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Effects of Naloxone on Ethanol Induced Alterations of Locomotor Activity in C57BL/6 Mice[1]

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MIDDAUGH, L. D., E. READ AND W. O. BOGGAN. *Effects of naloxone on ethanol induced alterations of locomotor activity in C57BL/6 mice.* PHARMAC. BIOCHEM. BEHAV. 9(2) 157–160, 1978.—Ethanol (2 g or 3 g/kg) or water vehicle was injected intraperitoneally into C57BL/6 mice 15 min after injections of naloxone, a narcotic analgesic antagonist, or its saline vehicle. Locomotor activity was monitored for 60 min beginning 30 min (Experiment 1) or immediately (Experiment 2) following the ethanol injection. In both experiments, animals injected with the lower dose of ethanol were more active than controls during the second half of the activity test. Animals injected with the high dose of ethanol were less active than controls during the first half of the activity test but returned to control levels or above during the second half of the test. Naloxone at the doses used injected 45 min prior to the activity test (Experiment 1) did not alter locomotor activity and did not influence ethanol induced activity changes. When injected 15 min prior to testing (Experiment 2), however, naloxone alone produced a transient reduction in activity observed only during the first half of testing. During the second half of testing all animals injected with naloxone had activity levels similar to controls and lower than those of animals injected with ethanol in the absence of naloxone. Hence, it appears that naloxone at a dose and time period which does not alter the locomotor activity of mice is capable of blocking ethanol induced excitatory effects.

Naloxone Ethanol Locomotor activity C57BL/6 mice

RECENT reports [11,12] indicate that morphine and alcohol have a common effect in reducing calcium concentrations in brain tissue of rats. In addition, the calcium reduction can be prevented by pretreating the animals with naloxone and can be alleviated by injections of naloxone following exposure to either ethanol or morphine.

Although naloxone is best known for its properties as a narcotic antagonist, there is some evidence that this drug can influence the effects of other psychoactive drugs. For example, naloxone has been reported to attenuate d-amphetamine induced increases in avoidance behavior and locomotor activity of rats [7]; and to enhance chlorpromazine induced reduction in key pecking by pigeons [8]. Since the naloxone attenuation of ethanol effects has potential value for helping to understand the action of alcohol on brain function and may have some practical value in the treatment of alcoholism, we have been conducting studies to determine if naloxone is capable of altering some of the other effects noted following injections of ethanol.

Another effect common to both narcotics and ethanol is altered locomotor activity following injections into mice. Low doses of these drugs elevate activity whereas higher doses decrease activity [9,14]. Elevated activity produced by these drugs appears to depend upon adequate stores of catecholamine since pretreatment with α-methyltyrosine, a tyrosine hydroxylase inhibitor, attenuates the effect [2, 6, 9]. In the present study we have used doses of ethanol which either elevate or decrease activity of mice to determine if these effects can be altered by pretreatment with naloxone.

METHOD

Animals and Procedure

Male C57BL/6J mice 60–90 days of age were used. They were maintained on a 12 hr light:dark cycle with lights on at 0700 hr and had access to Wayne Mouse Breeder Blox and water ad lib. Locomotor activity of individual mice was tested between 1000 hr and 1500 hr in one of three transparent polycarbonate mouse cages (32×21×13 cm) enclosed in sound attenuated cabinets (80×56×40 cm). Exhaust fans provided ventilation and masking noise. Light was provided from 6 W bulbs located 35 cm above the middle of the mouse cage. Each cage was divided into quadrants by two photobeams and activity was automatically monitored by counting switch closures of photocells produced by the mouse interrupting the photobeam directed on the cell. The counts were cumulated and printed out at 5 min intervals. On the day of

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testing, animals were brought into the testing room in groups of three and after receiving appropriate injections were placed in one of the three cages for activity assessment. The treatments were evenly distributed across the three activity monitoring devices. The cages were cleaned after each test to eliminate the influence of odor from prior test mice on activity.

In the first experiment, 54 mice were injected intraperitoneally with either naloxone (3 mg/kg) or equivalent volumes of saline (0.01 ml solution/g body weight). Fifteen min later they received a second injection of either ethanol (2 g or 3 g/kg) or water (0.02 ml solution/g body weight). Thirty min after the final injection, each animal was placed into one of the activity cages and activity was assessed for 1 hr. The design allowed a comparison of six groups: Saline+Water (SW); Saline+Ethanol at 2 g/kg (SE_2); Saline+Ethanol at 3 g/kg (SE_3); Naloxone+Water (NW); Naloxone+Ethanol at 2 g/kg (NE_2); and Naloxone+Ethanol at 3 g/kg (NE_3).

The second experiment used 36 mice and was identical to the first except that activity testing began immediately rather than 30 min after the final injection.

Data Analysis

Data for each experiment were analyzed in 2·(Naloxone)×3(Ethanol)×2(Time) analyses of variance with repeated measures on the time factor. Statistical significance of differences between group means was assessed using Newman-Keuls tests for multiple comparisons.

