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Butanediols: Selection, Open Field Activity, and NAD Reduction by Liver Extracts in Inbred Mouse Strains

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GOLDBORT, R., C. W. SCHNEIDER AND R. A. HARTLINE. *Butanediols: selection, open field activity and NAD reduction by liver extracts in inbred mouse strains.* PHARMAC. BIOCHEM. BEHAV. 5(3) 263–268, 1976. – Mice from the high-ethanol preferring C57BL strain and the low-ethanol preferring DBA strain were tested for their preference for butanediols. The C57BL strain showed a significantly higher preference for a 10% (v/v) solution of 1,3-butanediol than the DBA strain. The C57BL strain also showed a significantly greater consumption of 1,2- and 2,3-butanediol, but the separation between strains was much smaller than with 1,3-butanediol. Both strains uniformly avoided 1,4-butanediol. Tolerance for 1,3-butanediol was tested in an open-field monitor at 3 doses. At the lowest dose the DBA strain was hyperactive and the C57BL were unaffected. At the highest dose both strains were equally depressed. The specific activity of NAD reduction on incubation of liver extracts with 1,3-butanediol and ethanol as substrates was higher with both compounds in extracts from the C57BL strain.

Mice Butanediols Tolerance Preference NAD reduction Activity

DIFFERENCES in ethanol self-selection among inbred mouse strains were first demonstrated by McClearn and Rodgers [12]. With a choice between 10% ethanol and water, the C57BL strain drink an average of 75% of their daily fluid from the ethanol bottle, while most animals from the DBA strain almost totally avoid the ethanol solution after an initial sampling. Efforts to gain insight into the nature of the factors underlying self-selection have involved determinations of interstrain differences in metabolic capacity, such as those found between high and low-drinking strains in their ability to clear ethanol and its toxic metabolite, acetaldehyde, from the blood. It has been suggested that ethanol-avoiding strains, such as the DBA mice, do so because they learn to avoid the ill effects of accumulated acetaldehyde in their blood [18].

Less frequently considered as a factor in ethanol selection has been the effect of the alcohol on the site where all alcohols exert their most pronounced effects as narcotics, the central nervous system. The recent findings of Schneider *et al.* [23,24] that at least 3 low-ethanol-selecting strains show a jaw-jerk reflex that is more sensitive than the high-selecting C57BL strain could indicate that tolerance to and selection of ethanol may be positively related. A positive relationship between open field activity and self-selection was also found using the alcohol and central nervous system depressant 1,2-propanediol [29] which, unlike ethanol, is not converted to a toxic metabolite that could produce illness [21]. Hillman and Schneider [6] found the C57BL and 3 low alcohol drinking strains (CBA, DBA/2, BALB) to be widely separated in their tolerance, as indicated by open field activity, and selection of 1,2-propanediol. Furthermore, the difference was in the same direction as for ethanol. Subsequently, Strange *et al.* [29] in an investigation of C_3 alcohols found a positive relationship between apparent sensitivity to and selection of 1,2-propanediol and 2-propanol. The purpose of this study is to examine further the effects of diols on the high and low alcohol preference mouse strains by examining the selection tendencies for C_4 diols and behavioral sensitivity in an open field test and liver NAD reducing activity for the alcohol yielding the largest separation between strains in selection.

METHOD

Animals

A total of 300 male mice, half from the C57BL/6J strain and half from the DBA/2J strain, were obtained from the Jackson Laboratory, Bar Harbor, Maine. All animals were 10–12 weeks of age at the time of testing. Animals were housed in standard metal cages with wire floors and fronts, and were fed standard mouse chow. A laboratory light cycle of 8 a.m. to 5 p.m. light on was maintained and temperature was constant at 68° F.

[1] This investigation was completed in partial fullfilment of the requirements for a Master's degree under the supervision of the second and third authors.

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Chemicals

All chemicals were obtained from commercial sources and were used without prior examination for purity; 1,3-butanediol and 1,4-butanediol, Eastman-Kodak Company; 2,3-butanediol, J. T. Baker Chemical Company; 1,2-butanediol, a gift from Dow Chemical Company, Midland, Michigan; Bovine serum albumin, ARMOUR; NAD, Sigma Chemical Company.

