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Alcohol Dehydrogenase (ADH) Independent Ethanol Metabolism in Deermice Lacking ADH[1]

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SHIGETA, Y., F. NOMURA, M. A. LEO, S. IIDA, M. R. FELDER AND C. S. LIEBER. *Alcohol dehydrogenase (ADH) independent ethanol metabolism in deermice lacking ADH.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 195–199, 1983.—To assess the importance of non-ADH ethanol metabolism, ADH-negative (ADH^-) and ADH-positive (ADH^+) deermice were fed for 2–4 weeks liquid diets containing ethanol or isocaloric carbohydrate. They consumed progressively increasing amounts of ethanol. Blood ethanol clearance (BEC) increased significantly in both strains. It remained almost unchanged at low ethanol concentrations (5–10 mM), but at high levels (40–70 mM) BEC was strikingly increased with significant differences between ethanol-fed and control animals. Kinetics were consistent with the activity of a non-ADH high K_m system such as the microsomal ethanol-oxidizing system (MEOS). Naive ADH^- had a more active MEOS and more abundant SER than naive ADH^+. After ethanol feeding, MEOS was increased 3–4 times in both strains. There was striking proliferation of SER and cytochrome P-450 was enhanced significantly. Expressed per P-450, MEOS activity was higher in ADH^- than ADH^+. Thus despite absence of ADH, ADH^- deermice can consume large amounts of ethanol: this is associated with increased BEC, SER proliferation, enhanced MEOS activity and quantitative and qualitative changes of cytochrome P-450.

Alcohol dehydrogenase MEOS Ethanol Deermice

IT is generally assumed that ethanol metabolism proceeds primarily via alcohol dehydrogenase (ADH), which is located in liver supernatant fraction. We reported that, in addition to liver ADH, hepatic microsomes are also capable of oxidizing ethanol [8]. It has been reported that the microsomal ethanol-oxidizing system (MEOS) can be distinguished from a catalase-mediated H_2O_2-dependent reaction by using catalase inhibitors [10]. In addition, MEOS was isolated by DEAE cellulose column chromatography and its catalase-independent nature was shown [13,19]. Moreover, Ohnishi and Lieber [14] reported the reconstitution of MEOS and showed that partially purified cytochrome P-450 from ethanol-fed rats was more active for alcohol oxidation than the control preparation in the presence of excess NADPH-cytochrome c reductase and phospholipid.

However, the assessment of the respective roles of these non-ADH pathways in vivo has been hampered by the fact that, heretofore, experiments had to rely largely on the effects of inhibitors. A new tool for the assessment of non-ADH pathways became available when Burnett and Felder [2] described a strain of deermice, Peromyscus maniculatus, which totally lacks ADH genetically. Burnett and Felder showed that, despite the absence of ADH in these animals, they did consume ethanol. These previous studies were hampered, however, by the fact, as in the case of other animal species, deermice have an aversion for ethanol when presented in drinking water. We overcame this natural aversion for ethanol by incorporating the ethanol in a totally liquid diet as we have done successfully for rats and baboons in the past.

In the present paper, we describe ethanol metabolism in these animals in vivo and the contributions of MEOS to it.

METHOD

Animals used in the current studies had the following genotype, for liver alcohol dehydrogenase (ADH): ADH^F/ADH^F (ADH-positive) and ADH^N/ADH^N (ADH-negative). They were pair-fed nutritionally adequate liquid diets containing ethanol or isocaloric carbohydrate [9].

