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The Effects of Opiate Antagonists on the Discriminative Stimulus Properties of Ethanol

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ALTSHULER, H. L., E. APPLEBAUM AND T. S. SHIPPENBERG. *The effects of opiate antagonists on the discriminative stimulus properties of ethanol.* PHARMAC. BIOCHEM. BEHAV. 14(1) 97–100, 1981.—The effects of naloxone HCl (1.0 mg/kg, 10.0 mg/kg) and naltrexone HCl (1.0 mg/kg, 10.0 mg/kg) on the discriminative stimulus properties of ethanol were measured in order to assess the role of opiate pathways in that behavioral property of ethanol. Forty-eight Sprague-Dawley rats were trained to perform ethanol: saline discriminations on a DRL 10″ schedule of reinforcement in a double lever operant paradigm. Discrimination training for 170 days established 0.6 mg/kg IP ethanol doses as a discriminative stimulus producing at least 80% of all responses as drug appropriate lever choices during 10 min test sessions. After that performance criterion was achieved the effects of the opiate antagonists on the discrimination were assessed by administering naloxone (1.0 mg/kg, IM, 10.0 mg/kg IM) or naltrexone (1.0 mg/kg, IM, 10.0 mg/kg, IM) 15–30 min before the ethanol test dose. Neither antagonist produced significant changes in the performance of the ethanol-saline discrimination. These data demonstrate that the discriminative stimulus properties of ethanol do not require intact opiate pathways. That result implies that the neuropharmacological mechanisms mediating ethanol's stimulus properties in rodents are different from the mechanisms mediating many other behavioral actions of ethanol, including its reinforcing properties.

Ethanol Drug discrimination Naloxone Naltrexone Opiate mechanisms Discriminative stimulus

SINCE the discovery of endogenous opiate receptors [27, 29, 30] and related peptide ligands [4,10] in the central nervous system of many species, there have been many studies of their potential role as substrates of behavior or drug action [9, 14, 15]. Reports about the possible involvement of opiate mechanisms in the actions of alcohol (EtOH) have been inconsistent [9, 15, 17, 19, 20, 22, 23, 24]. We reported [2] that the opiate antagonist, naltrexone HCl (NLTRX) attenuated EtOH self-administration in rhesus monkeys, implicating endogenous opiate mechanisms in the reinforcing effects of EtOH.

It has been suggested (Overton, personal communication, [5, 6, 12, 14, 25]) that compounds which are self-administered are also capable of serving as discriminative stimuli (DS). Several investigators have reported that EtOH can serve as a DS in rodents [7, 11, 17, 18, 24, 26]. A number of behavioral and pharmacological contingencies underlying the DS properties of EtOH have been assessed as have many other behavioral actions of EtOH [3, 7, 9, 13, 21, 22, 28, 31, 32].

Although the endogenous opiate-like compounds have not been implicated as mediators of the DS properties of EtOH [31] opiate-like by-products of EtOH metabolism have been reported [8] that could, in theory, be involved in many actions of the drug.

This study was designed to evaluate the possibility that the DS properties of EtOH are mediated by opiate compounds, loci or pathways in the rodent.

METHOD

Animal Subjects

Forty-eight male Sprague-Dawley rats, ranging in initial weight from 140–190 g were used in this investigation. The animals were housed individually in 25×18×17 cm wire cages and maintained on a 12 hour light (7:00 a.m.–7:00 p.m.), 12 hour dark (7:00 p.m.–7:00 a.m.) daily cycle. Water was available ad lib throughout the study. The animals were fed standard laboratory rat chow (Wayne-Blox) and maintained at 80% of their free feeding weight based on published [1] growth curves for this strain of rats. The animals had not received any drugs prior to the start of study.

Apparatus

All training and testing were performed in rodent operant chambers enclosed in sound attenuated boxes. Each chamber was illuminated by a 15 W bulb and equipped with two response levers and a feeding cup. Reinforcement schedules and data recording were controlled by a Grason-Stadler solid state behavioral control unit. In addition, the time related pattern of operant responding was recorded with a double pen cumulative recorder.

