas the total doses of the other compounds were about 21 mmol/kg of body weight.

In previous studies, we have shown that the formation of NNN metabolites in cultured rat esophagus increased over a period of 48 hr (15). In the present study, most of the esophagus culture experiments were carried out for 24 hr, because the formation of metabolites, even in cases in which inhibition occurred, was sufficient to allow accurate determination of radioactivity. Some experiments were also carried out with incubation times of 4 or 6 hr, and the observed extents of inhibition were similar to those reported for the 24-hr incubations (data not shown). The concentrations of [2′-^{14}C]NNN used in these experiments were in the linear portion of the substrate concentration *versus* product formation curve. The concentration of NPYR and incubation time for the liver microsomal assays were determined based on previous results (1).

Table 1 summarizes some representative data obtained upon

Table 1

Metabolism of NPYR to 4-hydroxybutyraldehyde with liver microsomes from treated and control rats

Rats were treated by gavage with chemical (1 mmol/kg) in corn oil, 2 hr prior to sacrifice. Liver microsomes were prepared and incubated with NPYR as described in "Materials and Methods."

Chemical treatment	4-Hydroxybutyraldehyde (nmol/mg of protein)[a]	
	Treated	Control
Allyl isothiocyanate	24.0 ± 4.0[b]	48.7 ± 4.5
Phenethyl isothiocyanate	26.0 ± 6.0	48.7 ± 4.5
Sodium thiocyanate	45.0 ± 5.0	48.7 ± 4.5

[a] 4-Hydroxybutyraldehyde was measured as its 2,4-dinitrophenylhydrazone derivative. Average microsomal protein content was 7.4 mg/ml.

[b] Mean ± S.D. of preparations from 3 rats. All assays were performed in duplicate.

[4] S. Carmella, and S. S. Hecht. Optimized high-performance liquid chromatographic analysis of metabolites of *N′*-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, manuscript in preparation.

Table 2

Metabolism of NNN with cultured rat esophagus from treated and control rats

Rats were treated by gavage with chemical (1 mmol/kg) 2 hr prior to sacrifice. The esophagus was removed and cultured with [2′-^{14}C]NNN (1.82 μM), as described in "Materials and Methods." Each value is the mean of experiments with 3 rats and represents the amount of metabolite in 5 ml of incubation medium.

Chemical treatment	Metabolites[a]	nmol/culture dish		nmol[a]/mg of dry esophagus[b]	
		Treated	Control	Treated	Control
Allyl isothiocyanate	Hydroxy acid	0.29	0.75	0.18 ± 0.04[c]	0.59 ± 0.02
	Keto acid	0.46	0.98	0.29 ± 0.05	0.76 ± 0.04
	Keto alcohol	0.55	1.04	0.36 ± 0.02	0.82 ± 0.03
Phenethyl isothiocyanate	Hydroxy acid	0.29	0.75	0.17 ± 0.02	0.59 ± 0.02
	Keto acid	0.48	0.98	0.28 ± 0.04	0.76 ± 0.04
	Keto alcohol	0.50	1.04	0.30 ± 0.02	0.82 ± 0.03

[a] Normalized to the total nmol in the sample analyzed.
[b] Average weight of dry esophagus was 22.5 mg.
[c] Mean ± S.D.

incubation of NPYR with hepatic microsomes from rats which had been treated with allyl isothiocyanate, phenethyl isothiocyanate, or sodium thiocyanate, and Table 2 summarizes data obtained upon metabolism of [2′-^{14}C]NNN by esophagus organ cultures from the same rats treated with allyl and phenethyl isothiocyanates. These data and all other data obtained in this study were converted to an index of inhibition or induction and are presented as such in Tables 3 and 4.

