Elevation of Extrahepatic Glutathione S-Transferase and Epoxide Hydratase Activities by 2(3)-tert-Butyl-4-hydroxyanisole

Ann M. Benson, Young-Nam Cha, Ernest Bueding, Henry S. Heine, and Paul Talalay

Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine (A. M. B., E. B., P. T.) and Department of Pathobiology, The Johns Hopkins University School of Hygiene and Public Health (Y-N. C., E. B., H. S. H.), Baltimore, Maryland 21205

ABSTRACT

Investigations in these and other laboratories have established that administration of 2(3)-tert-butyl-4-hydroxyanisole (BHA) to rodents: (a) protects a variety of target tissues against the production of tumors by a wide range of chemical carcinogens; (b) reduces the levels of mutagenic metabolites produced from benzo(a)pyrene and numerous therapeutic agents in vivo; (c) elevates the hepatic activities of microsomal epoxide hydratase and cytosol glutathione S-transferase; (d) alters the activities of other hepatic enzymes and affects the levels of some hepatic catalytic constituents; and (e) increases the concentrations of nonprotein thiol compounds in liver and several other tissues. Addition of BHA to the diet resulted in elevated glutathione S-transferase and epoxide hydratase activities in multiple extrahepatic tissues of female CD-1 mice and male Sprague-Dawley rats. In mice, glutathione S-transferase specific activities in cytosol doubled in lung and stomach and increased to 15 times control levels (with 1,2-dichloro-4-nitrobenzene as substrate) in small intestine. Microsomal epoxide hydratase specific activity toward styrene oxide doubled in mouse colon and stomach and increased to nearly 6 times control levels in small intestine. Enhanced activities of these enzymes were observed in several other mouse tissues and in rat small intestine, kidney, and lung. Dietary administration of BHA to mice led to elevations of the concentrations of nonprotein sulfhydryl compounds in the mucosa of several digestive tract tissues as well as in the urinary bladder. Epoxide hydratase is a detoxifying enzyme which inactivates numerous mutagenic epoxides. However, elevation of epoxide hydratase activity may not necessarily exert a protective function in the case of all arene oxides since at least some forms of this enzyme catalyze an essential step in the formation of carcino- genic diol-epoxides of benzo(a)pyrene. The enhancement by BHA of extrahepatic glutathione S-transferase activities and nonprotein sulfhydryl levels, and possibly also the enhancement of extrahepatic epoxide hydratase activity, may be important factors in the mechanisms by which this antioxidant protects against chemical carcinogenesis.

INTRODUCTION

Reactive forms of chemical carcinogens are, in general, electrophilic derivatives resulting from the metabolic activities of the tissues of the host on the parent procarcinogens (41). The ultimate fate of these reactive intermediates depends not only upon their ability to reach and to react with critical sites on informational macromolecules, but also upon the rates of inactivation by competing enzymatic reactions. Our studies are concerned with 2 major types of host enzymes which metabolize electrophilic species: the soluble glutathione S-transferases (EC 2.5.1.18) (25) and the microsomal membrane-bound epoxide hydratase (EC 4.2.1.63) (46). We have demonstrated (6, 7, 14, 15) that the specific activities of these enzymes are dramatically elevated in the livers of mice and to a lesser degree in rats receiving diets supplemented with BHA under conditions where the activities of the hepatic mixed-function oxidation activation enzymes are relatively unchanged (13, 14). Furthermore, these elevations in enzyme levels are accompanied by increases in acid-soluble sulfhydryl compounds in mice (4). These observations may be of special interest since the administration of BHA and certain other antioxidants effectively suppresses the production of tumors in a variety of rodent tissues by chemical carcinogens of quite diverse structures (63). The latter phenomenon, which was first systematically investigated by Wattenberg (61, 62), is potentially of considerable theoretical and practical importance since BHA is one of a class of antioxidants widely used as food additives and is noted for its low toxicity (11, 23).