Initial Training

After the rats had been reduced to 80% of their free feeding weight, they were trained to press one of the two response levers to obtain 40 mg Noyes food pellets on a FR-1 schedule of reinforcement. Then the subjects were trained to perform a DRL 10" schedule of reinforcement during daily 30 min training sessions. After the rats were receiving at least 60 reinforcements/DRL-10 training session, they were trained to perform EtOH-saline (SAL) discriminations. Fifteen minutes prior to each test or training session, EtOH (1.0 g/kg, 10% w/v) or SAL (1.0 ml/kg) was administered IP to the animals in their home cages. One lever was the active lever associated with SAL pretreatment and the other lever was active following EtOH. The position of these levers was not altered throughout the study. Discrimination training with alternate day presentations of EtOH or SAL continued for 118 days. Extinction tests in which the animals were not reinforced for the first 10 min of each session were conducted every third day. Reinforcements were delivered during the second 10 min of sessions that began with an extinction test. Training was considered completed when the animals achieved the extinction test performance criterion of 80% of all responses on the drug appropriate lever.

Generalization Curve

After the animals had reached the performance criterion of 80% drug appropriate responding, a generalization (dose-response) curve was generated to determine the range of sensitivity of the discrimination. SAL was administered 15 min prior to each test. The order of presentation of the various doses to the animals was randomized with a table of random numbers. Test sessions were conducted every third day, preceded by a SAL training session on one day and an EtOH (1.0 g/kg, IP) training session on the other day. After the generalization curve was established for an EtOH training dose of 1.0 g/kg, the training dose was gradually reduced to 0.6 g/kg IP in order to increase the sensitivity of the discrimination [23].

Opiate Antagonist Testing

Experiments were conducted to assess the possible interactions between opiate antagonists and the DS properties of EtOH and SAL. Regular EtOH and SAL training and test sessions were continued during this phase. Every fifth session consisted of an extinction test with EtOH (0.6 g/kg IP) or SAL (1.0 ml/kg IP) when naloxone (NLX) HCl (1.0 and 10.0 mg/kg), NLTRX (1.0 and 10.0 mg/kg) or SAL (1.0 ml/kg) had been administered intramuscularly prior to the IP EtOH or SAL doses. NLX or SAL (IM dose) was administered in the animals' home cage 15 min before the DS dose, thus they were administered 30 min before testing. NLTRX was given 30 min before the stimulus dose, thus 45 min before testing. No experiments were conducted the day after a NLTRX session in order to avoid the possibility of the prolonged actions of NLTRX interfering with the test on those days.

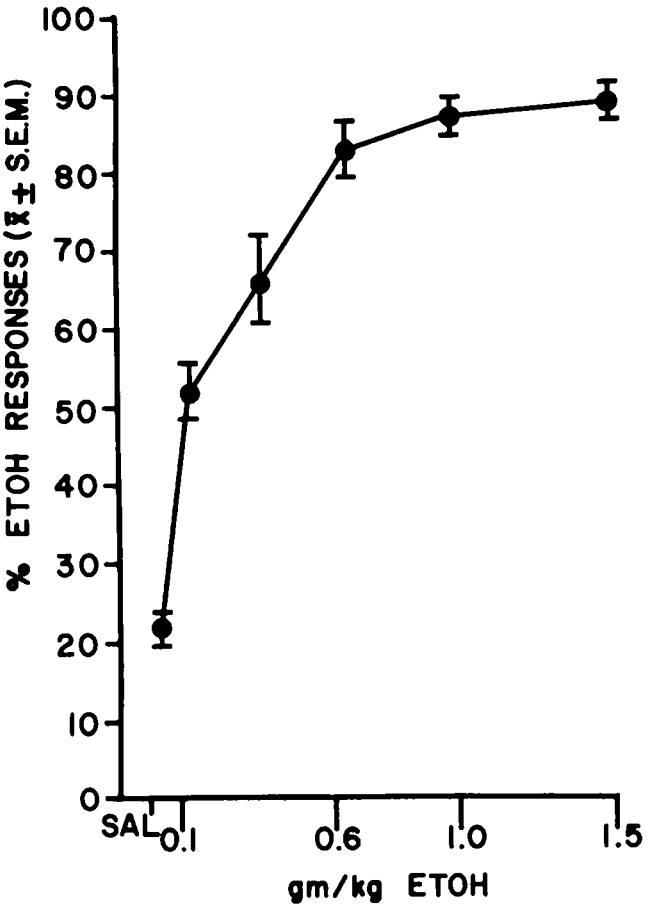

FIG. 1. Generalization (dose-response) curve. This figure summarizes the dose-response relationships and generalization curve for doses of ethanol ranging from 0.1 mg/kg IP to 1.5 g/kg IP. Drug-appropriate (percent correct) lever responding is represented along the ordinate and the ethanol test dose shown along the abscissa. Fifty percent correct responding would be indicative of random responding. The acceptable performance criterion for this response was 80% drug appropriate responding following the 0.6 g/kg training dose.

