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LIVER MICROSOMAL DRUG METABOLISM IN ETHANOL-TREATED HAMSTERS

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Abstract—Administration of ethanol in drinking water to Syrian golden hamsters for 1–3 weeks caused alterations of microsomal cytochrome P-450-dependent monooxygenase activities in the liver accompanied by a slight elevation in cytochrome P-450 content. Ethanol treatment resulted in an increase in the activities for ethanol oxidation, aniline *p*-hydroxylation and dimethylnitrosamine *N*-demethylation. In particular, when dimethylnitrosamine was used as a substrate, the rate of formaldehyde formation was enhanced by 2- to 2.7-fold, while ethanol oxidation and aniline *p*-hydroxylation were increased by 1.5- to 2- and 1.2- to 1.3-fold, respectively. On the other hand, the activities of 7-ethoxycoumarin *O*-deethylase, benzphetamine *N*-demethylase and benzo[*a*]pyrene 3-hydroxylase were apparently decreased after ethanol treatment. These results for hamsters were significantly different from those reported for rats.

Ethanol is a pharmacologically interesting compound because it has a variety of effects on biochemical systems throughout the body. Chronic ingestion of ethanol has been shown to cause alterations of drug metabolizing enzyme activities in liver microsomes [1–6] as well as a marked proliferation of hepatic smooth endoplasmic reticulum [7]. In addition, chronic ethanol consumption has long been recognized epidemiologically as one of the risk factors for human cancers of the upper respiratory tract [8–10], upper alimentary tract [9, 11–13] and liver [14, 15]. However, the mechanisms of the involvement of ethanol in alterations of the drug metabolism or chemical carcinogenesis are not fully understood.

It is well established that a wide variety of chemicals, carcinogens, pollutants and drugs as well as endogenous substrates are oxidatively metabolized by the action of the cytochrome P-450-dependent monooxygenase system in liver microsomes [16, 17]. Chronic administration of ethanol to rats or rabbits seems to cause the induction of a specific form of cytochrome P-450 which has a high affinity and catalytic activities for aniline and ethanol [18–20]. Recently, Peng *et al.* [21] reported that the treatment of rats with ethanol also resulted in an enhanced capability of the liver microsomes to metabolize dimethylnitrosamine (DMN). It is unknown at present whether or not a single form of cytochrome P-450 is involved in the metabolism of all these substrates.

Hamsters have become popular as an experimental animal for the study of chemical carcinogenesis or drug metabolism. The hamster liver has a higher capability of metabolically activating carcinogens such as DMN and *N*-acetylaminofluorene as compared with rats [22]. At present, however, little is known about the drug metabolizing enzyme system in hamsters. These facts led us to study the mechanism of drug metabolism in the liver microsomes using hamsters as a model animal. We examined the effect of administration of ethanol to hamsters on the cytochrome P-450-dependent monooxygenase activities in the liver microsomes. In this paper, we present data showing that ethanol treatment of hamsters for 1–3 weeks results in the enhancement or decrease of hepatic drug metabolism.

MATERIALS AND METHODS

Chemicals. DMN and 7-ethoxycoumarin were purchased from Aldrich Chemical Co., Milwaukee, U.S.A. Benzo[*a*]pyrene, aniline and ethanol were purchased from the Wako Pure Chemical Industries, Ltd., Osaka, Japan. NADPH and benzphetamine were obtained from Oriental Yeast Co. Ltd., Tokyo, Japan and Upjohn Co., Kalamazoo, U.S.A., respectively. All other chemicals were also of the highest purity commercially available.

Animals. Male Syrian golden hamsters were obtained from Shizuoka Laboratory Animal Center, Shizuoka, Japan. Animals were housed one per cage and given a commercial laboratory chow (Oriental MF diet) and water *ad libitium*. At 6 weeks of age the ethanol-treated groups received 10% (v/v) ethanol in drinking water for appropriate periods. For the control groups water was given in place of ethanol. All the animals were killed by decapitation around 9–10 a.m. on the same day to avoid changes in activities due to circadian variation.

Preparation of microsomes. The liver from each animal was separately homogenized in 10 mM potassium phosphate buffer (pH 7.25) containing 150 mM KCl and 1 mM EDTA and was centrifuged at 9000 *g* for 20 min. The supernatant fraction was centrifuged at 105,000 *g* for 60 min. The pellet was suspended in the same buffer and centrifugation at 105,000 *g* was repeated to obtain the microsomal fractions for the enzyme assay.

