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MECHANISM OF THE EFFECT OF ACUTE ETHANOL ON HEXOBARBITAL METABOLISM*

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Abstract—The effect of acute ethanol treatment on hepatic metabolism of hexobarbital (Hb) was studied in the rat. Oral administration of 3 g/kg of ethanol (15% w/v) inhibited Hb hydroxylase activity 45–50 per cent. A dose–response relationship was found for ethanol inhibition of Hb metabolism. The overall hepatic microsomal protein content was not affected, but the hepatic cytochrome P-450 level was reduced approximately 42 per cent by this ethanol treatment. Corticosterone (12.5 mg/kg, i.p.) inhibited Hb hydroxylase activity 43 per cent. The combination of ethanol and corticosterone treatment further inhibited Hb hydroxylase activity. Study *in vitro* showed that corticosterone inhibited Hb metabolism competitively. Ethanol caused a 3-fold increase in the plasma corticosterone level but had no effect on plasma corticosterone of adrenalectomized rats. Hexobarbital metabolism was not affected by ethanol in adrenalectomized rats. Thus, the inhibition of hepatic Hb metabolism by acute ethanol was caused by the increased release of corticosterone induced by ethanol.

Alcohol has been found to interact with many drugs [1–3]. In rats, ethanol (5 g/kg, orally) doubled the half-life of pentobarbital in the plasma [4]. Normal human volunteers were given labeled meprobamate and pentobarbital orally, after which blood levels of these drugs were measured for 16 hr. After the administration of ethanol, the half-life of pentobarbital was doubled and that of meprobamate was prolonged two to five times [5]. Thus, it has been demonstrated that, in the presence of ethanol, drug metabolism was inhibited in both the rat and man. In preliminary studies, we found that hexobarbital (Hb) metabolism in the rat liver 9000 *g* supernatant fraction and microsomal fractions was inhibited an average of 45 and 25 per cent, respectively, 1 hr after the administration of 3 g/kg of ethanol orally, and that the inhibition of Hb metabolism occurred in the microsomal fraction and not in the cytosol [6]. This paper presents a possible mechanism for ethanol-induced inhibition of Hb metabolism in the rat.

METHODS

Treatment. Adult male Sprague–Dawley rats, 180–220 g, were maintained in a temperature-controlled room with a 12-hr light–dark cycle for a minimum of 5 days before they were used. The animals were fed with Purina rat chow and water *ad lib.* All experiments were scheduled so that animals were treated between 9:00 and 10:00 a.m.

Ethanol (3 g/kg) was administered in a 15% solution by oral intubation. Control animals received an equal volume of water.

Tissue preparations. The animals were sacrificed by cervical dislocation. The livers were perfused with ice-cold 1.15% KCl, rapidly excised, blotted dry, and weighed. The livers were cut into small pieces and homogenized in 3 vol. (w/v) 1.15% KCl with a motor-driven coaxial Teflon pestle and glass homogenizer for 3 min at 1000 rev/min. Fractionation of cell components was performed in a Beckman model L-2 ultracentrifuge with a No. 30 rotor at 2°. The whole homogenate was centrifuged at 9000 *g* (ave) for 30 min. The 9000 *g* (ave) supernatant fraction was either used directly or further centrifuged at 78,000 *g* (ave) for 90 min to separate microsomes and cytosol. The microsomal pellets were washed once by resuspension in 1.15% KCl and centrifuged at 78,000 *g* (ave) for 60 min. The washed microsomal pellets were resuspended in pH 7.4 KCl–Tris buffer (150 mM KCl and 50 mM Tris).

Metabolism studies. All experiments were performed in fresh tissue. The standard reaction mixture consisting of 2 μmoles NADP, 10 μmoles $MgCl_2 \cdot 6\ H_2O$, 50 μmoles nicotinamide, 20 μmoles glucose 6-phosphate, and 2 I.U. glucose 6-phosphate dehydrogenase was added to 25-ml Erlenmeyer flasks. The remaining components of the incubation mixture included 1 ml liver fraction (333 mg of wet tissue), 4 μmoles hexobarbital and sufficient pH 7.4 KCl–Tris buffer (0.2 M) to make a final mixture of 4 ml. After addition of substrate, the samples were shaken in a Dubnoff metabolic incubator for 10 min at 37°.

Tritiated sodium hexobarbital (New England Nuclear Corp.) was added to non-radioactive sodium hexobarbital (Winthrop Laboratory), and a solution in water having a specific activity of 55 μCi/m-mole was used. The amount of unchanged hexobarbital or its metabolites was determined by the method of Kupfer and Rosenfeld [7].