Data Analysis

The primary data analysis consisted of the daily computation of the percentage of the total responses during each session that occurred on the drug appropriate lever. The individual rat data from any test session in which that animal emitted less than 10 responses was excluded from the final analysis.

The control data used for the assessment of the effects of opiate antagonists on the EtOH discrimination was the overall group mean percent correct (drug appropriate) responses from all EtOH test sessions that preceded the opiate antagonist phase of the study. In addition, the effect of SAL pretreatment (1.0 ml/kg, IM) on EtOH appropriate responding provided the control data for the evaluation of the effects of the antagonists on the EtOH DS. Since the data from all test sessions tended to be normally distributed about the mean, all statements about the significance of the differences

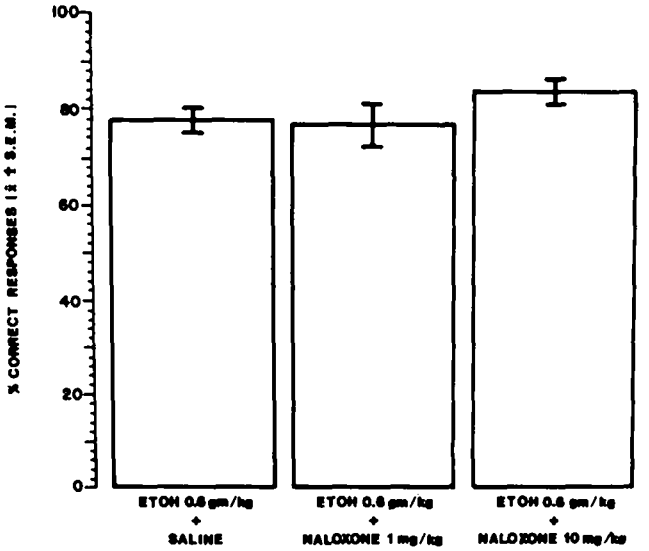

FIG. 2. The effect of naloxone (1.0 g/kg, 10.0 g/kg, IM) on ethanol correct responding. This figure summarizes the effects of naloxone (1.0 g/kg and 10.0 mg/kg, IM) or saline pretreatment on drug appropriate responding following ethanol (0.6 g/kg IP) pretreatment. The percent correct responding (mean ± SEM) is represented by the bars (ordinate) and the treatment condition indicated on the abscissa.

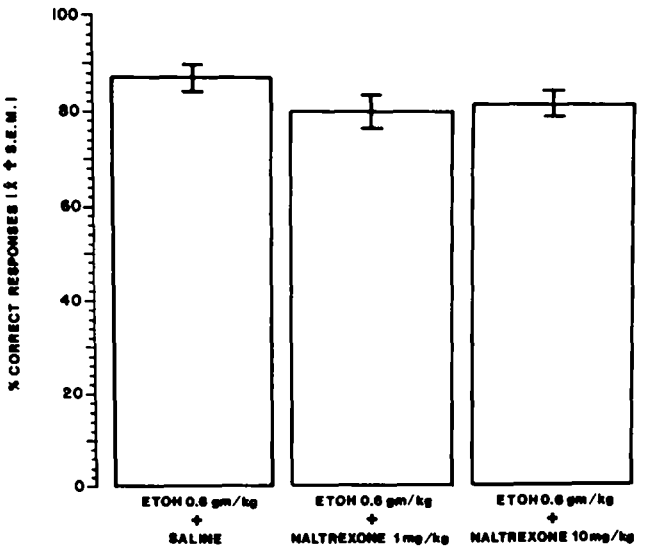

FIG. 3. The effects of naltrexone on ethanol appropriate responding. This figure summarizes the effects of naltrexone (1.0 g/kg and 10.0 g/kg, IM) on ethanol appropriate responding following ethanol (0.6 g/kg, IP) treatment. Percent correct responding is indicated on the ordinate and treatment condition on the abscissa.

reported here are based on parametric statistical analysis (Student's *t*) of the significance of differences between the mean percent correct responses observed during the various treatment conditions.

RESULTS

Acquisition

The acceptable performance criterion of 80% of all responses on the drug appropriate lever was met within 118 training sessions, when EtOH 1.0 g/kg, IP was the training dose. Performance improved slightly during 52 subsequent sessions using 0.6 g/kg. Near random responding (50% correct) occurred after 0.35 g/kg IP, 81% after 0.6 g/kg and 84% after 0.75 mg/kg (Fig. 1).

