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BRIEF COMMUNICATION

Alcohol Consumption by C57BL/6, BALB/c, and DBA/2 Mice in a Limited Access Paradigm

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LÊ, A. D., J. KO, S. CHOW AND B. QUAN. *Alcohol consumption by C57BL/6, BALB/c, and DBA/2 mice in a limited access paradigm.* PHARMACOL BIOCHEM BEHAV **47**(2) 375–378, 1994.—Alcohol consumption by three inbred mice strains in a limited access condition was examined. Access to "Richter" tubes containing alcohol solution was restricted to 60 min per day in a drinking cage. Alcohol solution was given in escalating concentrations starting at 3% and ending at 12% w/v over several days. During the 12% phase, C57 mice consumed an average of 1.68 g/kg, while BALB and DBA mice consumed an average of 0.66 and 0.25 g/kg, respectively. The C57BL/6 mice achieved an average blood alcohol level (BAL) of 60 mg%, whereas the other two strains displayed negligible levels. The relationship between alcohol intake in a continuous and limited access as well as the utility of the limited access paradigm are discussed.

Alcohol consumption Limited access Genetic C57BL/6, BALB/c, and DBA/2 mice

VOLUNTARY alcohol consumption in experimental rodents has been examined under two continuous access conditions. In one condition, alcohol solution is available for 23–24 h/day. In a second condition, access to alcohol solution is restricted to only a few hours or a short period per day (13–16). Alcohol consumption in the unrestricted access paradigm has been commonly measured with the 24-h two-bottle-choice technique (16). In the limited access paradigm, alcohol consumption has been investigated with operant procedures employing Skinner boxes (16). However, a number of investigators (5,7,15,16) recently showed that such drinking can be assessed using Richter tubes and drinking cages.

A number of variations have been employed in the limited access paradigm, ranging from prandial drinking (4,16) to sucrose fading (13) to restricted access to food and/or water (4,12), for either operant or two-bottle choice. Regardless of the procedure employed, the common feature in this paradigm is that the animals consume alcohol at a high rate and that the amount of alcohol consumed results in blood alcohol levels (BALs) which are pharmacologically relevant (16,17). This feature of the limited access paradigm is quite useful for investigation into the effects of various pharmacological agents on alcohol consumption. Moreover, this paradigm is particularly useful for investigation of drugs with short half-life or when the duration of drug action is short due to intracranial administration.

Although operant procedures offer a direct measurement of the reinforcing effects of alcohol, their uses are limited because specialized skills and equipment are required, hence limiting the number of animals that can be assessed economically. The successful adaptation of alcohol drinking in limited access condition, from operant procedure to two-bottle-choice home-cage drinking, has facilitated the testing of the effects of various agents as well as various behavioral and genetic factors on alcohol drinking (15–17).

Most of the studies concerning alcohol drinking in a limited access paradigm have been restricted mainly to the rat. In the present study, we examined alcohol drinking by various mice strains in a limited access condition using modified "Richter" tubes. Mice of C57BL/6, BALB/c, and DBA/2 strains were chosen for this study because differences in alcohol drinking among these three strains in a continuous access condition have been well documented (9,10).

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MATERIALS AND METHODS

Animals

Male C57BL/6, DBA/2, and BALB/c mice (18 from each strain) weighing 26–28 g and approximately 10–11 weeks old were obtained from Charles River Laboratories (Quebec). Mice of the same strain were housed in groups of four to five in a shoebox plastic cage with food and water available ad lib. Ambient temperature was maintained at 21 ± 1°C, and lights were on from 0700 to 1900 daily throughout the entire experiment.

Experimental Procedure

Mice were removed from their home cages daily and placed in individual drinking cages located in the same room. The drinking cage was constructed from stainless steel with a wire-mesh floor. The dimensions of the cage were 9.5 × 4.0 × 5.0 in. (L × W × H). Alcohol and water solutions were offered in "Richter" tubes mounted on the front of the drinking cage.