Assay of plasma corticosterone and adrenalectomy. The plasma level of corticosterone was determined by a fluorometric method according to Zenker and Bernstein [8]. Adrenalectomy was performed on the

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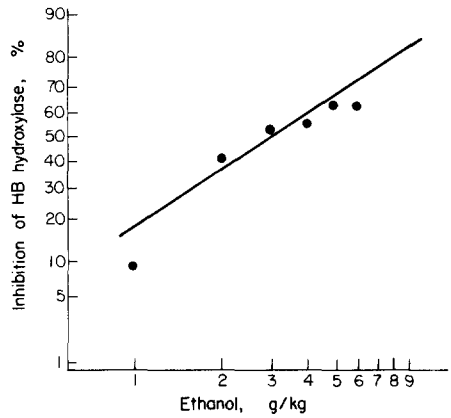

Fig. 1. Effect of ethanol on hexobarbital hydroxylase activity in the rat liver 9000 *g* supernatant fraction. Doses of ethanol were administered orally 1 hr before sacrifice. Each point represents the mean of four determinations.

rats under light ether anesthesia. A flank incision was made directly in front of either the palpated left or right kidney. The adrenals were approached retroperitoneally. The kidney and fat masses around it were then gently manipulated until the adrenal could be visualized and removed by blunt dissection. The wound was then closed by wound clips and the animals were allowed to recover for a period of 3 days. The animals were allowed food and a solution of 0.9% NaCl plus 0.1% KCl as drinking water *ad lib.* during the recovery period. The adrenalectomized rats were used for studies day 4 after surgery.

Determination of hepatic microsomal cytochrome P-450 content. Hepatic microsomal cytochrome P-450 was determined by the method of Omura and Sato [9] as described by Gillette *et al.* [10]. Microsomal protein concentration was measured according to Lowry *et al.* [11].

Analysis of results. Results were compared by analysis of variance or by Student's *t*-test. Differences were considered significant when the probability was less than 0.05.

RESULTS

A dose–response curve for the inhibitory effect of ethanol on hexobarbital hydroxylase (HbH) activity in the rat liver 9000 *g* supernatant fraction was constructed (Fig. 1). Figure 1 is a probit plot and the line was drawn by regression analysis. The results show that increasing the dosage of ethanol caused an increase in inhibition of HbH activity. The concentration of ethanol which caused a 50 per cent inhibition of HbH activity was estimated to be about 3 g/kg. Since 3 g/kg was an effective dose and did not cause an anesthetic effect on the rats, this dosage was used throughout the entire study.

Table 1. Effect of acute ethanol treatment on hepatic microsomal protein content and cytochrome P-450 level

Treatment	Protein content*	P-450 level†
Control	18.75 ± 2.95	0.55 ± 0.03
Ethanol (3 g/kg)	18.10 ± 0.96	0.32 ± 0.02‡

* Values are means ±S. E. M. (mg protein/g of liver) of six rats/group.

† Values are means ±S. E. M. (nmoles/mg of protein) of six rats/group.

‡ Significantly different from control, $P < 0.05$.

Table 2. Effect of acute ethanol treatment on plasma levels of corticosterone of non-adrenalectomized and adrenalectomized rats

	Treatment	N*	Corticosterone† (μg/100 ml)
Expt. A	Control (water intubated)	6	8.85 ± 2.78
	Ethanol (3 g/kg)	6	26.13 ± 5.16‡
Expt. B	Control (water intubated)	4	9.74 ± 1.69
	Adrenalectomized rats		
	Control (water intubated)	4	1.07 ± 0.58§
	Ethanol (3 g/kg)	4	0.93 ± 0.58§,‖

* Number of animals used.

† Values are means ±S. E. M.

‡ Significantly different from control in Expt. A, $P < 0.05$.

§ Significantly different from control in Expt. B, $P < 0.05$.

‖ Not significantly different from adrenalectomized control.

Hepatic microsomal protein content and cytochrome P-450 levels of the ethanol-treated rats were determined. Although the overall microsomal protein content did not change 1 hr after ethanol administration (3 g/kg), the hepatic microsomal cytochrome P-450 level was reduced 42 per cent (Table 1). These results suggest that ethanol may not affect the overall microsomal enzyme level, but has an effect on the microsomal drug-metabolizing system.

It has been found that ethanol administered either intraperitoneally or by gavage to rats and other experimental animals produces a marked decrease in adrenal ascorbic acid levels and cholesterol levels, which has been interpreted as evidence of stimulation of adrenocortical hormone release [12–14]. Therefore, the plasma level of corticosterone was determined 1 hr after the administration of ethanol (3 g/kg). The results in Table 2 show that a 66 per cent increase in the plasma level of corticosterone occurred in the ethanol-treated animals. This was tested in adrenalectomized rats and it was found that ethanol treatment did not significantly increase the plasma level of corticosterone in comparison with the control adrenalectomized rats (Table 2). Hb metabolism in adrenalectomized rats was not affected by ethanol treatment (Table 3).

