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# Selection of $C_3$ Alcohols by High and Low Ethanol Selecting Mouse Strains and the Effects on Open Field Activity[1]

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STRANGE, A. W., C. W. SCHNEIDER AND R. GOLDBORT. *Selection of $C_3$ alcohols by high and low ethanol selecting mouse strains and the effects on open field activity.* PHARMAC. BIOCHEM. BEHAV. 4(5) 527–530, 1976. – Mice of the high-ethanol selecting C57BL/6j strain consume significantly larger amounts of 10% solution of 1,2-propanediol and 1-propanol than the low-ethanol selecting DBA/2j strain. Both strains uniformly avoid a 10% solution of 1,3-propanediol and 2-propanol. Open field activity was tested 30 min after an IP injection of 3 different equimolar doses of each alcohol. An increase in activity was produced in the DBA/2j strain by high (0.003 ml/mg) and middle (0.0015 ml/lg) doses of 1,2-propanediol and by a low dose (0.0005 ml/mg) of 2-propanol. The C57BL/6j strain were unaffected by these doses. High doses of 2-propanol produced sleep in both strains with the DBA/2j strain sleeping significantly longer, and 1,3-propanediol produced depression in both strains. Death resulted in all animals following injections at the high (0.002 mg/gm) and medium (0.001 ml/gm) doses of 1-propanol while the low dose (0.0005 ml/gm) produced slight depression.

Mice strains  $C_3$ alcohols  Alcohol selection  Open field activity

THE tendency of C57BL/6j mice to choose an ethanol solution over water, while other inbred strains such as the BALB/c, DBA/2, and CBA reject ethanol is well established [9]. Differences in consumption between high and low drinking strains are large, and the mechanism underlying the behavior is not well understood.

At the present time two factors have been implicated as playing a possible role in the selection of ethanol, i.e., differential metabolic capacity [13] and differential neural sensitivity [3, 5, 11, 12]. With regard to metabolic differences between high and low drinking strains in the rate of conversion of alcohol to acetaldehyde the results have generally been found to be quite small or equivocal [3, 5, 10, 11]. However, results of investigations of metabolism of acetaldehyde suggest that the high drinking strains oxidize it more rapidly than the low drinking strains [11,14]. This being the case, one might expect the development of a conditioned aversion if the low drinkers became ill after an accumulation of the toxic substance, and that could account for the rejection of ethanol. Recently, Schneider *et al.* [11] have pointed out that is is highly unlikely that low drinkers form a conditioned aversion resulting from the toxic effects of acetaldehyde because of the extremely small amount of ethanol consumed even during their initial exposure.

While metabolic differences between high and low drinking strains seem to be quite small, differences between these strains with regard to tolerance to ethanol have been found to be quite large. Kakihana *et al.* [5] found much longer sleep times in low drinker strains after an anesthetic dose of the drug, and these findings were essentially confirmed more recently [3,8]. Schneider [11,12] in two separate investigations found that it took twice as long to produce a 50% decrement in the amplitude of the jaw-jerk reflex in a high drinker strain than in three low drinker strains even when metabolic differences were overwhelmed by infusion of ethanol at twenty times the metabolic rate.

Schneider [11] sought to explore this parallel between tolerange and selection further by examining another alcohol. The candidate was 1,2-propanediol, a $C_3$ alcohol that is very low in toxicity but, like ethanol, is a CNS depressant. The selection ratios were like those obtained with ethanol. Subsequently, Hillman and Schneider [4] demonstrated that three strains of low drinking mice were significantly more depressed by 1,2-propanediol than the high drinking C57BL strain.

In the present investigation we sought to determine if similar tolerance and selection relationships existed for other $C_3$ alcohols as well. It seems likely that most alcohols share the same characteristic, that is they are CNS depressants, and barring gustatory or olfactory aversions we might expect to find the previously observed parallel between selection and CNS sensitivity.