Because of the low volume of fluid that can be consumed by the mouse, the "Richter" tubes were custom made from 5-ml pipettes so that volume consumed could be measured to the nearest 0.05 ml. One hour after the alcohol became available the volumes of alcohol and water consumption were recorded and animals were returned to their home cages. The assessment of alcohol drinking was conducted daily between 1300 and 1600, with nine animals from each strain carried at a time.

To acclimate the animals to the taste of alcohol, alcohol solution was offered in escalating concentrations over several days. Alcohol was offered as a 3% (w/v) solution for the first 8 days. When the rate of alcohol consumption appeared to reached an asymptote, the concentration of alcohol was increased to 6% (days 9–20). On day 21, the 6% of alcohol was increased to 12% for the remaining duration of the experiment (days 21–36). The weights of the animals were recorded every second day for the estimation of alcohol drinking per body weight.

On day 34 of the experiment, a blood sample (50 μl) was taken from the tip of the tail of each mouse within 5 min after the termination of the drinking session for the determination of BAL. Blood ethanol levels were determined by gas-liquid chromatography technique with *n*-butanol as internal standard (6).

RESULTS

The amount of alcohol (g/kg) consumed by the three mice strains across different alcohol concentrations during the 1-h access period to alcohol is shown in Fig. 1. It is clearly seen from the figure that the amounts of alcohol consumed by the