It has been shown that the plasma level of corticosterone reached the peak concentration 15 min after corticosterone (12.5 mg/kg in propylene glycol solution) was administered i.p. in rats [15]. Table 4 shows that, when rats were given 12.5 mg/kg of corticosterone (i.p.), the HbH activity was inhibited by 43 per cent. When ethanol (3 g/kg, p.o.) and corticosterone

Table 3. Effect of acute ethanol treatment on hexobarbital hydroxylase activity in liver 9000 *g* supernatant fraction of adrenalectomized rats

Treatment	Hexobarbital hydroxylase activity*
Control rats	6.01 ± 0.54
Adrenalectomized rats	
Control	6.10 ± 0.44
Ethanol (3 g/kg)	6.12 ± 0.40

* Values are means ± S. E. M. (μmoles/g of liver/10 min) of four rats/group.

(12.5 mg/kg, i.p.) were combined, the HbH activity was inhibited by 57 per cent. These results suggested that the inhibitory effect of ethanol may be a result of ethanol-induced corticosterone release rather than of ethanol itself.

Figure 2 shows a double reciprocal plot of the inhibitory effect of corticosterone on hepatic microsomal metabolism of Hb. Two concentrations of corticosterone (2×10^{-5} M and 4×10^{-5} M) were used and the results show that corticosterone inhibited Hb metabolism competitively. Corticosterone and hexobarbital have the same V_{max} (9.09 μmoles/g of liver/10 min) but a different K_m.

DISCUSSION

The metabolism of ethanol is generally known to be mainly in the cytosol by alcohol dehydrogenase, an enzymatic system quite different from that of the mixed-function oxidase system. On the other hand, Hb has been found to be metabolized by the microsomal mixed-function oxidase system [16]. Therefore, the metabolism of ethanol would not have a direct effect on the metabolism of Hb. Although it has been reported that a hepatic microsomal ethanol-oxidizing system (MEOS) can oxidize ethanol *in vitro* [17, 18], a substantial amount of evidence has suggested that this enzyme system plays no significant role *in vivo*, after either acute or chronic administration of ethanol [19–21]. Thus, the acute ethanol-induced inhibition of microsomal metabolism of hexobarbital may be derived from an indirect mechanism.

The above evidence shows that acute administration of ethanol produced inhibition in hepatic metabolism of Hb, and that this inhibition was not caused by the direct action of ethanol. This inhibition was associated with the release of corticosterone induced by ethanol. This study suggests that corticosterone is released by ethanol and serves as an alternate substrate in the hepatic microsomal drug-metabolizing system competitively inhibiting the metabolism of Hb.

Table 4. Effect of ethanol, corticosterone and ethanol–corticosterone combination treatments on hexobarbital hydroxylase activity in rat liver 9000 *g* supernatant fraction

Treatment	Hexobarbital hydroxylase activity*	% Inhibition
Control	6.41 ± 0.40	
Ethanol (3 g/kg)	3.87 ± 0.31†	40
Corticosterone (12.5 mg/kg)‡	3.66 ± 0.17†	43
Ethanol + corticosterone§	2.74 ± 0.13†	57

* Values are means ± S. E. M. (μmoles/g of liver/10 min) of six rats/group.

† Significantly different from control, $P < 0.05$.

‡ Corticosterone in propylene glycol (10 mg/ml) was administered i.p. and animals were sacrificed 15 min after injection.

§ Corticosterone in propylene glycol (12.5 mg/kg, i.p.) was adminstered 45 min after ethanol (3 g/kg, orally) and the animals were sacrificed 15 min after corticosterone injection.

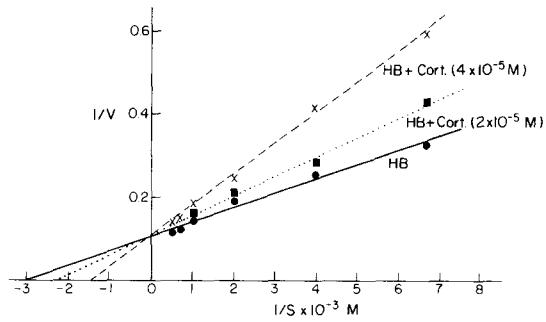

Fig. 2. Double reciprocal plot of the inhibitory effect of corticosterone (cort.) on microsomal metabolism of hexobarbital (Hb). Each point represents the mean of four determinations. Key: s = concentration of HB; v = velocity of HB metabolism (μmoles/g of liver/10 min).

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