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Effect of Naloxone on Behavioral Changes Induced by Subchronic Administration of Ethanol in Rats

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ALVAREZ, C., M. PRUNELL AND J. BOADA. *Effect of naloxone on behavioral changes induced by subchronic administration of ethanol in rats.* PHARMACOL BIOCHEM BEHAV **59**(4) 961–965, 1998.—Endogenous opioid peptides appear to be involved in acute behavioral effects induced by single doses of ethanol. However, its role in repeated ethanol exposure has not been studied. In the present study ethanol was given to rats at the doses of 2 and 4 g/kg by a stomach gauge for 15 days, and its effects on spontaneous motility, open-field activity, and active avoidance behavior recorded on the 3rd, the 6th and the 15th days. Then the effect of naloxone (0.5 and 2 mg/kg by intraperitoneal route) was tested against a challenge ethanol dose, administrated by oral route, on the 16th day. Control animals received tap water and saline instead of ethanol or naloxone, respectively. Both doses of ethanol induced a decrease in spontaneous motility that was antagonized by naloxone. Open-field ambulations were increased by the high dose of ethanol, low-dose lacking effect; naloxone did not modify these ethanol effects. The low dose of ethanol shortened latency time in shuttlebox, the high dose causing escape and freezing responses; none of these effects were modified by naloxone. Therefore, endogenous opioid peptides appear to play a limited role in the chronic effects of ethanol in rats; particularly its effects in tests inducing an increase in the level of anxiety were resistant to naloxone. © 1998 Elsevier Science Inc.

Ethanol Subchronic intoxication Naloxone Behavioral changes

THE fact that opioid antagonists reversed effects of ethanol (EtOH) on the central nervous system suggested an involvement of endogenous opioid peptides in the action mechanism of this drug (12). However, most of data were obtained from experiments in which EtOH had been given as a single dose or from clinical observations in acutely intoxicated patients. Therefore, the role of opioid peptides in chronic EtOH effects remains unknown. On the other hand, although naltrexone seems to be an useful agent in alcohol withdrawal, its action mechanism has not been clarified (1). Consequently, the aims of the present study were, first, to determine the time course of behavioral changes induced in rats by repeated exposure to ethanol, and second, to assess the effect of naloxone against a challenge dose of EtOH given at the end of the intoxication period. Nx was employed at a low and a high dose because at the relatively high doses producing reversion of ethanol intoxication, this compound may cause GABAergic blockade as well (9). Therefore, a low, more specific opioid antagonist dose (18) was also used.

METHOD

Male Sprague–Dawley rats, weighing 250–320 g, bred in our laboratory, were used. The animals were housed in groups of four to six in standard Makrolon cages (60 × 40 × 20 cm) under regulated conditions (light period from 0800 to 2000 h) and had free access to food and water until the beginning of the experiments.

Behavioral Tests

Three behavioral tests were performed following the procedures employed in a previous report (16) in which an acute effect of ethanol was studied.

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Motor activity. Motor activity was measured in an activity meter (Varimex, Columbus Instruments), provided with horizontal electromagnetic sensors, which employed two standard plastic rat cages lined with a layer of ground corn cob bedding. According to Tyler and Tessel (21), one animal was placed in each cage for 1 h, but the activity was recorded only for the second half hour. Thus, initial exploratory motility was not computed.

Open-field behavior. The open field consisted of a white circular base (80 cm diameter) subdivided in 16 zones by two red concentric circumferences and eight diameters of the same color. A white cylindrical wall (60 cm height) and a central illumination (100 W) completed the apparatus. Crossover behavior was measured by counting, for a period of 2 min, the lines crossed by the animals. An ambulation was noted when the animal crossed a line with both hindpaws.