Procedure

Preference testing. Preference testing was carried out in a windowless room with the light cycle and temperature held constant. Sixty naive mice from each strain were tested with 10% (v/v) solutions of the four alcohols (15 mice from each strain per alcohol). Each animal was housed individually for a 4 day acclimation period prior to a 10 day testing period. Testing consisted of the standard two-choice arrangement with the alcohol solution and distilled water available. Testing tubes were 15 ml Kimax centrifuge tubes graduated in 0.1 ml increments and each tube contained a stainless steel spout with a 2 ml orifice. Measurements of the amount of fluid consumed from each tube were taken every 24 hr at the same time each day, (10 a.m.) and the position of the tubes was alternated daily to control for position effects. The preference index was derived for each animal by dividing the amount of alcohol consumed by the total amount of fluid consumed and a mean index for each group was derived by determining the average for the 10 day test period. Error due to spillage or evaporation was determined to be less than 0.2 ml and was not corrected for in the calculations.

Activity tests. Activity was monitored in an open field apparatus previously described [6]. Animals were tested for 15 min exactly 30 min after an IP injection of 1,3-butanediol or saline, and monitoring began immediately upon introduction of the animal into the open field. All activity testing was carried out between 7 and 12 p.m.

Forty-five naive animals (15 per dose) from each strain were tested at either 0.0025, 0.0035 or 0.0045 ml/gm body weight. Injection volume was held constant at 0.2 ml with 0.9% saline. Saline controls (40 per strain) received a 0.2 ml injection. An index of activity for each animal was derived as a percentage of the activity of the saline controls of that strain.

Preparation of the liver homogenate. Five animals from each strain were sacrificed by cervical dislocation and the livers removed, weighed, and homogenized in 9 volumes of cold 0.25 M sucrose for 2 min at 5°C with a Potter-Elevhjem homogenizer. The homogenate of each liver was centrifuged at 5°C for 20 min at 12,000 × g and the supernatant immediately assayed for NAD reducing activity.

Assay of NAD reduction. Assaying crude extracts of liver for dehydrogenase activity with a substrate such as 1,3-butanediol and its presumed immediate metabolic oxidation product β-hydroxybutyraldehyde poses two problems that make attempts to determine individual dehydrogenase activities no more informative than evaluating the ability of the extracts to reduce NAD with the alcohol as the substrate. One, since specificity studies on purified alcohol [30] and aldehyde [26] dehydrogenases have not included 1,3-butanediol or β-hydroxybutyraldehyde, it is not possible to know whether the activity measured is due to these alcohol and aldehyde dehydrogenases, other dehydrogenases, or both. Two, since it is not presently possible to specifically prevent oxidation of β-hydroxybutyrate in a crude extract, attempts to measure dehydrogenase activity with β-hydroxybutyraldehyde as a substrate would be obscured by the presence of a dehydrogenase (not alcohol dehydrogenase) [30] oxidizing the β-hydroxybutyrate product to acetoacetate. Therefore, no attempt was made to determine the individual contributions by alcohol dehydrogenase using semicarbazide to trap formed aldehydes [30] and by aldehyde dehydrogenase using aldehydes as substrates [26]. Instead, the ability of extracts from the two strains to reduce NAD with each alcohol as a substrate was evaluated by means of an arbitrarily selected set of assay conditions. Since the comparison is between the extracts of the two different strains with each alcohol and not between the two different alcohols, knowledge of the exact number of steps assayed is not essential for meaningful data evaluation.