The inference that elevations of glutathione S-transferase and epoxide hydratase activities may play a role in the suppression of carcinogenicity by antioxidants is further strengthened by the recent findings in these laboratories (3, 4) that the administration of dietary BHA or 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (ethoxyquin) to mice resulted in a marked reduction of the levels of mutagenic metabolites of benzo(a)pyrene (and of a number of heterocyclic therapeutic agents) in the urine and in the peritoneal cavities of mice treated with these antioxidants. Moreover, the direct addition of glutathione and purified rat liver glutathione S-transferase A or B to the mutagenesis assay system of Ames (1) depressed the mutagenic activity of urinary benzo(a)pyrene metabolites (6, 7). Other investigators have shown that purified epoxide hydratase preparations obliterated the mutagenicity of several benzo(a)pyrene oxides (16, 18, 33, 47-49, 69-71) but that certain of the resulting dihydrodiols yielded highly potent mutagens upon further oxidative activation (69, 70).

The glutathione S-transferases are a family of highly versatile catalytic and binding proteins which are present in the cytosols of many tissues and species (25, 50). They are characterized by overlapping yet distinct substrate specificities and have been purified from human and rat livers where they constitute under basal conditions an extraordinarily high proportion (estimated variously as 3 to 10%) of the cytosolic proteins and play a major role in the detoxification of foreign compounds (25). The transferases enhance the nucleophilicity of glutathi-
one and, among other reactions, promote thioether formation from hydrophobic compounds as a first step in mercapturic acid formation (10, 25). These enzymes conjugate a variety of arene oxides with glutathione (5, 10, 24, 26, 43–45) and under some conditions may also play a sacrificial role by reacting covalently with specifically bound alkylating agents such as metabolites of 3-methylcholanthrene (35) and 4–dimethylna noazobenzene (31). Some of the relationships between the glutathione S-transferases and chemical carcinogenesis have been the subject of a recent review (54).

The microsomal membrane-bound epoxide hydratases have broad specificity in the cleavage of hydrophobic oxiranes (alkyl epoxides or arene oxides) which are converted to the corresponding trans dihydrodiols (46). Homogeneous preparations of epoxide hydratase have been obtained from rat liver microsomes (37, 48). In such preparations, the activity with styrene oxide, which is commonly used as a substrate for epoxide hydratase, appeared to reside in the same catalytic protein as that acting on polycyclic hydrocarbon derivatives, including the epoxides of benzo(a)pyrene (37, 48). Levin et al. (34) have shown that the effects of modifiers of epoxide hydratase activity depend on the substrate studied. Epoxide hydratase activities are elevated to a modest extent by administration of phenobarbital and hydrocarbon inducers to the host animals (26, 46). Evidence for the existence of multiple forms of epoxide hydratase in rat liver microsomes includes differential induction of activities toward several substrates (12) as well as chromatographic resolution of immunologically distinct proteins with hydratase activity toward styrene oxide (21).

Although the liver is quantitatively the most important organ for the metabolic activation and detoxification of various chemicals in vivo, chemical carcinogens are often highly selective with respect to their target organs and tissues. There is mounting evidence that tissue susceptibility to carcinogens may be in part controlled by the metabolic patterns of specific target tissues (2). BHA and other antioxidants have been found to inhibit chemical carcinogenesis in a variety of tissues (63), and the influence of these compounds on the detoxification capacities of extrahepatic tissues may be relevant to the protective effects. This report is concerned with the effects of dietary administration of BHA on the levels of glutathione S-transferase and microsomal epoxide hydratase activities and on the concentrations of nonprotein thiol compounds in a number of extrahepatic tissues of rodents.

**MATERIALS AND METHODS**

**Treatment of Animals.** Seven-week-old female CD-1 mice (Charles River Breeding Laboratories, Wilmington, Mass.) and 7-week-old male Sprague-Dawley rats (Madison, Wis.) were housed in stainless steel wire cages (4 mice or 1 rat per cage) and were fed ad libitum either a powdered diet of Purina laboratory chow (Ralston-Purina Co., St. Louis, Mo.) or the BHA diet. The BHA (Sigma Chemical Co., St. Louis, Mo.) was incorporated into the powdered laboratory chow at a final concentration of 7.5 g/kg by mixing in a Liquid-Solids Blender (Patterson-Kelley Co., East Stroudsburg, Pa.). Mice and rats were sacrificed by cervical dislocation and decapitation, respectively.

