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The Excitatory Effect of Ethanol: Absence in Rats, No Tolerance and Increased Sensitivity in Mice[1]

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MASUR, J., M. L. OLIVEIRA DE SOUZA AND A. P. ZWICKER. *The excitatory effect of ethanol: Absence in rats, no tolerance and increased sensitivity in mice.* PHARMACOL BIOCHEM BEHAV **24**(5) 1225–1228, 1986.—Three questions related to ethanol's stimulating effect (ESE) were studied. The first referred to the reported absence of tolerance to ESE in mice. It was determined whether tolerance would develop if the period of ethanol treatment were extended significantly beyond those normally found in the literature. No evidence of tolerance to ESE was found over a 5-month period of treatment. The second issue related to the possibility that mice not only do not develop tolerance but actually become more responsive to ESE after chronic exposure. A dose of ethanol that acutely did not produce a significant activating effect did induce a marked excitation after the animals were chronically treated with ethanol. Finally, the issue was addressed of whether the absence of ESE in some strains of rats could in part be due to a masking effect by the depressant component of this drug. To test this possibility rats were treated with ethanol for a 4-month period. Tolerance to the depressant effect was observed but no ESE was detected.

Ethanol and locomotor activity Ethanol's stimulant effect Tolerance to ethanol Genetics and ethanol

IN the last years the stimulant action of ethanol has been the object of increasing attention [5, 7, 8, 12, 14, 17, 19, 20, 22]. Two major possibilities have been considered concerning the mechanisms underlying ethanol's activating effects. One possibility is that ethanol-induced behavioral excitation results from depression of inhibitory mechanisms, while a second one would be that the depressant and the stimulant actions of ethanol are under the control of independent mechanisms. According to the latter view ethanol would have direct activating effects (see [18] for review).

Within this framework it becomes important to determine whether the phenomenon of tolerance, which is well known to develop to the depressant action of ethanol, also occurs in relation to ethanol's activating effects. If ethanol-induced depression and excitation share a common mechanism, one would expect both to show similar tolerance development. Hunt and Overstreet [11] reported, in rats, a parallel development of tolerance to the depressant and hyperactivating effects of ethanol. In contrast, Masur and Boerngen [15] showed in a study with mice that no development of tolerance to the locomotor stimulating action was reached after 60 days of ethanol treatment. This lack of tolerance to the excitatory component of ethanol in mice was confirmed by other authors [6,23]. However, as suggested by Tabakoff and Kiianmaa [23], it could well be that more extended periods of chronic treatment may be necessary to produce tolerance to ethanol's stimulating effects. The present study addressed this question by extending the period of ethanol treatment to 5 months. We found no evidence of tolerance to ethanol's activating effect during this period.

Another point raised in the literature relates to the possibility that mice not only do not develop tolerance but actually become more responsive to ethanol's stimulating effect after chronic exposure. Masur and Boerngen [15] observed that mice treated with ethanol for 15–60 days showed an increase in locomotor activity when compared to the first day of drug administration. This finding was not corroborated by Tabakoff and Kiianmaa [23] who concluded that the stimulatory effects of ethanol were not significantly accentuated after chronic exposure. In contrast to Tabakoff and Kiianmaa [23] and in agreement with Masur and Boerngen [15], Crabbe *et al.* [6] reported that there was a tendency for DBA mice to develop an increased responsiveness to the activating effect of ethanol after chronic treatment. This controversy was addressed in the present study by acutely injecting mice with a dose of ethanol that, although within the stimulant range, was insufficient to produce a significant increase in locomotor activity. The same dose, initially inef-

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fective to produce activation, induced a marked stimulation after the animals were chronically treated with ethanol. The hypothesis of an increased sensitivity to the excitatory action was therefore strengthened.

A third question relates to interspecies differences in the sensitivity to the stimulant action of ethanol, rats being described to show less behavioral activation [9,10]. Data from this and previous studies show that chronic treatment with ethanol leads, in mice, to an enhancement of the ethanol's activating effect [6,15]. One interpretation of this finding is based on the view of the excitatory and inhibitory effects of ethanol as two independent processes. It is conceivable that initially the excitatory effect is partially or totally masked by the depressant effect. With the development of tolerance to the latter, the excitatory response would be uncovered. If this were the case, one would expect rats to show an increased sensitivity to ethanol's activating effect after chronic, but not necessarily after acute exposure to ethanol. This possibility was also addressed in the present study. After 4 months of daily treatment with ethanol rats showed no evidence of increased behavioral activation.

