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Persistence of Tolerance to a Single Dose of Ethanol in the Selectively-Bred Alcohol-Preferring P Rat[1]

G. J. GATTO, J. M. MURPHY,[2] M. B. WALLER, W. J. McBRIDE, L. LUMENG AND T.-K. LI

Departments of Psychiatry, Biochemistry and Medicine
The Institute of Psychiatric Research
and The Regenstrief Institute
Indiana University School of Medicine
and The Richard L. Roudebush Veterans Administration Medical Center
Indianapolis, IN 46223

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GATTO, G. J., J. M. MURPHY, M. B. WALLER, W. J. McBRIDE, L. LUMENG AND T.-K. LI. *Persistence of tolerance to a single dose of ethanol in the selectively-bred alcohol-preferring P rat.* PHARMACOL BIOCHEM BEHAV **28**(1) 105–110, 1987.—The persistence of tolerance to a single dose of ethanol was examined in the selectively-bred alcohol-preferring P line of rats. Tolerance was measured by a test that required trained rats to jump onto a descending platform to avoid footshock. On day 0, each trained rat received a single IP injection of 2.5 g ethanol/kg body weight and was tested every 15 minutes for recovery to a criterion of 75% of pre-alcohol training performance. The second ethanol injection of 2.5 g/kg and testing were carried out seven days later for one group (n=12), and 14 days later for another group (n=12). Tolerance was assessed by the differences in time required to recover to criterion performance and blood alcohol concentrations (BACs) at time of recovery on day 0 vs. day 7 and day 14. The mean recovery times and BACs on day 0 were 156±5 minutes and 222±6 mg%, respectively. The group injected on day 7 exhibited shorter recovery times of 113±4 minutes and higher BACs at recovery of 261±4 mg%, while the group injected on day 14 did not show any significant differences from the values obtained on day 0. In a second experiment, the persistence of tolerance in P rats was compared with that of rats from the alcohol-nonpreferring NP line and of stock Wistar rats (n=6/group). All rats were trained and tested for recovery to criterion after 2.5 g ethanol/kg on day 0 as described for the first experiment. The rats were then injected with ethanol and tested for tolerance on three subsequent occasions. For the P rats, injections were administered after ethanol-free periods of 7, 10 and 14 days, while 3, 7 and 14 day intervals were used for the NP and Wistar rats. The P rats still exhibited tolerance 7 and 10 days (but not at 14 days) following the first dose of ethanol. The NP and Wistar rats, by contrast, showed no significant differences in either recovery times or BACs at time of recovery when injected and tested 3, 7 or 14 days apart. The findings demonstrate that the persistence of acute behavioral (neuronal) tolerance after even a single ethanol exposure is under genetic control and suggest a positive association of this persistence with alcohol drinking preference.

Alcohol preference P and NP rats Acute ethanol Persistence of tolerance

INBRED strains and selected lines of experimental laboratory animals have been raised over the years that exhibit extremes of genetic variability in a number of responses to ethanol [2, 18, 19, 22–24]. In our laboratory, we have selectively bred two lines of rats that differ greatly in their voluntary consumption of ethanol [11]. The P line, in the presence of food, water, and 10% (v/v) ethanol ad lib, voluntarily consumes greater than 5 g of ethanol/kg body wt./day. Under identical testing conditions, the NP line exhibits an aversion for the 10% alcohol solution. The study of innate differences in the effects of ethanol between the P and NP lines provides an approach to discovering potential biological and genetic factors that contribute to alcohol-seeking behavior.

Previous studies have shown that P and NP rats differ not only in alcohol drinking preference, but also differ in other alcohol-evoked behaviors. Compared with the NP rats, the P

[1]Supported by HHS AA-03243
[2]Requests for reprints should be addressed to James M. Murphy, Institute of Psychiatric Research, Indiana University School of Medicine, 791 Union Drive, Indianapolis, IN 46223.