The effects on NPYR metabolism of chronic administration of compounds to rats are illustrated in Table 3. Only *p*-methoxyphenol showed moderate inhibitory activity. Butylated hydroxyanisole and *o*-hydroxycinnamic acid had no effect on NPYR metabolism, and *p*-hydroxycinnamic acid, coumarins, and indoles were inducers. The 2 indole derivatives, indole-3-carbinol and L-tryptophan, were particularly effective inducers of NPYR metabolism. In view of these results, the effects of cinnamic acids, coumarins, and indoles were not investigated further in this study.

The potential inhibitory activities of organic isothiocyanates and related compounds on NPYR and NNN metabolism were evaluated in both acute and chronic studies. The results are summarized in Table 4. For NPYR in the acute studies, inhibitory activities were observed for all organic isothiocyanates and related compounds. However, sodium thiocyanate was inactive. In the chronic studies, benzyl isothiocyanate and benzyl thiocyanate had virtually no effect on the metabolism of NPYR. Sodium thiocyanate was, however, a good inhibitor, in contrast to the results obtained in the acute study.

Table 4 also shows that all of the organic isothiocyanates demonstrated considerable inhibition of NNN metabolism, ranging from 2- to 3-fold, in the acute studies. Except for benzyl thiocyanate, structurally related chemicals were essentially inactive. Two phenols, *p*-methoxyphenol and butylated hydroxyanisole, had little effect on the metabolism of NNN when the acute treatment protocol was used. In the chronic studies, benzyl isothiocyanate, phenyl isothiocyanate, and benzyl thiocyanate retained, at least partially, their inhibitory activity on NNN metabolism. Allyl and phenethyl isothiocyanate and other related compounds were essentially inactive. Interestingly, sodium thiocyanate displayed inhibitory activity in the chronic study but had no effect in the acute study, as observed with NPYR.

Table 3

Chronic effects of some dietary compounds on the metabolism of NPYR in rat liver microsomes

For 2 weeks prior to sacrifice, rats consumed NIH-07 diet containing the appropriate compound. Liver microsomes were used for the metabolism of NPYR to 4-hydroxybutyraldehyde.

Compound	Index[a]
Butylated hydroxyanisole	1.0[b]
p-Methoxyphenol	0.7[c]
o-Hydroxycinnamic acid	1.0
p-Hydroxycinnamic acid	1.5[d]
4-Hydroxy-3-methoxycinnamic acid	1.5[d]
Coumarin	1.6[d]
Umbelliferone	1.6[d]
Limetine	1.7[d]
Indole	1.4[c]
Indole-3-carbinol	2.8[d]
Indole-3-acetonitrile	1.5[d]
L-Tryptophan	2.2[d]
N-Acetylcysteine	0.9[c]

[a] Mean of the treated rat values (nmol of 4-hydroxybutyraldehyde/mg of protein) divided by the mean of the control rat values. For each compound, 3 or 4 treated rats were used. The same number of rats was used for each control group. Incubations were carried out at least in duplicate for each rat liver microsomal preparation.
[b] Average standard deviation for all values, 14%.
[c] $p < 0.05$ compared to control.
[d] $p < 0.01$ compared to control.

DISCUSSION

In previous studies, several nondietary compounds have been shown to inhibit the metabolism of certain nitrosamines such as *N*-nitrosodimethylamine and NPYR. For example, pyrazole, 3-amino-1,2,4-triazole, aminoacetonitrile, pregnenolone-16α-carbonitrile, and disulfiram inhibited metabolism of *N*-nitrosodimethylamine (10, 12, 30, 31), and pyrazole and disulfiram were also inhibitors of NPYR metabolism. (5). Disulfiram, pregnenolone-16α-carbonitrile, and aminoacetonitrile have been shown to reduce the hepatotoxicity and hepatocarcinogenicity of *N*-nitrosodimethylamine in rats (11, 12, 34). In contrast to studies in polynuclear aromatic hydrocarbon carcinogenesis, limited information is available on the inhibition of nitrosamine carcinogenesis, and even less is known about its inhibition by dietary-related compounds. Only 2 dietary-related compounds, butylated hydroxyanisole and indole, have been reported to inhibit nitrosamine carcinogenesis. Butylated hydroxyanisole inhibited lung tumor induction in mice by *N*-nitrosodimethylamine, and indole inhibited bladder carcinoma induction in hamsters by *N*-nitrosodibutylamine (24, 36).

