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# Initial Sensitivity and Acute Tolerance to Ethanol in the P and NP Lines of Rats[1]

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WALLER, M. B., W. J. McBRIDE, L. LUMENG AND T.-K. LI. *Initial sensitivity and acute tolerance to ethanol in the P and NP lines of rats.* PHARMACOL BIOCHEM BEHAV 19(4) 683–686, 1983.—We recently reported that selectively bred, alcohol-preferring (P) and alcohol-nonpreferring (NP) rats differ in sensitivity to a single sedative-hypnotic dose of ethanol, as measured by performance in the jump test. The present study examines the contributions of initial sensitivity and acute tolerance development to this difference. Initial sensitivity, assessed by brain alcohol content upon loss of the aerial righting reflex, was not significantly different between P and NP groups given 3 g ethanol/kg body weight intraperitoneally. Acute tolerance was indexed from blood alcohol concentrations (BAC) upon recovery of jumping performance following two successive ethanol doses. Practiced P and NP rats were required to jump 35 cm to a descending platform following the IP injection of 2.0 g ethanol/kg. The NP group took significantly longer (74 min) than the P (33 min) group whereupon $BAC_1$ of NP rats (234 mg%) was significantly lower than that of P rats (250 mg%). A second injection (1.0 g/kg) was given immediately after the animals reached the 35 cm criterion. Again, NP rats took significantly longer (124 min) than P rats (52 min) to jump 35 cm and $BAC_2$ of NP animals was lower (295 mg%) than that of P rats (343 mg%). The difference between $BAC_2$ and $BAC_1$, the measure of tolerance development, was significantly larger for P rats (90 mg%) than for NP rats (61 mg%). No significant differences in blood ethanol elimination were observed between the groups. The data indicate no difference in initial sensitivity between P and NP animals but that P rats develop acute tolerance more rapidly and/or to a greater degree than do NP rats. The results are consistent with a relationship in these selectively bred lines of rats between alcohol preference and the development of acute tolerance.

Alcohol-preferring rats  Initial sensitivity to ethanol  Acute tolerance to ethanol  Alcohol-nonpreferring rats

A NUMBER of studies in mice and rats have shown convincingly that there are genetic differences in sensitivity to the intoxicating effects of ethanol. Sensitivity, broadly defined as the responsiveness of an animal to a single, administered dose of ethanol, has been assessed with a variety of behavioral and physiological measures, and the extent of the observed differences appears to be not only species-specific but also test-specific. Among the methods employed, it is important to distinguish those that specifically measure impairment of function from those that measure recovery of function, because of the phenomenon of acute tolerance (tolerance occurring within the time course of a single session of testing). Tests that quantify change alone, e.g., brain ethanol content at loss of righting reflex or balance, might best be operationally defined as measures of "initial sensitivity" [14]. On the other hand, tests that quantify time of recovery and blood alcohol concentration (BAC) at time of recovery, e.g., in jump performance or "sleep time" (regain of righting reflex), may encompass elements of both initial sensitivity and acute tolerance. Differences in sensitivity to a single, administered dose of ethanol may, therefore, arise from a difference in initial sensitivity, acute tolerance development or both [15].

We reported that the selectively bred, alcohol-preferring (P) and alcohol-nonpreferring (NP) lines of rats differ in sensitivity to single, sedative-hypnotic doses of ethanol, as measured by the jump test [7]. Performance of the P rats recovered within a shorter period of time than that of the NP rats and BAC during the recovery phase was higher in the P than in the NP rats. There was no difference in alcohol metabolic rate between the lines. To what extent initial sensitivity and acute tolerance development contributed to the difference could not be assessed with the experimental design employed in that study. The experiments described here examine separately whether the P and NP rats differ in initial sensitivity or the rapidity of development of acute tolerance through measurements of brain ethanol content at time of loss of aerial righting reflex (ARR) and by comparisons of BACs at times of recovery of jumping performance following two doses of ethanol given in succession.

