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ROLE OF ALCOHOL DEHYDROGENASE IN THE SWIFT INCREASE IN ALCOHOL METABOLISM (SIAM)

STUDIES WITH DEERMICE DEFICIENT IN ALCOHOL DEHYDROGENASE*

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Abstract—Previous studies have shown that rates of ethanol metabolism increase markedly 2–4 hr after the administration of ethanol in rats and in four inbred strains of mice. This phenomenon, called the swift increase in alcohol metabolism (SIAM), also exists in humans. To determine whether alcohol dehydrogenase (ADH) is necessary for the SIAM response, we compared ethanol metabolism in two strains of the deermouse, *Peromyscus maniculatus*. One strain lacks alcohol dehydrogenase (ADH-negative), whereas the other strain has normal ADH levels (ADH-positive). Rates of ethanol elimination were determined after a single intraperitoneal injection of ethanol at different doses (0.5 to 3.0 g/kg) and also after both strains were exposed to various levels of ethanol vapor for 4 hr. The ADH-positive strain exhibited up to a 72% increase in the rate of ethanol elimination after exposure to ethanol vapor compared to the ethanol-injected controls. In contrast, treatment with ethanol vapor did not alter rates of ethanol elimination in the ADH-negative strain. These data demonstrate clearly that ADH is required for SIAM in the deermouse. In addition, in both the ADH-positive and the ADH-negative strain, rates of ethanol elimination increased in both the ethanol-injected and vapor-treated groups 2- to 3-fold as the dose of ethanol was increased from 100 to 500 mg/100 ml. Thus, it is concluded that this "concentration effect" of ethanol on rates of ethanol metabolism does not involve ADH in the deermouse.

It has been shown that acute treatment with ethanol produces a swift increase in alcohol metabolism (SIAM) in rats [1], in four inbred strains of mice [2], in perfused rat livers [3] and in humans [4]. Studies with 4-methylpyrazole, an inhibitor of alcohol dehydrogenase (ADH), suggest that ADH is involved in the mechanism of SIAM [3]. SIAM most likely involves an acceleration of the conversion of NADH to NAD^+, the obligatory cofactor for ADH, as a result of activation of mitochondrial electron transport by ADP following ethanol treatment [3]. However, this conclusion should be viewed cautiously, since studies employing inhibitors are often open to alternative interpretations.

A strain of the deermouse, *Peromyscus maniculatus*, which lacks hepatic ADH activity was identified by Burnett and Felder [5]. The ADH-positive strain contains the Adh^F allele of the structural gene for ADH at the *Adh* locus, while the ADH-negative strain contains the null allele, Adh^N. Even though ADH is absent, ethanol is still eliminated in ADH-negative deermice, but at rates considerably lower than in the ADH-positive strain [5, 6] via mechanisms that require elucidation. This ADH-negative strain provides a unique opportunity to test the hypothesis that SIAM is dependent on the presence of ADH without the use of metabolic inhibitors that may have multiple effects.

METHODS

Deermice. Male ADH-positive and ADH-negative deermice were obtained from a colony maintained at the University of North Carolina established from breeding pairs obtained from Dr. Michael Felder of the University of South Carolina. All deermice were well-fed adults (15–20 g) at times of study. The liver weight : body weight × 100 ratio was 4.0 to 4.5 in both groups of deermice.

Determination of breath ethanol concentrations. Blood ethanol concentrations were calculated from measurements (Fig. 1) of breath ethanol and rates of ethanol elimination were calculated by standard procedures [7]. Individual deermice were forced to breathe in a 2.75-ml closed vessel for 17 sec to ensure maximal equilibration between breath and vapor in the chamber. After equilibration, a 1.0-ml sample of exhaled breath was analyzed for ethanol by injection into a Hewlett–Packard model 5720 gas chromatograph equipped with a carbowax 60/80 column and a flame ionization detector. The operation parameters were: oven, 110°; detector, 315°; injection port, 300°; and carrier gas flow rate, 80 ml/min. A peak corresponding to ethanol with a retention time of about 50 sec was compared with ethanol standards

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[8]. Ethanol concentrations in blood correlated linearly with breath ethanol (data not shown). The concentration of ethanol vapor in the inhalation chamber was also measured routinely by gas chromatography.