In this study, 21 dietary and related compounds have been evaluated for potential inhibitory activities against the carcinogenic effects of NPYR or NNN by using *in vitro* metabolic assays in target tissues. We have shown that isothiocyanates can inhibit the metabolic α-hydroxylation of both NPYR and NNN using the acute treatment protocol. However, in the chronic experiments,

Table 4

Effects of dietary and related compounds on the metabolism of NPYR in rat liver microsomes and NNN in cultured rat esophagus

Rats were treated with the appropriate isothiocyanate by either the acute or chronic protocol described in "Materials and Methods." After sacrifice, liver microsomes or esophagus organ cultures were used for metabolism of NPYR to 4-hydroxybutyraldehyde or NNN to hydroxy acid, keto acid, and keto alcohol, respectively, as described in "Materials and Methods." Average standard deviation for all values: for NPYR, 18% for the acute study and 16% for the chronic study; and for NNN, 13% for the acute study and 16% for the chronic study.

	Index[a]							
	NPYR		NNN					
			Acute			Chronic		
Compound	Acute (4-hydroxybutyraldehyde)	Chronic (4-hydroxybutyraldehyde)	Hydroxy acid	Keto acid	Keto alcohol	Hydroxy acid	Keto acid	Keto alcohol
Benzyl isothiocyanate	0.5[b]	1.0	0.3[b]	0.4[b]	0.5[b]	0.6[c]	0.6	0.8
Allyl isothiocyanate	0.5[b]	0.7[b]	0.3[b]	0.4[b]	0.4[b]	0.9	1.0	1.0
Phenethyl isothiocyanate	0.5[b]	0.5[b]	0.3[b]	0.4[b]	0.4[b]	0.9	1.0	1.5[b]
Phenyl isothiocyanate	0.5[b]	0.6[b]	0.6[b]	0.6[b]	0.9	0.4[b]	0.5[b]	0.7[b]
Sodium thiocyanate	1.0	0.5[b]	0.9[c]	1.0	1.1	0.3[b]	0.6[b]	0.4[b]
Phenyl isocyanate	0.5[b]	0.7[b]	0.9[b]	0.9	1.2[c]	0.9	0.9	1.2
Benzyl isocyanide	0.6[b]	ND[d]	0.7[c]	0.9	1.0	ND	ND	ND
Benzyl thiocyanate	0.4[b]	1.2	0.4[b]	0.5[b]	0.6[b]	0.7[c]	0.8	0.7[c]
N-Acetylcysteine	ND	0.9[c]	1.0	1.0	0.9[b]	0.9	1.0	1.1
Butylated hydroxyanisole[e]	ND	1.0	1.0	1.0	1.1	ND	ND	ND
P-Methoxyphenol[e]	ND	0.7[c]	1.1	1.0	1.2	ND	ND	ND

[a] Mean of the treated rat metabolite values divided by the mean of the control rat values. Three or 4 treated rats were used for each compound and, in each control group, incubations were carried out at least in duplicate for each rat liver microsomal preparation.
[b] $p < 0.01$ compared to control.
[c] $p < 0.05$ compared to control.
[d] ND, not determined.
[e] NNN values are results from 4-hr incubation. NPYR values are from Table 3.

the isothiocyanates were not consistently inhibitory. An inhibitory activity could be accounted for by at least 2 mechanisms: inactivation of enzymes involved in metabolism and/or a scavenging effect. In this case, the enzyme inactivation mechanism is supported by several observations. First, the inhibitory activity of a compound was often associated with an increase in the amount of unmetabolized NNN in the postincubation medium as compared to that of the control experiments; secondly, in the NNN metabolism study, no additional peaks which might have resulted from scavenging of activated intermediates were observed in the radiograms; finally, *N*-acetylcysteine, which is known to protect biological systems from adverse effects because of its nucleophilicity (4), had no apparent effects on NNN metabolism.