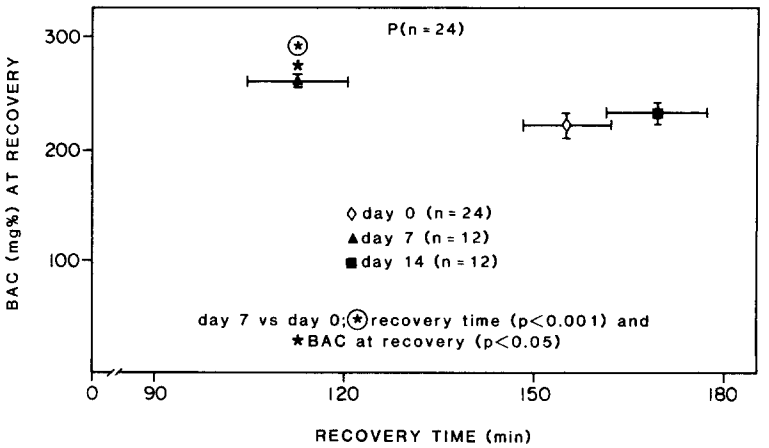

FIG. 1. Blood alcohol concentrations (BAC) and recovery times in minutes of P rats on the jumping test. Ethanol (2.5 g/kg body wt.) was administered 7 or 14 days after day 0.

rats are less sensitive to the hypothermic effects of ethanol and to a single sedative-hypnotic dose of ethanol, as measured by performance in a shock-motivated avoidance task [12]. The P, but not NP, rats exhibit an excitatory response to low doses of ethanol [30], and the lines differ in the steady-state concentrations of certain monoamines in several brain regions [15]. Additionally, while both P and NP rats have the capacity to develop acute tolerance to the depressant effects of ethanol, tolerance appears more rapidly and/or to a greater degree in the P rats than in the NP rats [29]. These latter studies with the P and NP rats [29] are in agreement with results obtained with HS/Ibg mice [4] and the selectively-bred AA and ANA rats [13]. The combined results of the three studies are consistent with a positive association between voluntary ethanol consumption and the acquisition of acute tolerance [4]. However, whether the rate of dissipation of acute tolerance relates inversely with alcohol preference has not been demonstrated. Therefore, the present experiments were undertaken to examine whether the time required for dissipation of tolerance developed to a single sedative-hypnotic dose of ethanol differs between the P and NP rats.

METHOD

Animals

Male Wistar rats (Harlan Industries, Inc., Indianapolis, IN) and P and NP rats from the S23 generation, weighing 350–500 g, were housed in a temperature- and humidity-controlled environment with a 12 hour day-night cycle beginning at 0600 hours (lights on). The selectively bred P and NP lines originated from a randomly bred Wistar colony at the Walter Reed Army Insitute of Research [10]. Standard solid laboratory food (Purina Lab Chow No. 5001) and water were freely available throughout the experiment unless otherwise noted.

Assessment of Tolerance

A descending jumping platform described in detail previously [12, 26, 29] was used to assess behavioral tolerance. Briefly, a rat is placed on the grid floor of the apparatus and is trained to avoid a 0.5 mA constant-current AC scrambled footshock by jumping onto a platform descending at a rate of 1 cm/sec. After ten days of training, all rats avoided the shock and jumped to a criterion of 50 cm on every trial. Training and subsequent testing on the apparatus were performed between 1200–1700 hr.

After the rats were trained, they were required to jump to a test criterion of 37.5 cm (75% of the pretest baseline height) following a single intraperitoneal (IP) injection of 2.5 g ethanol/kg body weight (day 0). The ethanol was given as a 12 g% solution in saline. A dose of 2.5 g ethanol/kg was chosen since a previous study demonstrated that this amount produced approximately the same degree of impairment in both P and NP rats within an experimentally reasonable recovery time [12]. Prior to the administration of ethanol, food but not water was removed at 0600 hours. Also at this time, rats received an additional five training trials on the jumping apparatus. Following the injection of ethanol, rats were tested every 15 minutes until they could jump to the criterion height of 37.5 cm. At this time, a blood sample was drawn from the retro-orbital sinus [20] and its alcohol content (BAC) was determined as previously described [12,29].

In the first experiment, alcohol-naive P rats were divided into two groups (n=12/group) following the initial testing on day 0. One group was retested seven days later, while the second group was retested 14 days later. Both groups were subjected to the same testing conditions as on day 0. The first and second injections of ethanol were delivered in different and novel environments. Alcohol preference drinking scores were determined for the animals at the end of the experiment, using a previously published procedure [11].