## METHOD

### *Animals*

A total of 400 male mice were used in the investigation.

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This included 200 mice of the high ethanol-selecting C57BL/6j strain, and 200 of the low ethanol-selecting DBA/2j strain. All animals were obtained from the Jackson Laboratory, Bar Harbour, Maine, and were approximately 10 weeks old at the beginning of the investigation.

*Procedure*

Ten naive mice from each strain were used to determine the selection index for each 10% (v/v) alcohol solution. Prior to the preference testing each animal was placed in an individual cage for 3 days of adaptation with food and water available. The room temperature was held constant at 68° and light–dark cycle contained an 8 a.m. to 5 p.m. light on period. Preference testing followed the traditional two-choice paradigm with appropriate controls for position preference. Measures of consumption were made every morning between 9 and 11 a.m. for 10 days, and the selection index was obtained each day by dividing the amount of fluid consumed from the alcohol bottle by the total amount of fluid consumed. A mean index for each group was derived each day and a grand mean index for the 10 day period was determined at the end of testing.

Tolerance was tested by determining open field activity levels (apparatus previously described, [4]) after an IP injection of the drug or saline. Ten naive animals from each strain were tested at each dose of each alcohol. The saline control group included 5 animals at each dose/alcohol condition making a total of 60 saline controls from each strain. Experimental animals received equimolar doses of the four alcohols in saline with volume held constant at 0.2 ml, and three dose levels were employed. Exactly 30 min after an injection mice were removed from an individual holding cage and placed in the center of the open field apparatus where they were photometrically monitored immediately for a period of 15 min. All activity tests were run between 6 and 12 p.m.

## RESULTS

Table 1 contains the mean consumption values and the selection ratios for the four alcohols. Consumption of all the alcohols were remarkably stable for the 10 day testing period, therefore, a mean for each group was derived from the ten day period.

The C57BL strain consumed larger amounts of 1,2-propanediol and 1-propanol than the DBA strain. Statistical analysis of the selection indexes using a *t*-test of differences between means [15] yielded highly significant results ($p<0.001$) for both alcohols. The other two alcohols were selected at a very low level by both strains.

Table 2 contains the measures of activity derived from the 15 min period following the open field test 30 min after injection. Also shown are death and sleep time where appropriate. The means and standard deviation derived from the saline controls not shown in the table, were: C57BL = 626.32 ± 175.73 and the DBA = 262.73 ± 93.83. Since the baselines are different for the two strains and direct comparisons impossible, all of the values were converted to the proportion above or below their control baseline by dividing the control mean into each individual value and determining the mean proportion. These values are shown in the table with a + for above control and a - indicating below control. The two strains were then compared by analysis with *t*-tests on the differences between strain proportions.

Significant differences between strains in the effects of the alcohols were obtained with the high and medium dose of 1,2-propanediol and all doses of 2-propanol, while 1,3-propanediol and 1-propanol yielded results that were equivocal. Both strains exhibited a decrease in activity at the high dose of 1,3-propanediol and although the DBA strain appeared more depressed than the C57BL strain the difference was not significant. However, there were readily observable qualitative differences between the strains after an injection of this alcohol. The DBA strain showed disorientation, staggering movements and partial loss of control over the hind legs, none of which were evident in the C57BL strain. The high and medium doses of 1-propanol proved to be very toxic and the lowest was ineffective. Although there was a significant separation between strains in the consumption of this alcohol its apparent narrow margin of safety would seem to preclude its usefulness.

Since anesthesia was produced by 2-propanol at the highest dose, sleep time was employed to determine whether or not strain differences were apparent. A *t*-test was run and the DBA strain showed a significantly longer sleep period. Of particular interest was the significant increase in activity of the DBA at the lowest dose of this alcohol, paralleling the results obtained with high and medium doses of 1,2-propanediol.