The Effects of Opiate Antagonists on Ethanol Discrimination

Neither NLX (1.0 mg/kg, 10.0 mg/kg) nor NLTRX (1.0 mg/kg, 10.0 mg/kg) altered the DS properties of EtOH or SAL. Figure 2 summarizes the effects of SAL (1.0 ml/kg) and NLX (1.0 or 10.0 mg/kg) pretreatment on the DS properties of EtOH (0.6 mg/kg IP). This comparison demonstrates that NLX pretreatment had no effect on EtOH discrimination. The slight apparent increase in correct responding following the 10.0 mg/kg NLX pretreatment was not statistically significant.

Figure 3 summarizes the effects of NLTRX (1.0 or 10.0 mg/kg) or SAL (1.0 ml/kg) pretreatment on the DS properties of EtOH (0.6 g/kg). The slight apparent decrease in correct responding observed after both NLTRX doses was not statistically significant. These data demonstrate that like NLX, NLTRX had no effect on the DS properties of EtOH.

Figure 4 summarizes the effects of NLX (10.0 mg/kg) and NLTRX (10.0 mg/kg) pretreatment on the DS properties of SAL when the animals were tested for SAL appropriate responding. The slight decrease in correct responding after NLX pretreatment was not statistically significant.

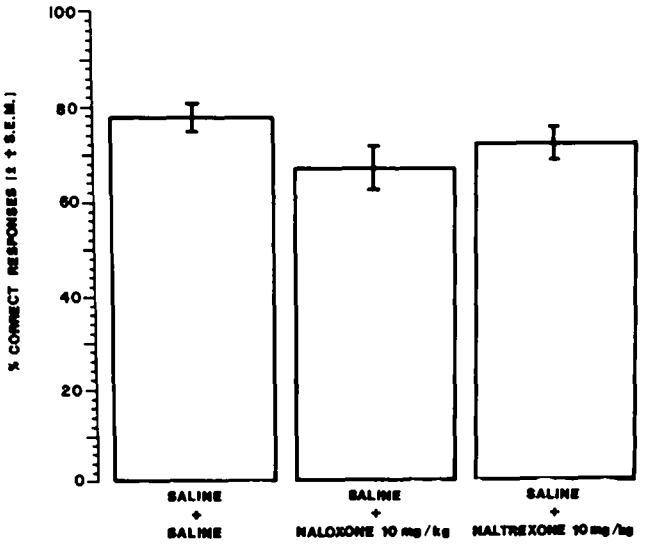

FIG. 4. The effects of naloxone or naltrexone pretreatment on saline appropriate responding. This figure summarizes the effects of pretreatment with naloxone (10.0 g/kg, IM) or naltrexone (10.0 mg/kg, IM) on saline appropriate responding following saline (1.0 ml, IP) injections.

DISCUSSION

These experiments demonstrate that blockade of opiate receptors by opiate antagonist drugs did not alter the DS properties of EtOH. Neither NLX nor NLTRX resulted in

significant changes in the performance of the EtOH vs SAL discrimination.

It is important to emphasize that the dose of both antagonists was well within the dose range that has been shown to block opiate receptors in rats [15, 21, 29, 30] and generally used [2, 9, 15] in studies similar to this one. Furthermore, it should be noted that the prolonged shaping phase of the study and subsequent reduction of the EtOH training dose of 0.6 g/kg provided a sensitive assay of the DS properties of EtOH. The 0.6 g/kg dose was lower than doses reported by Winter [31,32] or Overton [24].

These results are distinctly different from our findings [2] with a self-administration model, where we demonstrated that opiate antagonists attenuated EtOH self-administration by Rhesus monkeys. We interpreted that finding to suggest that the reinforcing effects of EtOH were mediated by endogenous opiate systems. The present findings with a drug discrimination task suggest that the DS properties of EtOH are not mediated by endogenous opiate systems. These data also suggest that the DS property of EtOH is probably mediated by different neuropharmacological mechanisms than its reinforcing effect, a finding contrary to the traditional view [25]. A complete assessment of that possibility must include an evaluation of the species specificity of both the self-administration data [3] and these DS data. There is, however, little evidence to suggest that there are important differences between subhuman primates and rats in the neuropharmacological mechanisms underlying the reinforcing effects of other drug reinforcers. If the premise that drugs that are reinforcers must also be discriminative stimuli, and that similar neuropharmacological mechanisms mediate both properties is a correct premise, then the results of this study suggest that there are significant species differences in the mechanism of EtOH action. Although it is clear that under the experimental conditions described opiate receptor blockade by NLX or NLTRX did not alter the DS properties of EtOH in rodents, the possibility that there could be marked differences in the outcome of similar studies with other species is a possibility that should be considered and investigated.

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