Assay for microsomal monooxygenase activities. Benzo[*a*]pyrene 3-hydroxylase activity was assayed according to the method of Nebert and Gelboin [23]. The assay for 7-ethoxycoumarin *O*-deethylation was

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carried out according to the procedure of Guengerich [24] with the following minor modifications: the reaction mixture contained, in a final volume of 1.0 ml, 50 mM potassium HEPES buffer (pH 7.7), 15 mM $MgCl_2$, 0.25 mM sodium deoxycholate, 0.3 mM NADPH, and 20 μg of microsomes. After preincubation at 37° for 5 min, 7-ethoxycoumarin was added to the reaction medium at a final concentration of 0.5 mM. Incubation was carried out for 5 min. The fluorometric intensity of 7-hydroxycoumarin formed was measured (extinction at 360 nm, emission at 460 nm). Benzphetamine *N*-demethylase activity was determined by measuring the rate of formation of formaldehyde according to the method of Prough and Ziegler [25]. The activity for aniline *p*-hydroxylation was determined by measuring the rate of formation of *p*-aminophenol [26]. The rate of ethanol oxidation to acetaldehyde was measured by the assay system using the semicarbazone trapping method of Lieber and Decarli [27]. The oxidative demethylation of DMN was determined by measuring the rate of formation of formaldehyde at a low DMN concentration (4 mM), which was performed by the method of Nash [28]. All the reactions were carried out in duplicate with shaking in the incubator. Under these assay conditions, the amounts of products formed were proportional to the amount of protein used and the incubation time. Protein concentration was determined by the method of Lowry *et al.* [29]. Cytochrome P-450 content was determined by the method of Omura and Sato [30]. Statistical analysis was performed by Student's *t*-test.

RESULTS

The effect of ethanol administration on body and liver wt is shown in Table 1. These results suggest that hamsters readily consume ethanol when it is administered as a 10% solution in drinking water. The animals in this study constantly consumed about 13–17 ml of ethanol per day during the treatment. However, the administration of ethanol to hamsters caused a slight loss in body and liver wts, when compared with results for control animals. For example, the body and liver wts after a 3-week treatment were decreased by 2 and 15%, respectively, and the ratio of liver wt to body wt was also significantly decreased by ethanol consumption. The decrease in both body and liver wts appears to be due to a decrease in food intake during the treatment. On the other hand, the gross content of microsomal cytochrome P-450 in the liver was slightly increased by ethanol consumption, namely, an increase of about 20% in the cytochrome P-450 content was observed except with a 2-week treatment group. In both control and ethanol-treated groups, the CO-reduced form of microsomal hemoprotein gave an absorption maximum around 449 nm.

Next, the effect of ethanol treatment on the microsomal monooxygenase activities was examined. As shown in Table 2, a decrease in enzyme activity with benzphetamine as a substrate was observed. After a 3-week treatment, 7-ethoxycoumarin *O*-deethylase and benzo[*a*]pyrene 3-hydroxylase activities were also significantly decreased as compared with the control values. On the other hand, an enhancement of enzyme activities was observed with ethanol, aniline and DMN as the substrate. In particular, the activity of DMN *N*-demethylase showed an increase by 2- to 2.7-fold during the period of ethanol administration. The enhancement of aniline *p*-hydroxylase activity was lower than that of DMN *N*-demethylation or ethanol oxidation. The rate of monooxygenation of these substrates were already enhanced 1 week after ethanol administration. The marked enhancement of ethanol oxidizing activity was observed with microsomes prepared from hamsters treated for 2 weeks.

Several lines of evidence for the involvement of cytochrome P-450 in the metabolism of ethanol, aniline and DMN have been presented for rats or mice [31, 32]. In hamsters, we also reconfirmed the involvement of cytochrome P-450 in the monooxygenation of these substrates: namely, these drug metabolizing enzymes essentially required NADPH as a cofactor, and CO bubbling for 2 min before incubation caused about 70% inhibition of the monooxygenase reactions (data not shown).

DISCUSSION

It is of great importance to investigate the involvement of ethanol in drug metabolism using a suitable model animals, because alcoholic beverages are commonly used by humans, and ethanol is thought to be one of the risk factors of human cancers. The results presented here demonstrated that hamsters are suitable for studying the effect of ethanol treatment on drug metabolism.