**Collection and Processing of Tissues for Enzyme Studies.** Mouse tissues were perfused in situ. The portal vein was severed, and cold 0.15 M KCl containing 2 mM ethylenediaminetetraacetic acid (final pH 7.5) was injected through the left ventricle. When necessary, the livers were perfused again after excision. Rat and mouse small intestines and mouse colons were slit longitudinally, and the mucosal surface was scraped with a blunt-edged spatula. The material so obtained was shown by histological examination to consist largely of mucosa and submucosa and to contain rather small amounts of muscular elements. The forestomach and glandular stomach were analyzed as a single organ. All tissues were excised, chilled, weighed, and frozen in liquid nitrogen within minutes of sacrifice, and were stored at —80° until further processing.

**Preparation of Cytosol Fractions and Microsomes.** All steps were carried out at 0 to 4°. Tissues were homogenized in 3 ml of 0.25 M sucrose per g of tissue, with the exception of rat intestine (2 ml/g) and mouse thymuses and bladders (5 ml/g). Some of the smaller mouse tissues were pooled before homogenization; thus, there were 4 bladders or 2 uteri, thymuses, or spleens per homogenate. The procedure for the preparation of microsomes was based on that of Schenkenman and Cinti (52). To the supernatant fluid obtained by centrifugation at 9,000 × g for 15 min was added 0.2 volume of 0.1 M CaCl₂. Centrifugation at 20,000 × g for 15 min or at 9,000 × g for 30 min then yielded the microsomal pellet and a clear cytosol fraction. The microsomes were resuspended in a volume of 0.25 M sucrose equivalent to one-half the volume of the homogenate from which they were derived and resedimented by centrifugation for 15 min at 37,000 × g. The washed microsomes were resuspended in 0.25 M sucrose at a protein concentration of 1 to 5 mg/ml, frozen in liquid nitrogen, and stored at —80°.

**Determinations of Enzyme Activities.** Glutathione S-transferase activities of the cytosol fractions were determined with 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, and p-nitrobenzylchloride, all of which were obtained from Eastman Organic Chemicals (Rochester, N. Y.), and glutathione (Sigma Chemical Co.) as substrates. The initial velocities of glutathione conjugate formation were measured spectrophotometrically at 25° according to the procedures of Habig et al. (22). Epoxide hydratase activity of microsomes was assayed at 37° with [8-14C]styrene oxide (New England Nuclear Corporation, Boston Mass.) essentially according to the thin-layer chromatography micromethod of Jerina et al. (27). Precise details of the assay procedures have been given elsewhere (15). The glutathione S-transferase and epoxide hydratase specific activities are based upon the protein concentrations of cytosol fractions and microsome suspensions, respectively, determined by the method of Lowry et al. (36) with crystalline bovine serum albumin as a standard.

**Determination of the Concentrations of Acid-soluble Thiol Compounds in Mouse Tissues.** Tissues were excised and placed on a glass plate over wet ice. Gastrointestinal tissues were slit longitudinally. Residual food and fecal matter were removed, and the mucosa and submucosa (see above) was collected by gentle scraping with a blunt-edged spatula. Tissues were homogenized in 5% trichloroacetic acid (20 ml/g of tissue). After centrifugation of the homogenates, unprecipitated thiols were determined by the spectrophotometric procedure of Grassetti et al. (20) as previously described (4). The results are expressed in terms of pmol of thiol per g of wet tissue.
RESULTS

Glutathione S-Transferase and Epoxide Hydratase Levels in Extrahepatic Tissues of Rodents Receiving BHA. Dietary administration of BHA to mice and rats resulted in significant elevations of the specific activities of microsomal epoxide hydratase and cytosol glutathione S-transferases in most of the tissues examined.