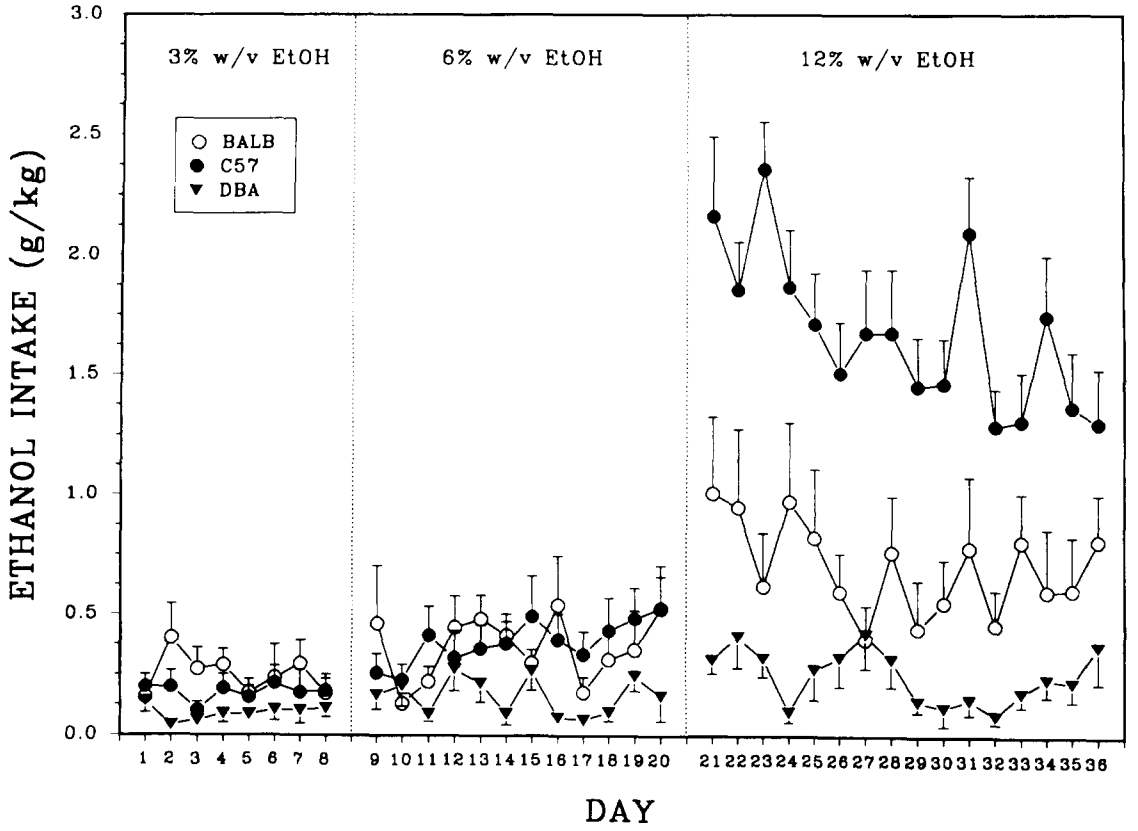

FIG. 1. Alcohol intake (g/kg) by C57BL/6, BALB/c, and DBA/2 mice during the 1-h daily access to alcohol solution. The concentration of alcohol solution was 3% w/v for the first 8 days, 6% for the next 12 days, and 12% for the remaining 16 days. N = 17–18 mice per strain. Vertical lines indicate positive or negative halves of the SEs.

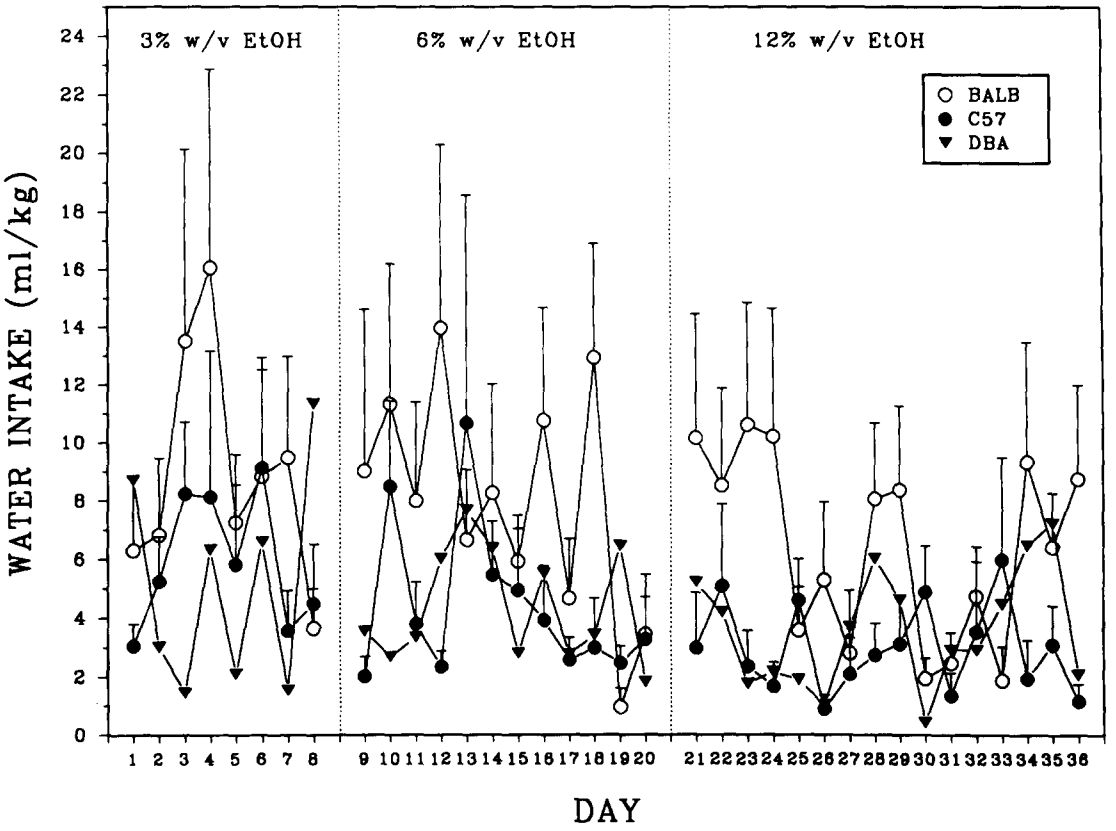

FIG. 2. Amount of water consumed (ml/kg) by C57BL/6, BALB/c, and DBA/2 mice during the 1-h daily access to water and alcohol solutions in the drinking cages across the 36 experimental days. N = 17–18 mice per strain. Vertical lines indicate positive or negative halves of the SEs.