Active avoidance behavior. A shuttlebox (Letica) was used. Rats were trained to avoid electric shock according to the following schedule learning: once the animal was introduced into the apparatus a 300 Hz auditory signal was delivered for 5 s (conditioned stimulus) followed by a foot shock for 3 s (unconditioned stimulus). To reduce excessive discomfort caused by foot shock, its intensity was adapted to the sensitivity of each animal using the same apparatus. To this end, the first shock delivered had in all cases an intensity of 0.25 mA (60 Hz); this was then increased or lowered in the following trials, depending on the response obtained (between 0.1 and 0.4 mA, habitually 0.25 mA was suitable). These individually adjusted shock intensities do not introduce bias into the results, because no relationship between shock intensity and speed of avoidance learning has been found (7). Each animal was given 10 trials daily, 30 s elapsing between consecutive trials. The intertrials were not punished. Three types of responses were considered: escape, avoidance, and freezing (no response). The training sessions continued daily until a 100% of avoidance responses was attained (which happened within the following 7 days) and then 4 more consecutive days to assure learning.

Drug administration. Two groups of 24 animals each received by a stomach gauge a daily dose of EtOH (Absolute ethanol Panreac, 20% v/v), 2 and 4 g/kg, respectively, for 15 days. These doses were identical to those used in acute experiments in this laboratory (see the introductory paragraphs). On the 16th day rats received an additional dose of EtOH but preceded, 10 min before, by the administration of Nx 0.5 mg/kg or 2 mg/kg intraperitoneally in saline (Naloxone Chlorhydrate, Laboratorios Abelló), these doses being also identical to those used in acute experiments. To this end, each group of 24 animals was conveniently subdivided into two halves. Control animals ($n = 36$) received tap water by a stomach gauge for 15 days, the volume being similar to that used to administrate EtOH 2 or 4 g/kg. On the 16th day, one-third of the animals received saline by intraperitoneal route in a volume similar to that used to administrate Nx, 10 min before water. Another two groups of 12 animals each received Nx 0.5 and 2 mg/kg, respectively, 10 min before water.

Experimental schedule. Prior to any drug or water administration, rats were successively tested in the open field, an activity-meter, and the shuttlebox. Shock avoidance training continued until optimum response was attained which occurred within 10 days in all cases. Three days after ending behavioral tests, EtOH or water administration was started, behavioral tests being repeated 3, 9, and 15 days afterwards. On the 16th day, tests were repeated to study the effect of Nx alone or in combination with a challenge dose of EtOH. In all cases behavioral tests started 30 min after giving EtOH or water.

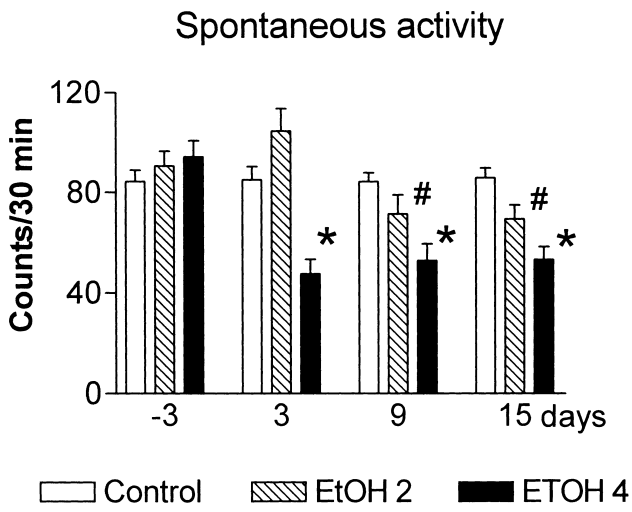

FIG. 1. Effects of EtOH 2 and 4 g/kg on spontaneous activity. Vertical lines on bars express standard error of mean. *$p < 0.001$ at least as compared with base line values. #$p < 0.01$ compared with the third day value.

To assess whether or not the animals suffered from alcohol dependence, possible convulsions elicited by noise key was explored daily for a week following EtOH withdrawal.

EtOH blood level. Blood EtOH level was measured in separate groups of animals by using Blood EtOH Combination Test kit (Boehringer–Mannheim). Blood samples (0.5 ml) were obtained by heart punction 30 min after a single dose of EtOH 2 or 4 g/kg given by a stomach gauge (six animals per dose) as well as 30 min after the 16th dose of EtOH 2 or 4 g/kg.