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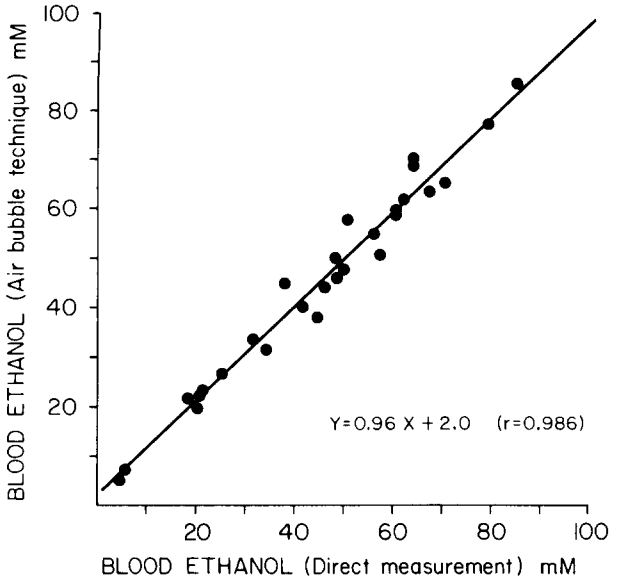

FIG. 1. Relationship of blood ethanol levels measured directly or determined by the air bubble technique.

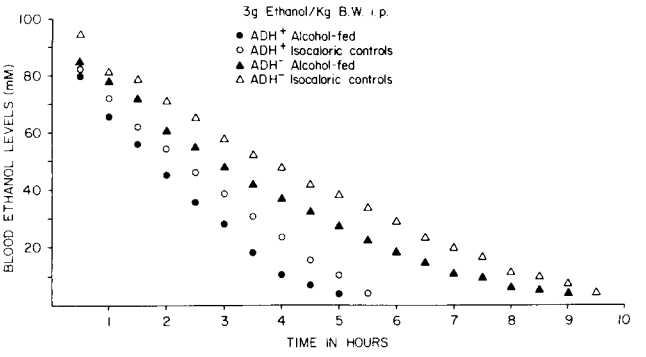

FIG. 2. Effect of chronic ethanol feeding on blood ethanol elimination rate in ADH-positive and ADH-negative deermice. Four kinds of animals were studied. ADH-positive ethanol-fed and pair-fed controls and ADH-negative ethanol-fed and pair-fed controls. Each point is the mean value of at least five animals fed for 2 to 4 weeks.

Ethanol concentration was gradually increased from 1% (w/v) to a final 4 to 5%.

In order to study rates of blood ethanol elimination, we applied the air bubble technique that Lester *et al.* [7] had developed in the rat for the measurement of blood ethanol disappearance. After 2 to 4 weeks on the diet, animals were subjected to the blood ethanol elimination study. In the ethanol-fed animals, the ethanol containing diet was replaced by control diet 16 hours before the ethanol injection in order to assure absence of blood ethanol at the start. Three grams per kg body weight of ethanol (10% w/v in physiological saline solution) was injected intraperitoneally. Then 3 to 5 ml of air was injected on the back of the animal subcutaneously in order to make an air bubble. The ethanol content in the air bubble was equilibrated for about 15 to 20 minutes. Animals were put under a heat lamp to maintain the body temperature of at least 35°C. Then 0.5 or 1.0 ml of bubble air was drawn with an air tight syringe with a 26 ga needle every 30 minutes and injected into pre-sealed vials. Ethanol was analyzed by head space gas chromatography (Perkin Elmer F40 or F42). The values were compared to an ethanol standard curve and converted into blood ethanol levels using the water-air partition coefficient of ethanol and correcting for body temperature [5]. In a preliminary study, ethanol from orbital sinus blood measured by the PCA method and calculated blood ethanol levels (by the air bubble technique) correlated well (Fig. 1).

Animals were sacrificed at the end of the blood ethanol elimination study or on the following day. Liver was dissected and homogenated with isotonic sucrose phosphate buffer pH 7.4. Cytochrome P-450 contents were measured in total homogenates and microsomes according to Omura and Sato [16] The preparative losses of microsomes are corrected according to Greim [4]. Cytosolic ADH activity was determined by the method of Bonnichsen and Brink [1]. Catalase activity was measured according to Lück [12] as modified by Cohen *et al.* [3]. MEOS activity was measured by the method of Ohnishi and Lieber [15]. Microsomal protein was determined by the method of Lowry *et al.* [11], using crystalline bovine albumine as standard. Results are given as mean±S.E.M. Significance of differences was calculated by Student's group or paired *t*-test.

