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Does Tolerance Develop to the Activating, as Well as the Depressant, Effects of Ethanol?

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TABAKOFF, B. AND K. KIIANMAA. *Does tolerance develop to the activating, as well as the depressant, effects of ethanol?* PHARMAC. BIOCHEM. BEHAV. 17(5) 1073–1076, 1982.—Genetically determined differences were demonstrated in the response of mice to low doses of ethanol. Ethanol (1.35 g/kg) produced an increase in locomotion in DBA/2 and BALB/c mice, but did not alter the locomotor activity of C57Bl/6 mice. Chronic administration of ethanol produced tolerance to the sedative/hypnotic effects of high doses of ethanol in DBA/2 and BALB/c mice, but the equivalent chronic ethanol administration paradigm produced no tolerance to the activating effects of ethanol in these animals. C57Bl/6 mice became tolerant to the hypnotic effects of ethanol, but no change in the behavior of these mice, given a low dose of ethanol, was noted after the mice were withdrawn from chronic feeding with ethanol-containing diets. The results indicate the presence of different mechanisms for tolerance development to the activating and depressant effects of ethanol, and indicate that strain-dependent differences in the activating effects of ethanol are not determined by an animal's greater sensitivity to the sedating effects of this drug.

Activation Ethanol Genetics Sedation Tolerance

ETHANOL is characterized as a depressant of CNS function, but its spectrum of action has been shown to include an excitatory component (see [11] for review). The mechanism by which ethanol produces behavioral excitation is yet unknown, but two theories have been put forth to explain this phenomenon. The first proposal suggests that ethanol may, at lower doses, directly activate certain neurons, and at higher doses, depress neuronal activity [12]. The second proposal suggests that the excitatory aspects of ethanol's action result from neuronal disinhibition produced by ethanol's suppression of the activity of inhibitory neuronal systems [14]. If ethanol is acting solely to depress neuron activity, one may expect that the tolerance which develops to the depressant effects of ethanol would also be reflected in changes in the activating effects of this drug. A number of investigations have demonstrated the development of tolerance to the sedative or depressant effects of ethanol (see [15] for review), but little work has appeared regarding the development of tolerance to ethanol's stimulating effects. Only two studies [8,4], to our knowledge, have addressed this issue, but quite opposite conclusions have been derived from these studies.

Mazur and Boerngen [8] have concluded from their studies with mice that chronic ethanol administration produces tolerance to the depressant effects of ethanol, but not to the locomotor-stimulating effects of ethanol. As tolerance to ethanol's depressant effects developed in their animals, the behavior-stimulating effects of ethanol became more pronounced, and the authors suggested that, "As tolerance develops to the depressant component, excitatory responses are uncovered." These authors, therefore, classified ethanol with other drugs with which tolerance to depressant, but not stimulatory, effects could be demonstrated [2,7].

On the other hand, Hunt and Overstreet [4] found that rats fed a liquid diet containing ethanol for several weeks, developed tolerance to both the stimulatory and incoordinating effects of ethanol in a parallel fashion and postulated that a similar mechanism may underlie the development of tolerance to the activating and depressant effects of ethanol.

These dichotomous findings indicated a need for further examination of tolerance development to the stimulatory and depressant effects of ethanol for the purpose of clarifying whether tolerance develops to both these ethanol effects simultaneously. Recent studies in our [10] and other laboratories [3,6] have also shown that tolerance to ethanol may, under certain conditions, be a learned compensatory response to the physiologic effects of this drug, but under alternative conditions, could develop in paradigms where learning would play a minimal role [16]. The prior studies did not consider this issue, and did not consider the fact that the genetic composition of the experimental subjects may influence the results obtained in studies of tolerance.

We performed our studies with three strains of mice

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TABLE 1

EFFECT OF AN ACUTE DOSE OF ETHANOL (1.35 g/kg, IP) ON THE LOCOMOTOR ACTIVITY IN C57Bl/6, DBA/2 AND BALB/c MICE

Strain	Treatment	
	Saline	Ethanol
C57Bl/6	1,235 ± 126 (14)	1,068 ± 93 (14)
DBA/2	691 ± 67 (8)	1,581 ± 102 (10)*
BALB/c	759 ± 89 (11)	1,388 ± 97 (10)*

Results are expressed as locomotor activity counts ±S.E.M. accumulated over a 30-minute monitoring period. Number of animals used to obtain each value is shown in parentheses.

*$p<0.001$, when compared to the saline-injected control (Newman-Keuls).

