

Effects of Tetraethylthiuramdisulfide (Antabuse) on the Metabolism and Consumption of Ethanol in Mice

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Acetaldehyde accumulation following ethanol administration and the effects of Antabuse on ethanol metabolism were studied in C57BL/Crgl and DBA/2Crgl mice. In alcohol preference tests, C57BL/Crgl mice consistently prefer alcohol to water, whereas DBA/2Crgl mice avoid alcohol. C57BL/Crgl mice were found to accumulate significantly less acetaldehyde than DBA/2Crgl mice following ethanol administration. Antabuse was found to increase acetaldehyde accumulation in both strains, but the effect was significantly greater in C57BL/Crgl than in DBA/2Crgl mice. Antabuse inhibited ethanol- $1-^{14}\text{C}$ metabolism in mice of both strains, but again this effect was significantly greater in C57BL/Crgl than in DBA/2Crgl mice. The effects of Antabuse on voluntary ethanol consumption in C57BL/Crgl, RIII/Crgl, and C3H/Crgl mice was studied: Antabuse treatment decreased alcohol consumption in animals of all three strains.

EVIDENCE for the involvement of genetic factors in the determination of alcohol preference behavior has been gathered for the rat and for the mouse. This evidence comes from selective breeding experiments¹⁻³ as well as from strain comparison studies.⁴⁻⁶ Several

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attempts have been made to explain these behavioral differences observed in different strains of mice. Rodgers and McClearn,⁷ for example, have reported a correlation between the activity of the enzyme alcohol dehydrogenase in liver and alcohol preference behavior in six inbred strains of mice. Since alcohol dehydrogenase is presumed to be the rate-limiting enzyme in the metabolism of alcohol⁸ the findings of Rodgers and McClearn⁷ suggested that alcohol preference behavior might be related to an organism's ability to metabolize alcohol rapidly. Schlesinger *et al.*⁹ attempted to test this hypothesis and found that two sublimes of C57BL mice metabolized alcohol significantly faster than two sub-

lines of DBA/2J mice; C57BL mice prefer alcohol to water in a two-bottle free-choice situation, whereas DBA/2 mice avoid alcohol in this situation. This result was, perhaps, not too surprising since McClearn *et al.*¹⁰ had previously shown that alcohol dehydrogenase activity in liver was significantly greater in C57BL/Crgl than in DBA/2Crgl mice. Nevertheless, the differences in the rate of alcohol metabolism between C57BL and DBA/2 mice, although statistically significant, are quite small (approximately 10%). The differences in alcohol preference behavior, on the other hand, are of great magnitude. It is, therefore, very likely that the ability of animals to metabolize alcohol rapidly is only one of many factors contributing to the manifestation of alcohol preference behavior.

The present experiments are further attempts at explaining strain differences in alcohol preference in mice in terms of differences in alcohol metabolism. The metabolism of alcohol involves a series of metabolic steps. Alcohol is first metabolized to acetaldehyde, a reaction which is catalyzed by the enzyme alcohol dehydrogenase. Acetaldehyde is then oxidized to acetic acid, a reaction which is most likely catalyzed by the enzyme aldehyde dehydrogenase. Acetaldehyde, the first metabolic product of alcohol metabolism, is extremely toxic and its accumulation during alcohol metabolism leads to unpleasant circulatory and respiratory symptoms, as detailed by Jacobsen.¹¹ It was, therefore, thought possible that the consumption of alcohol by mice of low preference strains leads to acetaldehyde accumulation and that this, in turn, discourages further drinking. Consequently, it was hypothesized that DBA/2Crgl mice would accumulate significantly more blood acetaldehyde than C57BL/Crgl mice following alcohol administration. It was also hypothesized that tetraethylthiuramdisulfide (Antabuse), a drug which inhibits aldehyde

dehydrogenase, would lead to a decrease in voluntary alcohol consumption in animals of normally high preference strains.