A second experiment was conducted to (a) compare the persistence of tolerance in P, NP and stock Wistar rats (n=6/group), (b) determine if tolerance was present in the NP group at an interval shorter than 7 days, and (c) obtain a better estimate of the length of time tolerance persisted in the P rat. All three groups were subjected to the same paradigm up to and including day 0 as described in the first experiment. Following day 0, all rats were tested three more times on the apparatus. The sequence of ethanol administration and testing for the P rats after the first ethanol injection on day 0 was as follows: the second injection, 10 days later (day 10); the third injection, an additional seven days later (day 17); and the fourth injection, an additional 14 days later (day

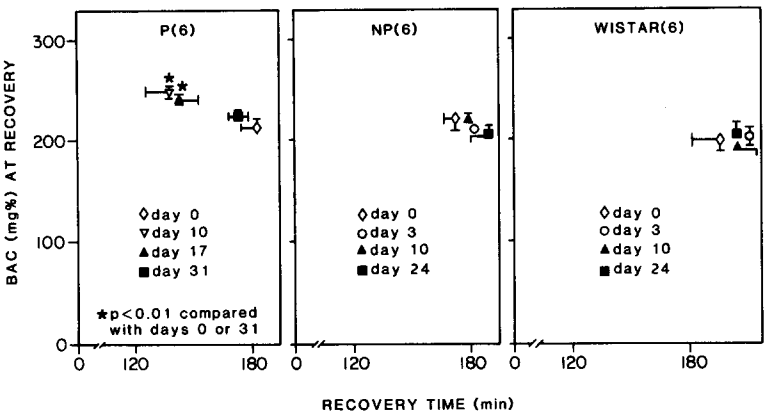

FIG. 2. Blood alcohol concentrations (BAC) and recovery times in minutes for P, NP and Wistar rats on the jumping test following an ethanol injection of 2.5 g/kg body wt. given after consecutive ethanol-free intervals of 10 (day 10), 7 (day 17) and 14 (day 31) days for the P rats and 3 (day 3), 7 (day 10) and 14 (day 24) days for the NP and Wistar rats.

31). The sequence for the NP and Wistar rats after the first injection on day 0 was: the second injection, three days later (day 3); the third injection, an additional seven days later (day 10); and the fourth injection, an additional 14 days later (day 24). The rats were not tested in the jump apparatus between testing days. Again, to minimize environment-dependent learning [25], no two ethanol injections were administered in the same environment. The P and NP rats used in the second experiment had been tested for alcohol preference when they were nine weeks of age, but had been alcohol-free for at least two months prior to this study.

Statistical Analysis

The results are expressed as mean values ±SEM. A paired-*t* was used for within group comparisons (i.e., to compare the performance of the same animal at two different occasions), while a repeated measure ANOVA and Newman-Keuls post-hoc tests were employed for multiple comparisons.

RESULTS

The relationship between mean recovery time and mean BAC at time of recovery for the 24 P rats in the first experiment is shown in Fig. 1 The time to recover and BAC on day 0 were 156±7 minutes and 222±6 mg%, respectively. Compared with day 0, the group injected and tested 7 days later exhibited a shorter recovery time, 113±7 minutes ($p<0.001$), and higher BAC at time of recovery, 261±4 mg% ($p<0.05$). The group injected 14 days later demonstrated no significant difference in recovery times (169±9 minutes) or BACs (225±4 mg%) when compared with day 0. When subsequently tested for the free-choice consumption of 10% (v/v) ethanol in the presence of food and water [11], the P rats used in this experiment consumed 6.8±0.4 g ethanol/kg/day.

The relationship between mean recovery times and BACs at time of recovery for the P, NP and Wistar rats in the second experiment are shown in Fig. 2. The recovery times on day 0 for the P, NP and Wistar rats were 185±10, 175±8 and 205±16 minutes, respectively, and the BACs at time of recovery were 217±10, 218±6 and 202±11 mg%, respectively. The times of recovery and the BACs at time of recovery on day 0 were not significantly different among the three groups. The P rats were injected and tested on days 10, 17 and 31 after day 0, i.e., after ethanol-free intervals of 10, 7 and 14 days, respectively. Compared with day 0, the P rats injected with alcohol 7 and 10 days apart exhibited significantly shorter recovery times of 144±9 and 138±12 minutes, respectively, and significantly higher BACs at time of recovery of 243±5 and 244±4 mg%, respectively, $Fs(3,15) \geq 10.89$, $p<0.001$; Newman Keuls, $p<0.05$. However, after an interval of 14 days between injections (day 31), the recovery time (175±5 minutes) and BAC (222±4 mg%) for the P rats were not significantly different from day 0.

The NP and Wistar rats were injected and tested on days 3, 10 and 24 after day 0, i.e., after sequential intervals of 3, 7 and 14 days, respectively. The NP and stock Wistar rats did not differ significantly from the day 0 recovery time or BAC at time of recovery at any of the intervals (Fig. 2).