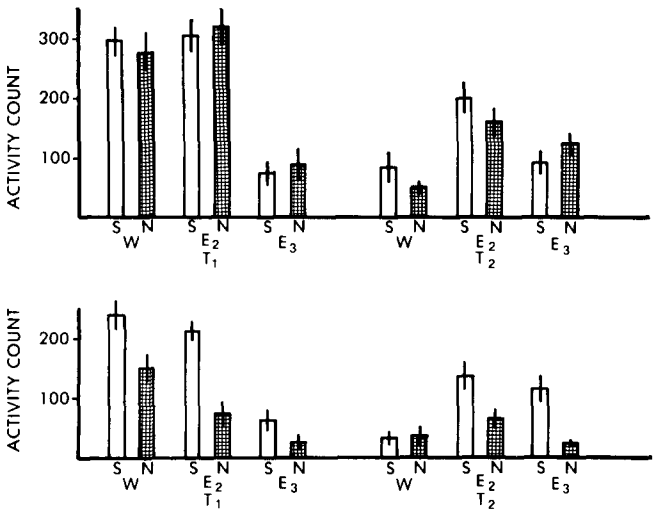

FIG. 1. The two graphs summarize activity counts generated by C57BL/6J mice during the first (T_1) and last (T_2) 30 min of a 60 min activity test. The bars represent means (N=9/group, upper graph; N=6/group, lower graph) and the vertical lines, the standard error of the mean associated with each group. In the upper graph data were obtained from mice injected first with naloxone 3 mg/kg (solid bars) or saline (open bars) 45 min before activity testing and then with water (W), ethanol at 2 g/kg (E_2) or 3 g/kg (E_3) 30 min prior to activity testing. In the lower graph, data were obtained from mice treated identical to those above except that activity assessment began 15 min after the naloxone and immediately after the ethanol injection.

RESULTS

The results of both experiments are summarized in Fig. 1. Data from the first experiment are summarized in the upper graph. An analysis of variance of the data obtained over the 60 min test period revealed that two of the main factors, Ethanol, $F(2,48)=22.11$, $p<0.01$, and Time, $F(1,48)=70.41$, $p<0.01$, were significant sources of variance. Thus, activity varied as a function of ethanol dose and time in the testing cage. Naloxone, however, did not influence activity under these conditions. Of particular interest was a significant Ethanol×Time interaction, $F(2,48)=30.31$, $p<0.01$, indicating that the change in activity across time varied according to the dose of ethanol injected. The different patterns of change in activity contributing to this interaction can be appreciated by comparing activity levels during the first period (T1) with that during the second period (T2) for the three ethanol conditions. Animals injected with either water (NW+SW) or with the low dose of ethanol (NE_2+SE_2) had similar activity levels during both periods. Other interactions in this analysis were not significant.

Since the Ethanol×Time interaction was significant, we analyzed the data within each 30 min period with 2(Naloxone)×3(Ethanol) analyses of variance. During the first period (T1), activity varied as a function of Ethanol dose, $F(2,48)=29.73$, $p<0.01$. Newman-Keuls tests statistically confirmed the group differences during this period suggested by inspection of the graph. Activity of animals injected with the high dose of ethanol (SE_3+NE_3) was significantly lower than that of animals injected with water (SW+NW, 29%) or with the low dose of ethanol (SE_2+NE_2, 26%).

During the second 30 min period (T2), activity again varied as a function of Ethanol dose, $F(2,48)=13.55$, $p<0.01$. Comparisons of group means via Newman-Keuls' tests established that animals injected with the low dose of ethanol (SE_2+NE_2) had significantly higher activity than those in the other two groups (SW+NW or SE_3+NE_3).

Data from the second experiment are summarized in the lower graph. In this experiment naloxone was injected 15 min and ethanol immediately before activity tests. A 2(Naloxone)×3(Ethanol)×2(Time) analysis of variance on the data for the 60 min test period indicate that all three main factors were significant sources of variance: Naloxone, $F(1,30)=44.73$, $p<0.01$; Ethanol, $F(2,30)=14.60$, $p<0.01$; Time, $F(1,30)=40.25$, $p<0.01$. In addition the Ethanol×Time interaction was again significant, $F(2,30)=32.51$, $p<0.01$, as well as the three-way Naloxone×Ethanol×Time interaction, $F(2,30)=5.24$, $p<0.01$.

As in the first experiment, data were further analyzed within the two 30 min periods using 2(Naloxone)×3(Ethanol) analyses of variance. During the first 30 min period (T1), activity varied as a function of Ethanol dose, $F(2,30)=27.08$, $p<0.01$, in a manner similar to that observed in the first experiment. In this experiment activity also varied as a function of Naloxone treatment, $F(1,30)=28.44$, $p<0.01$, however, the Naloxone×Ethanol interaction was not significant. Newman-Keuls' test provided statistical confirmation of the apparent lower activity of animals injected with the high dose of ethanol (SE_3+NE_3) compared to that of animals injected with either water (SW+NW, 31%) or with the low dose of ethanol (SE_2+NW_2, 23%). The reduced activity of animals injected with naloxone ($NW+NE_2+NE_3$) compared to that of saline controls ($SW+SE_2+SE_3$) was also confirmed by a Newman-Keuls' test.

