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INDUCTION OF MOUSE LIVER GLUTATHIONE *S*-TRANSFERASE BY ETHANOL

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Abstract—The induction of hepatic glutathione *S*-transferase by ethanol was investigated in male Swiss–Webster mice using a liquid diet. After a 7-day feeding period, mice that received 18, 27 or 36% of their calories as ethanol exhibited significant increases in the specific activity of glutathione *S*-transferase when 1,2-dichloro-4-nitrobenzene (DCNB), *p*-nitrobenzylchloride (NBC) and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (ENP) were used as substrates. The observed increases in activity appeared to be related to the concentration of ethanol in the diet. Thus, mice fed a diet with 36% of the calories as ethanol exhibited the greatest increase in specific activity (DCNB, 75%; NBC, 60%, ENP, 34%). Pair-fed mice demonstrated similar changes in enzymatic activity. A time–course study indicated a 4-day feeding period was not sufficient to elicit significant induction, but a significant increase was apparent by day 7. This increase was maintained or increased through day 14. By comparison, 0.5 mg of phenobarbital/ml of diet produced a greater increase in enzymatic activity (DCNB, 449%; NBC, 227%; ENP, 219%). These results suggest that ethanol does induce glutathione *S*-transferase, but it is a relatively poor inducer of this enzyme.

The glutathione *S*-transferases (EC 2.5.1.18) are a group of catalytic and ligand binding proteins which play an important role in the protection of cellular constituents from the deleterious effects of a variety of agents. These proteins exert their effect by sequestering xenobiotics and binding them either covalently [1] or noncovalently [2]. In addition to functioning as intracellular binding proteins, the glutathione *S*-transferases mediate the first step of mercapturic acid synthesis [3]. These proteins are capable of conjugating many structurally diverse electrophilic compounds such as benzyl halides [1], aromatic halides [4], α,β-unsaturated ketones and reactive intermediates formed by cytochrome P-450 [5]. Thus, these enzymes play a key role in xenobiotic detoxification.

Although they are cytosolic detoxification enzymes, the glutathione *S*-transferases appear to share the characteristic of inducibility with the microsomal drug-metabolizing enzymes. The glutathione *S*-transferases have been reported to be induced by phenobarbital [6–10], benzo[*a*]pyrene [6, 7] and butylated hydroxyanisole [11], all of which are known inducers of cytochrome P-450. The ability of ethanol to induce cytochrome P-450 has long been recognized [12]. Therefore, it is conceivable that ethanol, like the other inducers of cytochrome P-450, may also enhance glutathione *S*-transferase activity. Two previous reports have appeared that indicate that glutathione *S*-transferase is induced in rats given ethanol [13, 14]. However, no experiments have been performed to determine the time course of induction, dose–response relationship, or if the induction occurs in species other than rats. Accordingly, the induction of glutathione *S*-transferase was studied in mice. The influence of the level and the duration of alcohol consumption on the induction of murine glutathione *S*-transferase was also examined.

MATERIALS AND METHODS

Chemicals. The following chemicals were purchased from the Eastman Kodak Co. (Rochester, NY): 1,2-dichloro-4-nitrobenzene (DCNB), *p*-nitrobenzylchloride (NBC) and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (ENP). Reduced glutathione was purchased from the Sigma Chemical Co. (St. Louis, MO). The following dietary ingredients were purchased from the U.S. Biochemical Co. (Cleveland, OH); vitamin-free micropulverized casein, methionine, cystine, preservative-free olive and corn oil, linoleic acid, choline chloride, vitamin mixture, and Hegsted salt mixture. Sucrose was obtained as a commercial preparation from a local distributor and Viscarin (sodium carrageenate) was a gift from the FMC Corp. (Philadelphia, PA).

Animals. Male Swiss–Webster mice (Laboratory Supply Co., Indianapolis, IN) were used throughout the study. The mice were housed four to five animals per cage, provided with wood chip bedding, and maintained on Purina Lab Chow and tap water during the 14 to 18-day acclimation period. Animals were housed individually when pair-fed experiments were performed.