Statistical calculations. ANOVA with repeated measures analysis followed by Tukey's multiple comparison test for post hoc analysis was carried out with the data obtained for locomotor activity, open-field behavior, and shuttlebox for EtOH 2 g/kg. For EtOH 4 g/kg in shuttlebox, in view of the data obtained, χ^2 test was used. As a repeated measures design could not be used to study the effect of Nx on EtOH, because the ef-

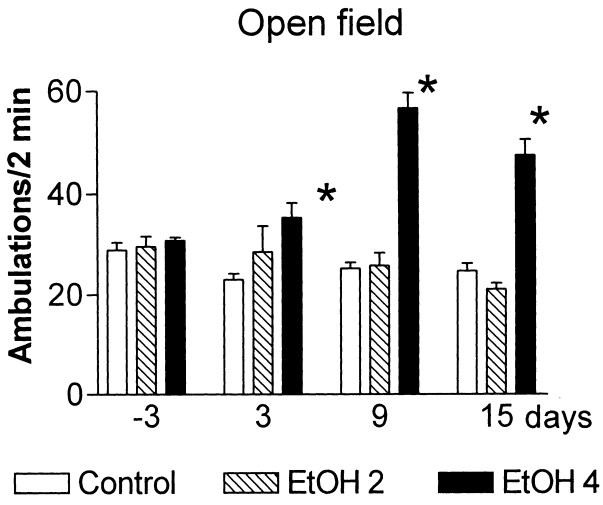

FIG. 2. Effects of EtOH 2 and 4 g/kg on open-field behavior. Vertical lines on bars express standard error of mean. * = as in Fig. 1.

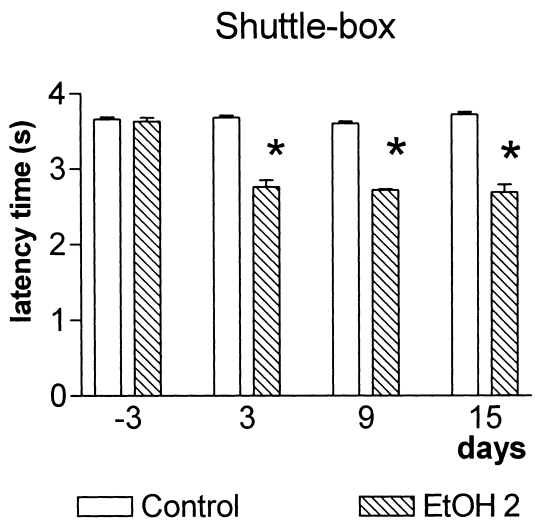

FIG. 3. Effects of EtOH 2 on active avoidance behavior. Vertical lines on bars express standard error of mean. * = as in previous figures.

fect of Nx alone had to be compared with the effect of EtOh alone in different animals, and, on the other hand, the number of animals was not the same in all experimental groups, one-way ANOVA was employed in this case; in the case of shuttlebox, χ^2 test with *p-v*alue adjusted for the number of comparisons made, was carried out. All calculations were performed by using GraphPad Software Version 2.00 (license GPA-21486-418).

RESULTS

Effects of Subchronic Administration of EtOH

Spontaneous activity. EtOH 2 g/kg did not induce significant changes in this variable compared with baseline values. However, compared with the third-day values a significant hypomotility was observed at the 9th and 15th days, $F(3, 23) = 6.82$, $p < 0.006$, ANOVA for repeated measures; post hoc Tukey's test $p < 0.01$ between the 3rd and the 9th or 15th day values. EtOH 4 g/kg caused a consistent and significant diminution of motility in all observations, $F(3, 23) = 12.34$, $p < 0.0001$, ANOVA for repeated measures, matching pair effective; post hoc Tukey's test $p < 0.001$ between control and the 3rd, the 9th, and the 15th day values) (Fig. 1).

Open-field ambulations. EtOH 2 g/kg did not produce significant changes in this variable throughout the observation period (Fig. 2). EtOH 4 g/kg gave rise to a clear increase in ambulations that was significant from the nineth day onwards, $F(3, 23) = 23.76$, $p < 0.0001$, ANOVA for repeated measures, matching pair effective; post hoc Tukey's test $p < 0.001$ between control and the 9th and 15th day values).