The ability of liver extracts to reduce NAD with 1,3-butanediol or ethanol as substrates was measured by reduction of NAD at 340 nM using the conditions for determining aldehyde dehydrogenase activity described by Sheppard *et al.* [26]. The assay was in 1 cm path-length silica cuvettes on a Cary Model 14 recording spectrophotometer using the 0.0 to 0.1 absorbancy expanded scale. The reaction temperature was 23 to 25°C. Reactions were initiated by addition of 0.1 ml of the alcohol (3.9% v/v 1,3-butanediol or 2.0% v/v ethanol) to a solution of 0.1 M sodium pyrophosphate buffer (pH 9.6) containing NAD at 2.0 mM and 0.1 ml of liver extract in a final volume of 3.0 ml. One unit of activity is defined as the amount of enzyme necessary to catalyze the formation of 1 μmole of NADH per minute. Specific activity is expressed in units per mg of protein. Protein was determined by the method of Lowry [10].

RESULTS

Strain preference ratios for each alcohol are presented in Fig. 1. The C57BL strain had a higher mean preference ratio for all of the alcohols except 1,4-butanediol which was uniformly avoided by both strains. A two-tailed t test indicated that differences in preference for 1,2- and 2,3-butanediol were highly significant ($p<0.005$) with no overlap in preference between the 2 strains except for 1,4-butanediol. The largest difference between strains in preference occurred with 1,3-butanediol and the C57BL strain consumed significantly greater ($p<0.001$) amounts of this alcohol than any of the other alcohols. The daily average consumption pattern for 1,3-butanediol is shown in Table 1. Both strains show a characteristic drop in consumption of the alcohol after Day 1, but at no time do the two strains approach each other in consumption. Higher consumption on the first day was observed with all of the alcohols, including 1,4-butanediol even though that alcohol was avoided by both strains after the initial exposure. (Table 2) At no time did the consumption of an alcohol return to the Day 1 level. Total consumption appears unaffected by exposure to the different alcohols.

The effects of 1,3-butanediol on open field activity are shown in Fig. 2. Because the saline controls of the 2 strains differ (C57BL = 451 ± 47.8 and DBA = 331 ± 53.3) the raw scores were divided by the mean activity score of their saline control and expressed as a percentage above or below control in order to facilitate comparison of the 2 strains. There was a pronounced differential effect on the activity

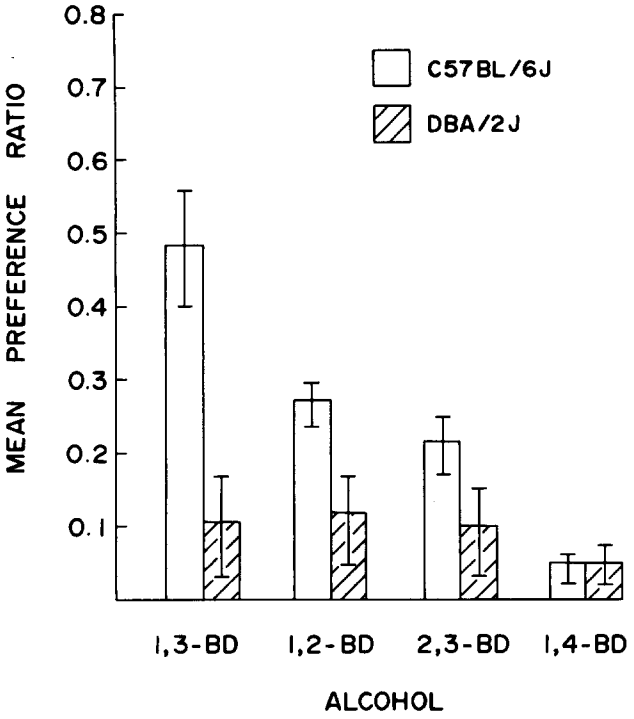

FIG. 1. Preference ratios for four 10% (v/v) solutions of butanediols obtained by finding the mean of each strain for a 10 day period. Each alcohol was tested on 15 naive mice per strain. Vertical lines inside bars indicate SD.