Mouse Tissues. Two groups of mice were examined. Eight mice received the BHA diet for 14 days, and 8 mice received the control diet. The specific activities of epoxide hydratase (with styrene oxide) in the microsomal fractions and of the glutathione S-transferases (with 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, and p-nitrobenzylchloride) in the cytosol fractions from 10 tissues of these mice are shown in Table 1. The effects of BHA on the hepatic enzyme levels were similar to those which we reported previously (6, 7, 14, 15); in the BHA group, the epoxide hydratase specific activity was 11 times as great as that in the control group, and glutathione S-transferase specific activities with the various substrates were 8 to 11 times those of the controls. Among the extrahepatic tissues examined, the most dramatic response to BHA administration was observed in the small intestine. The microsomal epoxide hydratase specific activity was elevated to nearly 6 times control levels, and increases in glutathione S-transferase activities to 4.4 times control levels with 1-chloro-2,4-dinitrobenzene and to 14.9 times control levels with 1,2-dichloro-4-nitrobenzene, were observed. Sizable increases in these enzyme activities were also found in other tissues of the digestive tract. BHA treatment led to doubling of epoxide hydratase specific activities in stomach and colon; glutathione S-transferase activities increased 60 to 120% in stomach, but only 40% in colon. Both enzymes increased in kidney, although the degree of elevation of the glutathione S-transferase activities was more substantial (45 to 72%) than that of epoxide hydratase (26%). More selective effects on these enzyme activities were observed in 2 tissues. In lung, glutathione S-transferase activity doubled in response to the BHA treatment, whereas epoxide hydratase activity increased only 17%. Conversely, in the thymus, epoxide hydratase activity increased to 258% of control levels, whereas glutathione S-transferase activities showed no response to BHA. Not enough data were obtained for statistical evaluation of the effects of BHA on the levels of these enzyme activities in mouse bladder. However, it is apparent that even in untreated mice the specific activities of epoxide hydratase and of the glutathiones S-transferases are extremely high in bladder in comparison to the activities in the other tissues examined and are at least several times those in liver.

Rat tissues. Of the 6 extrahepatic rat tissues examined, 3 exhibited elevations of enzyme specific activities in response to dietary BHA administration (Table 2). The tissue response was qualitatively identical for epoxide hydratase and glutathione S-transferase activities. We previously reported that in response to BHA, rat liver epoxide hydratase activity increased to 277% of control levels, and glutathione S-transferase activities rose to about 200% of control levels. The data on these hepatic enzyme activities have been published elsewhere (7, 15) but are included in Table 2 to facilitate comparison with the values for other tissues. The increases in epoxide hydratase and glutathione S-transferase specific activities in the small intestine to 255 and 160% of controls, respectively, approached those observed in liver, whereas more modest but statistically significant increases were observed in kidney and...

Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Diet</th>
<th>N</th>
<th>Epoxide hydratase specific activity in microsomes (nmol/min/mg)</th>
<th>Glutathione S-transferase specific activities in cytosol fractions (nmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1-chloro-2,4-dinitrobenzene</td>
<td>1,2-dichloro-4-nitrobenzene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p-nitrobenzylchloride</td>
</tr>
<tr>
<td>Liver</td>
<td>BHA</td>
<td>8</td>
<td>11.72 ± 0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13840 ± 417&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td></td>
<td>1.06 ± 0.05</td>
<td>1669 ± 87</td>
</tr>
<tr>
<td>Kidney</td>
<td>BHA</td>
<td>8</td>
<td>0.82 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>856 ± 44&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
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<td></td>
<td>0.65 ± 0.05</td>
<td>1526 ± 13</td>
</tr>
<tr>
<td>Lung</td>
<td>BHA</td>
<td>8</td>
<td>2.57 ± 0.12&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1664 ± 216&lt;sup&gt;d&lt;/sup&gt;</td>
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<td></td>
<td>2.20 ± 0.20</td>
<td>774 ± 51</td>
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<tr>
<td>Stomach</td>
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<td>8</td>
<td>1.67 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1292 ± 35&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td></td>
<td>0.83 ± 0.06</td>
<td>788 ± 38</td>
</tr>
<tr>
<td>Small intestine</td>
<td>BHA</td>
<td>8</td>
<td>1.94 ± 0.23</td>
<td>2285 ± 236&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Control</td>
<td>8</td>
<td></td>
<td>0.33 ± 0.04</td>
<td>521 ± 23</td>
</tr>
<tr>
<td>Colon</td>
<td>BHA</td>
<td>4, 8</td>
<td>1.66 ± 0.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>706 ± 17&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>4, 8</td>
<td></td>
<td>0.81 ± 0.02</td>
<td>517 ± 20</td>
</tr>
<tr>
<td>Uterus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>BHA</td>
<td>4</td>
<td>Not measurable</td>
<td>424 ± 30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td></td>
<td>Not measurable</td>
<td>295 ± 10</td>
</tr>
<tr>
<td>Thymus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>BHA</td>
<td>4</td>
<td>1.42 ± 0.12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>195 ± 26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td></td>
<td>0.55 ± 0.12</td>
<td>175 ± 10</td>
</tr>
<tr>
<td>Spleen&lt;sup&gt;a&lt;/sup&gt;</td>
<td>BHA</td>
<td>4</td>
<td>Not measurable</td>
<td>198 ± 4&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td></td>
<td>Not measurable</td>
<td>176 ± 8</td>
</tr>
<tr>
<td>Bladder&lt;sup&gt;b&lt;/sup&gt;</td>
<td>BHA</td>
<td>2</td>
<td>9.02 ± 7.83</td>
<td>7092, 7402</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td></td>
<td>4.00, 9.13</td>
<td>5356, 6014</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E.
<sup>b</sup> Significantly different from controls (p < 0.001).
<sup>c</sup> Significantly different from controls (p < 0.05).
<sup>d</sup> Significantly different from controls (p < 0.01).
<sup>e</sup> Not significantly different from controls (p > 0.05).
<sup>f</sup> Colon cytosols from 8 individual mice (N = 8) and microsomes from 4 groups of 2 mice (N = 4) were examined.
<sup>g</sup> Uteri, thymuses, and spleens were pooled in pairs.
<sup>h</sup> Four bladders were pooled for each homogenate, resulting in only 2 preparations for each group of mice. The individual experimental values are given.

AUGUST 1979
A. M. Benson et al.

**Table 2**

Microsomal epoxide hydratase and cytosol glutathione S-transferase specific activities of tissues from rats fed control and BHA diets

The tissues were obtained from male Sprague-Dawley rats which had received the powdered diet or the same diet containing 0.75% BHA for 8 days. Microsomes and cytosols were prepared by the calcium precipitation procedure.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Diet</th>
<th>Epoxide hydratase specific activity in microsomes (nmol/min/ mg)</th>
<th>Glutathione S-transferase specific activities in cytosol fractions (nmol/ min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1-chloro-2,4-dinitrobenzene</td>
</tr>
<tr>
<td>Liver</td>
<td>BHA</td>
<td>5.52 ± 0.02&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;,&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1800 ± 84&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.99 ± 0.10</td>
<td>918 ± 18</td>
</tr>
<tr>
<td>Small intestine</td>
<td>BHA</td>
<td>1.89 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>427 ± 48&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.74 ± 0.04</td>
<td>248 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidney</td>
<td>BHA</td>
<td>1.20 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>117 ± 0.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.91 ± 0.03</td>
<td>971 ± 26&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Lung</td>
<td>BHA</td>
<td>0.41 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>960 ± 15</td>
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<tr>
<td></td>
<td>Control</td>
<td>0.29 ± 0.02</td>
<td>176 ± 5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Testis</td>
<td>BHA</td>
<td>1.12 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>170 ± 2&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Control</td>
<td>1.13 ± 0.03</td>
<td>960 ± 15</td>
</tr>
<tr>
<td>Brain</td>
<td>BHA</td>
<td>0.21 ± 0.01&lt;sup&gt;f&lt;/sup&gt;</td>
<td>176 ± 5&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Control</td>
<td>0.22 ± 0.03</td>
<td>170 ± 2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spleen</td>
<td>BHA</td>
<td>0.34 ± 0.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>960 ± 15</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.27 ± 0.02</td>
<td>170 ± 2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> These data for liver have been published previously (7, 15).
<sup>b</sup> Significantly different from controls (p < 0.01).
<sup>c</sup> Mean ± S.E.
<sup>d</sup> Significantly different from controls (p < 0.02).
<sup>e</sup> Significantly different from controls (p < 0.01).
<sup>f</sup> Not significantly different from controls (p > 0.2).

