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Ethanol Intoxication as a Function of Genotype Dependent Responses in Three Inbred Mice Strains

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ELSTON, S. F. A., K. BLUM, L. DeLALLO AND A. H. BRIGGS. *Ethanol intoxication as a function of genotype dependent responses in three inbred mice strains.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 13–15, 1982.—Three strains of mice, ICR Swiss, DBA/2J and C57Bl/6J were compared for initial sensitivity, recovery from intoxication, and acute tolerance development to ethanol. The C57Bl/6J mice were found to be less sensitive and to recover more rapidly from the effects of the same dose of ethanol than the other two strains treated. None of the strains tested demonstrated acute tolerance to ethanol when given the same dose 3 hours later.

Ethanol intoxication Genotype responses Inbred mice

THE present study was designed to further evaluate differential sensitivty to acute intoxicating effects of ethanol, in three inbred strains of mice. Recently, our laboratory published a paper concerned with a new method to evaluate the effects of remarkably low doses of central depressants including ethanol [3]. This is the first report concerned with utilization of our new method to study differential sensitivity in various inbred strains of mice using very low doses of ethanol.

A review of the literature strongly indicates that sensitivity to ethanol has genetic determinants [1, 5, 11]. The conclusion that there is a genetic influence on sensitivity is based upon results showing that different inbred strains respond differently to alcohol and that differential sensitivity between lines may be achieved by selective breeding programs [5].

Evidence from studies with mice is derived from the work of Kakihana *et al.* [6]. These authors showed that mice of the Balb/cCrgl slept over 3.5 times as long as did mice of the C57Bl/Crgl strain. In other studies, Randall *et al.* [10] reported that C57Bl/6J mice showed a dose-dependent decrease in locomotor activity with ethanol at a range of 0.75 to 2.25 grams/kilogram body weight, whereas at the same ethanol doses, Balb/J strain showed an increase in activity.

Work with the non-alcohol preferring mice DBA/2J includes the finding by Danjanovich and MacInnes [4] that sleep-time induced by relatively high ethanol doses was longer in both DBA/2J and Balb/cJ compared C57Bl/6J over the same ethanol doses. Other work by the MacInnes [7] group revealed that C57Bl/6J mice were relatively unaffected by doses of alcohol that seriously interfered with the performance of DBA/2J mice.

Along these lines, the best evidence indicating the initial sensitivity to alcohol is under genetic control, comes from the research on selectively bred mice for long or short sleep-time following a hypnotic dose of ethanol (4.1 grams/kilogram). After eighteen generations of selective breeding, McClearn *et al.* [9] found virtually no overlap in sleep-time between the long-sleep (LS) and short-sleep (SS) lines.

METHOD

Our laboratory has modified the "pencil test" utilized by Belknap, *et al.* [2] to test intoxication induced by CNS depressants. A detailed description of the technique has been submitted elsewhere [3].

An apparatus consisting basically of a metal bar (6.4 mm diameter) fixed between 2 metal plates and suspended 27 cm above an electrified grid was used to evaluate ethanol intoxication in mice. The day before assessment of ethanol intoxication, mice from three strains, ICR Swiss, DBA/2J, and

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C57Bl/6J, were trained to remain on the bar every 30 minutes for a total of 5 hours, resulting in 33 trials per mouse. Before training, each mouse was placed on the grid to determine the minimum threshold of shock to which it would respond by squeaking, jumping and withdrawing its paws. The same voltage, usually between 15–35 volts, for both plates and grid was used for each mouse every time it was placed on the bar thereafter. The method of scoring ethanol intoxication was similar to that of Belknap *et al.* [2]. A ten second holding time trial was used for each training session.

On the day after training, an individualized shock level was again determined for each mouse. The animals received an intraperitoneal injection of ethanol 4.5 to 12% v/v dissolved in saline and doses of ethanol ranged from 0.9 to 2.4 grams/kilogram of body weight. Each animal received .0263 ml ethanol solution/gram of body weight. The concentration of ethanol was adjusted for each dose; for example, animals receiving 1.5 grams/kilogram were given 7.5% ethanol v/v, while animals receiving 2.4 grams/kilogram received injection of 12% ethanol v/v.

Five minutes after injection, each animal was placed on the bar for three trials. Intoxication scores were obtained by subtracting up to ten, the number of seconds they remained on the bar from the number ten. Thus, short bar holding times resulted in high intoxication scores. The lowest score of the trials was recorded. The mice were also scored at intervals of 30 minutes following injection for 3 hours. At the third hour, the intoxication procedure was repeated in order to assess the possible development of short-term tolerance. Each group of mice received the same dose of ethanol as before and intoxication was subsequently scored.