During the second 30 min period (T2), the analysis of variance established that activity varied as a function of

Ethanol dose, $F(2,30)=12.35$, $p<0.01$, and Naloxone, $F(1,30)=22.42$, $p<0.01$. In addition, the Ethanol×Naloxone interaction was a significant source of variance, $F(2,30)=6.38$, $p<0.01$. In light of the significant interaction, the saline and naloxone groups were not combined for subsequent group comparisons. Newman-Keuls test confirmed that mean activity was higher for both ethanol groups (SE_2 or SE_3) not pretreated with naloxone compared to water injected controls (SW). Mean activity for animals injected with naloxone and either dose of ethanol (NE_2 or NE_3), was lower than that for ethanol only groups (SE_2 or SE_3) and could not be distinguished from water vehicle control animals (NW).

DISCUSSION

Three useful bits of information were obtained from this study. First, locomotor activity of C-57 mice injected with ethanol can be either higher or lower than that of controls; the effect being heavily influenced by activity levels of control animals. Second, naloxone (3 mg/kg) injected into C-57 mice can reduce locomotor activity for up to 45 min after injection. Finally, naloxone at a dose and time period which does not alter locomotor activity can prevent ethanol induced elevations in this activity.

The finding that ethanol injected animals have either higher or lower activity than control animals depending upon how long the animal had been in the apparatus may help resolve some of the apparent discrepancies in the literature. In our experiments we observed elevated activity only during the last 30 min of a 60 min testing session whether ethanol was injected immediately or 30 min prior to testing. This was due primarily to a large reduction in activity of control animals during the last half of the session. The lower activity of animals injected with the high dose of ethanol was observed only during the first half of the session when activity of control animals was relatively high. Some previous studies [2, 3, 13] have reported elevated activity in mice injected with the low dose of ethanol used in the current study (2 g/kg). In each of these studies, activity was monitored over a time period where we obtained elevated activity in ethanol injected mice. In the study most comparable to our own [2], ethanol was injected immediately prior to activity testing but data was recorded only during the last 30 min of a 60 min testing session. Ethanol injected animals in that study had activity levels approximately 3.5 times higher than controls which is similar to the 4-fold increase observed during the last half of testing in the second experiment of the current study. In another study [13] the same dose of ethanol was injected but activity was monitored over the entire 60 min period. In this case activity of ethanol injected animals was approximately 1.5 times higher than controls which is similar to the 1.4 time increase observed in our study when total 60 min activity scores were analyzed. In addition, reduced activity for mice injected with this dose of ethanol has also been reported [10]. Activity in that study was sampled for 20 min beginning 10 min after injection. Although we did not obtain reduced activity with the 2 g/kg dose during this time period, this is the only time period where we observed a reduction with the 3 g/kg dose. Although most of these studies measured activity for groups of mice which may yield different results due to drug treatment, it is apparent that the results obtained on activity measures following injections of ethanol are extensively influenced by the particular time period of activity sampled. Our data suggest that this is due primarily to the performance of control animals since activity was elevated in ethanol injected animals only during the last half of the session whether it was injected immediately prior to or 30 min prior to activity assessment.

Our study demonstrates that naloxone, at the dose used, can reduce activity of C-57 mice. This is one of the few studies where naloxone alone has been observed to alter behavior of mice. In an earlier study [7], the activity of mice was not reduced by injections of naloxone unless very high doses (100 mg/kg) were given. In the present experiment, however, reduced activity was observed following a relatively low dose of naloxone (3 mg/kg). It appears that the effect is of rather short duration since the reduction was observed during the first 30 min of activity assessment when naloxone was injected 15 min prior to testing and was completely absent if the drug was injected 45 min before testing. Certainly, this result is limited due to the use of only one dose of naloxone. It is offered only as a direction for further research since there has been a renewed interest in the pharmacology of naloxone along with the recent interest in endogenous opiate-like pentapeptides in brain tissue [4, 5, 15]. Although inspection of group means during the initial 30 min of testing suggests that ethanol may be potentiating the naloxone induced reductions in activity, the lack of a significant Naloxone×Ethanol interaction prevents statistical support for this suggestion.

The third observation from these experiments is that naloxone appears to block the elevated activity observed in animals injected with ethanol. This is clearly demonstrated in the second experiment. During the second 30 min of the activity session, the locomotion of animals injected with naloxone (NW, NE_2, NE_3) was similar to that of vehicle controls (SW). Animals injected with either dose of ethanol (SE_2 or SE_2) had higher activity than vehicle controls (SW) or their naloxone pretreated counterparts (NE_2 or NE_2). This result provides further evidence that naloxone can attenuate ethanol induced changes in brain function as indicated by previous reports [11,12] that the drug attenuates ethanol induced reductions in brain calcium concentration.

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