Treatment with ethanol. Adult male deermice received various doses of ethanol intraperitoneally. Basal rates of ethanol elimination were determined by taking breath samples every 20–30 min (Fig. 1). Other groups of deermice were placed in Plexiglass chambers (50 × 50 × 40 cm) with free access to food and water and exposed to ethanol vapor for periods ranging from 0 to 8 hr (see figure legends for details). At the end of the vapor treatment periods, rates of ethanol elimination were determined without injecting additional ethanol (Fig. 1).

For some experiments, groups of deermice were given 20% (v/v) ethanol in saline either by injecting different doses (0.5 to 3.0 g/kg) intraperitoneally or by varying the flow rate of ethanol vapor into the inhalation chamber. Blood ethanol ranges of 50 to 500 mg/100 ml were obtained with these procedures. Statistical comparisons were made using Student's *t*-test for non-paired data.

RESULTS

Basal rate of ethanol metabolism. The basal rate is defined as the rate of ethanol elimination determined from multiple breath samples following a single intraperitoneal injection of ethanol [7]. The decline in breath ethanol correlates well with changes in the blood ethanol level with time [2]. As shown in Fig. 1, the decline in blood ethanol (the basal rate) was nearly 2-fold higher in ADH-positive than in ADH-negative deermice. When deermice were given increasing doses of ethanol to increase the blood ethanol concentration in steps up to 500 mg/100 ml, the basal rate increased about 2- to 4-fold in the ADH-negative and 4-fold in the ADH-positive deermouse strains, respectively. Similar increases in basal rates of ethanol elimination have been observed at high ethanol concentrations in mice [2], rats [8], and deermice *in vivo* [6] as well as in perfused rat livers [10] and isolated hepatocytes [11].

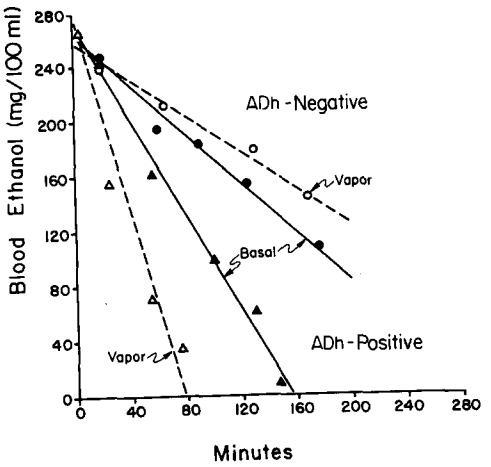

Fig. 1. Basal and vapor-treated ethanol elimination curves for ADH-positive (triangles) and ADH-negative (circles) deermice *in vivo*. Deermice from both strains received 3.0 g/kg of 20% (v/v) ethanol in saline intraperitoneally. Blood ethanol concentrations were determined by taking breath samples every 20–30 min for gas chromatographic analysis of ethanol (Methods). Closed symbols: elimination curves for untreated animals. Open symbols: elimination curves for deermice of both strains placed in a Plexiglas chamber (50 × 50 × 40 cm) with free access to food and water and exposed to ethanol vapor [9] for 4 hr to produce blood ethanol values in the same range as that achieved by injection. Representative experiments.

