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DURATION OF THE ENHANCED ACTIVITY OF THE MICROSOMAL ETHANOL-OXIDIZING ENZYME SYSTEM AND RATE OF ETHANOL DEGRADATION IN ETHANOL-FED RATS AFTER WITHDRAWAL*

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Abstract—The activities of ethanol-oxidizing enzymes, drug-metabolizing enzymes and rates of ethanol disappearance from the blood were determined in rats after 14 days of ethanol feeding and after withdrawal. The microsomal ethanol-oxidizing system, cytochrome P-450, aniline hydroxylase and the rates of ethanol disappearance from the blood were enhanced by ethanol feeding. Alcohol dehydrogenase remained unchanged. After withdrawal, the rates of ethanol disappearance from the blood returned to control values in 2 days, while the activities of the microsomal enzymes did not fall to control values until 7 days. The lack of parallelism in the fall of the rates of ethanol disappearance from the blood and the microsomal enzymes suggests that factors other than the enhanced activity of the microsomal ethanol-oxidizing enzyme system are responsible for the increases in rates of ethanol degradation after ethanol feeding.

IT IS well established that the administration of ethanol to rats enhances the activities of microsomal enzyme systems that metabolize ethanol[1,2] and other drugs,[3] as well as the rates of ethanol disappearance from the blood.[2,4] Alcohol dehydrogenase, present in the soluble fraction of liver homogenates, is the enzyme principally responsible for the oxidation of ethanol.[5,6] Studies of the effect of ethanol administration on this enzyme have yielded varying results: increases,[7,8] no change[1,2,9] and even decreases[10] in alcohol dehydrogenase have been reported. However, while the role *in vivo* of the microsomal ethanol-oxidizing system remains uncertain,[11] that of alcohol dehydrogenase is supported by the similarities of the Michaelis–Menten constant obtained for ethanol oxidation by alcohol dehydrogenase *in vitro*, and that calculated for the rates of ethanol disappearance from the blood *in vivo*.[12] In addition, pyrazole, a potent inhibitor of alcohol dehydrogenase[13] but not of the microsomal ethanol-oxidizing system,[4] greatly reduces ethanol oxidation *in vitro*.[14]

In the present study both the activities of the ethanol-oxidizing enzymes and the rates of ethanol degradation were determined in rats after maximum induction by ethanol feeding and after withdrawal, in order to determine the duration of induction and to compare the changes in the activities of the ethanol-oxidizing enzymes with those in the rates of ethanol disappearance.

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METHODS

Animals and diets. Twenty-eight male albino Wistar rats, weighing between 180 and 200 g were studied. The animals were fed a semi-liquid diet in which either ethanol or sucrose made up 36 per cent of the calories. The final caloric compositions per 100 g of the diets were: protein 14·2 per cent (7·72 g); amino acids 7·6 per cent (3·97 g); fat 6·3 per cent (1·43 g); carbohydrate 35·7 per cent (18·79 g); and either ethanol (11·4 g) or sucrose (19·5 g), 36·2 per cent. Vitamins and minerals were present in adequate amounts.[2] Twenty-four of the rats were fed *ad lib.* the ethanol-containing diet for a period of 14 days, followed thereafter by the sucrose-containing diet. Four control animals were paired from the onset of the feeding with four of the ethanol-fed rats, and pair fed the sucrose-containing diet. The eight paired rats were sacrificed at the end of 14 days of feeding. The remainder of the rats were sacrificed in groups of four each after 2, 4, 7, 11, 14 and 21 days of discontinuation of the ethanol and institution of the sucrose-containing diet.

The mean intake of the ethanol-containing diet was 25·6 $\pm$ 7·0 (S.D.) g/day, which corresponded to a mean intake of 13·9 $\pm$ 3·8 (S.D.) g of ethanol per kg of body weight per day. The mean intake of the sucrose-containing diet after ethanol withdrawal was 29·6 $\pm$ 5·2 (S.D.) g/day. The weight gain of the 24 ethanol-fed and four control sucrose rats was similar: 12·5 $\pm$ 4·1 and 11·6 $\pm$ 3·0 g (mean $\pm$ S.D.) per 7 days respectively. The mean weight gain of the rats after discontinuation of the ethanol and institution of the sucrose-containing diet was 12·8 $\pm$ 3·4 (S.D.) per 7 days.