EXPERIMENT 1: AN ATTEMPT TO OBSERVE ETHANOL'S LOCOMOTOR STIMULATION IN RATS

METHOD

Male Wistar rats from our own colony were used. After weaning at 25 days of age they were housed in groups of six in wooden cages measuring 50×35×20 cm and kept at a room temperature of 23±2°C on a 12 hour light-dark cycle.

In a pilot experiment several doses of ethanol were acutely tested in independent groups of rats. None of them induced an increase in locomotor activity. A decrease in activity began to be observed with 1.0 g/kg. To test whether after being chronically exposed to ethanol and developing tolerance to its depressant effect rats would become activated by this drug, the dose of 1.0 g/kg was chosen.

Fifty-four 3-month-old rats were randomly assigned to three groups of 18 animals each. The EE group was chronically treated for 120 days with IP injections of ethanol 1.0 g/kg diluted with saline to a concentration of 10% w/v and tested after the same dose of ethanol. The ES group received the same chronic treatment of the EE group but was tested under saline. The CON group was chronically treated with, and tested after, saline.

Activity Testing

Immediately after 1.0 g/kg of ethanol or saline injection on day 1, the animals were introduced in a 70×35×30 cm cage equipped with 3 photocells. Numbers of light beam interruptions were cumulatively recorded at 10 min intervals over a period of 60 min. This test was repeated on the 20th, 40th, 80th, 100th and 120th day. Activity testing was always carried out between 1:00 and 4:00 p.m.

The values of locomotor activity after drug administration were compared for each time interval using the Duncan's new multiple range test.

RESULTS

The mean (±SD) weights of the EE, ES and CON groups at the beginning of the experiment were 341±25 g, 338±21 g and 337±20 g, respectively. At the end of the experiment, 4 months later, they weighed 350±31 g, 357±23 g and 381±34 g, respectively.

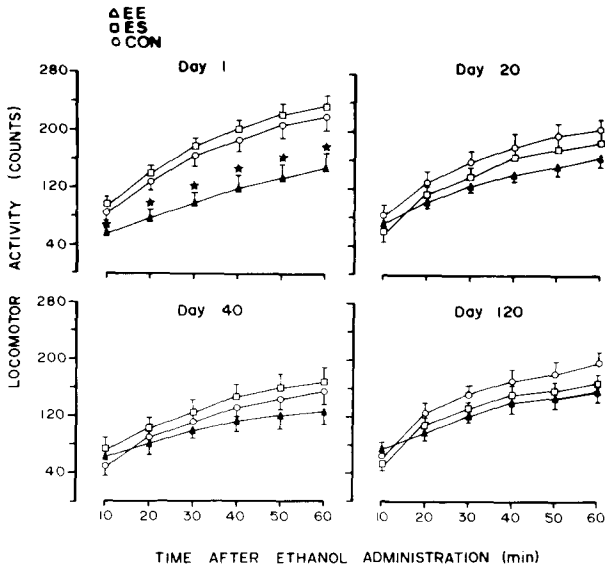

FIG. 1. Effect of ethanol on locomotor activity in rats treated chronically with ethanol for 120 days. Locomotor activity was recorded for 60 minutes. Mean cumulative activity counts±SEM of each group are given on the ordinate. The EE group was chronically treated with ethanol 1.0 g/kg IP and tested after the same dose. The ES group was chronically treated with 1.0 g/kg IP ethanol and tested after saline. Group CON was chronically treated with, and tested after, saline. Asterisks indicate significant differences when EE is compared to CON and ES ($p<0.05$; Duncan's new multiple range test). No significant differences were found between CON and ES.