Our results also suggest that ethanol is a potent

Table 1. Effect of ethanol administration on body and liver wts and cytochrome P-450 content in hamster liver

Period of administration (week)	Treatment	Ethanol consumption (ml/day)	P-450 Content (nmoles/mg protein)	Body wt (g)	Liver wt (g)	Liver wt / Body wt ($\times 10^{-2}$)
0			1.32 ± 0.01	87 ± 1.5	4.30 ± 0.12	4.94 ± 0.19
1	Control	—	1.36 ± 0.11	103 ± 1.7	4.67 ± 0.08	4.53 ± 0.03
	Ethanol	16.5 ± 0.1	1.65 ± 0.10*	94 ± 2.9*	3.89 ± 0.10*	4.16 ± 0.10*
2	Control	—	1.47 ± 0.05	114 ± 0.9	4.92 ± 0.18	4.32 ± 0.13
	Ethanol	12.6 ± 0.9	1.49 ± 0.08	100 ± 2.0*	3.88 ± 0.11*	3.90 ± 0.12*
3	Control	—	1.43 ± 0.02	111 ± 4.4	5.32 ± 0.14	4.51 ± 0.05
	Ethanol	14.9 ± 1.3	1.62 ± 0.04*	109 ± 1.9	4.51 ± 0.08*	4.14 ± 0.06*

Each value of control and ethanol-treated groups is mean ± S.E. of three and six animals, respectively.
* $P < 0.05$ compared to control.

Table 2. Effect of ethanol administration on liver microsomal monooxygenase activities

Period of administration (week)	Treatment	Benzphetamine *N*-demethylation	7-Ethoxycoumarin *O*-deethylation	Benzo[*a*]pyrene 3-hydroxylation	Aniline *p*-hydroxylation	Ethanol oxidation	DMN *N*-demethylation
		(nmoles products formed/min/nmole P-450)					
0		7.31 ± 0.15	2.35 ± 0.21	0.34 ± 0.02	0.77 ± 0.01	2.56 ± 0.38	1.54 ± 0.07
1	Control	7.64 ± 0.17	2.06 ± 0.15	0.19 ± 0.03	0.71 ± 0.04	2.36 ± 0.39	1.38 ± 0.05
	Ethanol	5.64 ± 0.14*	1.94 ± 0.11	0.22 ± 0.03	0.93 ± 0.05*	3.83 ± 0.50*	3.66 ± 0.30*
2	Control	6.05 ± 0.30	1.63 ± 0.19	0.21 ± 0.01	0.68 ± 0.04	2.72 ± 0.07	1.42 ± 0.09
	Ethanol	5.71 ± 0.15*	1.65 ± 0.11	0.12 ± 0.06	0.83 ± 0.03*	5.16 ± 0.59*	3.11 ± 0.25*
3	Control	6.05 ± 0.20	2.43 ± 0.08	0.35 ± 0.07	0.72 ± 0.03	2.51 ± 0.25	1.64 ± 0.08
	Ethanol	4.90 ± 0.16*	1.76 ± 0.07*	0.12 ± 0.02*	0.88 ± 0.04*	3.85 ± 0.58*	3.73 ± 0.35*

Each value of control and ethanol-treated groups is mean ± S.E. of three and six animals, respectively.
* $P < 0.05$ compared to control.

inducer of hamster liver microsomal cytochrome P-450 involved in the metabolism of ethanol, aniline and DMN, and that such enhancement of these activities is consistent with previous reports for rats and rabbits [18–21]. In light of DMN-inducible cancer of the liver, it is interesting that the metabolic activation of DMN to the methylating agent was enhanced by ethanol consumption.

Since several lines of evidence including our results show that nitrosamines such as DMN are metabolized by the action of the cytochrome P-450-dependent monooxygenase system, the increase in DMN demethylase activity may be attributed to the induction of a unique form of cytochrome P-450 responsible for DMN *N*-demethylation. In our experiment the microsomal cytochrome P-450 from ethanol-treated hamsters had a higher catalytic activity toward DMN than that from phenobarbital- or 3-methylcholanthrene-treated animals (data not shown). This fact has led to the hypothesis that ethanol treatment of hamsters results in the induction of a unique form of cytochrome P-450 unlike that induced by phenobarbital or 3-methylcholanthrene. Recently, Ingelman-Sundberg and Hagbjörk have shown that ethanol-inducible rabbit microsomal cytochrome P-450 catalyzes the oxidation of ethanol by mediating the hydroxy radical [33]. In hamsters the hydroxy radical derived from ethanol-inducible cytochrome P-450 may be also involved in DMN *N*-demethylation as is the case with ethanol-treated rabbits. In any case further purification of microsomal fractions is required to demonstrate the presence of cytochrome P-450 specific for DMN and its induction by ethanol treatment.

The effects of ethanol administration on the cytochrome P-450-dependent monooxygenases in hamster liver were somewhat different from those reported for Sprague–Dawley and Wistar rats [18, 34]. In rats the ethanol-induced form of cytochrome P-450 appears to have a high affinity for 7-ethoxycoumarin [17], but in hamsters 7-ethoxycoumarin *O*-deethylase activity was significantly decreased by ethanol administration. This discrepancy may be due to a species difference in cytochrome P-450 between rats and hamsters. It would be interesting to examine how many forms of cytochrome P-450 are present in hamster liver microsomes. Further purification of microsomal fractions is now in progress.

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