Tissue preparation. The animals were sacrificed by a blow on the head after a 14-hr fast. The livers were immediately removed, washed in ice-cold physiologic saline solution, weighed, minced with scissors and then homogenized in a Potter–Elvehjem homogenizer for 3 min with a volume of buffer equivalent to two times the liver weight. The composition of the buffer was as follows: tris–HCl (0·05 M), KCl (0·08 M), $MgCl_2$ (0·01 M), sucrose (0·25 M) pH 7·8. The homogenate was centrifuged at 9000 *g* for 10 min in a Sorvall refrigerated centrifuge. The resulting precipitate was discarded and the supernatant centrifuged at 106,000 *g* for 60 min in a B-50 International refrigerated ultracentrifuge (Rotor 211A). The supernatant fraction obtained at this point was separated for the analysis of alcohol dehydrogenase activity, and the microsomal pellet washed with 8 ml of the above buffer and then recentrifuged at 106,000 *g* for 60 min. The washed microsomal pellet finally was resuspended in 8 ml of 0·1 M NaH_2PO_4–K_2HPO_4 buffer, pH 7·4.

Enzyme determinations. Alcohol dehydrogenase activity was determined by the method of Bonnichsen and Brink,[15] the microsomal NADPH-dependent ethanol-oxidizing activity as described by Lieber and DeCarli,[1] aniline hydroxylase according to Imai and Sato,[16] and cytochrome P-450 according to Omura and Sato.[17] Protein concentration was determined by the method of Lowry *et al.*[18] with bovine serum albumin used as a standard.

Rates of ethanol disappearance. The rates of ethanol disappearance from the blood were determined in all the animals over a 6-hr time interval prior to sacrifice. Ethanol (4 g/kg of body weight) was given as a 20 per cent solution in water by stomach tube. Eighty μl of blood were obtained hourly for 6 hr from the retro-orbital plexus of each animal with a heparinized capillary tube. After centrifugation at 2000 *g* for 10 min, the separated plasma samples were analyzed for ethanol concentration by gas–liquid chromatography.[19] Ethanol concentrations in the plasma, when plotted against time,

followed a linear function. The rate of ethanol disappearance from the plasma was obtained from the slope of the regression line calculated by the method of least squares[20] and expressed in milligrams of ethanol cleared per 100 ml of plasma per hour.

RESULTS

The effect of ethanol feeding and withdrawal on liver weight and microsomal protein concentration is shown in Table 1. There were no significant changes in liver weight; by contrast, microsomal protein concentration was increased by ethanol

TABLE 1. EFFECT OF ETHANOL FEEDING AND WITHDRAWAL ON LIVER WEIGHT AND MICROSOMAL PROTEIN CONCENTRATION*

Experimental groups	Days after withdrawal from ethanol	Relative liver wt. (g/100 g body wt.)	Microsomal protein (mg/g wet wt. liver)
Control		3·5 ± 0·2	26·8 ± 2·1
Ethanol-fed		3·5 ± 0·1	31·6 ± 1·3†
Ethanol-fed	2	3·6 ± 0·1	30·8 ± 4·3
and withdrawn	4	3·5 ± 0·3	28·8 ± 4·8
	7	3·7 ± 0·4	27·1 ± 3·8
	11	3·5 ± 0·1	28·3 ± 0·7‡
	14	3·6 ± 0·3	26·3 ± 1·7§
	21	3·4 ± 0·1	25·4 ± 2·2§

* The values are expressed as mean ± standard deviation of four animals.
† Significant difference from control ($P < 0·01$).
‡ Significant difference from ethanol-fed ($P < 0·01$).
§ Significant difference from ethanol-fed ($P < 0·001$).