Ethanol-induced rates of ethanol metabolism (the SIAM phenomenon). Figure 1 shows that the rate of ethanol elimination was increased significantly in ADH-positive deermice following 4 hr of exposure to ethanol vapor. This is a typical SIAM response. This increase in the rate of ethanol elimination due to ethanol vapor (SIAM) in the ADH-positive strain was dose-dependent in a complex manner (Fig. 2,

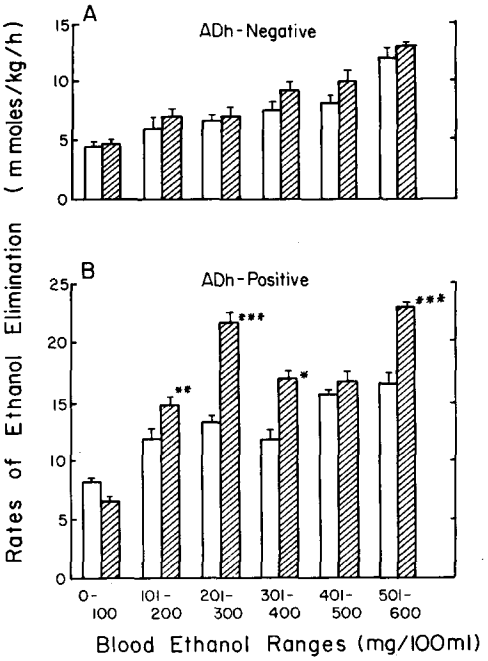

Fig. 2. Effect of blood ethanol levels on rates of ethanol elimination and SIAM in ADH-positive and ADH-negative deermice. ADH-negative (panel A) and ADH-positive (panel B) deermice were injected with various doses of ethanol intraperitoneally to achieve six blood ethanol ranges as denoted. Deermice were also exposed to ethanol vapor for 4 hr as described in Fig. 1 to obtain blood ethanol ranges from 50 to 550 mg/100 ml. Basal (open bars) and vapor-treated (hatched bars) rates of ethanol elimination were calculated from the linear decline of ethanol concentration per unit time (Fig. 1) according to the method of Widmark [7]. Rates are presented as mean ± S.E.M. for the midpoint of each blood ethanol range, four to thirteen animals per group. In the ADH-negative deermice, statistically significant increases in basal rates of ethanol elimination were observed for all comparisons between the 0–100% blood ethanol range and all other blood ethanol ranges, with the exception of the 101–200 mg/100 ml blood ethanol ranges ($P < 0.001$ or greater); all ranges were statistically significantly greater than the 0–100 range in the treated groups. In the ADH-positive deermice, (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$ for comparisons between untreated and treated rates of ethanol elimination at any given range of blood ethanol.

panel B). The increase was 27% in the 101–200 mg/100 ml ethanol range; 64% in the 201–300 mg/100 ml dose range; 45% with 301–400 mg/100 ml; 7% with 401–500 mg/100 ml; and 40% with 501–600 mg/100 ml (mean of values). Rates were not altered, however, in ADH-negative deermice receiving the same treatment. Thus, SIAM is not present in the ADH-negative deermouse.

DISCUSSION

Role of ADH in SIAM. The SIAM phenomenon exists in ADH-positive deermice, but it was not observed at any level of blood ethanol in the strain which lacks ADH (Fig. 2, panel A). It is therefore concluded that the mechanism of SIAM has an absolute dependence on the presence of ADH. A less likely hypothesis is that these two strains also differ in other variables such as changes in the volume of distribution of ethanol. Since the slope of the ethanol elimination curve (β of Widmark [7]) is steeper after treatment with ethanol vapor, this alternative seems unlikely.

Our theory of the metabolic sequences most likely responsible for SIAM is as follows. First, hormone-stimulated glycogenolysis leads to a depletion of glycogen, which in turn causes rates of glycolysis to decline. Since glycolysis converts ADP to ATP, the ADP that is not phosphorylated by glycolysis enters the mitochondria and is phosphorylated by the electron transport chain. This causes NADH to be reoxidized to NAD^+ at a faster rate. The increased turnover of NAD^+ leads to an increased rate of oxidation of ethanol via ADH resulting in a faster rate of ethanol elimination, i.e. the SIAM response [3]. Since SIAM could not be demonstrated in the ADH-negative strain, these studies show that ADH is involved in this proposed sequence of events (Fig. 2).