To test these hypotheses three experiments were conducted. The purpose of the first experiment was to establish that Antabuse inhibits in-vivo rates of ethanol metabolism in mice, an effect previously established in several other species. The purpose of the second experiment was to determine the amount of blood acetaldehyde accumulation in high- versus low-preference strains following the administration of a standard amount of alcohol. The effects of Antabuse on blood acetaldehyde accumulation following alcohol administration in high- versus low-preference strains was also studied. The purpose of the third experiment was to determine the effects of Antabuse on voluntary alcohol ingestion.

Methods

Subjects

For the first experiment 30 male mice from each of strains C57BL/Crgl and DBA/2Crgl were used. All animals were between 74 and 87 days of age. For the second experiment 40 male mice from each strain were used. All animals were between 60 and 80 days of age. For the third experiment 59 male mice were used: these came from strains C57BL/Crgl (20 mice), RIII/Crgl (20 mice), and C3H/Crgl (19 mice). All animals were 90 days of age at the beginning of this experiment. The origin and degree of inbreeding of these strains has been described.¹²

All animals were maintained in population cages of 3-5 animals per cage. Food and water were available *ad lib*.

Procedures

Determinations of rates of ethanol metabolism: Ethanol-1-¹⁴C (New England Nuclear Corporation) was diluted appropriately with water and inactive ethanol to give solutions containing 10 or 20 vol. per 100 ml. of alcohol and a final specific activity of approximately 5 μ c./ml. Animals were

randomly assigned to one of two conditions: *experimental* animals were injected intraperitoneally with 0.01 ml. per gram of body weight of Antabuse suspension (0.1 mg. of Antabuse per gm. mouse); *control* animals were injected intraperitoneally with equivalent volumes of distilled water. Three hours later the mice were again removed from their cages, weighed to the nearest 0.1 gm., and injected intraperitoneally with either 1.4 or 2.8 μ l. of radioactive ethanol per gram. The mice were immediately placed into a small metabolism chamber through which passed a continuous air-stream. $^{14}\text{CO}_2$ in the effluent air-stream was determined in a ^{14}C respiration analyzer which continuously monitored and recorded the rate of $^{14}\text{CO}_2$ excretion from an animal which had been given the radioactive ethanol. This method has been described in great detail by Tolbert *et al.*¹³ Measurements were made for at least 2½ hr., by which time the $^{14}\text{CO}_2$ excretion rates were less than 5% of the maximum rate. Sequencing of experiments by genotype, dosage, and condition was random.

The results of these experiments were analyzed as follows: The average rate of alcohol metabolism for the highest 30-min. period and the average time required to metabolize 40% of the injected ethanol- ^{14}C were determined. Both methods of analyzing the data gave essentially equivalent results and only those data for the maximum rate of ethanol metabolism are reported below.

Determinations of Blood Acetaldehyde

All mice used in these experiments were randomly assigned to two groups: *experimental* animals were injected intraperitoneally with 0.01 ml./gm. of Antabuse suspension (0.1 mg. of Antabuse/gm.) and *control* mice were injected intraperitoneally with equivalent volumes of distilled water. Three hours later all animals were injected intraperitoneally with 1.4 μ l./gm. of alcohol. Then, either 1 or 2 hr. after the alcohol injections, all animals were deeply anesthetized with ether and the thoracic cavity was opened. A drop of heparin was placed into the thoracic cavity and the interior vena cava was exposed by pushing the beating heart to one side. The vein was cut and the blood was allowed to flow into the

thoracic cavity. The blood was withdrawn by suction using disposable pipettes; typically, from 0.7 to 1.0 gm. of blood was obtained.

