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The Influence of Opioid Antagonists on the Discriminative Stimulus Effects of Ethanol

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SPANAGEL, R. *The influence of opioid antagonists on the discriminative stimulus effect of ethanol.* PHARAMACOL BIOCHEM BEHAV **54**(4) 645–649, 1996.—The aim of the present study was to investigate the role of different endogenous opioid systems in the expression of ethanol's discriminative stimulus effects in a two-lever operant drug discrimination paradigm. Wistar rats were trained to make differential responses following the administration of ethanol (1 g/kg, IP) or saline. The correct response (fixed-ratio schedule; FR10) resulted in the presentation of food. Once rats had acquired the discrimination an ethanol dose–response test was conducted. The effects of opioid antagonists on the discrimination were assessed by administering the μ-opioid receptor antagonists naloxone (0.5–20 mg/kg SC) and cyprodime (5–100 mg/kg SC) and the δ-opioid receptor antagonist naltrindole (0.1–25 mg/kg SC) 15–30 min before the discrimination test. Furthermore, the selective κ-opioid antagonist nor-binaltorphimine (5 mg/kg SC) given 24 h before the test session was examined. Results of generalization testing demonstrate that ethanol discrimination was dose dependent. Pretreatment with naloxone produced only at the highest dose a partial, but significant, antagonism, whereas cyprodime failed to alter the ethanol cue. This suggested the involvement of other opioid receptor subtypes. However, neither naltrindole nor nor-binaltorphimine had any effect on the ethanol-saline discrimination. These results demonstrate that the expression of the ethanol cue is only partly dependent on the function of endogenous opioid systems.

Ethanol Opioid antagonists Drug discrimination Discriminative stimulus

THERE is increasing evidence that endogenous opioid systems play a role in the rewarding effects of ethanol in laboratory animals and humans and subsequently in the addictive properties of this drug. Thus, numerous studies showed that opioid antagonists reduce ethanol self-administration in laboratory animals under various conditions (2,6,7,22) as well as alcohol drinking in humans (11,12). Besides the rewarding effects of ethanol the discriminative stimulus properties of this agent is thought to be a major factor in the development and maintenance of addictive behavior (20). It is suggested that the discriminative stimulus effects of ethanol can modulate and direct ethanol-seeking behavior because such behavior can become associated with previously percieved rewarding stimuli (20). Thus, it is speculated that endogenous opioid systems may also play a role in the expression of ethanol's discriminative stimulus properties.

Altshuler and co-workers (1) found no effect of either naloxone or naltrexone, even at high doses, upon the discriminative stimulus effects of ethanol, which was in line with earlier studies (3,23). However, the same investigators showed in a subsequent ethanol discrimination study that the excitatory phase, which takes place within the first few minutes following ethanol administration, depends on the function of endogenous opioid systems (15,16). It is important to note that naloxone and naltrexone used in these studies, have highest affinities for μ-opioid receptors but bind readily to other types of opioid receptors (10,13). Therefore, they are nonselective antagonists, and cannot differentiate clearly the roles of different opioid receptor types in ethanol discrimination.

The aim of the present study was to compare the roles of μ-, δ- and κ-opioid receptors in ethanol-saline discrimination by using selective receptor antagonists.

METHOD

Animals

Forty-eight male Wistar rats (Max Planck Institute of Psychiatry, Martinsried, Germany), weighing 250–270 g, were housed individually with free access to water. Their weights were maintained at about 80% of those under free-feeding conditions during the experimental period by restricting their daily food consumption. Animals received water ad lib and were kept in a climatically controlled room under a 12 L:12 D cycle, with the light phase commencing at 0700 h. At the end of the experiments the animals were killed with an overdose of halothane. The experiments were approved by the Committee on Animal Care and Use of the relevant local governmental body.

Apparatus

Standard operant chambers (Coulbourn Instruments, Lehigh Valley, PA) were used. Each chamber was equipped with two levers, one on either side and equidistance from a food cup. The chambers were contained in ventilated, sound-attenuated cubicles equipped with a houselight. The experiments were controlled by a computer connected to the chambers through LVB interfaces (Med Associates Inc., East Fairfield, VT) using a modified version of the software package (Operant Package for the Neurosciences, OPN) described by Emmett-Oglesby et al. (4) and Spencer and Emmett-Oglesby (19).