RESULTS

Both the ADH-positive and the ADH-negative strain of deermice consumed appreciable amounts of ethanol fed as a part of a liquid diet. Furthermore, upon chronic consumption of ethanol, the intake of ethanol increased considerably with time. After progressively increasing the concentration of ethanol in the liquid diet up to 4 to 5 percent, the ethanol intake exceeded 20 g/kg body wt./day after 2 to 4 weeks (after 3 to 5 days: ADH-positive 15.5±1.8; ADH-negative 13.1±1.2; after 2 to 4 weeks: ADH-positive 27.7±1.4; ADH-negative 22.7±1.2). Urinary and respiratory losses of ethanol amounted to less than 5% of total ethanol intake. The daily profile of the blood ethanol levels of ethanol-fed animals was approximately 40 mM in ADH-negative and 15 to 20 mM in ADH-positive ones.

Figure 2 shows examples of blood ethanol elimination curves after chronic ethanol feeding. As expected, the rate of ethanol disappearance was fastest in the ADH-positive animals receiving ethanol followed by the ADH-positive controls, the ADH-negative fed ethanol and the ADH-negative controls. It is noteworthy that even in the ADH-negative animals the rate of ethanol disappearance was substantial.

From the elimination curve of each animal, rates of ethanol disappearance were calculated both at high and at low ethanol concentrations (Table 1). At high ethanol concentrations (40 mM to 70 mM), which can be expected to fully saturate all ethanol metabolizing pathways, rates of ethanol disappearance were highest and were most significantly increased after chronic ethanol feeding. In ethanol-fed and pair-fed control ADH-positive deermice, the disappearance rates were 374±34 μmoles/l of blood/min and 283±13, respectively ($p<0.0125$, both n=8). In ethanol-fed and pair-fed control ADH-negative deermice, they were 240±13 and

TABLE 1

EFFECT OF CHRONIC ETHANOL FEEDING ON BLOOD ETHANOL ELIMINATION RATE IN ADH-POSITIVE AND ADH-NEGATIVE DEERMICE GIVEN ETHANOL (3 g/kg BODY WT. IP)

	(μmole ethanol/l of blood/min)			
	naive ADH$^+$	Alcohol-fed ADH$^+$	naive ADH$^-$	Alcohol-fed ADH$^-$
at "High" concentration (40–70 mM)	283 ± 13	374 ± 34*	188 ± 11†	240 ± 13*†
at "Low" concentration (5–10 mM)	181 ± 16	181 ± 18	97 ± 9†	99 ± 11‡

Data show mean ± SEM.
*Significant vs. each naive Control.
†Significant vs. naive ADH$^+$.
‡Significant vs. Alcohol-fed ADH$^+$.
Each value is significant between "High" concentrations and "Low" concentrations in each animal group.

TABLE 2

ADH, CYTOCHROME P-450 AND MEOS ACTIVITIES AFTER CHRONIC ETHANOL ADMINISTRATION IN DEERMICE

	naive ADH$^+$	Alcohol-fed ADH$^+$	naive ADH$^-$	Alcohol-fed ADH$^-$
ADH (μmole NAD reduced/g liver/min)	1.31 ± 0.09	0.87 ± 0.16*	absent	absent
P-450				
(nmole/mg protein)	0.67 ± 0.05	1.23 ± 0.19*	0.56 ± 0.11	1.69 ± 0.16*
(nmole/g liver)	26.67 ± 4.27	53.92 ± 4.36*	28.57 ± 2.81	97.36 ± 6.21*
MEOS				
(nmole acetaldehyde formed/ min/mg protein)	5.93 ± 0.73	19.45 ± 3.28*§	10.12 ± 1.86†	34.56 ± 3.52*
(/g liver)	262 ± 37	843 ± 71*§	525 ± 73†	1947 ± 342*
(/nmole P-450)	8.95 ± 1.23‡	15.80 ± 1.06§	21.57 ± 4.74	20.85 ± 2.85

Data show mean ± SEM.
*Significant vs. naive Control.
†Significant vs. naive ADH$^+$.
‡Significant vs. Alcohol-fed ADH$^+$, naive ADH$^-$ and Alcohol-fed ADH$^-$.
§Significant vs. Alcohol-fed ADH$^-$.

