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DIFFERENTIAL INDUCTION OF GLUTATHIONE *S*-TRANSFERASE IN RAT AORTA VERSUS LIVER

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Abstract—Polycyclic aromatic hydrocarbons, cigarette smoke components that induce atherosclerosis in animals, require metabolic biotransformation to electrophilic intermediates to exhibit atherogenic effects. The formation of reactive metabolites depends on both rates of cytochrome P450-catalyzed oxidation and rates of detoxification through conjugation with glutathione. Thus, changes in the activity of glutathione *S*-transferase in vascular tissue could affect the risk of polycyclic aromatic hydrocarbon-induced atherogenesis. We compared the effects of several exogenous chemicals on levels of glutathione *S*-transferase in aorta and liver. Male Wistar rats were treated with 3-methylcholanthrene, a polycyclic aromatic hydrocarbon, phenobarbital and butylated hydroxytoluene, an antioxidant known to have anti-atherogenic properties. In control animals, glutathione *S*-transferase activity was about 20-fold greater in liver than in aorta. Subunit expression was tissue specific. GST-Yp, for example, was the most abundant subunit in aorta but was undetectable in liver. In contrast, GST-Ya was barely detectable in aorta but was abundant in liver. Each of the xenobiotics caused induction of glutathione *S*-transferase but the extent of induction was greater in liver than in aorta. Phenobarbital, for example, caused 300% induction in liver but only 70% induction in aorta. By western blot analysis, differences in amounts of enzyme subunits corresponded to changes in enzyme activity. Thus, exogenous chemicals differentially regulate levels of glutathione *S*-transferase in the aorta and liver.

Key words: glutathione *S*-transferase; xenobiotic; atherosclerosis; antioxidant; aorta; liver

Several lines of evidence support the idea that xenobiotics can cause disease of the vascular system. An increased incidence of atherosclerotic plaques has been detected, for example, in birds [1–4] and rodents [5, 6] treated with PAHs§. Furthermore, cigarette smoke, which contains high levels of PAHs, is a known risk factor for atherosclerosis in humans. Although it is not clear whether PAHs induce atherosclerosis by a mutagenic mechanism, there is evidence that cytochrome P450-mediated bioactivation of PAHs modulates the severity of atherosclerosis [6, 7]. Moreover, there is evidence that metabolic biotransformation of PAHs to reactive, electrophilic intermediates is required for these molecules to bind to cellular macromolecules, including DNA and protein. However, the formation of adducts between cellular components and reactive metabolites of PAHs depends on the balance between rates of cytochrome P450-catalyzed oxidation and rates of detoxification of oxidation products via conjugation, primarily with glutathione and glucuronic acid. Inherited defects in conjugation reactions are known, for example, to predispose to DNA adduct formation in laboratory animals [8] and smoking-related cancer in humans [9, 10]. Conversely, there is evidence that glutathione can prevent the binding of PAHs to DNA in the aorta [11]. Thus, it is important to understand how levels of xenobiotic metabolizing enzymes are set in different tissues to develop strategies to modulate PAH-induced disease. To this end, we have compared the effects of several exogenous chemicals on the activity of glutathione *S*-transferase, a prototypical Phase 2 xenobiotic metabolizing enzyme, in rat aorta and liver. We have studied this enzyme because the glutathione *S*-transferases are involved in the metabolism of a large number of endogenous and exogenous compounds that interact with the vascular system including PAHs [12], nitroglycerin [13, 14], leukotrienes [15] and prostaglandins [16]. The data presented indicate that exogenous chemicals differentially regulate the expression of glutathione *S*-transferase in aorta and liver.

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§ Abbreviations: PAHs, polycyclic aromatic hydrocarbons; CDNB, 1-chloro-2,4-dinitrobenzene; BHT, 3,5-di-*tert*-butyl-4-hydroxytoluene and 3-MC, 3-methylcholanthrene.

MATERIALS AND METHODS

Materials. Glutathione, CDNB, 3-MC, phenobarbital and BHT were purchased from the Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade.

Animals and treatments with inducers. Male Wistar rats (225–275 g) were obtained from Charles

River (Wilmington, MA). Phenobarbital (80 mg/kg) dissolved in 0.9% NaCl was injected intraperitoneally on day 1 as described in Ref. 17. These rats were subsequently given phenobarbital (1 mg/mL) in their drinking water for 4 days (animals were killed on day 5). In the second experimental group, 3-MC (20 mg/kg) dissolved in corn oil: ethanol (9:1, v/v) was given by daily intraperitoneal injection on 5 consecutive days as described in Ref. 17. The animals were killed on day 6. Control animals only received the appropriate injection vehicle. In the third experimental group, powdered diet (U.S. Biochemical Corp., Cleveland, OH) containing 0.5% BHT was given to the experimental animals for 2 weeks as described previously [18]. The control animals received the same diet without BHT. On the day of the experiments, rats were killed by exposure to carbon dioxide followed by cervical dislocation.