Benzyl, allyl, and phenethyl isothiocyanates, as well as benzyl thiocyanate, are found as glucosinolates in cruciferous plants such as cabbage, brussels sprouts, and broccoli (19, 25). They are released in the free form upon hydrolysis by enzymes or *in vivo* (27, 35). The relative amounts of free isothiocyanates and the parent glucosinolates present in vegetables that humans consume depend largely on how the vegetables are prepared (6). Thus, further studies are necessary to determine whether vegetables containing the appropriate isothiocyanates or glucosinolates *per se* can inhibit NNN or NPYR metabolism.

Allyl isothiocyanate has been shown to be toxic and carcinogenic (9, 18). However, very little is known about the metabolism or pharmacokinetics of isothiocyanates. Chronic p.o. administration of allyl isothiocyanate to rats increased the serum thiocyanate level by 3- to 5-fold (20). The isothiocyanate group is reactive and is likely to interact with amino, hydroxy, and thiol groups of proteins to form addition products (8). α-Naphthyl isothiocyanate reduces the activity of hepatic cytochrome P-450 monooxygenases (7), and the isothiocyanates examined in the present study may have similar effects. The inhibitory activity of α-naphthyl isothiocyanate toward P-450 monooxygenases may be related to its anticarcinogenic effects toward *N*-2-fluorenylacetamide and *N*-nitroso-*N*-(4-hydroxybutyl)butylamine (17, 33).

In contrast to the isothiocyanates, the cinnamic acids, coumarins, and indoles enhanced hepatic α-hydroxylation of NPYR, and butylated hydroxyanisole had no effect on NPYR or NNN metabolism. While the effects of cinnamic acids and coumarins on microsomal enzyme activity have not been well established, indoles have been shown to be good inducers of microsomal enzymes (21, 29), and indole-3-carbinol is among the most potent inducers. These results are consistent with our observation of induction of NPYR metabolism by indoles, among which indole-3-carbinol demonstrated the most pronounced activity.

In conclusion, among all of the compounds tested in this study, isothiocyanates were generally the most effective in inhibiting the *in vitro* metabolism of both NPYR and NNN in their target tissues. However, the role these compounds may play in preventing the carcinogenicity of NPYR and NNN awaits the results of bioassay studies.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Betti Reiss, Carol Weiss, and Stella Spathopoulos for their extensive help with the organ culture studies.

REFERENCES

1. Chen, C. B., McCoy, G. D., Hecht, S. S., Hoffmann, D., and Wynder, E. L. High-pressure liquid chromatographic assay for α-hydroxylation of *N*-nitrosopyrrolidine by isolated rat liver microsomes. Cancer Res., *38:* 3812–3816, 1978.
2. Committee on Diet, Nutrition, and Cancer. Assembly of Life Sciences, National Research Council. Diet, Nutrition, and Cancer, *Chap. 15:* "Inhibitors of Carcinogenesis." Washington, DC: National Academy Press, 1982.
3. Committee on Diet, Nutrition, and Cancer. Assembly of Life Sciences, National Research Council. Diet, Nutrition, and Cancer, *Chap. 17:* "The Relationship of Diet to Cancer at Specific Sites." Washington, DC: National Academy Press, 1982.
4. Cottrell, R. C., Young, P. O. J., Walters, D. G., Phillips, J. C., Lake, B. G., and Gangolli, S. D. Studies of the metabolism of *N*-nitrosopyrrolidine in the rat. Toxicol. Appl. Pharmacol. *51:* 101–106, 1979.
5. Cox, P. J., and Abel, G. Cyclophosphamide-induced cystitis: its cause and possible clinical significance. Br. J. Cancer, *40:* 311, 1979.
6. Daxenbichler, M. E., Van Etten, C. H., and Spencer, G. F. Glucosinolates and derived products in cruciferous vegetables. Identification of organic nitriles from cabbage. J. Agr. Food Chem., *25:* 121–124, 1977.