Blood acetaldehyde levels were determined using the spectrophotometric technique described by Burbridge *et al.*¹⁴ Two ml. of 10% sodium tungstate and 2 ml. of 2/3N sulfuric acid were pipetted into the outer chamber of a Conway Diffusion Cell (5.1 cm. outside diameter). Two ml. of 0.006M semicarbazide buffer (pH 7.2) were pipetted into the inner chamber. Known quantities of acetaldehyde (for standard curve determination) or known amounts of freshly drawn blood were then added to the outer concentric chamber. The outer wall of the Conway cell was quickly greased with Lubri-Seal and the cell was sealed. The contents were mixed by gentle rotation and placed into an oven maintained at 28–29° C. for 90 min. The contents of the inner chamber (acetaldehyde-semicarbazone) were removed and diluted to a final volume of 5 ml. with distilled water. The optical densities of these solutions were read on a Beckman DU spectrophotometer at 224 m μ . All blood acetaldehyde determinations were made by reference to standard curves.

Determination of Alcohol Preference

A two-bottle free-choice preference test, previously described by McClearn and Rodgers,⁴ was used. All animals were placed into individual living cages and supplied with 2 inverted 25-ml. graduate cylinders fitted with silicon stoppers and standard metal drinking tubes. One of these cylinders contained a 10% solution of alcohol, and the other, tap water. Daily readings were made of the amount of fluid consumed from both cylinders. The positions of the cylinders were changed every 3 days to counteract possible position preferences.

After 14 days of alcohol preference testing the average amount of alcohol solution consumed per day per animal was calculated. Mice of each of the three strains were then divided into two matched samples on the basis of their scores on this measure. One of these groups was designated *experimental* and the other group was further subdivided, by matching scores on this measure, into 2 groups designated

placebo and *control*, respectively. The experimental groups were injected intraperitoneally with Antabuse on Days 15, 16, and 17 of the experiment; the placebo groups were injected with the same solution minus the Antabuse; the control groups were injected with distilled water. Experimental animals received 0.1 mg. of Antabuse/gm. Volumes of all injections were 0.01 ml./gm. Alcohol preference behavior was determined as before. No injections were given after the seventeenth day, but the consumption of liquid from the two graduate cylinders was recorded for an additional 4 days.

Results

Table 1 summarizes the results of the experiments comparing the effects of Antabuse on the metabolic rates of ethanol-1-¹⁴C metabolism in C57BL/Crgl and DBA/2Crgl mice. Antabuse inhibited the rates of alcohol metabolism at both doses of alcohol and in both strains. At the lower dose of injected alcohol, Antabuse inhibited ethanol metabolism by 65% in C57BL/Crgl and by 34% in DBA/2Crgl mice. At the higher dose of injected alcohol Antabuse inhibited ethanol metabolism by 38% in C57BL/Crgl and by 13% in DBA/2Crgl mice. These results suggest that Antabuse has differential effects in mice of these two strains.

Table 2 summarizes the experiments

comparing blood acetaldehyde accumulation in C57BL/Crgl and DBA/2Crgl mice either with or without previous Antabuse treatment. One hour after alcohol injections DBA/2Crgl mice had 27% more acetaldehyde per gram blood than did C57BL/Crgl mice. After 2 hr. this difference had decreased to approximately 5%. Antabuse increased blood acetaldehyde levels in both strains; this treatment increased blood acetaldehyde levels by 49% in C57BL/Crgl and by 14% in DBA/2Crgl mice when determinations were made 1 hr. after alcohol administration. Two hours after alcohol administration, Antabuse had increased blood acetaldehyde levels by 43% in C57BL/Crgl and by 17% in DBA/2Crgl mice.

The effects of Antabuse on voluntary alcohol consumption in C57BL/Crgl, C3H/Crgl, and RIII/Crgl mice are summarized in Table 3. The data are reported as follows: The column marked 1-14 days is the average amount of 10% alcohol solution consumed during the first 14 days of the experiment for the matched groups. The column marked 15-17 represents the average amount of alcohol consumed during the 3 days Antabuse was administered. The column marked 18-21 represents the average amount of alcohol consumed for the 4 days following Antabuse administration.