Some representative daily jumping performances during the test sessions for the P, NP and Wistar groups from the second experiment are shown in Fig. 3. In comparison with day 0, it is evident that the P rats showed better performance at almost all time points when injections were given ten days apart. The jumping performance of the NP rats was better in the first two hours of testing on day 3 compared with day 0. However, the NP rats still required approximately the same amount of time to jump to the criterion of 37.5 cm on day 3 as they did on day 0. When the interval was seven days between injections, the jumping performance of the NP rats was not different from their performance on day 0 at any time point. The Wistar rats did not differ in testing performance between day 0 and any of the subsequent test sessions, as exemplified by the results shown for day 3 (Fig. 3).

The P and NP rats used in the second experiment were tested for alcohol preference at nine weeks of age, two months prior to this study. The P rats voluntarily consumed 7.5±0.2 g ethanol/kg/day, whereas the NP rats consumed 0.4±0.1 g/kg/day. The Wistar rats were not tested and were alcohol-naive except for the ethanol injections given during the course of the second experiment.

DISCUSSION

We have shown previously that the alcohol-preferring P rats develop acute, within-session tolerance more rapidly

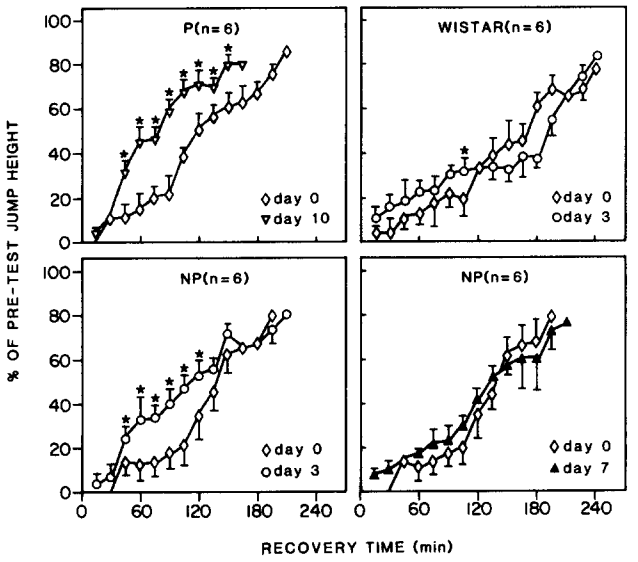

FIG. 3. Jumping performance as a function of time when the interval between ethanol injections was 10 days for P rats, 3 days for Wistar rats, and 3 or 7 days for NP rats. (*$p<0.05$ compared with day 0).

than do the alcohol-nonpreferring NP rats [29]. The present study has extended this earlier observation. Using a slightly higher dose of ethanol (2.5 vs. 2.0 g/kg) the present data demonstrate that the acute tolerance which the P rat develops to the first single dose of ethanol is still evident at 7 and 10 days and only dissipates by 14 days (Figs. 1–3). By contrast, the NP rats and heterogeneous stock Wistar animals fail to exhibit persistence of tolerance when tested 3 days after a single dose of ethanol (Fig. 2). It is likely that these two processes, i.e., the rapid acquisition of acute tolerance [29] and its persistence over many days, may have important bearing on the high voluntary alcohol drinking exhibited by the P rats. To date, we have tested only high doses (2.0 to 2.5 g/kg) of ethanol in the development and persistence of acute tolerance. It is not known whether lower doses (e.g., 0.5 g/kg) of ethanol can also produce prolonged acute tolerance in P rats. Studies are in progress to examine these relationships.

Although the possibility exists that the heightened capacity of the P rats to retain tolerance to a single dose of ethanol for a substantially longer period of time than the NP rats may be a fortuitous association with selective breeding for alcohol preference, it is more attractive to entertain the hypothesis that this characteristic may be mechanistically related to the high volitional intake of ethanol characteristic of the P rat [5,28]. We have reported several other genetically influenced behaviors which differentiate the P from the NP rat and these, too, may contribute to the high alcohol-seeking behavior of the P rat. Unlike the NP rats, the P rats find the pharmacological effects of ethanol rewarding [27], they are less sensitive to the sedative hypnotic effects of ethanol [12], and they exhibit locomotor stimulation to low doses of ethanol [30]. We postulate that the combination of the rewarding and stimulating effects of ethanol along with the persistence of acute tolerance may be important mediating factors in the high volitional intake of ethanol by P rats. The unusually low voluntary consumption by NP rats may be due to an innate sensitivity to the aversive actions of ethanol as well as to the failure to maintain acute tolerance over time. It would be interesting to test the validity of these mechanistic factors in man.