TABLE 1

MEAN DAILY CONSUMPTION OF 1,3-BUTANEDIOL AND H_2O BY 15 MICE FROM TWO STRAINS FOR 10 DAYS

	C57BL/6J		DBA/2J	
Day	H_2O	ALC	H_2O	ALC
1	2.6	4.1	3.8	1.4
2	3.8	2.5	4.6	0.7
3	3.0	2.5	4.5	0.4
4	2.6	3.0	5.4	0.4
5	3.4	2.4	4.5	0.3
6	2.7	3.1	5.0	0.7
7	3.7	2.6	5.2	0.5
8	2.6	3.2	5.3	0.4
9	3.6	2.3	5.1	0.4
10	2.8	3.1	5.6	0.4
Total $\bar{X}$ Consump.	5.9		5.5	

of the 2 strains at the lowest dose employed. A t test between strains showed the DBA strain to be significantly ($p<0.001$) more active than the C57BL strain that was apparently unaffected at that dose. A t test at the highest dose showed that both strains were significantly less active than their controls ($p<0.001$) but the strains did not differ significantly from each other in the percent of decline. The middle dose produced no apparent effect on the DBA strain, but the C57BL strain remained significantly less active than their controls ($p<0.001$).

Specific activities of NAD reduction with both alcohols were higher in extracts from the C57BL strain than in extracts from the DBA strain. The mean and S.D. of specific activities of NAD reduction with 1,3-butanediol were 13.54 ± 3.6 and 6.84 ± 2.19 for the C57BL and DBA strain, respectively. The values for ethanol were 1.612 ± 4.23 for the C57BL strain and 6.02 ± 1.94 for the DBA strain.

DISCUSSION

This study, an extension of a line of investigation initiated by Schneider *et al.* [24] and continued by Hillman and Schneider [6] and Strange *et al.* [29], examines selection and tolerance of C_4 short-chain aliphatic diols in high (C57BL/6J) and low (DBA/2J) ethanol preferring strains. The initial studies revealed that the C57BL strain consumes significant amounts of 1,2-propanediol [6,24] and ethanol [20] and moderate amounts of 1-propanol and 2-propanol [29]. The DBA strain rejects all of these alcohols except 1,2-propanediol which it consumes in moderate amounts [6,28]. Both strains totally reject 1,3-propanediol [29].

Of the 4 butanediols tested only 1,4-butanediol was rejected by both strains. The compound is catabolized to the potent depressant γ-hydroxybutyrate [3, 20, 21] which could account for its rejection by the C57BL strain, known to readily consume a variety of short-chain aliphatic alcohols [6, 24, 29]. The separation between strains in choice of 1,2- and 2,3-butanediol was significant but not as large as with 1,3-butanediol where selection is non-overlapping and parallels results obtained with ethanol [23] and 1,2-propanediol [6, 24, 29].

The determinants of acceptance or rejection of an alcohol are unknown and may not be the same for all alcohols. However, it would be most parsimonious to consider a similar mechanism underlying the choice of all alcohols. For example, toxicity presumably through accumulation of acetaldehyde has been implicated as a factor inhibiting consumption of ethanol by the DBA strain [18]. However, this would have to be ruled out as a factor in the rejection of 1,3-butanediol by the DBA strain since both it and its catabolite β-hydroxybutyrate possess very low toxicity [3, 27, 31]. In fact, β-hydroxybutyrate is one of several ketone bodies formed during fatty acid catabolism [7]. In this regard, 1,2-propanediol is also very low in toxicity [2, 21, 32] yet its consumption by the DBA strain is only moderate, while 1-propanol is consumed in moderate amounts by the C57BL strain even though it is extremely toxic [14,29].

It has been shown that choice of an ethanol solution may be influenced by the alteration of internal or external factors affecting taste and smell [18]. Nachman *et al.* [15] have suggested that at least one low drinker strain (BALB/cJ) may avoid ethanol because of its odor. While orosensory and olfactory cues may play a role in the selection of ethanol it is difficult to see how the taste or odor of all alcohols could be aversive to the DBA strain and not to the C57BL strain. It could be that the C57BL strain is generally less sensitive to the sensory cues provided by alcohols, but this seems unlikely since they show total

TABLE 2

MEAN CONSUMPTION OF ALCOHOLS AND H_2O FOR DAY 1 AND 2 BY 15 MICE FROM TWO STRAINS. NAIVE MICE WERE USED WITH EACH ALCOHOL