**DISCUSSION**

These studies are part of a continuing effort to elucidate the biochemical mechanisms by which several antioxidants protect against chemical carcinogenesis.

The anticarcinogenic effects of antioxidants are not restricted to a single strain, sex, species, or route of administration (63). Administration (p.o.) of BHA or the structurally related antioxidant, 3,5-di-tert-butyl-4-hydroxytoluene, has been shown to inhibit carcinogen-induced neoplasia in female rats of the Sprague-Dawley and CD SPF strains (57, 61) and male rats of CD SPF, Fischer, and F344 strains (57, 66). Protection has been achieved by administering BHA p.o. to female CD-1 mice (61, 62), p.o. and i.p. to female A/HeJ mice (60–62), and topically to female CD-1 mice (53). The use of female CD-1 mice in the present experiments has its origin in the extensive experience with this strain in the chemotherapy of schistosomiasis. The carcinogenicity of a chemotherapeutic nitrovinylfuran (SQ 18,506; trans-5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,4-oxadiazole) is markedly reduced in female CD-1 mice receiving dietary BHA. Furthermore, administration of BHA to these mice reduced or eliminated the mutagenic activities resulting from the metabolism of several antischistosomal compounds as well as of benzo(a)pyrene and other promutagens (4).

Further studies have disclosed that administration of BHA to CD-1 mice led to increased activities and/or levels of a number of hepatic enzymes and catalytic components directly or indirectly concerned with the metabolism of carcinogens (7, 13–15). Extension of these studies to male Sprague-Dawley rats revealed that enhancement of hepatic enzyme activities by BHA was not restricted to a single strain, sex, or species (7, 15).

The effects of antioxidants administered in vivo on the activities of enzymes, some of which are involved in the metabolism of carcinogens, have been the subject of a number of studies.  

<sup>h</sup> H. Dunsford, P. M. Dolan, and E. Bueding, unpublished observations.
(11, 17, 23, 29, 32, 39, 53, 55, 56, 64). In particular, Lam and Wattenberg (32) and Speier et al. (55, 56) observed that p.o. administration of BHA to mice resulted in alterations of patterns of metabolites produced from benzo(a)pyrene by hepatic microsomes in vitro with concomitant decrease in the binding to DNA and that these effects were observable within a few hours after a single dose of BHA. We reported recently (7, 15) that dietary administration of 2 antioxidants, BHA and ethoxyquin, to rodents resulted in dramatic elevations of hepatic glutathione S-transferase and epoxide hydratase activities, suggesting that some of the anticarcinogenic effects of these antioxidants may be attributable to enhanced capacity for enzymatic inactivation of reactive metabolites.

Although the liver is the principal site of tumor production by relatively specific carcinogens, such as azo-dyes (41) and aflatoxins (67), extrahepatic tissues are in general more susceptible to the action of a wide variety of carcinogens (9). Since BHA and other antioxidants protect not only the liver but at least several other target tissues from the effects of numerous chemically dissimilar carcinogens (63), the ability of BHA to elevate glutathione S-transferase and epoxide hydratase activities in a number of extrahepatic tissues suggests that increased enzymatic inactivation of carcinogens may be involved in the mechanism of protection by this antioxidant.