A number of studies have now indicated that ethanol preference may be related to a lower brain content of serotonin [7, 14, 15, 17, 33]; serotonin uptake inhibitors have been shown to reliably decrease alcohol intake in rats [1, 3, 16, 21]. Moreover, one of the most robust findings in studies on the P and NP lines has been that the P rats have a lower serotonin content in forebrain regions than the NP rats [15], resulting in an apparent up-regulation of serotonin receptors [32]. Some experimental studies on chronic tolerance indicate that depletion of brain serotonin delays the development of tolerance and accelerates its loss [8,10]. Although these findings appear inconsistent with the results obtained for the P and NP rats, it remains to be determined whether the acute tolerance seen in the present study involves the same mechanisms as chronic tolerance. Also, it is quite possible that sudden, drastic experimental reductions in brain serotonin may produce a qualitatively different effect on the functioning of serotonin systems than does the chronically lower content of 15–20% [15].

Variables other than genetic factors can influence tolerance, including age and a history of prior exposure to ethanol [6,9]. In the present study, there were no apparent effects of these two variables. The younger and lighter P rats used in the first experiment showed no difference in the persistence of tolerance when compared with the older rats used in the second experiment. Experiment 1 was the better controlled, since the rats were naive to alcohol and were tested only once for the persistence of tolerance. Owing to the limited supply of the P and NP rats, a different design was used for the second experiment. Sequential injections were given with varying intervals between injections. Also, the rats had been pretested for alcohol preference, but were alcohol-free for at least two months before the start of the experiment. Since the data from Experiments 1 and 2 are in agreement, the results for the second experiment apparently were not confounded by the sequential testing design or the history of prior exposure to alcohol drinking.

An examination of the performance on the jumping task as a function of time (Fig. 3) indicated that the NP rats did exhibit some persistence of acute tolerance for at least three days if the criterion for recovery was lowered to 50% or less of the pretest jump height. No tolerance was evident at seven days regardless of criterion. Although fatigue could have contributed to the failure of the NP rats to attain the 75% criterion after three days, this seems unlikely, since, in a previous study, both the P and NP rats had demonstrated tolerance even when a second injection of ethanol was given immediately following recovery from the first injection [12].

The marked difference in the dissipation of acute tolerance between the P line and two other groups of rats is not likely due to the development of conditioned tolerance (state-dependent learning produced by testing animals in the jump apparatus each time ethanol was injected). For one, there is no evidence of tolerance in the stock rat with an interval of 3 days between ethanol injections. The P and NP rats were originally derived from a colony of Wistar rats [11] and thus both lines might be expected to perform as stock rats in a behavioral test which does not relate to their selective breeding for alcohol preference or nonpreference. Thus, the NP rats, which have daily alcohol drinking scores that are closer to the drinking scores of stock animals than values

for P rats, also do not clearly show tolerance with a 3-day interval between injections (Fig. 2). Secondly , other studies have shown that learned tolerance in stock rats did not appear to develop until after a third injection of ethanol (2.0–2.2 g/kg) was given when the interval between injections was 4 days [31]. Third, in Experiment 2, there was no difference in jumping performance of the P rats between 10 days and 7 days even though the data obtained for day 7 was the third consecutive injection. Perhaps, if conditioned tolerance was a factor in the performance of the P rat then shorter recovery times should have occurred with subsequent injections. Finally, in Experiment 2, the performance of the P rat following the fourth injection given at an interval of 14 days was the same as the first injection on day 0. If conditioned tolerance was a major factor for the improved jumping performance (at day 10 and 7), then perhaps a complete loss of this improvement might not be expected to occur on day 14 after a fourth injection. Although the present experimental design does not completely rule out the retention of the learned behavior for a longer time by the P rat, the data do not favor this as a major factor. Furthermore, even if this were the case, it would still indicate a unique response to ethanol by the P rats.

In summary, the present study demonstrates that the selectively-bred alcohol-preferring P rats have a greatly enhanced capacity for retention of tolerance to a single hypnotic-sedative dose of ethanol as compared with the alcohol-avoiding NP rats or heterogeneous stock Wistar rats. These findings indicate that this persistence of tolerance is influenced by genetic factors and provide additional support for a positive relationship between acute tolerance and high volitional intake of alcohol [4, 13, 29].

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