	1, 2-Butanediol				2, 3-Butanediol				1, 4-Butanediol			
	Day 1		Day 2		Day 1		Day 2		Day 1		Day 2	
	ALC	H_2O	ALC	H_2O	ALC	H_2O	ALC	H_2O	ALC	H_2O	ALC	H_2O
C57BL/6J	1.9	5.1	1.6	5.4	1.8	4.4	1.5	5.3	0.6	5.0	0.3	6.0
Total $\bar{X}$	7.0		7.0		6.2		6.8		5.6		6.3	
DBA/2J	1.7	4.9	0.8	5.7	1.6	4.4	0.8	5.5	0.6	5.4	0.3	5.7
Total $\bar{X}$	6.6		6.5		6.0		6.3		6.0		6.0	

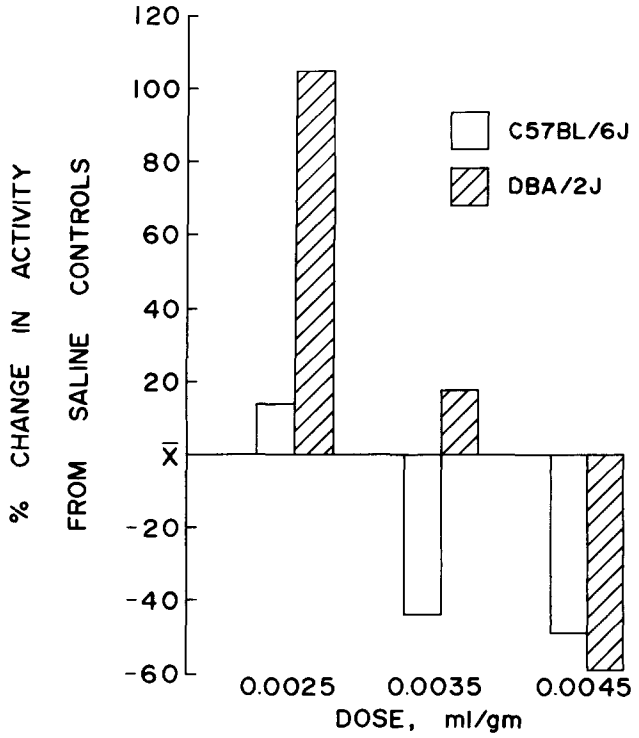

FIG. 2. Increase or decline in open field activity relative to control after an injection of 1,3-butanediol at various doses. Each strain included 15 naive animals at each dose.

aversion of some alcohols and moderate aversions of others. Furthermore, the selection level for many alcohols appears to be postingestional which does not lend support to the existance of an innate disposition to avoid the substances. Schneider *et al.* [24] have monitored lick rate and observed a decline in lick rate on Day 2 for ethanol and 1,2-propanediol. Hillman and Schneider [6] observed a decline in consumption of 1,2-propanediol after Day 1 by 3 low-drinker strains (DBA/2J, BALB/cJ and CBA/J). Strange [28] has observed a decline in consumption after Day 1 of a 10% solution of 1,3-propanediol and a 2.5% solution of 2-propanol by both C57BL and DBA strains. In this investigation the DBA strain showed a decline in consumption for every alcohol after the initial exposure. The C57BL strain displayed a pronounced decline in consumption after initial exposure only for 1,3-butanediol, the most preferred solution. The decline in consumption of 1,4-butanediol, the least preferred solution, was identical for both strains.

If we also consider the characteristics of the alcohols discussed above it becomes more difficult to understand the role played by sensory cues. The alcohols selected by the C57BL vary considerably in taste and odor; 1,2-propanediol has no odor and a bittersweet flavor while 1,3-butanediol has a pleasant odor and sweet flavor. The butanediols are all quite similar in odor yet are not equally consumed. Sensory cues may indeed play a role in the selection or avoidance of some alcohols, but it is not at all clear at this point how this could explain the results we have observed.