This theory does not explain why SIAM declined at higher doses of ethanol in the deermouse (Fig. 2, panel B). This phenomenon was also observed to varying degrees in four inbred strains of mice [2], and any theory concerning the mechanism of SIAM must also take these strain differences in dose dependence into account. One possibility is that more than one biological trigger for SIAM exists, one operating at low doses of ethanol and others operating at high doses.

Potentially, the SIAM phenomenon could be important in identifying factors important in alcohol-induced liver damage, since SIAM is linked to oxygen utilization. One theory to explain ethanol-induced liver damage is that ethanol metabolism causes hypoxia by metabolically depleting O_2 [12, 13]. Since enhanced ethanol elimination (e.g. SIAM) requires O_2 for the reoxidation of NADH, it may well be that the ADH-positive deermouse is more susceptible to hypoxia after treatment with ethanol.

Ethanol elimination in the ADH-negative deermouse. In spite of a complete absence of ADH [5], the ADH-negative deermouse does eliminate ethanol. Two questions need to be addressed in this strain: (a) the cause of ethanol elimination at low doses of ethanol, and (b) the cause of the increased rate of ethanol elimination at high doses of ethanol; i.e. the so-called "ethanol concentration effect." Numerous studies *in vivo* [6, 8] and *in vitro* [10, 11] have demonstrated clearly that ethanol elimination is faster at high blood levels of ethanol. Since the basal rate of ethanol elimination increased two to three times at the high blood ethanol concentrations in the ADH-negative deermouse (Fig. 2), we conclude that this dose-dependent response to ethanol is not dependent upon ADH.

The possibility that some of the ethanol elimination in the ADH-negative deermouse is not due to ethanol metabolism is quite realistic, especially at high doses of ethanol. The deermouse is a small animal with a high rate of respiration and metabolism, and excretion and expiration may play an important role in ethanol elimination especially when ADH is absent. To test the extent of this possibility, we are developing a perfusion technique to study livers from ADH-negative deermice *in vitro*.

Two other pathways also exist which could metabolize ethanol in the ADH-negative deermouse: catalase and cytochrome $P\text{-}450_{3a}$. Wendell and Thurman [8] showed that aminotriazole, an inhibitor of catalase, prevents the ethanol concentration effect in the rat *in vivo*. In addition, in perfused rat livers, much of the ethanol elimination at high doses of ethanol remaining after the administration of 4-methylyrazole, an ADH inhibitor, is blocked by aminotriazole [10]. These data suggest that the ethanol concentration effect involves catalase, which operates faster at high ethanol concentrations. In contrast, Shigeta *et al.* [6] failed to observe an effect of aminotriazole on ethanol elimination in the ADH-negative deermouse. However, in their experiments catalase was inhibited only 90–95%. Since catalase activity in liver is very large [14] and peroxidation of ethanol by catalase-H_2O_2 is limited by much slower rates of H_2O_2 production [15], 5–10% of normal catalase levels would probably be enough to metabolize all the H_2O_2 generated and to metabolize ethanol at near normal rates in their aminotriazole-treated deermice.

A cytochrome P-450 induced by ethanol-feeding, form $P\text{-}450_{3a}$, has been purified from rabbit liver [16, 17]. Since long-term ethanol feeding increased ethanol metabolism in the ADH-negative deermouse, Shigeta *et al.* [6] concluded that such a cytochrome is responsible for the concentration-dependent ethanol elimination in the deermouse. They reported that the metabolism of ethanol by microsomes is also increased markedly by ethanol feeding in the ADH-negative strain. However, catalase is a common contamination of microsomes, and microsomes produce H_2O_2 [15]. Thus, clear evidence quantitating either the catalase-H_2O_2- or the cytochrome $P450_{3a}$-dependent portion of ethanol metabolism in the ADH-dependent deermouse awaits further work.

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