

Enhancement of Liver Microsome Epoxide Hydratase Activity in Rodents by Treatment with 2(3)-*tert*-Butyl-4-hydroxyanisole¹

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ABSTRACT

Administration of the antioxidant 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) in the diet caused a marked increase in the specific activity of epoxide hydratase (EC 4.2.1.63) in hepatic microsomes of CD-1 mice. The increases in epoxide hydratase activities produced by BHA were far greater (11-fold) than were those produced by the administration of well-known enzyme inducers such as 3-methylcholanthrene, phenobarbital, and Aroclor 1254 (2- to 3-fold). The near-maximal increase in epoxide hydratase activity was observed after feeding of the BHA diet for 3 days. When BHA was administered by gastric intubation, the level of increase was only 75% of that attained by feeding BHA in the diet. The increase in epoxide hydratase activity produced by BHA treatment of Sprague-Dawley rats was not as pronounced (<3-fold) as that observed in CD-1 mice.

INTRODUCTION

The carcinogenic and mutagenic effects of many polycyclic aromatic hydrocarbons are generally believed to be mediated through epoxides (8, 12, 17). These epoxides, which are formed during hepatic microsomal oxidative metabolism, may undergo partial inactivation by hydration of the oxide ring to less toxic *trans*-dihydrodiols. This reaction is catalyzed by microsomal epoxide hydratase (EC 4.2.1.63). In this manner, the activity of epoxide hydratase may control, at least in part, the degree to which epoxides may accumulate and become available for covalent binding to DNA and other macromolecules, thereby exerting carcinogenic and cytotoxic effects.

Studies of Wattenberg *et al.* (20-23), Ulland *et al.* (19), and Weisburger *et al.* (24) have shown that dietary administration of antioxidants, *e.g.*, BHA² and 3,5-di-*tert*-butyl-4-hydroxytoluene, reduced the neoplastic effects of various types of chemical carcinogens. In subsequent studies these authors have determined the quantities of benzo(a)pyrene metabolites covalently bound to calf thymus DNA following incubation of the hydrocarbon with liver microsomes. With microsomes isolated from BHA-treated mice, the quantities of covalently bound metabolites were much lower than were those found after incubation of benzo(a)pyrene with microsomes from animals maintained on control diet (18). In addition, recent results from our laboratory have shown

that prior treatment with BHA reduces the levels of mutagenic metabolites of benzo(a)pyrene in mice (1, 2).

The mechanisms of these protective effects of BHA have not been elucidated. Although a direct interaction between BHA and the ultimate carcinogenic metabolites has been considered possible for such protective effects, selective activations and deactivations of the carcinogens or their metabolism could also be involved (23). Since epoxide hydratase catalyzes the hydration of certain polycyclic hydrocarbon epoxides, we examined whether the protective effects of BHA were associated with changes in this enzyme activity. In this report it is shown that BHA treatment was associated with a dramatic increase in epoxide hydratase activity; there were no concomitant increases in monooxygenase activities (6).

MATERIALS AND METHODS

Female CD-1 mice (Charles River Breeding Laboratories, Wilmington, Mass.), 6 to 8 weeks old, were housed in wire-bottomed stainless steel cages (4 mice/cage) with free access to water and powdered Purina laboratory chow (Ralston Purina Co., St. Louis, Mo.).

Mice were divided into 2 groups, one of which was fed the powdered diet containing 0.75% BHA (Sigma Chemical Co., St. Louis, Mo.) for specified durations (usually 10 days); they were sacrificed by cervical dislocation. Liver microsomes were prepared according to Schenkman and Cinti (15), and their protein concentrations were determined according to the method of Lowry *et al.* (11), with the use of bovine serum albumin as the standard. [8-¹⁴C]Styrene oxide (New England Nuclear, Boston, Mass.) was purified according to the method of Seidegard *et al.* (16) and diluted with acetonitrile and nonradioactive styrene oxide (Aldrich Chemical Co., Milwaukee, Wis.) to a specific activity of 510 dpm/nmol. Solutions for liquid scintillation counting were prepared with 0.75 g of POPOP (New England Nuclear), 24.7 g of PPO (New England Nuclear), 1 liter of scintillation grade Triton X-100 (Research Products International Corp., Elk Grove, Ill.), and 3 liters of toluene.