feeding and decreased significantly from the enhanced values of the ethanol-fed rats after 11 days of withdrawal from ethanol ($P < 0·001$). The microsomal enzymes were enhanced by ethanol feeding. Both aniline hydroxylase and cytochrome P-450 (Table 2)

TABLE 2. EFFECT OF ETHANOL FEEDING AND WITHDRAWAL ON CYTOCHROME P-450 AND THE ACTIVITY OF ANILINE HYDROXYLASE*

Experimental groups	Days after withdrawal from ethanol	Aniline hydroxylase (μmoles/g wet wt. liver/hr)	Cytochrome P-450 (mμmoles/g wet wt. liver)
Control		0·28 ± 0·04	27·2 ± 5·0
Ethanol-fed		0·70 ± 0·13†	59·3 ± 12·4‡
Ethanol-fed	2	0·54 ± 0·15	58·7 ± 14·6
and withdrawn	4	0·47 ± 0·16	44·0 ± 17·4
	7	0·34 ± 0·08§	23·2 ± 6·5§
	11	0·29 ± 0·09§	31·2 ± 2·9§
	14	0·37 ± 0·14‖	29·0 ± 4·5§
	21	0·23 ± 0·02¶	26·7 ± 2·7§

* The values are expressed as mean ± standard deviation of four animals.
† Significant difference from control ($P < 0·001$).
‡ Significant difference from control ($P < 0·01$).
§ Significant difference from ethanol-fed ($P < 0·01$).
‖ Significant difference from ethanol-fed ($P < 0·02$).
¶ Significant difference from ethanol-fed ($P < 0·001$).

TABLE 3. EFFECT OF ETHANOL FEEDING AND WITHDRAWAL ON THE ACTIVITIES OF THE MICROSOMAL ETHANOL-OXIDIZING SYSTEM AND ALCOHOL DEHYDROGENASE AND ON RATES OF ETHANOL DISAPPEARANCE FROM THE BLOOD*

Experimental groups	Days after withdrawal from ethanol	Alcohol dehydrogenase	Microsomal ethanol-oxidizing activity	Rates of ethanol disappearance from the blood (mg/100 ml plasma/hr)
		(μmoles/g wet wt. liver/min)		
Control		0·83 ± 0·07	0·28 ± 0·03	33·0 ± 3·2
Ethanol-fed		0·82 ± 0·13	0·48 ± 0·08†	46·5 ± 5·9†
Ethanol-fed and withdrawn	2	0·80 ± 0·10	0·40 ± 0·02	31·1 ± 3·0§
	4	0·76 ± 0·24	0·39 ± 0·08	33·1 ± 4·0§
	7	0·82 ± 0·21	0·31 ± 0·04‡	34·8 ± 4·9‡
	11	1·03 ± 0·10‡	0·28 ± 0·03§	32·0 ± 4·7§
	14	0·84 ± 0·09	0·29 ± 0·09‡	33·2 ± 3·6§
	21	0·79 ± 0·15	0·32 ± 0·04‡	32·9 ± 4·6‡

* The values are expressed as mean ± standard deviation for four animals.
† Significant difference from control ($P < 0·01$).
‡ Significant difference from ethanol-fed ($P < 0·05$).
§ Significant difference from ethanol-fed ($P < 0·01$).

decreased to control values after 7 days of withdrawal from ethanol. The microsomal ethanol-oxidizing activity (Table 3) also decreased to control values 7 days after withdrawal from ethanol. Alcohol dehydrogenase activity was not affected by ethanol feeding. After withdrawal, with the exception of an increase in activity at 11 days, there were no changes in alcohol dehydrogenase. The rates of ethanol disappearance from the blood were enhanced by ethanol feeding, and fell to control values 2 days after withdrawal from ethanol.