TABLE 1

MEAN CONSUMPTION IN ML FOR EACH STRAIN AND EACH ALCOHOL. ALSO INCLUDED ARE THE SELECTION RATIOS AND STANDARD DEVIATIONS

| Strain | Strain Consumption Means and Selection Ratios | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1,2-Propanediol | | | 1,3-Propanediol | | | 1-Propanol | | | 2-Propanol | | |
| | ALC | $H_2O$ | Ratio (SD) | ALC | $H_2O$ | Ratio (SD) | ALC | $H_2O$ | Ratio (SD) | ALC | $H_2O$ | Ratio (SD) |
| C57BL/6J ml consump. $\bar{X}$ and selection ratio (SD) | 6.73 | 1.74 | 0.79(0.03) | 0.91 | 5.56 | 0.14(0.03) | 2.76 | 4.00 | 0.41(0.07) | 1.14 | 5.85 | 0.16(0.03) |
| DBA/2J ml consump. $\bar{X}$ and selection ratio (SD) | 2.79 | 3.41 | 0.45(0.07) | 0.70 | 4.61 | 0.13(0.04) | 0.86 | 5.00 | 0.14(0.03) | 0.78 | 4.76 | 0.14(0.03) |

TABLE 2

CHANGE IN ACTIVITY OF HIGH AND LOW ETHANOL—PREFERRING MOUSE STRAINS 30 MIN AFTER AN INJECTION WITH $C_3$ ALCOHOLS. INCREASE (+) OR DECREASE (−) IN ACTIVITY RELATIVE TO CONTROL IS PRESENTED BELOW THE RAW MEANS. DOSES FOR 1 AND 2-PROPANOL ARE EQUIMOLAR WITH THE PROPANEDIOLS. ($LD_{100}$ = DEATH IN ALL ANIMALS; MIN = SLEEP TIME)

| | ALCOHOLS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1,2-Propanediol | | 1,3-Propanediol | | | 1-Propanol | | 2-Propanol | |
| Dose ml/gm | C57BL | DBA | C57BL | DBA | Dose ml/g | C57BL | DBA | C57BL | DBA |
| 0.003 | 631.6 ± 163.2 (+0.01) | 359.7 ± 104.5 (+0.37) | 256.4 ± 178.5 (−0.59) | 60.3 ± 37.8 (−0.77) | 0.002 | $LD_{100}$ | $LD_{100}$ | 54 min ± 47.6 | 93 min‡ ± 28.0 |
| 0.0015 | 663.8 ± 172.3 (+0.06) | 353.6 ± 69.4 (−0.36)* | 588.4 ± 151.5 (−0.06) | 249.8 ± 73.8 (−0.05) | 0.001 | $LD_{100}$ | $LD_{100}$ | 363.3 ± 134.3 (−0.42)† | 257.7 ± 76.8 (−0.02) |
| 0.00075 | 645.1 ± 104.1 (+0.03) | 256.8 ± 94.8 (−0.02) | 669.8± 71.5 (+0.07) | 266.6 ± 112.8 (+0.01) | 0.0005 | 537.7 ± 219.9 (−0.14) | 218.5 ± 81.4 (−0.17) | 579.6 ± 131.4 (−0.08) | 368.8 ± 134.0 (+0.40)† |

*$p<0.05$.
†$p<0.01$.
‡Sleeptime $p<0.01$.

## DISCUSSION

The positive relationship between the selection of and apparent sensitivity to the effects of 1,2-propanediol was clearly demonstrated in this investigation. Hillman and Schneider [4], using a higher dose of the alcohol to examine its effects on open-field activity obtained significantly greater depression in three low drinking strains than in the high drinking C57BL strain. With the lower doses employed in this experiment the higher sensitivity to the effects of alcohol in low drinkers manifests itself as a significant increase in activity.