## METHOD

Adult, male P and NP rats weighing 250–350 g were housed individually in a temperature- and humidity-controlled environment with a 12 hr day-night cycle (8:00

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a.m.–8:00 p.m., light and 8:00 p.m.–8:00 a.m. dark). A standard solid laboratory diet (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL) and water were freely available throughout the experiment. The ethanol preference scores were determined for all animals at the end of the experiment. The preference testing procedure has been previously described [6].

A jumping apparatus similar to that described by Tullis *et al.* [17] was used to assess the acquisition of acute tolerance. Briefly, the apparatus is designed such that a rat placed on a grid floor must jump onto a descending platform to avoid or escape shock. The animals were given 10 days of training on the apparatus. The height jumped, measured from the top of the grid floor to the top of the platform by a permanently mounted meter stick, was determined when the animal had at least three paws grasping the top of the platform. By this criterion, all the rats jumped at least 45 cm on every trial at the end of the training period. Training and testing on the apparatus was performed between 8:00 a.m. and 3:00 p.m.

After the rats were trained, they were required to jump to a height of 35 cm following an intraperitoneal (IP) injection of 2.0 g ethanol/kg ($I_1$). We have previously established that P rats would need at least 30 min to jump 35 cm at which time blood and brain alcohol concentration are similar after the IP injection of ethanol [7]. When this criterion was attained ($T_1$), a blood sample for alcohol content ($BAC_1$) was drawn from the retro-orbital sinus [12]. An additional 1.0 g ethanol/kg was then administered intraperitoneally ($I_2$). The animals were again tested in the apparatus until they could jump 35 cm. At this time ($T_2$), another blood sample ($BAC_2$) was drawn and the experiment terminated. Additional blood samples were taken from the NP rats 33 and 52 min after their $I_1$ and $I_2$, respectively, even though they could not jump 35 cm. This was done to compare the BAC in the NP group with that of the P group which could jump the criterion height at these postinjection times.

Loss of the aerial righting reflex (ARR) was induced, in a separate group of P and NP animals, by the IP injection of 3 g ethanol/kg. One minute after the injection, each rat was placed on its back in a Plexiglas restrainer and dropped onto a towel suspended 37 cm below the restrainer. Each animal was retested every 15 sec thereafter and was judged to have lost the righting reflex when it could not right itself on three consecutive trials. At this time, the rat was immediately decapitated with a guillotine and the head promptly immersed in liquid nitrogen. The time between loss of righting reflex and immersion of the head in liquid nitrogen was kept to less than four sec.

Ethanol elimination curves were determined in different groups of P and NP rats (n=5/group). The double injection paradigm used in the tolerance test was followed and $I_2$ was given at 30 min. A BAC sample was drawn at 30 min, immediately before $I_2$ was given. Following $I_2$, four blood samples were drawn at 20 min intervals, following which four additional samples were taken at 30 min intervals. Thus a total of nine BAC determinations were made over an elapsed time of 230 min.

Blood samples were collected in heparinized capillary tubes. After centrifugation, the plasma fraction was sampled for ethanol content by direct injection into a Hewlett-Packard 5730A gas chromatograph equipped with a flame ionization detector and a 3380A integrator. The glass columns were packed with 50% Porapak Q and 50% Porapak R (100/120 mesh) and the oven temperature was 105°. n-Propanol was used as the internal standard. Brain alcohol content was determined as previously described [7] except that in the present study, the head was frozen *in toto* and the brain extirpated when the tissue was frozen to a soap-like consistency.

TABLE 1

BRAIN ETHANOL CONTENT OF MALE P AND NP RATS AT TIME OF LOSS OF AERIAL RIGHTING REFLEX (3 g ETHANOL/kg BODY WEIGHT, IP)

| | N | Body Weight g | Time min | Ethanol Content mg/g |
|---|---|---|---|---|
| P | 8 | 301 ± 17 | 2.53 ± 0.10* | 2.89 ± 0.17 |
| NP | 8 | 284 ± 12 | 2.13 ± 0.05 | 3.00 ± 0.15 |

Mean ± SEM.
*$p < 0.005$, P vs. NP.