TABLE I. EFFECTS OF ANTABUSE ON THE RATES OF ETHANOL METABOLISM

Dose of injected ethanol (μ l./gm. mouse)	Strain	Treatment	No. mice	Max. rate of ETOH oxidation*	Inhibition (%)
1.4	C57BL/Crgl	Control	5	0.1708 \pm 0.021	
1.4	C57BL/Crgl	Antabuse	5	0.0594 \pm 0.019	65
1.4	DBA/2Crgl	Control	5	0.1518 \pm 0.034	
1.4	DBA/2Crgl	Antabuse	5	0.1012 \pm 0.011	34
2.8	C57BL/Crgl	Control	5	0.1498 \pm 0.018	
2.8	C57BL/Crgl	Antabuse	5	0.0928 \pm 0.038	38
2.8	DBA/2Crgl	Control	5	0.1605 \pm 0.018	
2.8	DBA/2Crgl	Antabuse	5	0.1402 \pm 0.020	13

Results are given as the average rate for the group \pm one standard deviation.

*Maximum rate of ethanol oxidation (μ M/min./gm. mouse) for a 30-min. period.

TABLE 2. EFFECT OF ANTABUSE ON BLOOD ACETALDEHYDE LEVELS

Strain	Condition	Time of determination (hr.)*	No. mice	Blood acetaldehyde ($\mu\text{gm./gm. blood}$)	Increase (%)
C57BL/Crgl	Control	1	10	1.13 \pm 0.089	49
C57BL/Crgl	Antabuse	1	10	1.84 \pm 0.162	
C57BL/Crgl	Control	2	10	0.97 \pm 0.025	43
C57BL/Crgl	Antabuse	2	10	1.69 \pm 0.122	
DBA/2Crgl	Control	1	10	1.54 \pm 0.033	14
DBA/2Crgl	Antabuse	1	10	1.80 \pm 0.216	
DBA/2Crgl	Control	2	10	1.02 \pm 0.040	17
DBA/2Crgl	Antabuse	2	10	1.29 \pm 0.119	

*Animals were injected with ethanol at Time 0 and blood for the determination was drawn either 1 or 2 hr. thereafter.

The data are averaged within strains over animals and days.

For statistical analyses the average amount of 10% alcohol consumed by each animal was determined for the 3 treatment days, and this score was subtracted from the average amount of 10% alcohol consumed by the animal on the previous 14 days. None of these difference scores between placebo and control animals were statistically significant; for this reason the control and placebo groups were combined into a single control group and the effects of Antabuse were determined relative to this combined control group.

For each of the 3 strains the average amount of 10% alcohol solution consumed while Antabuse injections were

given was less than the average amount taken on the 14 previous days. These differences were tested statistically by analysis of variance (Table 4). Antabuse was found to significantly decrease voluntary alcohol consumption. The absence of a significant strain effect or strain by treatment interaction shows that the strains do not respond differently to Antabuse in terms of voluntary alcohol intake.

Water consumption was also recorded: Control and experimental animals were compared on the basis of water intake during the 3 treatment versus the previous 14 days. Statistical analyses of these difference scores showed that water intake was not significantly affected by treatment with Antabuse.

TABLE 3. EFFECTS OF ANTABUSE ON VOLUNTARY ALCOHOL CONSUMPTION

Strain	Treatment	No. mice	Av. amt. of alcohol consumed (ml. of 10% alcohol/day)		
			Days 1-14	Days 15-17	Days 18-21
C57BL/Crgl	Control	10	3.51	3.44	3.99
C57BL/Crgl	Exper.	10	3.51	3.02	3.05
C3H/Crgl	Control	10	1.71	2.08	2.54
C3H/Crgl	Exper.	10	1.66	1.36	2.41
R111/Crgl	Control	9	1.37	1.06	1.41
R111/Crgl	Exper.	10	1.38	0.79	1.27

TABLE 4. ANALYSIS OF VARIANCE OF MILLILITERS OF 10% ETHANOL CONSUMED BY THREE STRAINS OF MICE UNDER TWO TREATMENT CONDITIONS

Source of variation	df	Mean squares	F
Strain	2	1.1047	1.49
Treatment	1	4.6817	6.33*
Strain \times Treatment	1	0.0400	<1
Error	53	0.7398	

* $P < .05$.

