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Delta-Opioid and 5-HT_3 Receptor Antagonist Effects on Ethanol Reward and Discrimination in C57BL/6 Mice

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MIDDAUGH, L. D., B. M. KELLEY, C. H. GROSECLOSE AND E. R. CUISON, JR. *Delta-opioid and 5-HT_3 receptor antagonist effects on ethanol reward and discrimination in C57BL/6 mice*. PHARMACOL BIOCHEM BEHAV **65**(1) 145–154, 2000.—The effects of the receptor antagonists MDL 72222 (MDL, 5-HT_3) and naltrindole (δ-opioid) on ethanol reward and its discrimination were examined in ethanol-preferring C57BL/6 (C57) mice. MDL attenuated lever responding for 12% ethanol delivered on a fixed-ratio 8 reinforcement schedule at a dose that did not influence responding for water reward, thus confirming a previous report that ICS 205-930 reduced ethanol reward for Long–Evans rats. Our study in combination with the reduced ethanol consumption reported for C57 mice injected with odansetron indicates that 5-HT_3 receptor systems are involved in mediating behavior directed toward obtaining ethanol as well as its consumption. By attenuating the rewarding effects of ethanol or of ethanol conditioned cues (e.g., the operant environment), 5-HT_3 antagonists may be useful in the treatment of alcohol abuse. The 5-HT_3 antagonist effects in this study are comparable with the effects of naltrexone on ethanol reward in C57 mice, although higher doses were required to reduce operant responding for ethanol reward. In contrast to the 5-HT_3 antagonist and naltrexone effects, naltrindole, an antagonist with greater specificity for the δ-opioid receptor, was without effect on ethanol reward. This result and recent reports for rats and monkeys suggests that the general antagonists might be more efficacious in attenuating ethanol reward. Both MDL and naltrindole produced only slight reductions in the ethanol discriminative cue, suggesting that the rewarding and discriminative effects of ethanol are not likely mediated by identical neural mechanisms as previously suggested. © 1999 Elsevier Science Inc.

MDL 72222 Naltrindole Ethanol reward Ethanol discrimination 5-HT_3 antagonist δ-opioid antagonist C57 mice

THE general opioid antagonist, naltrexone, which has varying degrees of affinity for μ-, δ-, and κ-receptor subtypes, has recognized potential as a therapeutic agent for alcoholism. Prior to clinical trials, the literature indicated that naltrexone, or the shorter acting naloxone, reduced ethanol consumption by mice (27), rats (15,16,21), and nonhuman primates (1). Additional reports indicated that naltrexone reduced lever responding by rats for ethanol reward (22,37,41,48), an effect also recently noted for ethanol-preferring C57BL/6 (C57) mice in experiments completed in our laboratory (34).

More recent literature suggests the possibility that compounds with greater neurotransmitter receptor subtype specificity might be as effective as, or more effective than naltrexone in reducing the consumption of ethanol. For example, the δ-opioid receptor antagonist, naltrindole, was reported to be equivalent to naltrexone in reducing ethanol consumption by HAD (16) or P (25) rats and by C57 mice (27). Further, at least for HAD rats, the δ_2-opioid antagonist, naltriben, was reported to be more selective than naltrindole for attenuating ethanol, relative to saccharin reward (26). Studies using rats not selectively bred for ethanol consumption are conflicting, with one report indicating that naltrindole reduced ethanol consumption by water-deprived SD rats (14), while the other indicated no influence on ethanol consumption by Wistar rats

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(44). Additionally, recent reports indicate no effects of naltrindole on lever-responding for ethanol reward by rhesus monkeys (50), or on the discriminative stimulus properties of ethanol in Wistar rats (43).