RESULTS

Training

It was found that C57Bl/6J and DBA/2J mice usually remained on the bar when first placed there during the training sessions, and scored consistent zeros thereafter when not under the influence of ethanol. The ICR Swiss mice did not initially hold on the bar as well as the other two strains, but by the end of the training sessions, these animals scored consistent zeros. It was concluded that bar holding scores above zero following alcohol treatment were the result of ethanol intoxication.

In another study, it was demonstrated that trained and untrained mice did not significantly differ in their response to various doses of ethanol. In fact, a similar inhibitory dose—50% ($IS_{5.0}$)—was observed for ICR Swiss [3].

Ethanol Sensitivity

Figure 1 illustrates intoxication scores ($IS_{5.0}$) for the three strains of mice 5 minutes after the initial ethanol injection. The response of the ICR Swiss and DBA/2J strains exhibited an $IS_{5.0}$ of 1.4 grams/kilogram. The C57Bl/6J mice exhibited significant ($p<0.05$) less initial sensitivity to ethanol as compared to the other strains. The dose response curves of both the ICR and DBA/2J mice are to the left of the C57Bl/6J group. The $IS_{5.0}$ for the C57Bl/6J mice was 1.7 grams/kilogram.

Recovery Bar Holding Response

Our studies also demonstrate the C57Bl/6J mice recovered from the effects of ethanol more rapidly than ICR and DBA/2J mice. Figure 2 illustrates the time course for the recovery of bar holding ability following an injection of 1.8 grams/kilogram in the three strains. The bar holding ability of all C57Bl/6J mice recovered with 30 minutes from this dose of ethanol which was significantly less ($p<0.05$) than obtained in all ICR (>60 min) and DBA/2J mice (>60 min). In addition, all C57Bl/6J mice recovered bar holding response within 60 minutes at a dose of 2.4 grams/kilogram of ethanol.

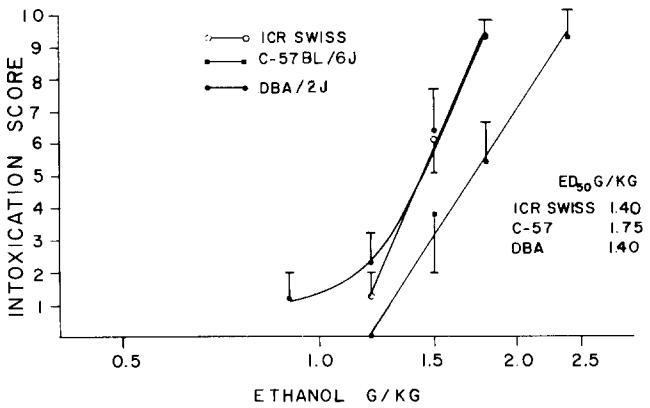

FIG. 1. Genotypic response to ethanol on bar holding time in three strains of mice, ICR Swiss ○——○, DBA/2J ●——● and C57 ■——■, five minutes after intraperitoneal injection of ethanol dissolved in saline. Abscissa indicates dose of ethanol in grams per kilogram of body weight. Bars illustrate the standard error of the mean. n=10 for each point.

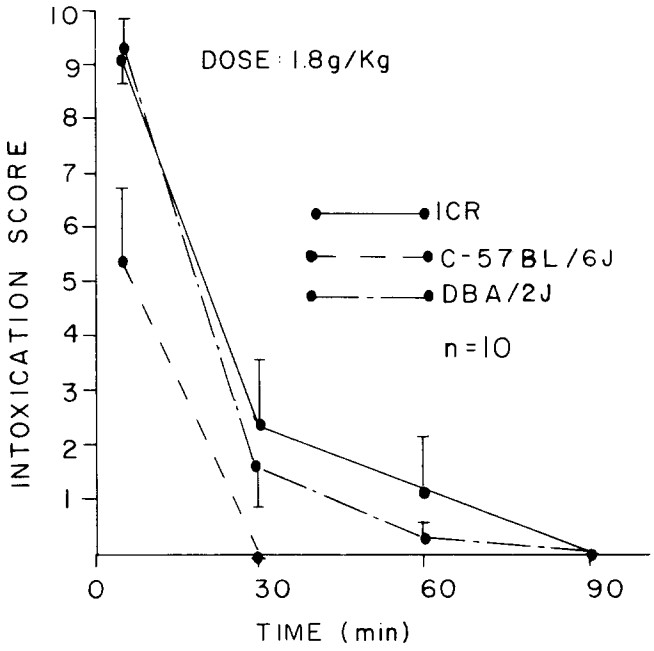

FIG. 2. Recovery of bar holding ability in three strains of mice, ICR Swiss ●——●, DBA/2J ●— - —●, and C57Bl/6J ●---● following intraperitoneal injection of ethanol dissolved in saline at a dose of 1.8 grams ethanol per kilogram of body weight. Abscissa indicates time in minutes. n=10 for each point.