7. Drew, R., and Priestly, B. G. Microsomal drug metabolism during α-naphthyl-isothiocyanate-induced cholestasis. Toxicol. Appl. Pharmacol., *35:* 491–494, 1976.
8. Drobnica, L., Nemec, P., Antos, K., Kristian, P., and Hulka, A. Reactions of isothiocyanates at molecular and cellular level. *In:* Natural Occurring Goitrogens and Thyroid Function. Symposium, Smolenice, Czechoslovakia, 1964, pp. 61–69.
9. Dunnick, J. K., Prejean, J. D., Haseman, J., Thompson, R. B., Gile, M. D., and McConnell, E. E. Carcinogenesis bioassay of allyl isothiocyanate. Fund. Appl. Toxicol., *2:* 114–120, 1982.
10. Fiume, L., Campadelli-Fiume, O. G., Magee, P. N., and Holsman, J. Cellular injury and carcinogenesis: inhibition of metabolism of dimethylnitrosamine by aminoacetonitrile. Biochem. J., *120:* 601–605, 1970.
11. Hadjiolov, D. The inhibition of dimethylnitrosamine carcinogenesis in rat liver by aminoacetonitrile. Z. Krebsforsch., *76:* 91–92, 1971.
12. Hadjiolov, D., and Mundt, D. Effects of aminoacetonitrile on the metabolism of dimethylnitrosamine and methylation of RNA during liver carcinogenesis. J. Natl. Cancer Inst., *52:* 753–756, 1974.
13. Hecht, S. S., Chen, C. B., and Hoffmann, D. Evidence for metabolic α-hydroxylation of *N*-nitrosopyrrolidine. Cancer Res., *38:* 215–218, 1978.
14. Hecht, S. S., McCoy, G. D., Chen, C. B., and Hoffmann, D. The metabolism of cyclic nitrosamines. *In:* R. A. Scanlan and S. R. Tannenbaum (eds.), *N*-Nitroso Compounds, American Chemical Society Symposium, Series, *Vol. 174:* pp. 49–75. Washington, DC: American Chemical Society, 1981.
15. Hecht, S. S., Reiss, B., Lin, D., and Williams, G. M. Metabolism of *N'*-nitrosonornicotine by cultured rat esophagus. Carcinogenesis (Lond.), *3:* 453–456, 1982.
16. Hoffmann, D., Adams, J. D., Brunnemann, K. D., and Hecht, S. S. Assessment of tobacco-specific *N*-nitrosamines in tobacco products. Cancer Res., *39:* 2505–2509, 1979.
17. Ito, N., Matayoshi, K., Matsumura, K., Denda, A., Kani, T., Arai, M., and Makiura, S. Effect of various carcinogenic and noncarcinogenic substances on bladder tumors in rats induced by *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine. Gann, *65:* 123–130, 1974.
18. Kasamaki, A., Takahashi, H., Tsumura, N., Niwa, J., Fujita, T., and Urasawa, S. Genotoxicity of flavoring agents. Mutat. Res., *105:* 387–392, 1982.
19. Kjaev, A. Naturally occurring isothiocyanates and their parent glycosides. *In:* N. Kharasch (ed.), Chemistry of Organic Sulfur Compounds, Vol. 1, pp. 409–420. Elmsford, New York: Pergamon Press, Inc.,1961.
20. Langer, P. Relations between thiocyanate formation and goitrogenic effect of foods. VI. Thiocyanate activity of allyl isothiocyanate, one of the most frequently occurring mustard oils in plants. Z. Physiol. Chem., *339:* 33–35, 1964.
21. Loub, N. D., Wattenberg, L. W., and Davis, D. N. Aryl hydrocarbon hydroxylase induction in rat tissue by naturally occurring indoles of cruciferous plants. J. Natl. Cancer Inst., *54:* 985–988, 1975.
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurements with the Folin reagent. J. Biol. Chem., *193:* 265–275, 1951.
