Pharmacology Biochemistry & Behavior, Vol. 16, pp. 757–760, 1982. Printed in the U.S.A.

Ethanol Elimination Rates in Mice: Effects of Gender, Nutrition, and Chronic Ethanol Treatment[1]

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Received 27 June 1981

ALLEN, D. L., R. G. LITTLE II, J. E. THEOTOKATOS AND D. R. PETERSEN. *Ethanol elimination rates in mice: Effects of gender, nutrition, and chronic ethanol treatment.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 757–760, 1982.—Twenty male and 20 female mice of a heterogeneous stock were assigned to each of three groups. One group was administered ethanol in a liquid diet for 9 days, a second group was fed an isocalorically controlled diet containing no ethanol for the same length of time, and the third group was fed standard lab chow. Each animal was injected with a dose of ethanol equal to 3.5 g/kg body weight at the time corresponding to 6 hr post-withdrawal for the ethanol-treated group. Blood ethanol elimination rates were determined at 1, 2, and 3 hr post-injection. Neither gender, nutritional state, nor chronic ethanol treatment was found to affect ethanol elimination rates.

Alcohol Ethanol Mice Ethanol dependence Gender differences Ethanol elimination rates

SEVERAL factors influence ethanol elimination rates. Nutritional deficiencies lower the ethanol elimination rates in humans [1,18], dogs [9,26] and rats [16, 26, 28]. Studies using inbred strains of mice have demonstrated that genotype, age, and their interaction significantly affect ethanol elimination rates [2,29]. The effects of other factors are not so clear. For example, chronic ethanol treatment has been found to increase ethanol elimination rates in humans [16], rats [7,15], and mice [19,20]; decrease ethanol elimination rates in rats [27]; and not affect ethanol elimination rates in humans [18], rats [10], and mice [8]. The female mouse has been found to eliminate ethanol faster than the male in two studies [2,6], but not in a third [8]. The primary objective of the present investigation was to assess the influence of gender, nutrition, and chronic ethanol treatment on ethanol elimination rates in mice.

METHOD

Sixty male and 60 female mice, aged 60±5 days, from a heterogeneous stock (HS) maintained at the Institute for Behavioral Genetics were randomly assigned to three diet groups. Chronically treated mice (chronic) were fed a liquid diet containing ethanol; mice in a second group were pair-fed an isocalorically controlled (isocal) liquid diet containing no ethanol; and animals to be acutely treated with ethanol (acute) were fed standard lab chow (Wayne Sterilizable Lab Blox).

The acute animals were housed with their same-sex siblings in large plastic cages with free access to tap water and lab chow until the testing day, when they were placed individually in metal cages with aspen shavings as bedding and tap water provided. Chronic and isocal animals were individually housed in metal cages throughout the treatment period; Lieber-DeCarli liquid diet (Iso-Cal, Bio-Serv, Inc.) was accessible through a glass sipper tube extending from an inverted 25-ml cylinder into each cage, and tap water was provided ad lib. Isocal animals were fed the #711-PRC diet. The diet to be administered to the chronic animals was prepared as follows: First, 95% ethanol was added to the #711-PRA diet to produce a concentration of 35% ethanol-derived calories (e.d.c.). Then, this 35% e.d.c. diet was diluted with appropriate amounts of the #711-PRC diet to produce diets that contained either 20% e.d.c. or 10% e.d.c. This dilution method insured that the diets remained isocaloric over the three ethanol concentration conditions.

Presentation of the liquid diet to the chronic and isocal mice was initiated at 0700 hr on day 1 of the 9-day treatment period, and fresh diet was provided every 12 hr throughout treatment. The chronically treated animals received increasing concentrations of ethanol as follows: On days 1 and 2, they were fed the 10% e.d.c. diet; on days 3 and 4, the 20% e.d.c. diet was presented; on days 5 through 9, they received the 35% e.d.c. diet. Other studies (e.g., [8]) have found that this progressive schedule successfully produces ethanol withdrawal symptoms in mice.

[1]This research was supported in part by NIAAA grant AA-03527.

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TABLE 1

MEAN ETHANOL ELIMINATION RATES BY TREATMENT AND GENDER

	Females	Males
Chronic	97.3 ± 7.0 (N=19)	83.2 ± 8.2 (N=19)
Isocal	95.0 ± 7.6 (N=19)	91.6 ± 10.7 (N=18)
Acute	98.8 ± 9.6 (N=19)	87.3 ± 10.1 (N=17)

Values are mean mg/dl/hr ± SEM. None of the treatment or gender differences is significant.