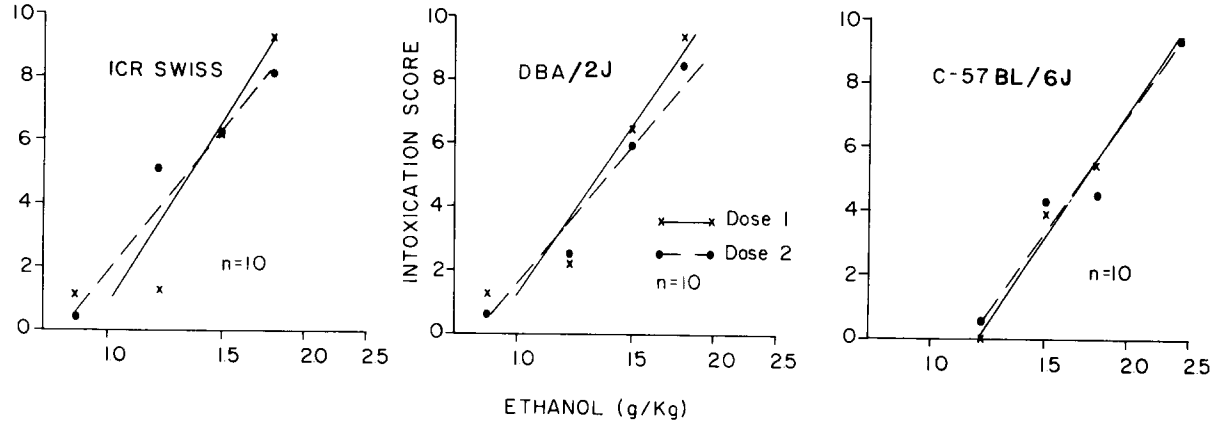

FIG. 3. Lack of single dose tolerance to ethanol in three strains of mice, ICR Swiss, DBA/2J and C57Bl/6J. Ordinates illustrate intoxication scores, abscissas illustrates doses of ethanol in grams ethanol per kilogram of body weight. Dose response curves illustrate response to first dose X—X, and response to the second dose ●---● in group of mice receiving two identical doses of ethanol spaced three hours apart.

Tolerance

Figure 3 depicts dose response curves to the first and second doses of ethanol given 3 hours apart in all three strains. It must be noted that all animals retested with a second dose of ethanol completely recovered ability to hold onto the bar. The response to both injections of ethanol was not significantly different, indicating that tolerance did not develop under these conditions and with relatively low ethanol doses.

DISCUSSION

The results of this systematic investigation utilizing three inbred strains of mice reveal that initial sensitivity to acute administration of rather low doses of ethanol may be a function of genotype. This is supported by the finding that C57Bl/6J mice were found to be less sensitive to ethanol's intoxicating action compared to both ICR Swiss and DBA/2J strains. This finding by itself is in agreement with the work of Kakihana *et al.* [6], who reported that the Balb/Crgl strain of mice slept over 3.5 times as long as did mice of the C57Bl/Crgl strain. In their study, C57Bl/Crgl strain regained the righting response ("time of awakening") when blood ethanol levels were considerably higher than the awakening time for the Balb/cCrgl strain. Other work by Danjanovich and MacInnes [4] showed difference in fall time between C57Bl/6J, DBA/2J and Balb/cJ which could be explained by differential rates of alcohol absorption and by differing rates of alcohol metabolism. However, the best evidence indicating that initial sensitivity to alcohol is under genetic control coming from the development of selective breeding experiments performed by McClearn and Kakihana [8]. They found that after 17 generations of selective breeding, the sleep-time of the short-sleeplines (SS) was 11 minutes compared to approximately 140 minutes for the long-sleep (LS). Furthermore, no differences in alcohol dehydrogenase and aldehyde dehydrogenase activity were detected, and *in vivo* rates of ethanol metabolism were similar in the two lines. These data taken together with our data of a rapid recovery time from ethanol in C57Bl/6J mice relative to ICR Swiss and DBA/2J strains, indicate neuronal sensitivity to intoxicating doses (low in our experiments) and to hypnotic doses (high in sleep-time experiments) may be under genetic control.

Our work shows that acute tolerance did not develop following a second full dose response to ethanol in the three strains of mice.

The findings of differential initial sensitivity to ethanol in various strains of mice utilizing this newly developed method to assess intoxication warrants further investigation with regard to other central depressants.

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