23. Magee, P. N. (ed.) Nitrosamines and Human Cancer. Banbury Report 12. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982.
24. Matsumoto, M., Oyasu, R., Hopp, M. L., and Kitajima, T. Suppression of dibutylnitrosamine-induced bladder carcinomas in hamster by dietary indole. J. Natl. Cancer Inst., *58:* 1825–1829, 1977.
25. Miller, L. P. Glycosides. *In:* L. P. Miller (ed.), Phytochemistry, Vol. 1, pp. 197–375. New York: Van Nostrand Reinhold Co., 1973.
26. Mirvish, S. S. Formation of *N*-nitroso compounds: chemistry, kinetics, and *in vivo* occurrence. Toxicol. Appl. Pharmacol., *31:* 325–351, 1975.
27. Oginsky, E. L., Stein, A. E., and Greer, M. A. Myrosinase activity in bacteria as determined by the conversion of progoitrin to goitrin. Proc. Soc. Exp. Biol. Med., *119:* 360–364, 1965.
28. Ohshima, H. and Bartsch, H. Quantitative estimation of endogenous nitrosation in humans by monitoring *N*-nitrosoproline excreted in the urine. Cancer Res., *41:* 3658–3662, 1981.
29. Pantuck, E. J., Pantuck, C. B., Garland, W. A., Mins, B., Wattenberg, L. W., Anderson, K. E., Kappas, A., and Conney, A. H. Effects of dietary brussels sprouts and cabbage on human drug metabolism. Clin. Pharmacol. Ther., *25:* 88–95, 1979.
30. Philips, J. C., Lake, B. G., Gangolli, S. D., Grasso, P., and Lloyd, A. G. Effects of pyrazole and 3-amino-1,2,4-triazole on the metabolism and toxicity of dimethylnitrosamine in the rat. J. Natl. Cancer Inst., *58:* 629–63, 1977.
31. Schmähl, D., and Krüger, F. W. Influence of disulfiram on the biological actions of *N*-nitrosamines. *In:* W. Nakahara, S. Takayama, T. Sugimura, and S. Odashima (eds.), Topics in Chemical Carcinogenesis, pp. 199–201. Baltimore: University Park Press, 1972.
32. Sen, N. P., Seaman, S., and Tessier, L. A rapid and sensitive method for the determination of nonvolatile *N*-nitroso compounds in foods and human urine: recent data concerning volatile nitrosamines in dried foods and malt-based beverages. *In:* H. Bartsch, K. O'Neill, M. Castegnaro, and M. Okada (eds.), *N*-Nitroso Compounds: Occurrence and Biological Effects. IARC Scientific Publication 41, pp. 185–197. Lyon, France: International Agency for Research on Cancer, 1982.
33. Sidransky, H., Ito, N., and Verney, E. Influence of α-naphthyl isothiocyanate on liver homogenesis in rats ingesting ethionine and *N*-2-fluorenylacetamide. J. Natl. Cancer Inst., *37:* 677–686, 1966.
34. Somoggi, A., Conney, A. H., Kuntzman, R., and Solymoss, B. Protection against dimethylnitrosamine toxicity by pregnenolone-16α-carbonitrile. Nat. New Biol., *237:* 61–63, 1972.
35. Van Etten, C. H., Daxenbichler, M. E., and Wolff, J. A. Natural glucosinolates (thioglucosides) in food and feed. J. Agric. Food Chem., *17:* 483–491, 1969.
36. Wattenberg, L. W. Inhibition of carcinogenic effects of diethylnitrosamine and 4-nitroquinoline-*N*-oxide by antioxidants. Fed. Proc., *31:* 633, 1972.
37. Wattenberg, L. W. Inhibition of chemical carcinogenesis. Adv. Cancer Res., *26:* 197–226, 1978.