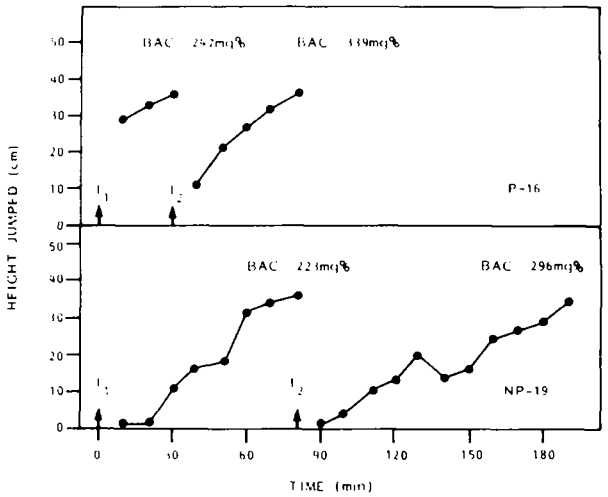


FIG. 1. Typical patterns of jumping performance for a P and NP rat. Each animal received 2.0 g ethanol/kg ($I_1$) at $T_0$ and 1.0 g ethanol/kg ($I_2$) when the jump test criterion (35 cm) was met. BAC samples were drawn from the retro-orbital sinus when the jump criterion was achieved after $I_1$ and $I_2$.

The results are expressed as mean values±SEM. Either an independent or a paired *t*-test was used to determine the statistical significance of the differences between the means.

## RESULTS

Both P and NP rats lost the ARR within 3 min of the IP injection of ethanol, 3 g/kg body weight. Although the NP rats responded more quickly than did the P rats, the brain alcohol content at the time of loss of ARR was not significantly different between the two groups (Table 1). Hence, the initial sensitivities of the P and NP rats are not different. The difference in the time of loss of ARR may indicate a small difference between the two groups in the initial rate of ethanol absorption and/or distribution.

Figure 1 compares the jumping performance of a P and an NP rat following the injection of two successive doses ($I_1$=2.0 and $I_2$=1.0 g/kg) of ethanol. The P rat recovered to the criterion level of 35 cm following $I_1$ in 30 min and BAC at this time ($BAC_1$) was 247 mg percent. $I_2$ was given immediately thereafter and recovery from the second dose took

TABLE 2

EFFECTS OF IP INJECTIONS OF ETHANOL, 2.0 g/kg FOLLOWED BY 1.0 g/kg, ON THE TIME TO JUMP TO A HEIGHT OF 35 cm AND ON THE BAC OF P AND NP RATS

| | Mean ± SEM | | | | |
|---|---|---|---|---|---|
| | $I_1$ (2.0 g/kg) | | $I_2$ (1.0 g/kg) | | |
| Group (N) | $T_1$ (min) | $BAC_1$ (mg%) | $T_2$ (min) | $BAC_2$ (mg%) | $BAC_2$-$BAC_1$ |
| P (10) | 33 ± 0.5 | 250 ± 5 | 52 ± 5§ | 343 ± 6§ | 90 ± 7 |
| NP (11) | 74 ± 6* | 234 ± 5* | 124 ± 4*§ | 295 ± 6*§ | 61 ± 5§ |

*$p$ < 0.001 and ‡$p$ < 0.01 and †$p$ < 0.05, P vs. NP with independent $t$; §$p$ < 0.05 with paired $t$, $T_2$ vs. $T_1$ and $BAC_2$ vs. $BAC_1$.

52 min ($T_2$). $BAC_2$ at time of recovery was 339 mg percent. In the NP rat, recovery to criterion from $I_1$ took 82 min ($T_1$) and $BAC_1$ was 223 mg percent. $I_2$ was given immediately thereafter and recovery to criterion from the second dose took 112 min ($T_2$). $BAC_2$ was 295 mg percent.