Discrimination Training

Rats were shaped to lever press for food using a progressive fixed ratio (FR). Once animals had reached a fixed ratio of 10 responses for each food pellet (FR10) (45 mg pellets, Bioserve, Frenchtown, NJ), drug and vehicle training sessions began. Training sessions began 6 min after injection of either ethanol (1.0 g/kg IP; 12.5% v/v solution) or the appropriate volume of saline and terminated after 10 min (15). Responses on the correct lever were reinforced and those on the incorrect one were only recorded. The left-hand lever was designated as the drug lever in 50% of the animals and right-hand in the remainder. During each training session, the first 10 presses on either lever designated the selected lever, a measure used to ascertain acquisition of stimulus control. Rats received a randomised sequence of training sessions (one session per day) with a maximum of three consecutive drug or vehicle training sessions. Criterion for stimulus control was set at eight correct lever selections out of the last 10 with at least 90% drug- or vehicle-appropriate responding during these sessions.

Discrimination Testing

Tests were conducted twice weekly, with either drug or vehicle training during the intervening days. The day prior to testing all rats were trained with saline. Test sessions terminated either after one completed fixed ratio (10 presses) or 5 min had elapsed. No responses were reinforced during these sessions. Testing commenced once the subjects were placed into the chambers 6 min following either ethanol or saline administration. Two measures of discrimination were obtained. A quantal measure, which was derived from the percentage of animals tested that selected the ethanol lever, and a graded measure that was calculated from the number of responses accumulating on the drug lever against the total number of responses on both levers until the first fixed ratio was completed (first 10 presses on either lever designated it as the selected lever). In addition to obtaining discrimination data, the time taken to complete the first ratio (selection latency) served as a measure of rate of responding. Following tests were conducted: (a) ethanol dose–response test: following acquisition of the discrimination, generalization tests were conducted with four doses of ethanol (0.25, 0.5, 1.0, 1.5 g/kg, IP) to obtain a dose–response relationship for the discrimination. All doses were tested in a randomized order. (b) Opioid receptor antagonist tests: animals were injected either with naloxone (0.5, 1, 5, 10, 20 mg/kg, SC; pretreatment time: 15 min), cyprodime (5, 10, 50, 100 mg/kg, SC; pretreatment time: 15 min) or naltrindole (0.1, 1, 10, 25 mg/kg, SC; pretreatment time: 30 min). Nor-binaltorphimine (5 mg/kg, SC), a ultralong-lasting κ-opioid receptor antagonist (5,8,18), was injected 24 h prior testing to obtain a selective κ-opioid receptor blockade (5,9,18). All drugs and doses were injected in a randomized order with the exception of nor-binaltorphimine, which was injected as last drug to avoid interactions with other opioid receptors because of its ultra long-lasting effects.

Statistics

The graded measure of discrimination was used to test for statistical significance of data. Each percentage score was transformed to an arc-sine and a single-factor analysis of variance with repeated measures was employed. Post hoc tests were the Student–Newman–Keuls test or, when applicable, the Dunnett's test to identify significant differences between vehicle and opioid receptor antagonist pretreatment. The accepted level of significance was $p < 0.05$. A computer-generated formulation of Litchfield–Wilcoxon analysis (21) yielded ED_{50} values and confidence levels for ethanol dose–response curves.

Drugs

The compounds injected in this study: cyprodime (a generous gift of Dr. H. Schmidhammer, University of Innsbruck), naloxone (RBI, Cologne, Germany), naltrindole (a generous gift of Prof. A. Herz, Martinsried and RBI, Cologne, Germany), and nor-binaltorphimine (nor-BNI was provided by Dr. A. W. Lipkowski, University of Minnesota) were dissolved in distilled water.