188±11, respectively ($p<0.01$, both n=7). Thus, in the ADH-negative deermice, after ethanol administration, rates of ethanol elimination were almost as high as naive ADH-positive animals despite the total absence of ADH activity (Table 2). At low ethanol concentrations (5–10 mM), there was no significant difference in elimination rate between ethanol-fed and pair-fed control animals of both ADH-positive and ADH-negative strains.

Hepatic ADH activity was determined as shown in Table 2. In the ADH-positive animals, after chronic ethanol consumption, a decrease (1.31±0.09 μmoles NAD reduced/g liver/min to 0.87±0.16, $p<0.05$) was observed comparable to that found before in rats [8] and baboons [18]. In the ADH-negative animals, as expected, there was no ADH activity in naive animals; even after ethanol feeding, no ADH activity appeared.

With regard to liver catalase, its activity was not significantly changed by chronic ethanol consumption and it is noteworthy that in ADH-negative animals the activity of catalase (2908±422 Lück unit/g liver) was lower than in the ADH-positive animals (5760±425).

The administration of 3-Amino-1,2,4-Triazole (Aminotriazole) in order to block the catalase activity in vivo following the ethanol elimination study did not produce any signifi-

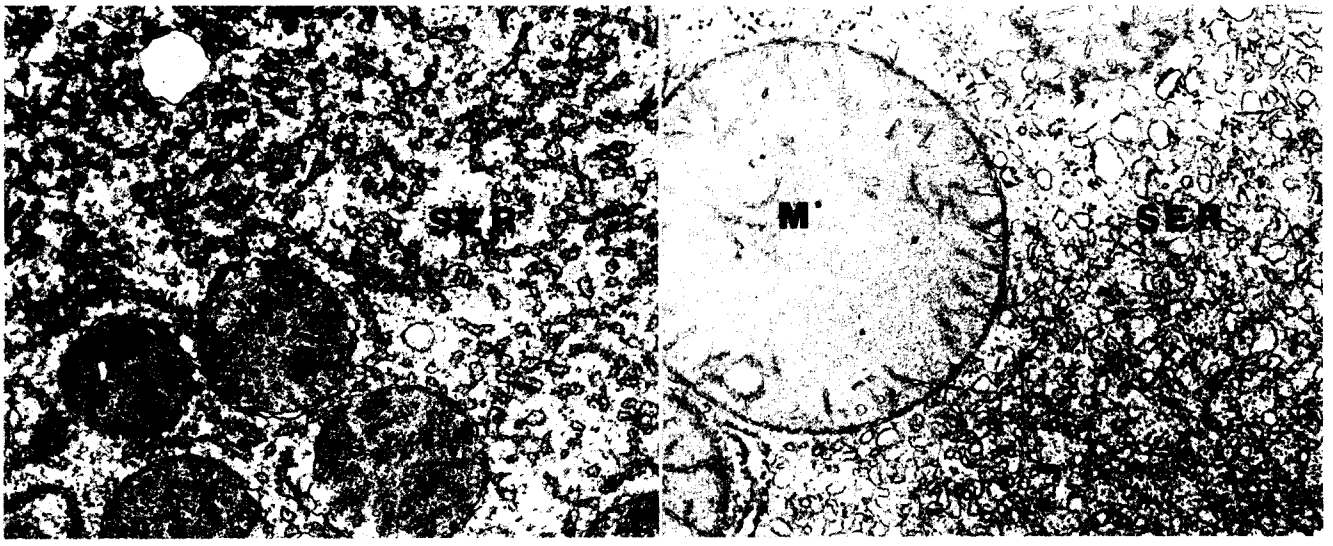

FIG. 3. Electron micrographs. Left: naive ADH-negative deermouse; note a somewhat abundant smooth endoplasmic reticulum (SER). Right: ADH-negative deermouse; after chronic ethanol consumption, the liver became literally packed with the proliferated membranes of the smooth endoplasmic reticulum (SER). A giant mitochondrion (M) is also seen (both uranyl acetate and lead citrate stained, ×24,000).

cant change in ethanol elimination rates in ADH-positive and ADH-negative animals although liver catalase activity was blocked 90 to 95%.