DISCUSSION

Previous studies from this laboratory have shown that maximal increases in rates of ethanol disappearance from the blood and in microsomal enzymes are obtained in male rats after 7 and 14 days of ethanol feeding respectively.[2] This is a shorter period of time than the 19–25 days of intraperitoneal administration of ethanol necessary to obtain maximal tolerance to ethanol in the performance of a complex motor task.[21] These findings are in contrast to the more rapid development of maximal induction of microsomal enzymes and decreased hexabarbitone sleeping times after 4 days of beginning barbiturate administration.[22,23] Ethanol also differs from barbiturates in that it fails to stimulate liver growth and it is a relatively weak inducer; the increases in enzyme activity after ethanol administration being of the order of 1-fold, in contrast to the 2 to 4-fold increases induced by barbiturates. These differences may be related to the observation that the administration of ethanol, unlike that of barbiturates, fails to increase microsomal protein synthesis, but rather results in decreases in protein degradation.[2]

The time required for the return of the microsomal enzymes to normal after withdrawal from ethanol was similar to the 6–7-day interval necessary for the return to normal of the microsomal enzymes and the hexobarbitone sleeping time after withdrawal from barbiturates.[22,23] Surprising was the rapid return of the rates of ethanol

disappearance from the blood to normal as early as 2 days after withdrawal. Similar findings were also obtained in chronic alcoholics in whom, after withdrawal from alcohol, the rates of ethanol disappearance from the blood returned to normal by 7 days while the activity of the NADPH dependent ethanol-oxidizing system did not reach control levels until 21 days.[24] While the increases in both the rates of ethanol degradation and the microsomal ethanol-oxidizing activity with no change in alcohol dehydrogenase would suggest that induction of the microsomal enzyme is responsible for the enhanced rates, the lack in parallelism in their fall after withdrawal from ethanol suggests that factors in addition to the activity of the microsomal enzyme are responsible for the regulation of the rates *in vivo* of ethanol disappearance from the blood. Both hepatic blood flow and the availability of coenzymes may regulate rates of ethanol degradation. The acute administration of ethanol has been shown to either increase[25,26] or decrease[27] hepatic blood flow, depending on the dose administered; however, the effect of chronic administration of ethanol on hepatic blood flow remains unknown. The availability of oxidized coenzyme is probably even more important. This is suggested by studies *in vitro* showing that the dissociation of the alcohol dehydrogenase—NADH complex is rate limiting in ethanol oxidation.[28] Also mitochondrial uncoupling agents, such as dinitrophenol, which increase the ability of the mitochondria to oxidize NADH, have been shown to increase the rate of ethanol metabolism by rat liver slices.[29] Furthermore, studies *in vivo* show that stimuli to increased re-oxidation of NADH, such as elevated pyruvate concentrations in children with type I glycogen storage disease,[30] or infusions of fructose in dogs[31] and man[32] enhance the rates of ethanol metabolism.

The data in this study also raise serious questions as to what the role of rates of ethanol degradation is on the acquisition and loss of tolerance to ethanol in animals and man. Although in the studies of tolerance to ethanol by LeBlanc *et al.*,[21] ethanol was administered by stomach tube, which is not strictly comparable to the administration of ethanol in the diet in our studies, it nevertheless appears that the development of tolerance to ethanol takes longer both to become maximal and return to normal after withdrawal than the time required for the rates of ethanol degradation to change similarly. The discrepancy is especially apparent after withdrawal where the above authors demonstrated that the increased tolerance to ethanol did not return to normal until after 14 days, while the rates of ethanol disappearance from the blood in the present study returned to normal within 2 days. Furthermore, LeBlanc *et al.*[21] ruled out increased metabolism as the cause of the increases in tolerance by showing that the effects on tolerance were apparent when expressed in relation to measured blood ethanol concentration, rather than to administered dose. It would, therefore, be reasonable to postulate that the changes in tolerance to ethanol are related not as much to the rates of ethanol degradation, but rather to adaptive changes in the central nervous system initiated by ethanol. This was also suggested by studies in human subjects showing that increased tolerance to alcohol in heavy drinkers, as compared with abstainers, was not related to rates of ethanol disappearance from the blood, but rather to an increase in the blood-alcohol threshold for impairment of sensory, motor and psychological functions.[33] Although ethanol and other depressants have been shown to have various biochemical and physiologic effects on the brain,[34,35] the mechanism underlying adaptation remains unknown.

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