Preparation of cytosol. Liver cytosol was prepared as previously described [19]. Resected aortas were chopped finely with a razor blade and then homogenized in 2 mL of 0.25 M sucrose with a polytron (The Virtis Co., Gardiner, NY). After filtering through cheesecloth, the homogenate was centrifuged at 10,000 *g* for 45 min at 4°. The pellet was discarded, and the supernatant was centrifuged at 100,000 *g* for 60 min at 4°. Cytosol was decanted and stored at −80°. Samples were used within 2 weeks of preparation.

Enzyme assay. Protein concentrations were determined by the method of Lowry *et al.* [20]. The activity of cytosolic glutathione *S*-transferase was determined by measuring the formation of the conjugate of glutathione with CDNB at 25° according to the method of Habig *et al.* [21]. The assay mixture contained 1.0 mM glutathione and 0.5 mM CDNB.

Western blot analysis. Polyclonal antibodies against GST-Ya, GST-Yb_1, and GST-Yp were obtained from Biotrin International (Dublin, Ireland). Sodium dodecyl sulfate – polyacrylamide gel electrophoresis was performed under reducing conditions on 10% polyacrylamide gels according to the method of Laemmli [22]. Proteins were transferred from slab gels onto nitrocellulose paper (Schleicher & Schuell, Keene, NH) as described by Towbin *et al.* [23]. The nitrocellulose paper was then incubated with antiserum for 3 hr. Subsequently, the nitrocellulose membrane was probed with goat anti-rabbit antibody conjugated to alkaline phosphatase (Promega, Madison, WI). The glutathione *S*-transferases were then detected by the alkaline phosphatase color reaction using 5-bromo-4-chloro-3-indolyl phosphate and nitro-blue tetrazolium as described in Ref. 24. Under these conditions, stain intensity accurately reflected protein concentration. Intensities of immunostained bands were measured with a computing densitometer (Molecular Dynamics, Sunnyvale, CA).

Statistics. Results are given as means ± SEM for a minimum of five animals in each treatment group. Comparisons between groups were made by Student's

Table 1. Effects of phenobarbital, 3-methylcholanthrene and butylated hydroxytoluene on glutathione *S*-transferase activity in rat aorta versus liver

	Glutathione *S*-transferase activity (nmol/min/mg protein)	
	Aorta	Liver
Control	43 ± 2	814 ± 76
Phenobarbital	74 ± 2*	2545 ± 72*
Control	39 ± 1	973 ± 16
3-Methylcholanthrene	52 ± 1*	1992 ± 66*
Control	39 ± 3	1002 ± 88
Butylated hydroxytoluene	33 ± 5	2656 ± 165*

Enzyme activity was measured using 0.5 mM 1-chloro-2,4-dinitrobenzene and 1.0 mM glutathione at 25°. Values are means ± SEM, N = 5.
* $P < 0.01$.

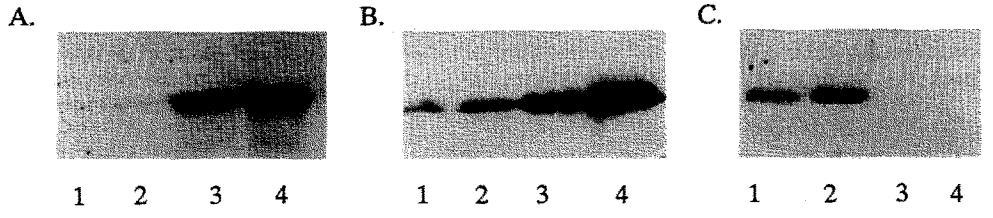

Fig. 1. Phenobarbital induction of glutathione *S*-transferase subunits in aorta and liver. Western blot analysis was carried out as described in Materials and Methods. Immunoblots were performed on pooled cytosol prepared from the aortas and livers of untreated rats and rats treated with phenobarbital. The lanes were loaded with 10 μg of aorta cytosolic protein and 2 μg of liver cytosolic protein. Lanes 1 and 3 represent cytosol from untreated aorta and liver, respectively. Lanes 2 and 4 represent cytosol from phenobarbital-treated aorta and liver, respectively. The nitrocellulose membranes were probed with anti-GST-Ya antiserum (panel A), anti-GST-Yb_1 (panel B) or anti-GST-Yp (panel C). Results of densitometry were as follows. Panel A: lane 1, 58 arbitrary units; lane 2, 173 arbitrary units; lane 3, 680 arbitrary units; and lane 4, 903 arbitrary units. Panel B: lane 1, 98 arbitrary units; lane 2, 292 arbitrary units; lane 3, 768 arbitrary units; and lane 4, 1260 arbitrary units. Panel C: lane 1, 135 arbitrary units; lane 2, 254 arbitrary units.