Active avoidance behavior. In Fig. 3 the effects induced by EtOH 2 g/kg are summarized. At this dose, animals continued to exhibit only avoidance responses, similar to control animals, but a significant and consistent shortening in latency time was observed throughout the observation period, $F(3, 23) = 27.90$, $p < 0.0001$, ANOVA for repeated measures, matching effective; post hoc Tukey's test, $p < 0.001$ between control and the 3rd, the 9th, and the 15th day values). On the other hand, EtOH 4 g/kg did cause qualitative and significant changes in the shuttlebox behavior, escape, or freezing responses being exhibited by a number of animals. In Table 1 results are presented as the proportion of each type of response seen.

Effects of Nx on Behavioral Changes Induced by Subchronic Administration of EtOH

Spontaneous activity. Nx by itself did not produce any change on spontaneous activity (Fig. 4) at any dose tested. However, the depressant effect seen after repeated EtOH administration for 16 days was antagonized by the two doses of Nx, this effect being more clearly noticed against the high dose of EtOH, $F(3, 80) = 24.32$, $p < 0.0001$.

Open-field ambulations. No effects were observed after administration of Nx alone (Fig. 5). On the other hand, this drug was not able to modify the increase in ambulations induced by the administration of EtOH 4 g/kg.

Active avoidance behavior. Latency time was not significantly changed by Nx alone at any dose (Fig. 6). Likewise, shortening in the latency time observed after 15 days of EtOH 2 g/kg was unaltered by Nx. Lastly, the modifications induced by EtOH 4 g/kg remained unchanged after Nx administration (Table 2).

EtOH Blood Level

Blood EtOH levels measured 30 min after a single dose of 2 and 4 g/kg were 1.3 ± 0.05 mg/ml (mean ± ES) and 2.05 ± 0.08 mg/ml, respectively. After 16 days of EtOH administration a diminution to 0.81 ± 0.08 mg/ml [$p < 0.001$, $t(10) = 12, 72$]; and 1.09 ± 0.13 mg/ml [$p < 0.001$, $t(10) = 15.41$], respectively, was found.

Alcohol Dependence

No signs of alcohol dependence were observed within a week after drug withdrawal.

TABLE 1

EFFECT OF EtOH 4 g/kg ON ACTIVE AVOIDANCE BEHAVIOR IN SHUTTLE BOX

Response	Control	%	Third Day	%	Ninth Day	%	15th Day	%
Avoidance	240	(100)	138	(57, 5)	170	(70, 8)	142	(59, 1)
Escape	0	(0)	60	(25)	46	(19, 1)	54	(22, 5)
Freezing	0	(0)	42	(17)	24	(10)	44	(18, 3)

Data express number of avoidance, escape, and freezing responses observed. Percentages are presented between brackets ($n = 24$)

Global p-value = 0.008 as determined by χ^2 test.

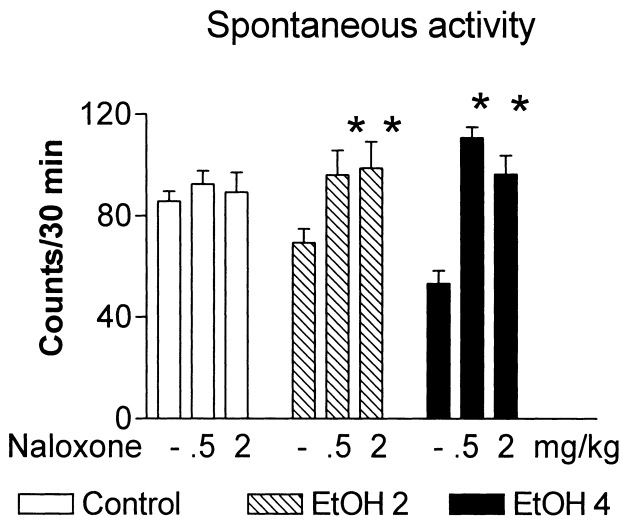

FIG. 4. Effects of naloxone on spontaneous activity changes induced by EtOH 2 and 4 g/kg. *$p < 0.05$ at least as compared with EtOH alone (15 days).