Table 2 summarizes the results of 10 P rats and 11 NP rats studied in the manner described above. The mean $T_1$ for the P rats was shorter ($p<0.001$) than that for the NP rats and the mean $BAC_1$ for the P rats was higher ($p<0.05$) than that for the NP rats. At the time that the P group had reached criterion after $I_1$ ($T_1$=33 min), the NP rats were still far below. However, the mean BAC value for the NP group at this time, 266±8 mg%, was not statistically different from the $BAC_1$ of the P group, 250±5 mg% (Fig. 2).

Following the second dose of ethanol ($I_2$), $T_2$ for the P rats was again significantly shorter than that for the NP rats and the mean $BAC_2$ for the P animals was again significantly higher than that for the NP rats (Table 2). Comparison of the BACs obtained 52 min after their respective $I_2$ again revealed similar values for the P and NP groups, 345±6 and 343±9 mg%, respectively (Fig. 2). $BAC_2$-$BAC_1$, taken as the measure of development of tolerance, indicates that both the P and NP rats had developed tolerance, but that the P animals developed tolerance to a greater degree and/or did so more quickly than did the NP rats.

Ethanol elimination curves were also determined in the P and NP animals following the double injection schedule. $I_2$ was given at 30 min after $I_1$ in both the P and NP animals and BACs were measured until a total of 230 min had elapsed (Fig. 2). No difference was discerned in blood ethanol elimination between the P and NP lines.

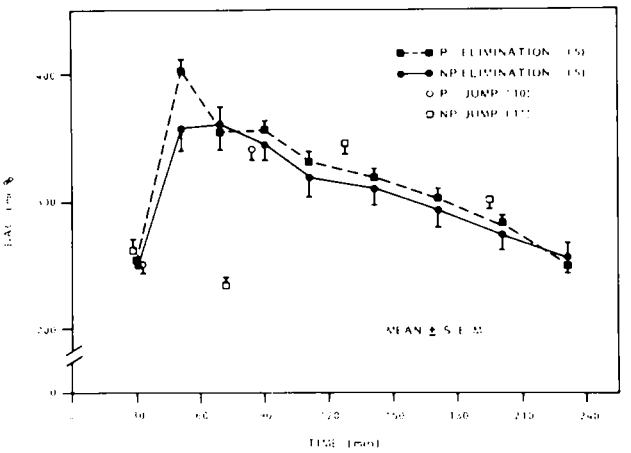


FIG. 2. Comparison of blood alcohol concentrations during an ethanol elimination study and during the jump test. In the blood ethanol elimination study, the P and NP rats received 2.0 g ethanol/kg at zero time and 1.0 g/kg at 30 min. In the jump test, blood samples were drawn when the jump criterion was achieved after $I_1$ and $I_2$. This occurred at 33 min and 85 min in the P rat and at 74 min and 198 min in the NP rat. Additional blood samples were taken from the NP group at 33 min and 126 min (52 min after $I_2$) for comparison with the P group.

## DISCUSSION

Studies in inbred strains of mice and selectively bred lines of rats have revealed a relationship between alcohol preference (or voluntary oral ethanol consumption) and sensitivity to ethanol. (As described in the Introduction, sensitivity to ethanol is defined as a response to alcohol occurring within the time course of a single session of testing, which encompasses both initial sensitivity and acute tolerance.) In inbred strains of mice, the alcohol-preferring C57BL strain exhibits a shorter sleep time following the administration of a hypnotic dose of ethanol than do the alcohol-nonpreferring DBA and BALB strains [11]. In rats selectively bred for alcohol preference, both the AA (alcohol-preferring) and ANA (alcohol-nonpreferring) lines raised in the Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki, Finland [1] and the P and the NP lines developed in our laboratory [4] exhibit differences in sleep-time, the hypothermic response to ethanol and duration of performance impairment [5, 7, 10, 13]. Both alcohol-preferring lines are less sensitive to the actions of ethanol than their corresponding nonpreferring lines.