Several studies also implicate the serotonergic systems in ethanol consumption as well as its reinforcing (13,20,24,46) and interoceptive effects (18,40,42). Some of the more recent work indicates that the 5-HT_3 receptor subtype antagonists can reduce ethanol reinforcement (20), consumption (13,24,46), and discrimination (18). The latter characteristic may provide an added dimension of therapeutic effectiveness. Structural differences in the 5-HT_3 receptor antagonists appear to be important in the interoceptive effects of ethanol because ethanol discrimination was blocked by the tropanyl compounds [MDL 72222 (MDL), ICS 205-930] but not by the benzamide, zacopride (18). Reduced ethanol consumption, however, has been noted in rats following injections of either zacopride, a benzamide 5-HT_3 antagonist (24), or MDL, a tropanyl type 5-HT_3 antagonist (13). Conclusions about the effects of 5-HT_3 antagonists on ethanol consumption and its stimulus discrimination from this limited literature are hampered by the use of different strains of rats with differing ethanol consumption characteristics (Sprague–Dawley; Long–Evans; Sardinian Ethanol-Preferring rats), and the use of a different species (e.g., pigeon) for the ethanol discrimination experiment.

The evaluation of both of these drug categories is still in the early stages. For example, with the exception of a report indicating that the δ-receptor antagonist, naltrindole, was as effective as naltrexone in attenuating ethanol consumption by mice (27), the studies have been confined to the rat species. In addition, even for the rat species the literature provides little information about the influence of either the δ-opioid or the 5-HT_3 receptor subclass antagonists on ethanol reward or discrimination. The present experiment was to examine the effects of the δ-opioid receptor antagonist, naltrindole, and the 5-HT_3 receptor antagonist, MDL, on ethanol reward and on discrimination of its interoceptive effects in the C57 mouse using operant procedures. The C57 mouse is an ethanol-preferring inbred strain. Reports characterize the strain's consumption of ethanol (2,4,6–8,29,33,35), its operant responding for ethanol reward (9–12,17,32,36), and its discrimination of ethanol's interoceptive cues (19,23,30,31), as well as providing information about the influence of naltrexone on ethanol reward and the discrimination of its interoceptive effects (34). The experiments in the present study were conducted under conditions identical to each other, and to our report on naltrexone effects (34), thus allowing a direct comparison of the experiments.

EXPERIMENT I: ANTAGONIST EFFECTS ON ETHANOL REWARD

Method

Subjects. Twenty-four experimentally naive adult male C57 mice (3 months old; 20–25 g) were obtained from The Jackson Laboratory (Bar Harbor, ME). Half of the mice were used to assess the effects of the δ-opioid receptor antagonist, naltrindole ($C_{26}H_{26}N_2O_3$, MW = 451), on ethanol reward; the other half were used to assess the effects of 5-HT_3 receptor antagonist, MDL ($C_{15}H_{17}$ Cl_2 NO_2, MW = 314). The mice were housed in an AAALAC-approved colony room, with the temperature maintained at 70 ± 3°F. Lighting was controlled on a 12 L:12 D cycle, with lights on at 0700 h. Each mouse was housed individually, and had unlimited access to water except as described below. Food intake was reduced to maintain the mice at 80% of their ad lib weight, and the animals were tested between 0800–1200 h. The experimental protocols included in this study were approved by the IACUC, Medical University of South Carolina (AR #1441), 11/17/98, and comply with the Public Health Policy on Humane Care and Use of Laboratory Animals and all applicable provisions of the Animal Welfare Act.

Apparatus. The animals were tested in six gray Plexiglas operant chambers with stainless steel grid floors (16 × 12 × 16 cm) enclosed in sound- and light-controlled boxes. One wall of the chamber contained a lever (Model SRL-003, BRS/LVE, Laurel, MD) located 3 cm above floor level and 3 cm to the right of a 2 × 2-cm opening, allowing access to a brass fountain. A house light (GTE, 1819) was located directly above the opening, and a stimulus light was positioned outside of the operant chamber directly above the fountain. Depression of the lever (6–10 g dead weight) served as a signal for input via interfacing (SG-215 input/output interface cards, MED Associates, Inc., Georgia, VT) to an IBM-286 computer. Lab-derived software controlled experimental events and data collection. Off-set of the house light, and on-set of the stimulus light signaled availability of liquid reward delivered to the fountain. Animal contacts with the brass fountain were detected via electronic contact sensors (ENV25a, MED Associates, Inc., Georgia, VT) that activated a solenoid (Honeywell, Inc.), allowing fluid to be delivered to the fountain tip from a 250-ml beaker. Verification of correct fluid delivery to the fountain was completed weekly, and the delivery system was flushed with each new concentration of ethanol (0–12%, v/v).