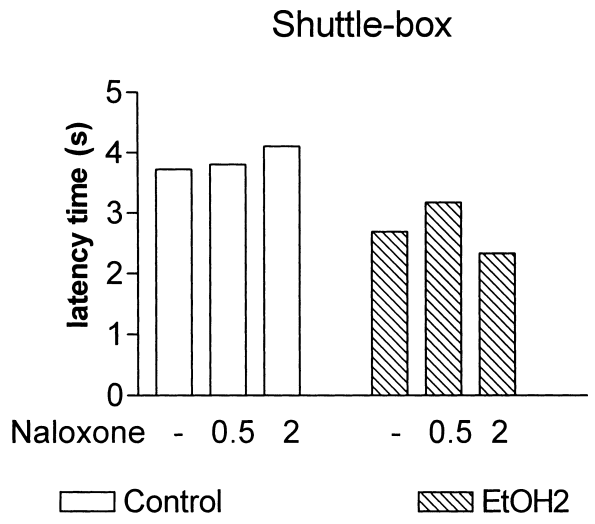

FIG. 6. Effects of naloxone on shuttlebox latency time shortening induced by EtOH 2 g/kg.

DISCUSSION

Subchronic administration of EtOH produced a decrease in locomotor activity, a phenomenon that was clearly seen with the high dose. Therefore, compared with acute experiments (16), an activating effect of EtOH was not observed in the present experiments. In spite of that, the experimental paradigm could mask this effect because the first measurement was performed 3 days after the beginning of EtOH intoxication, and, therefore, an early tolerance to this effect could not be ruled out; this is suggested by the hypomotility seen on the 9th and the 15th days compared with the third day but not with the baseline value. Tolerance to the depressant effect could not be seen, these results being in opposition with those reported by Tabakoff and Kiianmaa (19), who found tolerance to the depressant action of EtOH, but not to its stimulating effect. A definitive explanation is not available for this disagreement, but differences in experimental schedules are so relevant that comparing findings may be confusing. Thus, three strains of mice (DBA/2, BALB/c, and C57B1/6) known for their different hypnotic sensitivity to EtOH were used; the measurement of locomotor activity, used only for studying the activating effect, was started immediately after a challenge dose of EtOH given by intraperitoneal route (1.35 mg/kg) and continued for only 30 min; an EtOH-containing diet (EtOH 5% v/v for DBA/2 and EtOH 7% for the other two strains) was employed for giving EtOH; the period of intoxication was only 7 days; the depressant effect of ethanol was studied by measuring the duration of loss of righting reflex induced by a challenge dose of 3.5 g/kg of EtOH given by the intraperitoneal route. Lastly, blood ethanol levels were not measured. As to the main aim of the present study, Nx, at the two doses used, completely reverted hypomotility induced by EtOH. Although an acceleration of the hepatic metabolism of EtOH by Nx has been postulated (2), results of experiments performed in this laboratory did not confirm such hypothesis (3) and, therefore, an involvement of opioid system in EtOH effect on spontaneous motility may be suggested.

Open-field behavior was modified only by the high dose of EtOH, an increase of ambulations, with a peak on the ninth

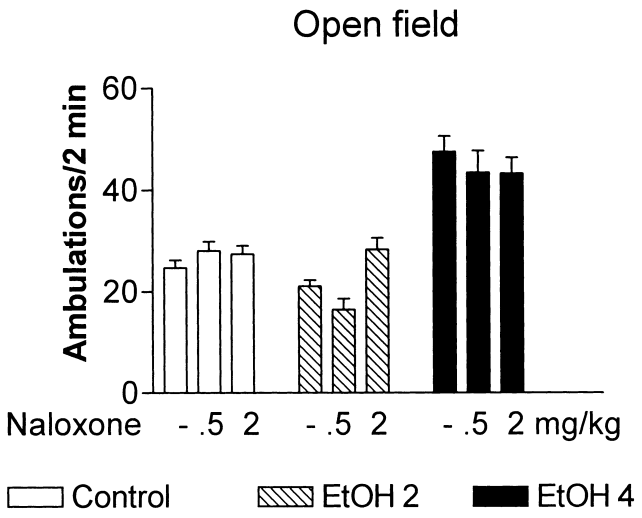

FIG. 5. Effects of naloxone on open-field modifications induced by EtOH 2 and 4 g/kg.