TABLE 2

DURATION OF LOSS OF RIGHTING REFLEX AFTER ADMINISTRATION OF AN ACUTE DOSE OF ETHANOL (3.5 g/kg, IP) IN C57Bl/6, DBA/2 AND BALB/c MICE

Strain	Duration of Loss of Righting Reflex (min)
C57Bl/6	37.5 ± 1.7 (30)
DBA/2	45.8 ± 1.7 (24)†
BALB/c	45.3 ± 2.5 (28)*

Results are expressed as mean ±S.E.M. Number of animals used to obtain each value is shown in parentheses.

*$p<0.05$; †$p<0.01$, when compared to C57Bl/6 mice (Newman-Keuls test).

(C57Bl/6, DBA/2 and BALB/c), and used an ethanol administration and tolerance testing paradigm in which conditioned compensatory responses could play but a minimal role.

METHOD

Male C57Bl/6, DBA/2 and BALB/c mice (21–25 g) were purchased from ARS/Sprague-Dawley (Madison, WI), and were housed five per cage in our laboratories (22° C, 12-hour light/dark cycle) for at least seven days with free access to Purina Lab Chow and water before they were used for experiments. Separate groups of animals were used for measuring initial sensitivity to ethanol and sensitivity to ethanol after consumption of the control liquid diet or the ethanol-containing liquid diet.

In order to study an animal's initial sensitivity to ethanol, we measured locomotor activity after a low dose of ethanol, and duration of ethanol-induced loss of righting reflex after a high dose of ethanol. The effect of ethanol on locomotor activity was studied by injecting the mice, IP, with 1.35 g of ethanol/kg. Ethanol solutions were administered in a volume of 0.2 ml/10 g of mouse and equal volumes of physiologic saline solutions were administered to control animals. Preliminary studies using doses between 0.8–3.0 g/kg demonstrated that the 1.35 g/kg dose of ethanol was most efficacious in stimulating locomotor activity in both DBA/2 and BALB/c mice. C57Bl/6 mice were not stimulated at any of the doses tested. Immediately after the injection of ethanol, the mice were placed individually in macrolon cages and their locomotor activity was recorded for 30 minutes using an electronic activity meter (Stoelting, Chicago, IL).

Ethanol hypnosis was induced by an IP injection of 3.5 g of ethanol/kg. The injected volume was 0.2 ml/10 g of mouse. The duration of hypnosis was defined as the time from the loss of the righting reflex to the time the righting reflex was regained. The mice were judged to have lost or regained their righting reflex when they could not or could, respectively, right themselves twice within 30 seconds after being placed on their backs.

Tolerance to the behavioral effects of ethanol was studied by testing the mice after chronic administration of ethanol to the animals. C57Bl/6 and BALB/c mice were fed a liquid diet containing 7% (v/v) ethanol, as previously described [13]. Because the DBA/2 mice did not accept the 7% diet, they were given a diet containing 5% ethanol. Control animals of the three strains received an equivalent diet in which sucrose in equicaloric quantities was substituted for the ethanol, and the quantity of the sucrose-containing diet consumed by the control mice was restricted to equal the daily intake of diet by the ethanol-consuming mice. After seven days of consuming the ethanol diet, the ethanol-consuming animals were given the sucrose-containing control diet for 24 hours and then tested for tolerance. For tolerance testing, the mice were administered a challenge dose of 1.35 or 3.5 g of ethanol/kg, and ethanol-induced changes in locomotor behavior or loss of righting reflex were monitored as described above.

Differences between the different strains and treatments were studies using the Student's t-test or analysis of variance, followed by the Newman-Keuls test.

RESULTS

Acute Effects of Ethanol

Locomotor activity. There was a significant difference among the three strains in the effect of ethanol on locomotion (strain and ethanol interaction: $F(2,61)=14.66$, $p<0.001$). An acute dose of ethanol (1.35 g/kg) stimulated locomotor activity significantly ($p<0.01$) in BALB/c and DBA/2 mice, but had no effect on the activity of C57Bl/6 mice (Table 1). As already noted, none of five different doses within the range of 0.8–3.0 g/kg had any stimulatory effect on the locomotor activity of C57Bl/6 mice.

Ethanol hypnosis. The data in Table 2 demonstrate that there also was a significant difference, $F(2,79)\times 5.54$, $p<0.01$, in the hypnotic effect of ethanol between the three strains. The duration of the loss of righting reflex in C57Bl/6 mice was significantly shorter than in BALB/c ($p<0.05$) and DBA/2 ($p<0.01$) mice. No difference in the duration of the loss of righting reflex was found between the latter two strains.