If there is a common factor underlying the consumption of alcohol it may be tolerance determined by metabolic capacity, neural sensitivity or both. The higher rate of NAD reduction by extracts from the C57BL strain in the presence of ethanol or 1,3-butanediol confirms and extends previous findings that the C57BL strain shows greater dehydrogenase activity than the DBA strain [1, 13, 19, 25]. Whether this is significant for the in vivo metabolism of 1,3-butanediol remains to be demonstrated, for differences in the rate of ethanol metabolism between high and low drinking strains have not been compelling [17, 22, 26, 33] even though in vitro studies have found large differences between high and low drinkers [13, 19, 25]. As alluded to earlier, differential acetaldehyde metabolism might play a role in tolerance and selection of ethanol [18], but this could not be a factor in selection or tolerance of 1,3-butanediol or 1,2-propanediol because of the absence of toxic catabolites [2, 3, 21, 27, 32]. Furthermore, Schneider *et al.* [23] have criticized this hypothesis on the grounds that low-drinkers could not accumulate enough of the aldehyde under natural conditions to produce ill effects.

It seems more likely that if tolerance and selection of an alcohol are related then neural sensitivity should play an important role. Previous studies have indicated that all of the alcohols consumed by the C57BL strain with the exception of 1-propanol tend to have less impact on their behavior than the effects observed on the DBA and other low-drinking strains [6, 23, 24, 29]. As indicated above, drinking strains will consume larger amounts of alcohols on their initial exposure than on subsequent days. In light of these findings it seems reasonable to suggest that all mouse strains may be attracted to many of the alcohols because of their novelty but are limited in their consumption by a predetermined tolerance level. Many investigators have obtained dramatic differences in the response of high and low drinkers to alcohols. Differential effects of ethanol on

sleep time are large [5, 6, 8, 9] with low-drinking strains sleeping twice as long as high-drinkers even though the brain alcohol levels are the same [8,9]. The C57BL strain suffers much less decrement in the amplitude of the jaw-jerk reflex than low-drinkers [23,24] when ethanol is infused at a rate overwhelming metabolic differences. Recently, McClearn [11] reported evidence for greater brain sensitivity in long-sleep (LS) versus short-sleep (SS) mice after injections of ethanol, methanol and butanol. These animals were selected from a genetically heterogeneous stock and bred for 14 generations to achieve differences in their sensitivity to the narcotic effects of alcohol. His findings and ours suggest that the differential neural sensitivity between strains and lines bred for narcotic sensitivity to ethanol may not only be ethanol specific but may apply to a variety of alcohols.

In this investigation the apparent differential sensitivity between the C57BL and DBA strain is less clear than that previously reported for other alcohols. At the highest dose there was no difference between strains in reduction of activity. The middle dose seemed to produce depression in the C57BL while the DBA was at control level. The lowest dose produced extreme hyperactivity in the DBA while the C57BL remained at control level. This finding is consistent with previous results utilizing low doses of ethanol [19], 1,2-propanediol and 2-propanol [29], and it has been suggested that the C57BL strain may be insensitive to the hyperactive effects of alcohol [16]. This is supported by the finding that lower doses of 1,3-butanediol than those reported here [4] have all failed to increase activity in the C57BL strain. Hillman and Schneider [6] have reported a hyperactive phase with a sample of 5 C57BL mice 30 min after an injection of 1,2-propanediol. However, in light of subsequent findings with other alcohols previously mentioned, their results bear reexamination with a much larger sample.

At the middle dose it is conceivable that the activity of the DBA strain does not drop to the level of the C57BL strain because of concurrent hyperactive and depressant effects. If these effects result from two distinct mechanisms the apparent no-effect of the middle dose on the DBA strain could represent antagonism between the two mechanisms [29]. The highese dose used in these studies is close to that producing anesthesia, and any differences in activity between the strains at this dose could be obscured. If differential sensitivity accounts for differences in preference between the two strains it is not unreasonable to think that in a natural drinking situation, where low levels are consumed at any one time, only the DBA strain experiences hyperactive phase. Therefore, differential sensitivity may best be studied at low rather than anesthetic doses of alcohols.

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