three strains over the whole experimental period are dependent on the concentration of alcohol solution. Analysis of variance, $F(2, 50) = 9.4, p < 0.01$, followed by Duncan multiple range tests indicates that during the 3% phase BALB/c mice consumed more alcohol ($p < 0.05$) than DBA/2 and C57BL/6 mice. The amounts of alcohol consumed by BALB/c and C57BL/6 mice during the 6% phase were essentially similar to one another (with an average mean of 0.368 and 0.377 g/kg for BALB/c and C57BL/6 mice, respectively); however, they are significantly higher than those consumed by DBA/2 mice (0.16 g/kg).

Alcohol intake by C57BL/6 mice increased markedly at the 12% phase, with an average intake of 1.68 g/kg, and was significantly higher ($p < 0.001$) than the intake of BALB/c (0.66 g/kg) and DBA/2 mice (0.25 g/kg). BALs measured on day 34 of the experiment show negligible levels in both BALB/c (0–10 mg%) and DBA/2 mice (0–5mg%). A range of 11–102 mg% BALs with a mean of 59 ± 8 mg%, however, was observed in the C57BL/6 mice.

The amounts of water consumed by the three strains of mice during the daily 1-h access to both alcohol and water solutions over the whole experimental period are shown in Fig. 2. Analysis of variance followed by post hoc tests show that the amounts of water consumed by the C57BL/6 or DBA/2 mice across different phases of alcohol concentration were not different ($p > 0.05$) from one another. The C57BL/6 and DBA/2 mice, however, consumed significantly lower amounts of water than BALB/c mice throughout the experimental period.

DISCUSSION

The present study demonstrates that C57BL/6 mice, under free access to food and water, consumed an average of 1.6 g/kg of alcohol during the daily 1-h access to alcohol solution of 12% w/v concentration, offered in modified "Richter" tubes. Such consumption produced a mean BAL of 60 mg%. Under the same experimental conditions, mice of BALB/c and DBA/2 strains consumed little alcohol and had negligible blood alcohol concentrations. Most of the alcohol intake occurred within the first 10 min of access to alcohol solution in a limited access condition (5,7). It is possible, therefore, that the blood alcohol attained in C57BL/6 mice might be higher than that attained if blood samples were collected at a time earlier than at 65–70 min after exposure to alcohol solution. One study has shown a BAL of 200 mg% measured at 30 min following consumption of 2.4 g/kg in C57BL/6 mice (1).

Using prandial drinking procedure, Elmer et al. (1) showed that C57BL/6 mice self-administered 2.5 to 5.6 g/kg of alcohol, depending on whether food was provided before or after a 30-min access to alcohol. With similar procedures, alcohol has also been shown to serve as a reinforcer in C57BL/6 mice but not in BALB/c mice (4). Food deprivation and prandial drinking are likely to be the main factors that account for a

higher intake of alcohol in these studies compared to the present one, in which animals had free access to food and water.

The rank orders of alcohol intake among the three strains of mice observed in the present study are consistent with those reported for these strains in a continuous access paradigm (9,10). Studies comparing ethanol intake in limited- and continuous-access conditions among different rat lines and rat strains have revealed interesting results. The AA and the P rats, which have been selectively bred for high alcohol intake, consumed much more alcohol than Wistar or Long-Evans rats under continuous access conditions (2,12). However, with a limited access paradigm there are no differences between the AA and Wistar or P and Long-Evans rats (2,12). Clearly, the mechanisms that mediate the different drinking in the two drinking paradigms may involve important theoretical factors.

In summary, the present work shows that alcohol intake in a limited access condition can be measured using a two-bottle-choice technique with modified "Richter" tubes. The availability of this technique can facilitate investigations of various strains of mice that have been selected for differences in various alcohol-related behaviors (11). As in the case of the rats, this technique also permits pharmacological investigation into the effects of various agents on alcohol intake in large numbers of animals. Finally, due to the relatively small body weight of mice the procedure is extremely economical for studies using expensive pharmacological agents.

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