Development of tolerance. Although the DBA/2 mice received a diet which contained less ethanol, calculation of the absolute amount of ethanol consumed each day by these animals revealed that ethanol consumption by DBA/2 mice was only 18% less than the ethanol consumption of BALB/c and C57Bl/6 mice. The average daily ethanol consumption during the last three days of the seven-day treatment was found to be 33.8±1.0 g/kg (mean±SEM; n=34) for C57Bl/6 mice; 33.3±1.0 g/kg (n=41) for BALB/c mice; and, 27.6±0.5 g/kg (n=39) for DBA/2 mice.

Figure 1 demonstrates that BALB/c and DBA/2 mice did, but C57Bl/6 mice did not, show an increase in locomotor activity when a challenge dose of ethanol (1.35 g/kg) was

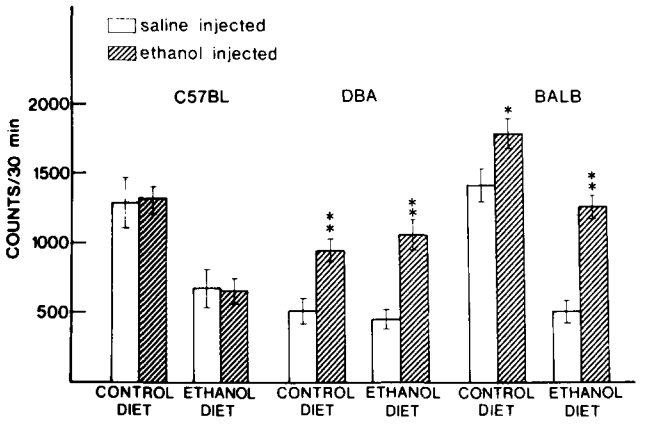

FIG. 1. Effect of a challenge dose of ethanol (1.35 g/kg, IP) on locomotor activity in C57Bl/6, DBA/2 and BALB/c mice treated chronically with ethanol for seven days. Locomotor activity was monitored for thirty minutes, and the mean accumulated activity counts ±SEM of 10–20 animals are given on the ordinate. The abscissa indicates the chronic dietary regimen which was administered prior to testing mice with a challenge dose of ethanol or saline. $*p<0.05$; $**p<0.01$, when compared to the corresponding saline-injected group (Newman-Keuls test).

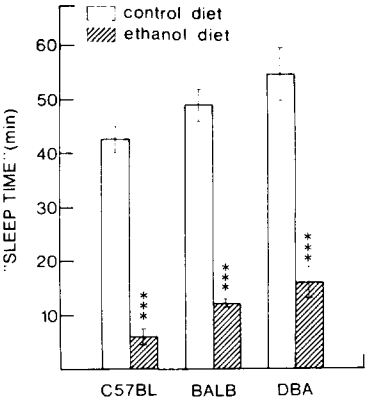

FIG. 2. Duration of loss of righting reflex induced by a challenge dose of ethanol (3.5 g/kg, IP) in C57Bl/6, DBA/2 and BALB/c mice. Animals received either a sucrose-containing (control) diet or an ethanol-containing diet for seven days prior to being tested with the 3.5 g/kg dose of ethanol. The duration of the loss of righting reflex (sleep time) was measured as described in the text and the results are expressed as the mean±SEM of values obtained from 6–13 mice. $***p<0.001$ (Student's t-test) when compared to the sucrose diet-fed (control) mice.

administered 24 hours after withdrawal of the ethanol-containing diet from the animals. The extent of the increase was comparable to the ethanol-induced increase in locomotion witnessed in chow-fed (Table 1) or control diet-fed (Figure 1) mice. Thus, neither the BALB/c nor the DBA/2 mice developed measureable tolerance to the locomotor activating effects of ethanol. The response of the C57Bl/6 mice to a challenge dose of ethanol was also not altered by the chronic ethanol treatment. The chronic ethanol treatment did lower spontaneous locomotor activity (measured after saline injection) in both the C57Bl/6 and the BALB/c animals (Fig. 1), but the effect of *ethanol* on locomotor activity in these mice was similar to the effect of ethanol in the control animals.

All three strains showed tolerance to the hypnotic effect of a high dose of ethanol (3.5 g/kg); the duration of ethanol-induced hypnosis in the mice treated chronically with ethanol was significantly shorter than in the chow-fed controls (Table 2), or control mice fed with the sucrose-containing diet (Fig. 2).