RESULTS

Aquisition of Stimulus Control and Ethanol Dose–Response Test

Forty-four out of 48 animals trained to discriminate ethanol (1 g/kg) from saline acquired stimulus control by meeting the criterion of correct lever selection after 60–90 training sessions with a mean ± SE of 72 ± 7. Data obtained in the ethanol dose–response testing (0.25–1.50 g/kg IP) revealed that discrimination of the ethanol stimulus was dose dependent (data not shown). The lowest dose of ethanol, which partly generalized to the ethanol training dose, was 0.5 g/kg. The ED_{50} value of ethanol was 0.48 g/kg. Lever selection latencies with doses lower than 1 g/kg ethanol did not differ from the training dose. However, a dose of 1.5 g/kg ethanol significantly increased the time taken for rats to select the lever ($p < 0.05$; $n = 12$).

Opioid-Antagonist Test

Figure 1 shows the effects of vehicle and the μ-opioid receptor antagonists naloxone (0.5–10 mg/kg SC) and cyprodime (5–100 mg/kg SC) pretreatment on the ethanol/saline discrimination. Vehicle pretreatment did not affect the discrimination task. Neither naloxone nor cyprodime had a pronounced effect upon the ethanol discrimiation; however, naloxone, given at the highest dose (10 mg/kg; the 20 mg/kg dose could not be evaluated because no animal completed one fixed ratio within 5 min), partly antagonized the ethanol cue. Compared to vehicle pretreatment statistical analysis revealed a significant difference for this naloxone dose ($p < 0.05$; $n = 15$). It is further important to note that discrimination of saline was not altered either by naloxone or cyprodime pretreatment. The highest dose of naloxone and cyprodime also significantly increased the time taken for rats to select the lever ($p < 0.05$; $n = 15$). Selection latencies with lower doses of naloxone or cyprodime did not differ from saline pretreatment (Fig. 1).

Pretreatment with the δ-opioid receptor antagonist naltrindole (0.1–25 mg/kg SC) 30 min prior testing had no effect

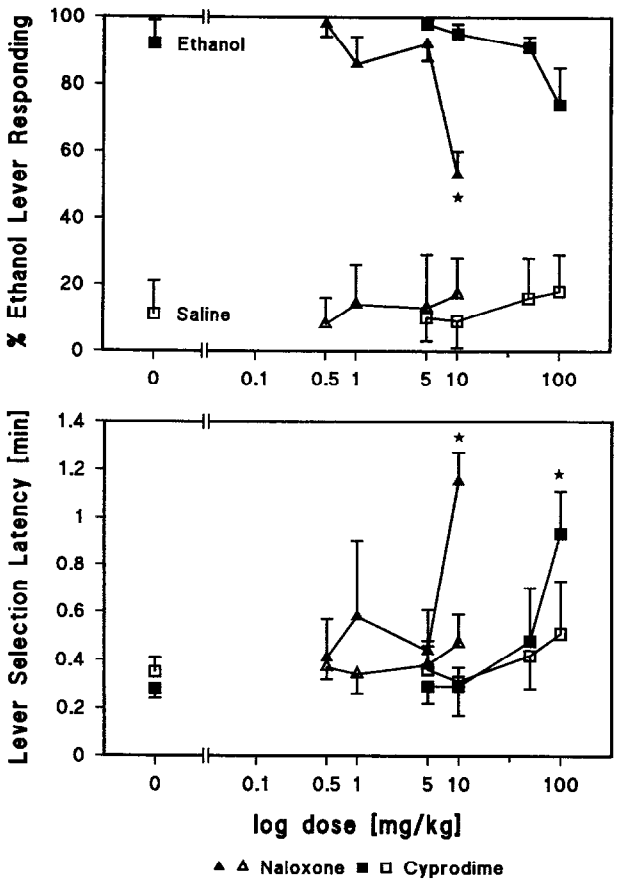

FIG. 1. Effects of the μ-opioid receptor antagonists naloxone and cyprodime upon the discriminative stimulus effects of ethanol in rats trained to discriminate ethanol (1 g/kg; IP) from saline. Top panel: naloxone and cyprodime were injected in a randomized order and the mean ± SE percentage of ethanol appropriate responding is given on the ordinate. Rates of lever responding following naloxone or cyprodime pretreatment are shown in the lower panel. Asterisks indicate significant differences from saline pretreatement; $p < 0.05$.

upon the ethanol discrimination (Fig. 2). In parallel, measurements of the lever response latency revealed no differences compared to vehicle pretreatment at the lower dose range of naltrindole; however, a 25 mg/kg dose of naltrindole significantly increased the time taken for rats to selecet the lever ($p < 0.05$; $n = 7$) (Fig. 2).