All mice were matched by gender and weight prior to the experiment, and the chronic and isocal mice were pair-fed to assure parallel weight loss in the two groups. (In a previous study in which we used a 9-day treatment period [8], body weight was found to be highly correlated with caloric intake; thus, parallel weight loss may be taken as an indication that caloric intakes of the chronic and isocal groups were essentially equal.) On day 10, the liquid diet of the chronic and isocal mice was removed and replaced with lab chow.

Each mouse received an intraperitoneal injection of 30% ethanol in saline in a dose equal to 3.5 g/kg body weight at a time corresponding to 6 hr post-withdrawal for the chronic group. The blood samples were taken from the retro-orbital sinus using heparinized 25-μl pipettes at 1, 2, and 3 hr post-injection, which corresponds to the pseudo-linear phase of ethanol elimination. The pipettes were inserted into the sinus until full, then placed in collection tubes with 0.975 ml of distilled, deionized water to yield 1 ml total solution. The samples were kept frozen until blood ethanol levels could be determined. After thawing, the solutions were incubated at 60°C for 15 min. One milliliter of the headspace gas in the tubes was subjected to gas chromatography [21]. By comparison with known blood ethanol standards, the experimental ethanol concentrations were plotted against hours after injection. The slope of this line was the calculated elimination rate (mg/dl/hr).

RESULTS

The mean ethanol elimination rates as a function of treatment and gender are presented in Table 1. A two-way analysis of variance revealed neither a gender difference, $F(1,105)=1.76$, $p>0.15$, nor any difference between treatments, $F(2,105)=0.07$, $p>0.90$. There was no significant interaction between gender and treatment $F(2,105)=0.197$, $p>0.80$.

Results of other studies have indicated that higher doses of ethanol result in more rapid ethanol elimination rates [19, 20, 22]. One of these studies [22] also found that length of treatment was not important when considered independently of dose. In our study, there were no significant correlations of ethanol elimination rates with total consumption (g ethanol/kg body weight) over the 9-day treatment period, $r(35)=0.11$, $p>0.25$; mean=164.28; range=119.85 to 200.97, or with consumption of ethanol on the last day of treatment, $r(35)=-0.20$, $p>0.10$; mean=17.21; range=4.24 to 26.06. These findings indicate that dose did not have a critical influence upon ethanol elimination rates in the present study.

Table 2 summarizes the data for original body weight, body weight at time of withdrawal, and percent of original body weight retained at withdrawal. Both males and females from the chronic and the isocal treatment groups weighed significantly less at withdrawal than they did at the beginning of the experiment, males: $t(35)=10.79$, $p<0.001$; females: $t(36)=6.62$, $p<0.001$. There was no difference between any of the treatment groups for original body weight, males: $F(2,53)=0.199$, $p>0.80$; females: $F(2,56)=0.294$, $p>0.70$, but the weight of the acute group did differ from the other two groups at time of withdrawal, males: $F(2,53)12.39$, $p<0.01$; females $F(2,56)=9.73$, $p<0.01$. A two-way analysis of variance for percent of body weight retained at withdrawal revealed no significant differences between genders, $F(1,71)=3.99$, $p<0.05$, or treatments, $F(2,71)=2.89$, $p<0.05$.

DISCUSSION

Our finding of no change in blood ethanol elimination rates after chronic ethanol treatment contradicts the reported increases in blood ethanol elimination rates after chronic treatment in mice [19,20]. This difference between our results and those of other studies may be due to differences in time of treatment, not found to be important for monkeys, rats, or humans (cf. [22]); to dosage; or to other

TABLE 2

SUMMARY OF BODY WEIGHT DATA

	Original body weight		Body weight at time of withdrawal		% of original body weight at time of withdrawal	
	Females	Males	Females	Males	Females	Males
Chronic*	21.1 ± 0.5	26.5 ± 0.6	19.1 ± 0.4	22.8 ± 0.5	91 ± 2	87 ± 2
Isocal*	21.0 ± 0.5	26.1 ± 0.6	19.4 ± 0.3	23.7 ± 0.4	93 ± 2	91 ± 2
Acute†	—	—	20.5 ± 0.6	25.9 ± 0.8	—	—

Body weight data are expressed as mean g ± SEM.

*Differences between original weight and weight at withdrawal are signficant for both males and females ($p<0.001$ for each gender) in both chronic and isocal groups.