Ethanol was administered via a liquid diet [15] and was introduced into the diet in increments until the desired percentage of total calories, as ethanol, was achieved. Control animals received an isocaloric

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diet prepared by substituting an isocaloric quantity of sucrose for ethanol.

Enzyme preparation. At the end of the feeding period all animals were weighed and killed by cervical dislocation. The livers were excised, weighed and homogenized in 2 vol. of cold 1.15% KCl using a Dounce homogenizer. The resulting homogenates were centrifuged for 20 min at 10,000 *g*. The supernatant fraction was decanted and centrifuged for 60 min at 105,000 *g*. At the end of the centrifugation, the supernatant fraction was decanted from the microsomal pellet and stored at −20° until assayed. Preliminary results indicated that no loss of activity occurred after storage at −20° for up to 1 month. Protein concentration was determined by the method of Lowry *et al.* [16] using bovine serum albumin as the reference standard.

Enzyme assays. Glutathione *S*-transferase activity was measured according to the method of Habig *et al.* [17], using DCNB, NBC or ENP as the substrate. All assays were performed on a Cary model 219 dual-beam spectrophotometer and were initiated by the addition of enzyme. All enzymatic rates are corrected for the contribution of the nonenzymatic reaction. Statistical significance was determined using either Student's *t*-test, paired-*t* test, or analysis of variance.

RESULTS

Induction of glutathione S-transferase in male mice

Five groups of male mice were placed on a liquid diet containing either 0, 9, 18, 27, or 36% of the calories as ethanol. At the end of the 7-day feeding period, the animals were killed and the livers were assayed for glutathione *S*-transferase activity using DCNB, NBC, and ENP as individual substrates (Table 1). The specific activities of the glutathione *S*-transferase of the animals on the 9% ethanol diet were not significantly different from the control group. However, the enzyme activities from the mice that received the 18, 27, or 36% ethanol diet were all significantly different from the control for all three substrates. It also appeared that glutathione *S*-transferase activity increased with increasing amounts of ethanol in the diet.

To exclude the possibility that the observed induction was the result of a difference in caloric intake between the control and experimental groups, a second experiment employing a 36% ethanol diet and pair-fed littermates was performed (Table 2). The rate of conjugation of DCNB and NBC with glutathione by the ethanol-treated group was significantly different from the control group, and the percentage increases were approximately the same as those observed in the previous experiment, 75 vs 71% for DCNB and 60 vs 33% for NBC. Unlike the previous experiment, the rate of conjugation of ENP was not statistically significant. The microsomal and cytosolic protein concentrations were not altered by the ethanol diet, but cytochrome P-450 was increased significantly. The concentration of P-450 in the control group was 0.80 ± 0.05 vs 1.36 ± 0.08 nmoles/mg microsomal protein for the animals on the ethanol diet.

To gain some appreciation of the absolute level of inducibility of glutathione *S*-transferase in mouse liver, a group of male mice was placed on a liquid diet, containing 0.5 mg/ml phenobarbital, for 7 days. The glutathione *S*-transferase activity in the phenobarbital-treated group was significantly different from control with all three substrates employed (Table 3). Also, the percent increase in specific activity observed using phenobarbital was much greater than the increase observed after induction with 36% ethanol.

Time course of induction

To determine the time course of induction for glutathione *S*-transferase after ethanol treatment, groups of mice were placed on an ethanol diet and killed after 4, 7, 11, or 14 days on the diet. As can

Table 1. Effect of ethanol concentration on induction of glutathione *S*-transferase

Group	Enzymatic activity* Substrate DCNB	NBC	ENP
Control diet (6)†	24 ± 1	110 ± 6	76 ± 4
Ethanol diet			
9% (4)	24 ± 1	99 ± 7	69 ± 10
18% (4)	29 ± 3‡	150 ± 10‡	93 ± 6‡
27% (4)	36 ± 2‡	170 ± 5‡	100 ± 3‡
36% (4)	43 ± 1‡	180 ± 7‡	100 ± 6‡

* Activity is expressed as nmoles substrate conjugated per mg protein per min ± S.E.M.
† Number of animals per group.
‡ Statistically significant difference, $P \leq 0.05$.