DISCUSSION

The present study substantiates numerous reports which indicate that the genotype of an animal influences the acute effects of ethanol [1]. A low dose of ethanol stimulated locomotor activity in BALB/c and DBA/2 mice, but had no effect on the activity of C57Bl/6 mice, suggesting the C57Bl/6 mice may not be sensitive to the activating effects of low doses of ethanol, or may be more sensitive to the depressant effects of ethanol than the other two strains. Greater sensitivity to the depressant effects of ethanol could mask the activating actions of ethanol in the C57Bl/6 mice. The results regarding the acute effect of ethanol on locomotor activity are partially in agreement with the findings of McClearn and Anderson [9], who reported an increase in open field activity of BALB/c mice and no change in the activity of C57Bl/6 mice after a 1.35 g/kg dose of ethanol. DBA/2 mice in our studies also demonstrated significant activation by ethanol at a dose of 1.35 g/kg, but McClearn and Anderson [9] did not note a significant activation in their DBA/2 mice. This discrepancy will require further analysis.

If one assumes that the inability of low doses of ethanol to activate C57Bl/6 mice is related to the greater sensitivity of these mice to the hypnotic effects of ethanol, the data in Table 2, and the work of other authors [5], would contradict this assumption. Given a hypnotic dose of ethanol, the C57Bl/6 mice regained their righting reflex significantly faster than did the BALB/c or DBA/2 mice. Thus, one cannot readily conclude that a greater sensitivity of the C57Bl/6 mice to the depressant effects of ethanol is masking the activating effect of this drug.

Our studies of the development of tolerance to the activating and depressant effects of ethanol also indicate that changes in an animal's sensitivity to the depressant effects of ethanol do not necessarily influence its response to the activating effect of this drug. Mazur and Boerngen [8] have suggested, from their studies with Swiss albino mice, that tolerance development to the depressant effects of ethanol would "unmask" ethanol's stimulatory effects. This was not found to be the case with the C57Bl/6 mice (Fig. 1). In addition, the stimulatory effects of ethanol in DBA/2 and BALB/c mice were not significantly accentuated after these mice developed tolerance to the hypnotic/sedating effects of ethanol.

A notable difference in experimental designs may account for the divergent results obtained in our present study and the study of Mazur and Boerngen [8]. Ethanol was administered, in the study by Mazur and Boerngen [8], by daily intraperitoneal injection and "tolerance" was also tested within the same paradigm of drug administration. The daily injection ritual within a consistent environment may have provided a situation wherein conditioning played an important role in the witnessed changes in ethanol's effects in an animal [3,10]. In the paradigm of chronic ethanol administration and tolerance testing used in our studies, learning or conditioning would be expected to play a minimal role and, therefore, the

differences between our results and those of Mazur and Boerngen [8] may be contingent upon the fact that the experimental designs predisposed the development of different types of ethanol tolerance (see [16] for further discussion).

Hunt and Overstreet [4], on the other hand, fed rats a liquid diet containing 6.5% v/v ethanol and measured ethanol-induced changes in locomotor activity and ethanol-induced incoordination after an IP injection of a test dose of ethanol. The experimental design used by Hunt and Overstreet [4] is, therefore, similar to the design of our studies. Results presented by Hunt and Overstreet [4] suggested a parallel development of tolerance to both the activating and incoordinating effects of ethanol, and if one equates the measures of incoordination used by these researchers to a measure of ethanol-induced sedation, one would conclude that tolerance to the sedative and activating effects of ethanol developed simultaneously under their experimental conditions.

The incoordinating effects of ethanol were measured by use of a "rotarod" apparatus, and this measure may be affected by changes in the rat's locomotor activity after ethanol is administered to the animal. Thus, if tolerance developed to the locomotor activating effects of ethanol, the diminished activation could, in itself, allow an animal to improve its performance on a rotating drum. Given this possibility, the issue of whether tolerance develops concurrently to the activating and sedating/depressant effects of ethanol would not be adequately resolved by the experiments performed by Hunt and Overstreet [4].

Our results (Figure 1) indicate that tolerance is not evident to the activating effects of ethanol within a period of chronic ethanol exposure that produces clear tolerance to the hypnotic effects of ethanol in DBA/2 and BALB/c mice. We would, therefore, contend that the mechanism by which tolerance develops to the sedative/hypnotic effects of ethanol is different from the mechanism by which tolerance develops to the activating effects of ethanol, and more extended periods of chronic ethanol treatment may be necessary to produce tolerance to ethanol's activating effects. Tolerance to the activating effects of ethanol may, however, not develop in mice, even if longer periods of ethanol administration are employed, and ethanol may actually fall into the class of drugs which do not produce tolerance to their activating effects while producing tolerance to their sedative properties.

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