Figure 1 shows the locomotor activity of rats injected for the first time with 1.0 g/kg of ethanol (group EE) or saline (groups CON and ES) as well as after 20, 40 and 120 days of daily treatment with this drug. Results obtained when animals were tested on days 80 and 100 are not depicted in the figure as they are essentially the same as for day 40. As can be seen in the first panel of Fig. 1 the ethanol-induced decrease in locomotor activity in group EE was significant over the 60 minute monitoring period on day 1. After twenty days of daily injections the rats became tolerant to the depressant action as no significant differences in activity were detected among rats tested under ethanol (EE) or saline (ES and CON). It is essential to observe that although the rats became tolerant to the depressant effect of ethanol, no evidence of the activating action of this drug was found over 4 months. Figure 1 also shows that the activity of the group daily treated with ethanol and tested after saline (ES) was similar to the CON group, no statistical difference being detected between both groups.

EXPERIMENT 2: AN ATTEMPT TO DETECT TOLERANCE TO THE ACTIVATING EFFECT OF ETHANOL IN MICE BY A CHRONIC EXPOSURE FOR 5 MONTHS

METHOD

Ninety-one albino Swiss female mice from our own colony, 80 days old were used. After weaning at 20 days of age, they were housed in plastic cages in groups of 20–25 under the same conditions as described for Experiment 1.

Groups EE (n=34) and ES (n=19) received a 5% and 10% ethanol—0.3% saccharin solution as sole source of fluid for 3 and 4 days, respectively, and then a 15% ethanol—0.3% saccharin solution for 5 months. The CON group (n=38) received an isocaloric sucrose solution for 5 months.

Activity Testing

All groups were tested on 40×25×20 cm Plexiglas cages fitted with 3 photocells. Light beam interruptions were cumulatively recorded at 10-min intervals for 60 minutes. Testing was done on day 1, 90 and 150. Group EE was tested immediately after a 2.0 g/kg IP injection of ethanol. From the results of a pilot experiment it was observed that a dose of 2.0 g/kg of ethanol IP would induce a slight but non-significant increase in locomotion. Groups ES and CON were tested immediately after an IP injection of saline.

For the EE and ES groups tap water substituted the ethanol solution 20 hr before testing which was always carried out from 1 to 4 p.m.

The values of locomotor activity after drug administration were compared for each time interval using the Duncan's new multiple range test. In the case when only two groups were compared the Student's *t*-test was employed.

RESULTS

At the beginning of the experiment the means (±SD) weight of the EE, ES and CON groups were 28±4 g, 28±3 g and 29±4 g, respectively. At the end of the experiment, 5 months later, the EE, ES and CON groups weighed 34±5 g, 33±3 g and 38±6 g, respectively. The average dose of ethanol taken daily by each animal ranged from 10.1–12.8 g/kg.

The data obtained when mice were chronically treated for 5 months with an oral solution of ethanol are shown in Fig. 2. The slight excitatory effect observed on day 1 for the EE group reached no statistical significance when compared to the two groups injected with saline (ES and CON). However, when tested again in the activity cage, after being treated with ethanol for 90 days, a marked stimulatory effect was observed for the EE group. This is shown by the significantly increased locomotor activity over by the 60-minute monitoring period when compared to the CON and ES animals. The picture remained the same after 150 days of ethanol administration as no alteration in the activating effect was observed. That is, no development of tolerance was observed. Group ES was not tested at day 150. As shown in Fig. 2 their activity on day 1 and day 90 did not differ from the CON group.

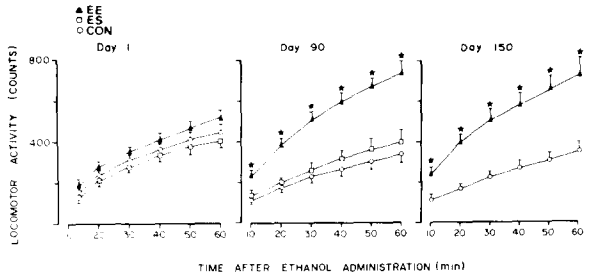

FIG. 2. Ethanol's effect on locomotor activity in mice chronically treated with ethanol for 150 days. Locomotor activity was recorded for 60 minutes. Mean cumulative activity counts±SEM of each group are given in the ordinate. The EE group was chronically treated with ethanol as the sole source of fluid, and tested after 2.0 g/kg ethanol IP. The ES group was chronically treated with ethanol and tested after an injection of saline. The CON group received an isocaloric sucrose solution as chronic treatment and was tested after saline. Asterisks indicate significant differences when EE is compared to ES and CON ($p<0.01$; Duncan's new multiple range test) on days 1 and 90, and to CON on day 150 (Student's *t*-test; $p<0.01$). No significant differences were found between ES and CON.