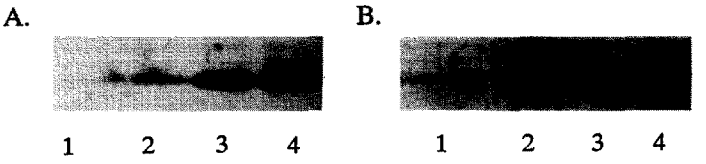

Fig. 2. 3-Methylcholanthrene induction of glutathione S-transferase subunits in aorta and liver. Immunoblots were performed on pooled cytosol prepared from the aortas and livers of untreated rats and rats treated with 3-methylcholanthrene. The lanes were loaded with 10 μg of aorta cytosolic protein and 2 μg of liver cytosolic protein. Lanes 1 and 3 represent cytosol from untreated aorta and liver, respectively. Lanes 2 and 4 represent cytosol from 3-methylcholanthrene-treated aorta and liver, respectively. The nitrocellulose membranes were probed with anti-GST-Ya antiserum (panel A) and anti-GST-Yb_1 (panel B), respectively. Results of densitometry were as follows. Panel A: lane 1, 31 arbitrary units; lane 2, 74 arbitrary units; lane 3, 148 arbitrary units; and lane 4, 181 arbitrary units. Panel B: lane 1, 60 arbitrary units; lane 2, 99 arbitrary units; lane 3, 110 arbitrary units; and lane 4, 120 arbitrary units.

t-test. A difference between groups of $P < 0.05$ was considered significant.

RESULTS

Effect of phenobarbital on glutathione S-transferase. The data in Table 1 show the effects of treatment with phenobarbital on the activity of glutathione *S*-transferase in rat aorta and liver. In the control animals, glutathione *S*-transferase activity was approximately 20-fold greater in liver than in aorta. Treatment with phenobarbital resulted in a 300% increase in the activity of glutathione *S*-transferase in liver but only a 70% increase in aorta. Immunoblotting was performed to examine the effect of phenobarbital on the levels of the Ya, Yb_1 and Yp subunits of glutathione *S*-transferase, which are representative of the alpha, mu and pi classes of glutathione *S*-transferase, respectively [25]. As shown in Fig. 1, treatment with phenobarbital caused increases in levels of the Ya and Yb_1 subunits in aorta and liver. Phenobarbital also caused an increase in the amount of the Yp subunit in aorta. The Yp subunit was not detected in liver cytosol.

Effect of 3-MC on glutathione S-transferase in aorta versus liver. The data in Table 1 show the effect of a polycyclic aromatic hydrocarbon, 3-MC, on the activity of glutathione *S*-transferase in aorta and liver. Treatment with 3-MC caused a 2-fold increase in the activity of glutathione *S*-transferase in liver but only about a 30% increase in aorta. Immunoblots (Fig. 2) revealed a positive correlation between the activity of glutathione *S*-transferase and levels of the Ya and Yb_1 subunits in aorta and liver. Thus, 3-MC caused increases in levels of both alpha and mu class subunits in aorta and liver. 3-MC had no effect on levels of the Yp subunit in aorta; the Yp subunit was not detected in the liver (data not shown).

Effect of the antioxidant 3,5-di-tert-butyl-4-hydroxytoluene on glutathione S-transferase in aorta and liver. The data in Table 1 show that BHT had no effect on the activity of glutathione *S*-transferase in aorta, although more than a 2-fold increase in activity was detected in liver. Antioxidants are known to induce the Ya subunit of glutathione *S*-transferase in liver via an antioxidant responsive element [26]. The Ya subunit is present in aortic cytosol in extremely low levels as compared with other subunits such as Yp. Thus, induction of the Ya subunit might not be detected by changes in enzyme activity using CDNB, a universal substrate (Table 1). Consequently, immunoanalysis was done to investigate the possibility that induction had occurred in the aorta. As shown in Fig. 3, the antioxidant BHT increased levels of the GST-Ya subunit in both aorta and liver. Butylated hydroxytoluene also induced levels of the GST-Yb_1 subunit in liver but not aorta; levels of GST-Yp were unaffected by treatment with BHT (Fig. 3).