Discussion

Antabuse retarded the rates of in-vivo ethanol metabolism in both C57BL/Crgl and DBA/2Crgl mice. This effect was significantly greater in C57BL/Crgl than in DBA/2Crgl mice. Blood levels of acetaldehyde were significantly greater in mice pretreated with Antabuse and then injected with ethanol than in mice not pretreated with Antabuse. This effect was significantly greater in C57BL/Crgl than in DBA/2Crgl mice. Thus, using two very different techniques for assessing the effects of Antabuse, it was found that mice of strain C57BL/Crgl were much more affected than DBA/2Crgl animals. Since Antabuse is an inhibitor of aldehyde dehydrogenase, this finding suggests that the metabolism of acetaldehyde is either quantitatively or qualitatively different in animals of these two genotypes. These data, however, do not permit exclusion of any of these several possible explanations: (1.) that different enzymes catalyze the oxidation of acetaldehyde in these two strains; (2.) that several enzymes catalyze this reaction in these two strains, that these are differentially inhibited by Antabuse, and that they are present in different amounts; and (3.) that the enzymes which catalyze this reaction are differentially active in animals of these two strains.

The hypothesis was tested that accumulation of blood acetaldehyde is great-

er in DBA/2Crgl than in C57BL/Crgl mice and that this could account for differences in alcohol consumption. This hypothesis was based on the fact that acetaldehyde is toxic and that its accumulation leads to detrimental effects and that these would result in lower alcohol consumption. The results supported this hypothesis. Blood acetaldehyde levels were determined in animals of these two genotypes both 1 and 2 hr. following injection of alcohol. In both cases, i.e., both 1 and 2 hr. after injection of alcohol, blood acetaldehyde levels were higher in DBA/2Crgl than in C57BL/Crgl mice.

If the accumulation of blood acetaldehyde does indeed lead to unpleasant consequences, then pretreatment with Antabuse should lower the voluntary consumption of alcohol in mice. It was impossible to test DBA/2Crgl mice in this experiment since these animals do not drink alcohol and any before-after treatment comparison would be meaningless. For this reason animals of three other strains, C57BL/Crgl, C3H/Crgl, and RIII/Crgl were tested. Antabuse was found to decrease voluntary alcohol intake in animals of all three strains; this decrease was small but statistically significant. The percentage decrease in alcohol consumption with Antabuse treatment was approximately the same for all three genotypes. In C57BL/Crgl mice, but not in the other strains, the decreased consumption of alcohol outlasted the treatment with Antabuse.

Summary

The purpose of this research was to determine whether strain differences in alcohol preference behavior in mice could be explained in terms of differences in acetaldehyde accumulation following alcohol administration in high-versus low-preference strains. Significantly smaller amounts of blood acetaldehyde levels were found in C57BL/

Crgl mice following alcohol administration than in DBA/2Crgl mice; mice of the former strain prefer a 10% solution of alcohol to water whereas DBA/2Crgl mice avoid alcohol in similar preference tests. Antabuse, a drug which inhibits the enzyme aldehyde dehydrogenase and leads to acetaldehyde accumulation following alcohol administration, was found to have quantitatively different effects in C57BL/Crgl and DBA/2Crgl mice. Antabuse inhibited the in-vivo metabolism of alcohol in both strains, but the inhibition was much greater in C57BL/Crgl than in DBA/2Crgl mice. Blood levels of acetaldehyde were found to be higher in mice pretreated with Antabuse and injected with alcohol than in animals not pretreated with Antabuse, this effect, again, was significantly greater in C57BL/Crgl than in DBA/2Crgl mice. Voluntary consumption of alcohol was depressed following Antabuse treatment in C57BL/Crgl, RIII/Crgl, and C3H/Crgl mice.

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