No shift in the ethanol dose–response curve was observed following the blockade of κ-opioid receptors by nor-binaltorphimine (Fig. 3). Furthermore, vehicle vs. nor-binaltorphimine–pretreated animals showed similar ED_{50} values of ethanol (0.44 vs. 0.38 g/kg, respectively). The pretreatment with nor-binaltorphamine had no consequences on the lever response latency (Fig. 3).

DISCUSSION

It is suggested that ethanol discrimination results partly from activation of endogenous opioid systems (15). The purpose of the present study was to retest this working hypothesis and to obtain in parallel a deeper insight into specific opioid receptor mechanisms involved in ethanol discrimination. Surprisingly, no pronounced effects of different opioid receptor antagonists selective for μ-, δ-, and κ-opioid receptor subtypes, respectively, could be found in the ethanol discrimination task. Only naloxone, given at a high dose, produced a partial antagonism of the ethanol cue. However, this effect seems not to be mediated via one specific opioid receptor, because all other opioid receptor antagonists failed to produce an antagonism of the ethanol discrimination.

In the opioid antagonist tests we found in accordance with other studies (15,16) that the ethanol cue is affected by naloxone pretreatment. However, in the present study only a high dose of naloxone partly antagonized the ethanol cue, whereas others reported that naloxone given at μ-opioid receptor selective doses (e.g., 1 mg/kg) is able to completeley antagonize the ethanol cue (15). A possible explanation for this discrepancy might be the use of different rat strains. Thus, it was recently shown that Sprague–Dawley rats, which were used in the former study, are more sensitive to opioids than Wistar rats (17). In contrast, when cyprodime, a selective μ-opioid receptor antagonist (14), was used in the discrimination test, this compound produced only a slight, nonsignificant effect on the ethanol cue. Thus, one might suggest that naloxone, when given at a 10 mg/kg dose, could also act via δ- and possibly

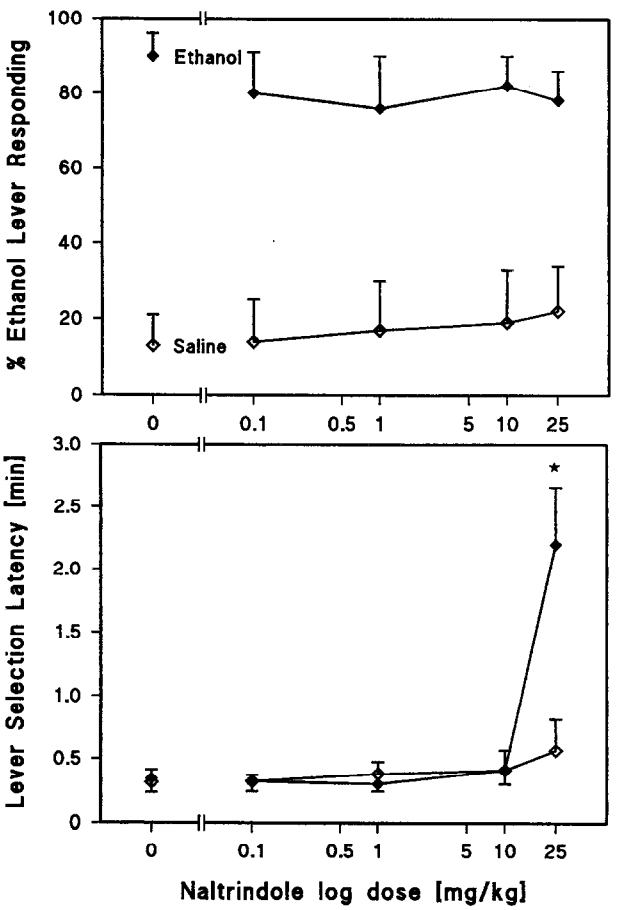

FIG. 2. Effects of the δ-opioid receptor antagonist naltrindole pretreatment upon ethanol/saline discrimination. Top panel: the mean ± SE percentage of ethanol appropriate responding following naltrindole pretreatment is given on the ordinate. Rates of lever responding following this treatment are shown in the lower panel. Asterisks indicate significant differences from saline pretreatement; $p < 0.05$.