TABLE 2

ACTION OF NX ON EtOH (4 g/kg)-INDUCED CHANGES IN SHUTTLE BOX

Response	EtOH 4 g/kg	%	EtOH 4 g/kg + Nx 0.5 mg/kg	%	EtOH 4 g/kg + Nx 2 mg/kg	%
Avoidance	152	(63.3)	70	(58.3)	90	(75)
Escape	48	(20.0)	27	(22.5)	14	(11.6)
Freezing	40	(16.6)	23	(19.1)	18	(15)

Data express number of avoidance, escape, and freezing responses. Nx 0.5 and 2 mg/kg did not modify the response pattern (100% avoidances). Percentages are presented between parentheses.

Global p-value = 0.1044 as determined by χ^2 test.

day, being observed. Again, this effect contrasts with that observed after the acute administration of EtOH—which was similar to that mentioned for locomotor activity. In this respect, literature data are controversial and scarce. Thus, Bond and Di Guisto (6), in agreement with our data, found that four 2-day periods of EtOH ingestion produced an increase in activity, whereas Keane and Leonard (14) observed a decrease in ambulations 16 days after giving a diet containing EtOH. Nevertheless, this discrepancy may be explained because rats received 12 g/kg of EtOH daily from the fifth day onwards, a dose that produced a blood EtOH level of 3.01 mg/ml, clearly higher than that obtained in our work; therefore, a more intense depressant effect might be produced. An increased open-field activity may be interpreted as a reduced level of anxiety (20,22,23), an effect that has been repeatedly described for EtOH by using specific tests (4,8,15,17). Nx, at any dose, was unable to modify this anxiolytic effect of EtOH, indicating that mechanisms other than opioid system activation could be involved. In this respect, although modifications in the anxiety level are commonly attributed to changes in the GABAergic system, in the present experiments the magnitude of the GABAergic blockade induced by a high dose of Nx was apparently not sufficient to antagonise the anxiolytic action of EtOH. In fact, to attain such effect doses over 5 mg/kg seem to be required (19).

As to the EtOH effects on active avoidance behavior, the shortening in latency time seen with low doses indicate a stimulating action resulting in an increase of alertness. The opposite effect was observed with high doses, escape, and freezing responses being noticed in a number of animals, and as in the case of open-field behavior, a reduced level of anxiety might be involved in this effect. Unfortunately, no literature data exist to compare our results, only studies concerned with residual effects observed in hamsters several days after intoxication being available (5,13). Similarly to open-field behavior, Nx failed to modify the EtOH effects in the shuttlebox. Therefore, these data represent a further support to the hypothesis that EtOH acts on anxiety through mechanisms other than the opioid system.

Taken as a whole, the data attained in the present study suggest that the opioid system could be involved in subchronic EtOH action when stressing stimuli are absent, that is, activity-meter measured motility. However, its action on high anxiety level derived from stressing stimuli (open field, foot electric shock), is apparently mediated by nonopioid mechanisms.

The fact that acute effects of single doses of EtOH, studied in the same three experimental tests, were antagonized by Nx (16) is of particular interest. Therefore, in addition to stressing stimuli, either repeated administration of EtOH or repeated test performance, or both must be involved in Nx lacking effect. In other words, activation of the opioid system could play a relevant role in acute effects of EtOH in unexposed animals, but its involvement in chronic effects could be more limited. In spite of that, naltrexone, another opioid antagonist, suppresses alcohol appetite in dependent subjects (10,11), an effect whose mechanism has not been definitively clarified. Probably, mechanisms regulating alcohol intake are different from those operating in behavior changes seen in our investigation. Lastly, the difference observed between subchronic and acute effects may explain, at least partially, the variability in effectiveness of Nx in clinical alcohol intoxication.

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