The biphasic response to ethanol has been recognized for some time [16], but it seems to be less obvious with other alcohols. We have also found the excitatory phase with the DBA strain in response to a low dose of 2-propanol. Recently, Goldbort and Hartline [1] obtained similar results with 1,3-butanediol, and Randall *et al.* [7] have observed an increase in activity in the low drinking BALB strain with low doses of ethanol. Evidently, the excitatory phase is difficult to obtain in the high drinking C57BL strain. Randall *et al.* [7] did not find it with ethanol and Goldbort and Hartline [1] did not find it with a low toxicity $C_4$ alcohol. We have not observed it with any of the $C_3$ alcohols in this investigation, but Hillman and Schneider [4] did observe an increase in activity in C57BL with a higher dose of 1,2-propanediol. Randall *et al.* [7] have suggested that the C57BL strain may either be insensitive to the excitation produced by ethanol or more sensitive to its depressant effects. It may be that an appropriate dose that could produce excitation in the C57BL has not been employed. Whatever the case, these findings lend further support to the assumption that there are inherent differences in the neural response to alcohol between high and low drinking mouse strains [10].

While the highest dose of 2-propanol we employed produced sleep and the lowest dose produced excitation, the middle range had no apparent effects on behavior of the DBA strain. Goldbort and Hartline [1] have obtained similar results with 1,3-butanediol. Apparently, this is not unique to these alcohols. Grenell [2] found that the cortical response of cats increased during low doses of ethanol, appeared at non-drug levels with a middle range of doses and were attenuated at high doses after direct electrical stimulation. This progression from agitation to an apparent non-drug state to depression is not uncommon with many anesthetics (M. B. Chenoweth, Personal Communication), and may indicate a range in which the drug has not yet reached an effective level to produce depression but is capable of inhibiting whatever the actions are underlying the excitatory phase.

It is difficult to know how our results may be affected by gustatory or other factors. Indications are, at least with the rejection of 2-propanol, that taste is not a factor. On the basis of the results we obtained from the activity studies, we postulated that a lower concentration of the solution used in the selection testing might yield the usual separation in choice observed with ethanol and other alcohols [1,11]. Therefore, we tested 10 animals from each strain with a 2.5% solution of 2-propanol and the selection index for the C57BL strain was 0.6 and that of the DBA strain was 0.1. A 2.5% solution of 2-propanol possesses a strong flavor and odor and one would expect that if rejection was due to taste aversion there would not be such a dramatic increase in the amount consumed. Thus the positive relationship found between the selection of other alcohols and their effect on the activity of high and low selecting strains was demonstrated with 2-propanol.

Monick [6] claims that 1-propanol is highly toxic, but a mild depressant. The results we obtained tend to support that claim. One might speculate that the differential selection we obtained is paralleled by a differential effect on activity but this would be extremely difficult to demonstrate because of the very narrow margin of safety that is evident for this alcohol. However, it seems unlikely that toxicity could be a factor limiting consumption in either strain. The DBA strain drank considerably less of the alcohol than the C57BL strain, yet there is no indication that they are more sensitive to the toxic effects of it. Consumption of 1-propanol by the C57BL strain during any one hour period might approach the toxic levels as determined by IP injection, but it is generally known that the oral toxicity is higher than it is via the IP route. In addition, consumption of an amount spread over an hour that is toxic in a single dose would be considerably less toxic since it would be readily metabolized. It would seem that the limitations mentioned above would preclude the usefulness of this alcohol in selection studies.

Similarly, 1,3-propanediol might also have a limited usefulness. It is a fairly potent depressant and no separation in tolerance or selection at any concentration could be obtained in this investigation.

Undoubtedly, a number of factors may influence selection or rejection of an alcohol: caloric utility, taste, odor, neural sensitivity, metabolic rate, toxicity and other unidentified factors. Indeed, the mechanisms underlying choice may be different for each alcohol thus compounding the difficulty in understanding the question of differential selection. One fact seems clear, the high and low alcohol drinking strains give evidence of possessing a differential neural sensitivity to some alcohols, and perhaps a better understanding of the mechanisms involved may lead us closer to understanding the mechanisms underlying alcohol tolerance.

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