Table 2. Glutathione *S*-transferase activity of male mice pair-fed 36% ethanol for 7 days

Group	Enzymatic activity* Substrate DCNB	NBC	ENP
Control diet (5)†	34 ± 2	96 ± 3	60 ± 2
Ethanol diet (5)	57 ± 8‡	130 ± 8‡	67 ± 4

* Activity is expressed as nmoles substrate conjugated per mg protein per min ± S.E.M.
† Number of animals per group.
‡ Statistically significant difference, $P \leq 0.05$.

Table 3. Glutathione *S*-transferase activity of male mice fed phenobarbital for 7 days

Group	Enzymatic activity* Substrate DCNB	NBC	ENP
Control (3)†	33 ± 3	99 ± 8	61 ± 3
Phenobarbital diet (5)	180 ± 4‡	320 ± 11‡	196 ± 6‡

* Activity is expressed as nmoles substrate conjugated per mg protein per min ± S.E.M.
† Number of animals per group.
‡ Statistically significant difference, $P \leq 0.05$.

Table 4. Time course of induction of glutathione S-transferase by ethanol

Days*	Diet	Enzymatic activity† Substrate DCNB	NBC	ENP
4	Control (4)‡	37 ± 4	101 ± 4	61 ± 5
	Ethanol (4)	39 ± 2	110 ± 1	58 ± 3
7	Control	35 ± 3	99 ± 7	68 ± 5
	Ethanol	56 ± 9§	160 ± 16§	66 ± 3
11	Control	29 ± 3	115 ± 4	64 ± 2
	Ethanol	53 ± 4§	180 ± 5§	75 ± 1
14	Control	31 ± 3	101 ± 7	65 ± 3
	Ethanol	55 ± 7§	160 ± 5§	85 ± 4§

* Number of days on diet.
† Activity is expressed as nmoles substrate conjugated per mg protein per min ± S.E.M.
‡ Number of animals per group.
§ Statistically significant difference, $P \leq 0.05$.

be seen in Table 4, no significant induction was observed after 4 days on the diet. However, by the end of 7 days glutathione S-transferase activity with DCNB and NBC had increased significantly and either remained elevated or increased until day 14. Glutathione S-transferase activity with ENP increased more slowly and appeared to lag behind the increase in activity observed with the other substrates.

DISCUSSION

The data from the present study indicate that glutathione S-transferase is induced in male mice by feeding ethanol. This observation had been reported previously for rats [13, 14]. There also appears to be a relationship between the amount of ethanol in the diet and the extent of glutathione S-transferase induction. This should not be construed as a strict dose–response relationship because no attempt was made initially to control total caloric intake. Pair-fed mice exhibited similar increases in glutathione S-transferase activity. Thus, differences in caloric intake do not appear to be responsible for the observed induction [14].

The increase in the specific activity of glutathione S-transferase with ENP appeared to lag behind that observed with the other two substrates, and the extent of induction was not as large. These phenomena may relfect a differential induction of one of the transferases. Four forms of glutathione S-transferase are reported to exist in mouse liver [18]. One of these glutathione S-transferases has a much higher specific activity with ENP than the other forms of the transferase. It is possible that ethanol produces a differential induction of the transferases which is responsible for the delayed increase in activity observed with ENP.

The extent of inducibility of glutathione S-transferase in the mouse is reported to be much greater than in the rat [11]. However, the extent of induction also appears to be very dependent on the inducing agent. Hetu *et al.* [13] reported a 22% increase in glutathione S-transferase specific activity with DCNB after maintaining female rats on a 36% ethanol diet for 3 weeks. Benson *et al.* [11] observed a 950% increase in the rate of conjugation of DCNB in mice and a 100% increase in rats after induction with butylated hydroxyanisole. These data suggest that the extent of induction of glutathione S-transferase is dependent not only on the species utilized for the study but also on the inducing agent. Although ethanol does significantly increase glutathione S-transferase activity, it appears to be a fairly poor inducer of glutathione S-transferase in mice.

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