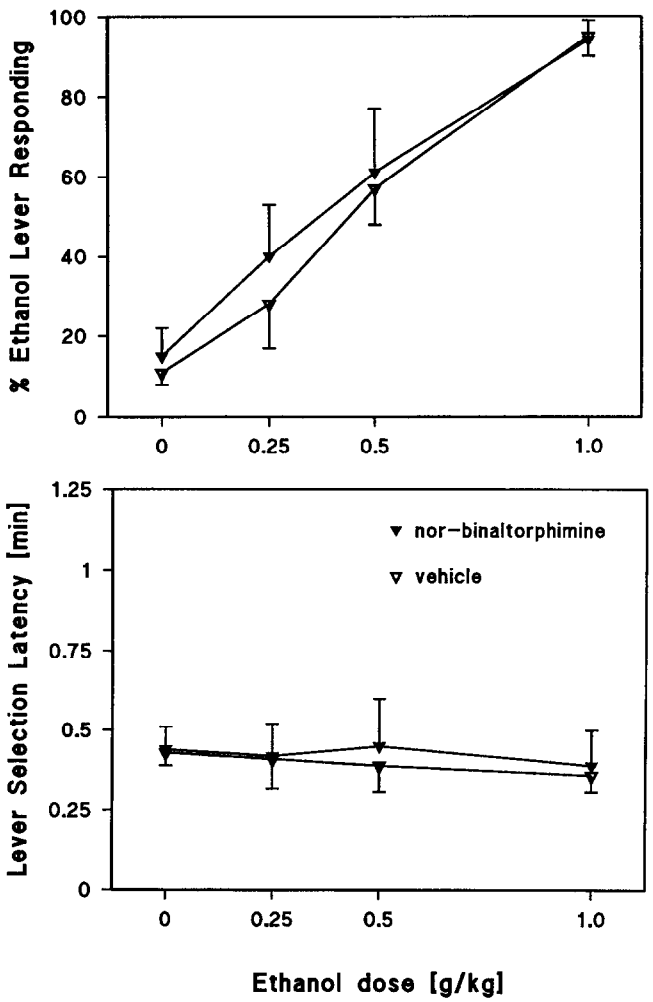

FIG. 3. Effects of the κ-opioid receptor antagonist nor-binaltorphimine (5 mg/kg; 24 h given before testing) on ethanol appropriate responding at various ethanol test doses (0.25–1.0 g/kg). Top panel: the mean ± SE percentage of ethanol appropriate responding following nor-binaltorphimine pretreatment is given on the ordinate. Rates of lever responding following this treatment are shown in the lower panel.

via κ-opioid receptors (10,13) and that the observed naloxone effects on the ethanol discrimination could be mediated by these opioid receptor types. Therefore, we examined the effects of the highly selective nonpeptide δ-opioid receptor antagonist naltrindole on the ethanol discrimination. However, naltrindole, when tested over a wide dose range, completley failed to alter the saline-ethanol discrimination. Further, the involvement of κ-opioid receptors was tested by nor-binaltorphimine. This κ-opioid receptor antagonist exerts extremely long-lasting antagonistic effects, for instance, at least for 1 month in the rat (5,8,18). To obtain a selective κ-opioid receptor blockade this compound was given 24 h before testing (5,9,18). Surprisingly, no shift in the ethanol dose–response curve could be seen after nor-BNI pretreatment. Out of these data one has to conclude that no specific μ-, δ-, or κ-opioid receptor effect is underlying the partial antagonism of naloxone upon the stimulatory ethanol cue. However, one should emphasize that naloxone given at lower doses (≥ 5 mg/kg) did not affect the ethanol cue, which is in line with other studies (1,3,23). Thus, the partial antagonism by a high naloxone dose observed in the present study might result from an opioid receptor unspecific action.

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