DISCUSSION

In this study, we compared the effects of several exogenous chemicals on levels of glutathione *S*-transferase in aorta and liver. The current experiments showed that glutathione *S*-transferase activity was much lower in aorta than in liver and that glutathione *S*-transferase subunit expression was tissue specific. GST-Yp, for example, was an abundant subunit in aorta but was undetectable in liver. GST-Ya was barely detectable in aorta but was abundant in liver. Thus, the capacity for glutathione conjugation is likely to differ in aorta and liver because of differences in both the composition and concentration of glutathione *S*-transferases. We also found that each of the three xenobiotics investigated induced glutathione *S*-transferase in aorta as previously shown in liver [18, 27, 28]. Phenobarbital caused induction of the GST-Ya and GST-Yb_1 subunits in aorta and liver. Since phenobarbital regulates levels of GST-Ya and GST-Yb at a transcriptional level in liver [29], the same mechanism is likely to be true for aorta. Polycyclic aromatic hydrocarbons including 3-MC are known to control the expression of multiple xenobiotic metabolizing enzymes including the glutathione *S*-transferases via the Ah receptor [29]. Thus, the finding that 3-MC upregulates the glutathione *S*-transferases in aorta suggests the presence of a functional Ah receptor in vasculature. This idea is also supported by the previous

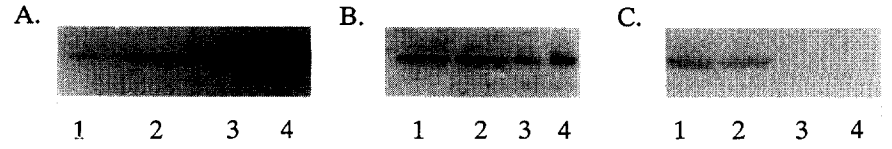

Fig. 3. Butylated hydroxytoluene induction of GST-Ya in aorta and liver. Immunoblots were performed on pooled cytosol prepared from the aortas and livers of untreated rats and rats treated with butylated hydroxytoluene. The lanes were loaded with 10 μg of aorta cytosolic protein and 2 μg of liver cytosolic protein. Lanes 1 and 3 represent cytosol from untreated aorta and liver, respectively. Lanes 2 and 4 represent cytosol from butylated hydroxytoluene-treated aorta and liver, respectively. The nitrocellulose membranes were probed with anti-GST-Ya antiserum (panel A), anti-GST-Yb_1 (panel B) and anti-GST-Yp (panel C). Results of densitometry were as follows. Panel A: lane 1, 31 arbitrary units; lane 2, 52 arbitrary units; lane 3, 105 arbitrary units; and lane 4, 163 arbitrary units; Panel B: lane 1, 82 arbitrary units; lane 2, 75 arbitrary units; lane 3, 22 arbitrary units; and lane 4, 48 arbitrary units; Panel C: lane 1, 77 arbitrary units; lane 2, 71 arbitrary units.

observation that aryl hydrocarbons induce cytochrome P450 monooxygenase isozymes in rabbit aorta [30]. We also showed that butylated hydroxytoluene, an antioxidant with known antiatherogenic properties [31], induced GST-Ya in aorta and liver. This observation is consistent with the recent report that GST-Ya subunit expression is regulated by an antioxidant responsive element in the 5′-flanking region of the glutathione *S*-transferase Ya subunit gene [26]. It remains to be shown, however, that a link exists between the antiatherogenic effects of butylated hydroxytoluene and the induction of glutathione *S*-transferase. It is also important to note that the extent of induction of glutathione *S*-transferase in aorta was less than in liver for each of the three xenobiotics tested. This difference in inducibility may reflect tissue specific differences in transcription factors. Clearly, changes in the levels of xenobiotic metabolizing enzymes in vasculature or liver could alter the metabolism of a wide variety of endogenous and exogenous molecules, including toxins and drugs. Benzo[*a*]-pyrene, for example, is converted by cytochrome P450 to a mutagen, but the amount of mutagen produced from a given amount of benzo[*a*]pyrene depends on rates of oxidation and on rates of conjugation of intermediates with glutathione. In the context of the above data, it is interesting to speculate that exposure to xenobiotics could affect the threshold for toxic injury by altering tissue levels of glutathione *S*-transferase. In addition, because glutathione *S*-transferase is important in the metabolism of nitroglycerin [13, 14], xenobiotic-induced changes in enzyme activity could alter the efficacy of this commonly used drug.

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