GENERAL DISCUSSION

Previous studies designed to test whether tolerance to the stimulating effect of ethanol in mice would develop utilized different routes of drug administration as well as different lengths of time of chronic drug exposure. Masur and Boerngen [15] employed a schedule of 60 daily IP injections while Tabakoff and Kiianmaa [23] fed mice with a liquid diet containing ethanol over a period of seven days. The mice used in the study by Crabbe *et al.* [6] were injected IP either once daily for 16 days or twice daily for 10–19 days. Despite these methodological differences, the data from these 3 studies are in agreement in that tolerance to the excitatory effect of ethanol was not observed. However, the question remained whether more extended periods of drug administration would render mice tolerant to ethanol's stimulating effect. The data presented here show that this hypothesis is unlikely as mice exposed to ethanol over a period of 5 months showed no development of tolerance to the ethanol-induced increase in locomotor activity.

Our findings that, in mice, a dose of ethanol that acutely had a slight but non-significant stimulatory effect induced a marked increase of activity after chronic exposure to this drug, confirms our previous statement on the increased response in mice to the stimulating effect of ethanol [15]. The mechanism(s) underlying this phenomenon remain(s) unclear. Among the alternatives is the possibility that with the development of tolerance to the depressant component of ethanol, a larger stimulant action, present from the beginning, would be unmasked.

Another hypothesis is that the increased behavioral activation would be consequent to an enhanced activation in central catecholamine mechanisms. This possibility is based on data showing that the stimulating effect of ethanol is dependent on central catecholamines [1, 3, 4, 16, 21] and that prolonged ethanol administration was described to produce an increased sensitivity of the dopamine receptors [13]. It is important to note in this context that our studies were performed with a randomly bred strain of mice. It remains to be investigated whether similar data would be obtained with genetically selected strains. An interesting question is whether strains with a low sensitivity to the stimulant action of ethanol would show activation after chronic exposure to this drug.

The data reported here for rats, showing that they did not become stimulated by ethanol either with an acute dose or after being chronically treated over a 4-month period, confirms and extends previous data on the low sensitivity of strains of this species to the activating effect of ethanol [9,10]. Nevertheless it could be argued that the reason why the rats did not become stimulated after chronic treatment is that the dose tested (1.0 g/kg) had an acute depressant action. This possibility is weakened by our previous observation showing that mice chronically exposed to an initially depressant dose presented clear signs of behavioral activation as early as 15–30 days into treatment [15].

Therefore our results do not support the hypothesis that,

in Wistar rats, an excitatory action of ethanol could be masked by its depressant effects. This is in contrast to data obtained by Breese *et al.* [2] in a study designed to test the possibility that the lack of an ethanol-induced stimulatory effect in Sprague-Dawley rats could be due to a masking of the stimulation by the depressant component of ethanol.

These authors reported that the coadministration of ethanol and thyrotropin releasing hormone (TRH) induced an increase in locomotion and suggested that TRH unmasked the stimulant action of ethanol by antagonizing its depressant effects. Differences in experimental design and strain used in the Breese *et al.* [2] and in the present study may account in part for the different results and conclusions reached.

In 1977, in a review article on the biphasic action of ethanol, Pohorecky [18] stated that it is difficult to attribute the large differences in the dosage required to induce motor stimulation entirely to species and/or strain variability, and suggested that aspects such as task differences and handling of animals could be important contributors to the large variance observed. In the last few years, however, evidence has been growing in favor of the notion that marked differences in the stimulation response induced by ethanol occurs even when confounding variables are tightly controlled (e.g., [10]). This suggests that the variability is mainly under genetic control. As the activating effect of ethanol is most probably an important determinant in the intake of alcohol by humans, this issue takes on increasing relevance and deserves further investigation.

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