Tabakoff and coworkers have shown that the sleep-time differences in mice may be caused predominantly by strain or line differences in initial sensitivity or acute tolerance development. In the C57BL and DBA strains, the former develops acute tolerance whereas the latter does not [15]. By contrast, both the selectively bred SS (short-sleep) and LS (long-sleep) lines of mice do not develop acute tolerance and the difference in sleep time can be entirely attributed to a difference in initial sensitivity [16]. In this regard, it is particularly significant that correlational studies in HS (heterogenous stock) mice have revealed a positive association between voluntary ethanol consumption (preference) and the acquisition of acute tolerance. It was suggested that

as much as 30 to 35 percent of the variance in voluntary ethanol consumption may be predicted by the acquisition of tolerance or vice versa. Interestingly, these measures did not appear related to "initial sensitivity" [2]. However, in this study, the regain of balance following the first dose of ethanol was employed as the measure of initial sensitivity. As already discussed, interpretation based on this measure alone can be confounded by the occurrence of rapidly developing acute tolerance. Nonetheless, the established difference in ethanol drinking preference of the C57BL and DBA strains [9], their initial sensitivity as measured by the loss of righting reflex [15], and their ability to develop acute tolerance [15] are consistent with the conclusions of the HS study.

Since the P and NP rats were developed from the bidirectional selection for ethanol drinking preference, it was of interest to discern whether or not the same or similar relationships as those seen in the HS, C57BL and DBA mice pertain in these rat lines. As measured by the brain ethanol content at time of loss of ARR, the P and NP rats do not appear to differ in initial sensitivity (Table 1). However, in the jump test, their $T_1$ and $BAC_1$ values differed significantly, suggesting that tolerance was developing in the P rats during this time period or that both the P and NP rats were developing tolerance, but at different rates and/or to a different degree (Fig. 1, Table 2). The results obtained with the second dose of ethanol indicate that, indeed, both lines developed tolerance, but the P line developed tolerance more rapidly and/or to a greater degree than did the NP lines (Table 2). The blood ethanol elimination curves of P and NP rats do not differ from each other either after a single 2 g/kg dose of ethanol [7] or after two successive doses, 2 g/kg followed 30 min later by 1 g/kg (Fig. 2).

In the studies with the heterogeneous stock and inbred mice, the alcohol-preferring mice showed little or no capacity for acute tolerance development [2,15]. Thus, while the relationship between acute tolerance development and voluntary ethanol consumption here demonstrated with the P and NP rats is, in the main, consistent with that seen in mice, they are not identical. This is perhaps not surprising in view of the probability that each of these and other alcohol-related traits is polygenic in nature and they share only subsets of the genes [8]. Alternatively, the associations may be fortuitous. This possibility is of concern with both inbred and selectively bred lines. The trueness of genetic correlations must be tested using genetically segregating offspring derived from phenotypically different parental lines.

Comparisons of this study with our earlier report [7] also revealed that both P and NP rats recovered more rapidly in this study than in the previous one. The animals employed in the previous study were older and heavier (350–400 g) than those used in the present study. The data suggest that perhaps older animals are less able to develop acute tolerance than younger ones. This relationship is currently under study. Conceivably, a closer correspondence between the data from this study and those in the mice could have been obtained if older P and NP rats had been employed.

Finally, it should be noted that, although the rapid acquisition of tolerance may be a factor that facilitates sustained high ethanol intake, it may have little or no mechanistic importance with regard to the ethanol drinking behavior of the animals. With free-choice drinking, the BACs of the P rats [4] and the C57BL mice [3] never approach those experimentally produced in studies of acute tolerance. However, it is of interest that low dose (0.0625 to 0.5 g/kg) ethanol has been found to produce stimulation in the P, but not in the NP, rats as measured by spontaneous motor activity [18]. The reinforcing actions of ethanol in drinking behavior should perhaps be sought in this and other effects of low dose ethanol.

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