Lung, stomach, and colon are among the major target tissues for carcinogenesis in which protection by BHA or the structurally related antioxidant, 3,5-di-tert-butyl-4-hydroxytoluene, has been demonstrated (63). Administration of BHA to mice led to doubling of epoxide hydratase specific activities in stomach and colon microsomes and of some of the glutathione S-transferase specific activities in lung and stomach cytosols. These increases in enzyme activities appear to represent significant enhancement of the ability of these tissues to inactivate a wide range of xenobiotics. The enhancement by BHA of glutathione S-transferase levels, as well as of the concentrations of nonprotein thiols (4), in mouse lung may be of particular interest since administration of BHA protects this tissue against the neoplastic effects of several carcinogens, including benzo(a)pyrene and other polycyclic aromatic hydrocarbons, diethylnitrosamine, nitroquinoline N-oxide, uracil mustard, and urethan (60, 62).

The most impressive elevations of enzyme activities in response to dietary BHA were observed in mouse liver (7, 15) and small intestine. In general, greater increases in the levels of these enzyme activities occurred in the mouse tissues than in the rat tissues examined. However, the experimental animals differed not only in species but in sex and in duration of BHA treatment. It is apparent that dietary administration of BHA results in elevation of glutathione S-transferase and epoxide hydratase activities in multiple tissues of both species and that this response occurs in both female and male rodents. Speier et al. (55) recently reported that administration of BHA by p.o. intubation 4 hr prior to each of 3 p.o. doses of benzo(a)pyrene (at 2-week intervals) yielded substantial protection against the formation of pulmonary adenomas in mice. Thus, it would be of interest to investigate the effects of intermittent doses of BHA on the levels of detoxification enzymes in the digestive tissues (since both BHA and the carcinogen were administered by p.o. intubation) as well as in liver and lung.

Although the activities of these enzymes generally lead to the formation of innocuous metabolites, recent studies have shown that epoxide hydratase catalyzes the formation of benzo(a)pyrene 7,8-dihydriodiol, a precursor of bay region dialkoxides which are believed to be ultimate carcinogenic forms of benzo(a)pyrene (28, 30). Similarly, the conversion of 1,2-dichloroethene to mutagenic metabolites was reported to be dependent upon its conjugation with glutathione (51). Styrene oxide and benzo(a)pyrene 4,5-oxide, both of which have been shown to be mutagenic (1, 19, 38, 42, 48, 68) and may be weakly carcinogenic (58, 59, 68), are converted into nonmutagenic dihydrodiols and glutathione conjugates by epoxide hydratase and glutathione S-transferases, respectively (5, 24, 26, 45, 48, 69, 70). Suggested explanations for the relative ineffectiveness of benzo(a)pyrene 4,5-oxide in initiating carcinogenesis include the possibility that this arene oxide may be subject to efficient enzymatic inactivation (65, 69). The widespread tissue distribution of epoxide hydratase and glutathione S-transferase activities, as well as their enhancement by BHA, may be significant in this respect.

Glutathione is normally the most abundant thiol compound in mammalian cells (40). Thus, the observations that administration of BHA to mice leads to increases in nonprotein thiol levels in liver, kidney, lung, and duodenum (4) as well as in the mucosa of stomach, jejunum, ileum, and colon, and in the urinary bladder, suggest enhanced conjugational capacity of the glutathione S-transferases as well as increased availability of noncritical nucleophiles for nonenzymatic inactivation of reactive electrophilic species in these tissues. The elevation by BHA of the levels of glutathione S-transferase and epoxide hydratase activities and of the concentrations of nonprotein thiol compounds, not only in liver but also in extrahepatic tissues, is a further indication that enhancement of detoxification of carcinogens and their metabolites may constitute part of the biochemical mechanisms by which this antioxidant protects against chemical carcinogenesis.

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Elevation of Extrahepatic Glutathione S-Transferase and Epoxide Hydratase Activities by 2(3)-\textit{tert}-Butyl-4-hydroxyanisole

Ann M. Benson, Young-Nam Cha, Ernest Bueding, et al.


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