†At time of withdrawal, males and females in the acute group weighed significantly more than those in both other groups ($p<0.001$ for each gender).

unknown causes. In one of the previous studies that used liquid diet of the same type we used [20], mice (all males) consumed an average of approximately 16–17 g/kg/day of 20% e.d.c. diet after 8 days of chronic treatment. There was no difference in the rate of ethanol elimination between ethanol-treated animals and their isocaloric controls at that time. In our study, the average consumption after 9 days of treatment was also about 17 g/kg/day, and we also found no difference in ethanol elimination rates between chronic and isocal animals. After 16 days, the treated mice in the previous study consumed about 20–23 g/kg/day of a 30% e.d.c. diet, and there was a 1.4- to 1.5-fold difference between their ethanol elimination rates and those of isocaloric controls.

The blood ethanol elimination rates after 9 days of treatment in our study are equal to the increased rates observed by Petersen and Atkinson [20] after 30 days of treatment. Their study used mice from the long-sleep (LS) and short-sleep (SS) lines selectively bred for ethanol sensitivity [12], whereas we used the heterogeneous stock which was the foundation population for these selected lines [13]. It is possible that selection has caused either (1) a decrease in ethanol elimination rates that can be overcome through an induction of the systems involved in ethanol metabolism, or (2) an increase in the inducibility of the systems involved in ethanol metabolism. Both liver ADH activity and the microsomal ethanol oxidizing system have been shown to be under genetic control and inducible by chronic ethanol treatment [3, 4, 19, 20, 23, 25]. Two of these studies [19,20] have shown the inducibility of the microsomal ethanol oxidizing system to be under genetic control. It could also be that a significant drop in mating individuals in generation 6 and a drop in the number of offspring in generation 7 of the selective breeding program that produced the LS and SS mice caused a chance fixation of the genes that affect ethanol elimination rates and/or the inducibility of the ethanol metabolizing enzyme systems. More work will be needed to investigate these possibilities.

The lack of gender differences found in this study is in agreement with previous results obtained using HS mice [8], but disagrees with the results of a study that used Swiss-Webster mice and three derived generations selected for handling-induced convulsions during ethanol withdrawal [6]. Gender differences in ethanol elimination rates in inbred mice after an acute injection have been shown to be influenced by genotype and age [2]. This could be due, however, to a random fixation or linkage of the genes affecting ethanol elimination rates during the course of inbreeding. Our data on HS mice seem to support such a hypothesis, since our acutely injected animals showed no gender difference. The inbred strains that have been found to show gender difference were used in the development of the HS mice [13]. Further research using heterogeneous mice as well as inbred strains and their derived F_1, F_2, and backcross generations may provide answers to questions raised by the discrepant results of studies of gender effects on ethanol elimination rate.

Previous studies have consistently found a correlation between deficiencies in nutrition and lower ethanol elimination rates [1, 9, 17, 18, 26, 28]. In one study using rats, a weight loss of only 6.7% over 24 hr caused a 30% reduction in ethanol elimination rates in acutely injected subjects and a 10% reduction in ethanol elimination rates in chronically treated, "dependent" animals [23]. On the other hand, we found that a weight loss of 7–13% of body weight, alone or in conjunction with a chronic ethanol treatment, did not significantly affect ethanol elimination rate. This discrepancy may be due to a species difference, a difference in rats of development of nutritional deficiency, or other unknown causes.

An investigation of the effects of dose vs length of ethanol treatment in monkeys revealed that only dose had a significant effect upon ethanol elimination rate [22]. In the same article, the authors reviewed the pertinent literature and found it to corroborate their results. Studies of humans and rats that showed an increase in ethanol elimination rates after chronic ethanol treatment used higher doses of ethanol (in g/kg/day) than those that showed no change, and the length of treatment appeared to be irrelevant. The present study did not find this dose effect in mice, i.e., no significant correlation was found between ethanol elimination rates and (1) the total amount of ethanol consumed over 9 days (in g/kg), or (2) the amount of ethanol consumed on the last day of treatment. It may be that the animals in our study never reached some critical threshold level of ethanol consumption necessary to produce a dose-related effect.

Taken together, these data suggest a possible buffering mechanism against changes in ethanol elimination rates in this heterogeneous stock of mice which may be lost or modified during selection and/or inbreeding. As homozygosity increases and genetic heterogeneity is lost, a population is more susceptible to the effects of environmentally induced stress (see [5], Chapters 14–16). The HS mice used in the present study are purposely maintained in heterozygous condition. Thus, from a genetic perspective, their susceptibility to the stress presumably induced by alcohol treatment and nutritional deficiencies should be less than that of either the inbred strains that were crossed to produce the HS stock or the LS and SS selected lines that were derived from the HS population.

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