The ADH-negative strain had a significant activity of the microsomal ethanol-oxidizing system (MEOS). Most interestingly, the activity of MEOS in ADH-negative naive animals (10.12 ± 1.86 nmole acetaldehyde formed/min/mg protein) was greater than in the ADH-positive naive deermice (5.93 ± 0.73, $p < 0.01$) (Table 2). The increase in MEOS activity was associated with a striking rise in cytochrome P-450, particularly in ADH-negative animals (almost 3 fold, Table 2). When one assesses the capacity of the cytochrome to sustain ethanol oxidation by expressing MEOS activity per unit of cytochrome P-450, this ratio was highest in the ADH-negative animals (approximately 20 nmole/nmole P-450 in both ethanol-fed and pair-fed control animals) but after chronic ethanol consumption, ADH-positive animals also seemed to acquire increased amounts of this apparently more effective form of cytochrome P-450.

The induction of MEOS activity in the microsomes of deermice by chronic ethanol consumption particularly in the ADH-negative ones has its morphologic counterpart. Figure 3 shows the electron micrograph of the hepatocyte of ADH-negative pair-fed control (left) and ADH-negative ethanol-fed deermouse (right). Even in the naive ADH-negative deermice, hepatic smooth endoplasmic reticulum (SER) is somewhat abundant compared to the ADH-positive animals. The most striking change was noted after chronic ethanol consumption in ADH-negative deermice. Their liver became literally packed with the proliferated membranes of the smooth endoplasmic reticulum (right).

DISCUSSION

There is much debate on the extent MEOS contributes to ethanol metabolism in vivo [6,20]. Contrasting with ADH-positive animals, in which there are three pathways to consider (ADH, MEOS and catalase), the strain of ADH-negative deermice is a unique animal model for the study of non-ADH mediated ethanol oxidation. In ADH-negative deermice, the only known pathways of ethanol metabolism are via MEOS and catalase.

Administration of 3-Amino-1,2,4-Triazole(Aminotriazole) blocked catalase activity but did not significantly affect the ethanol disappearance rate in ADH-positive and ADH-negative animals, suggesting that catalase does not significantly participate in ethanol metabolism in vivo in the deermice. Therefore, MEOS must play an important role, at least in the ADH-negative strain.

It is now recognized that there are multiple forms of cytochrome P-450 in the rat and we have shown that chronic ethanol consumption induces a form which is distinct from those induced by other drugs. It has a higher capacity to sustain ethanol oxidation in reconstituted system [14]. We have now gathered preliminary evidence that in deermice as well, after chronic ethanol feeding, the form of cytochrome P-450 which is induced may be different from the forms which normally prevail in ADH-positive animals. Our data show that the MEOS activity and cytochrome P-450 content correlate. When MEOS activity is expressed per P-450 content in ADH-positive animal, the values increased after chronic ethanol feeding to reach the levels found in ADH-negative animals, which seemed to have the most effective form of cytochrome P-450 in terms of ethanol oxidation.

The blood ethanol levels of ethanol-fed animals were approximately 40 mM, 20 to 25 mM higher in ADH-negative deermice than in ADH-positive animals, a level sufficient to effectively saturate MEOS for the oxidation of ethanol at high rates. In a baboon study, Pikkarainen and Lieber [17] found concentration-dependent ethanol elimination rates, indicating that a non-ADH system (such as MEOS) contributes significantly to ethanol elimination in vivo, especially in alcohol-fed baboons, and at high concentrations comparable to those we found in ADH-negative animals.

In conclusion, ADH-negative deermice consume ethanol at a rate almost $^2/_3$ that of ADH-positive animals despite the total lack of ADH. This is associated with a progressive increase in MEOS activity which is most likely responsible for the ethanol oxidation.

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