Assays for epoxide hydratase were performed with the use of a thin-layer chromatography micromethod of Jerina *et al.* (9). Samples were incubated at 37° in 0.4-ml capacity stoppered centrifuge tubes which contained, in a final volume of 80 μ l, 20 μ l of Tris buffer (0.5 M Tris hydrochloride: 0.1% Tween 80, pH 8.9), 35 μ l of distilled water, 20 μ l of microsomal suspension (120 to 240 μ g of protein), and [8-¹⁴C]styrene oxide (200 nmol; 100,000 dpm) in 5 μ l acetonitrile. Microsomes for the blanks were heated for 10 min in boiling water prior to addition to the incubation system. The experimental samples were equilibrated to 37° for 2

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² The abbreviation used is: BHA, 2(3)-*tert*-butyl-4-hydroxyanisole.

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min, and the reaction was started by addition of substrate. After incubation for 15 min, the reaction was terminated by addition of 20 μ l of saturated Ba(OH)₂ solution followed immediately by 5 μ l of 30% ZnSO₄. The mixture was centrifuged for 20 min at 600 \times g. A portion (50 μ l) of the supernatant was applied to Quanta-gram LQ6D thin-layer plates (Kontes Co., Vineland, N. J.) and were developed in chloroform:ethyl acetate (1:1, v/v) for a distance of 15 cm. The styrene diol metabolite cochromatographed with authentic 1-phenyl-1,2-ethanediol (Aldrich Chemical Co.), R_f 0.20. The product was located precisely by autoradiography with Kodak SB-54 X-ray film. Radioactivity of styrene diol removed from the plates was measured in a liquid scintillation counter with 10 ml of scintillation fluid. Sample counting efficiency (typically 95%) was determined by the external standard method, and values were corrected for quenching. Epoxide hydratase activities are expressed in nmol of product per mg protein per min.

RESULTS

The time course of changes in specific activity of hepatic microsomal epoxide hydratase was examined following initiation and subsequent discontinuation of BHA treatment (Chart 1). Three days after initiation of BHA feeding, the epoxide hydratase specific activity rose to 9 times that of control values. The specific activities continued to rise more slowly to 11 times those of control values with continued exposure to BHA for another 3 days. When the BHA diet was replaced by the control diet, enzyme activity fell to one-half of its peak value within 5 days and returned to control levels after another 15 days. In a separate experiment, BHA suspended in Emulphor EL-620 (a gift of GAF Corp., New York, N. Y.) was administered by gastric intubation once daily (750 mg/kg) for 4 days. The increase in the specific activity of epoxide hydratase was only 75% (7.1 nmol per mg protein per min) of that obtained when BHA was fed in the diet (data not shown). Hence, the maximal elevations of this enzyme activity may depend on continuous exposure to the antioxidant.

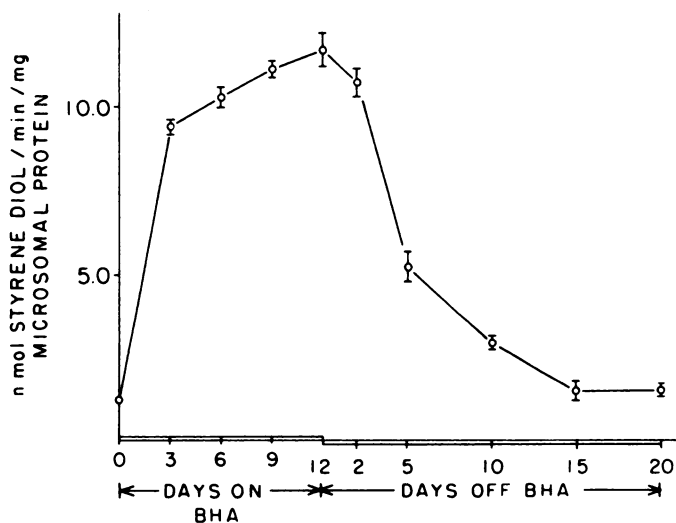


Chart 1. Effect of the initiation and discontinuation of feeding of a diet containing 0.75% BHA on the specific activity of epoxide hydratase in liver microsomes of female CD-1 mice. See text for experimental details.

The ability of BHA to increase the epoxide hydratase activity in mice was much greater than that of several well-known inducing agents such as 3-methylcholanthrene, phenobarbital, or Aroclor 1254 (Table 1). The increases in epoxide hydratase activity produced by BHA were not associated with an elevation of monooxygenase activity as measured by cytochrome P-450 content, benzo(a)pyrene hydroxylase, or aminopyrine demethylase (6).

The effect of BHA on epoxide hydratase activity in rats was studied in a separate experiment. Adult male Sprague-Dawley rats (250 to 310 g; Charles River Breeding Laboratories) were fed the same diet as that used for the mice for 10 days. This treatment resulted in a substantial increase in specific activity of epoxide hydratase (2.8-fold), but the effect was far less pronounced than in mice (10-fold; see Table 2).

DISCUSSION

In mammals highly reactive arene oxides are intermediates in the metabolic conversion of aromatic compounds to phenols. Epoxide hydratase plays a major role in these conversions (8). This microsomal enzyme was shown to be nonspecific and moderately inducible by treatment of animals with phenobarbital, 3-methylcholanthrene, and Aro-

Table 1

Comparative effects of BHA and various other inducers on the specific activity of epoxide hydratase in mouse liver microsomes

Liver microsomes obtained from mice that were treated with either 3-methylcholanthrene [dissolved in Mazola corn oil (5 mg/ml) and administered by daily i.p. injections at 25 mg/kg/day for 3 days], sodium phenobarbital [dissolved in H₂O (20 mg/ml) and injected i.p. at 100 mg/kg/day for 3 days], or Aroclor 1254 [diluted with corn oil (100 mg/ml) and administered as a single i.p. injection at a dose of 500 mg/kg]. The livers were removed in each case on the fourth day. BHA was administered in the diet (0.75%). Other experimental details are described in "Materials and Methods."

Treatment	No. of animals	Epoxide hydratase activity (nmol diol/mg protein/min)
Control	12	1.24 \pm 0.07 ^a
3-Methylcholanthrene	4	3.04 \pm 0.17
Phenobarbital	4	2.53 \pm 0.10
Aroclor 1254	4	3.23 \pm 0.10
BHA		
Fed for 3 days	5	9.40 \pm 0.16
Fed for 12 days	5	11.79 \pm 0.57

^a Mean \pm S.D.

Table 2

Effects of dietary BHA administration on epoxide hydratase activity of liver microsomes in mice and rats

The animals used for this experiment were 10-week-old female CD-1 mice and male Sprague-Dawley rats weighing 250 to 310 g. Both groups received 0.75% BHA in the diet for 10 days.

	Epoxide hydratase activity (nmol/mg protein/min)	
	Control	BHA-treated
Mouse	1.17 \pm 0.13 ^a (n = 12)	11.11 \pm 0.42 (n = 12)
Rat	1.99 \pm 0.15 (n = 6)	5.52 \pm 0.08 (n = 5)

^a Mean \pm S.D.

clor 1254 (14). In studies with rats treated with ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline), another antioxidant used as a preservative in animal food, Kahl and Netter (10) observed moderate increases of epoxide hydratase (4-fold) in the absence of any increase of benzo(a)pyrene hydroxylase activity. In mice treated with BHA, the 11-fold increase in epoxide hydratase was not accompanied by any changes in benzo(a)pyrene hydroxylase activities (6). Our studies not only support the hypothesis of Oesch (13) that these enzymes are under separate control but also indicate that these antioxidants, which are relatively nontoxic (5), are tools for selectively increasing the epoxide hydratase activity.

There is much evidence that the mutagenic and carcinogenic effects of benzo(a)pyrene are caused by the epoxide metabolites of this hydrocarbon (17). Furthermore, recent studies by Wood *et al.* (25, 26) have shown that the mutagenic effects of benzo(a)pyrene epoxides were essentially eliminated by treatment with purified epoxide hydratase. The results obtained in this study support the view that the antimutagenic (1) and anticarcinogenic (19-24) effects of BHA may, at least in part, result from increased activity of epoxide hydratase and more efficient inactivation of epoxides. However, attention should also be drawn to the finding in this laboratory that administration of BHA is associated with marked elevation of the levels of several glutathione S-transferases in hepatic cytosols of mice and rats. Furthermore, such cytosols in combination with glutathione profoundly depress the mutagenic activity of urines of benzo(a)pyrene-treated mice in the Ames test system. Purified glutathione S-transferase mimics the effects of the hepatic cytosols (3, 4).

Under certain circumstances, however, epoxide hydratase may contribute to the formation of products with greater carcinogenic and mutagenic properties through the formation of diol-epoxides (7, 17). Therefore, an increase of epoxide hydratase activity may, under certain circumstances, protect against or, under others, enhance carcinogenic effects. Since BHA and other antioxidants are widely used as food preservatives, the implications of these effects in relation to environmental carcinogenesis warrant further exploration.

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