Procedure. The experiment consisted of several phases that were common for both antagonists, and are detailed below. As a brief description, after acquiring lever responding for water rewards delivered on fixed-ratio (FR)1 and FR2 reinforcement schedules, food-deprived mice were given experience responding for ethanol reward solutions (3, 6, and 12%) during 30-min operant sessions. After the final response of each ratio run, 10 licks at the fountain delivered approximately 120 μl of liquid (i.e., 0, 3, 6, and 12% ethanol). During these procedures, the animals were tested beginning 1 h after being given their daily food allotment (postprandial tests, high thirst, low hunger motivation). Water was not available during this 1-h period, but was available at all other times. The reinforcement schedule was then increased from FR2 to FR8; the daily food ration was given after, rather than before the tests (preprandial tests; low thirst, high hunger motivation); and the session duration was reduced to 15 min. After determining ethanol concentration curves, the effects of naltrindole (12 mice) or MDL (12 mice) on responding for 12% ethanol were determined, initially establishing dose–response functions, and then the time course of drug action. Additional assessment of MDL effects on ethanol or water reward was determined during postprandial tests (high thirst, low hunger motivation) when free access to the alternative reward was available during testing. The number of lever presses and the number of contacts with the fountain were recorded during each session.

Phase 1: food deprivation, habituation, reward, and lever acquisition. Food was restricted to maintain body weights at 80% of ad lib feeding weights. During the last 2 days of the first week of food deprivation, the mice were acclimated to the operant chambers, with water available at the fountain but no access to the response lever. Over the next 2 weeks, the animals were fed their daily ration of food for 1 h prior to the operant session, with no water available, and were given water reward for lever responses, initially on an FR1 and then

an FR2. Water was available at all times other than during the feeding period prior to operant tests. During the next 3 weeks, the animals responded for different ethanol concentrations (3, 6, and 12%) delivered on an FR2 schedule. During week 7, 12% ethanol was delivered on an FR8 schedule. At the end of the week, blood was drawn from the infraorbital sinus to determine blood ethanol levels (BEL). Samples were collected after a 15-min session and assayed for BEL as previously described (32). The following 3 weeks were identical except that food was given after the operant session rather than before (preprandial tests).

Phase 2: Ethanol concentration curve determinations. Preprandial tests were given to establish ethanol concentration curves to ensure that responding was influenced by ethanol concentration, rather than just liquid availability. The first ethanol concentration curve was determined during week 12, with water given as reward on Mondays and Fridays, and increasing concentrations of ethanol (3, 6, and 12%) on the intervening days. After 2 additional weeks of responding for 12% ethanol, during which time we explored possible naltrindole or MDL doses (1.25–10 mg/kg), a second ethanol concentration curve was determined (week 15).

Phase 3: Antagonist effects on responding for 12% ethanol, a dose–response function. During week 16, the week following the second ethanol concentration curve, antagonist dose–response functions were determined. During these tests, the mice were reinforced with 12% ethanol 15 min following IP injections (0.01 ml/g body weight) of the antagonists with doses increasing daily (0, 0.31, 1.25, 2.5, and 5.0 mg/kg as the salt). Naltrindole hydrochloride ($C_{26}H_{26}N_2O_3 \cdot HCl$, MW: 450.6) was dissolved in saline. MDL 72222 ($C_{15}H_{17}C_{12}NO_2$; MW: 314.2) was dissolved in saline with three drops of 0.05 M acetic acid. The acid solution minus the drug was used as the vehicle control. The mice had water available at all times, and received their daily food allotment immediately after the operant sessions. This procedure was repeated during week 18 after an additional week of responding with vehicle injections. Additional doses of each antagonist were assessed during weeks 19 and 20 to complete the dose–response functions.

Phase 4: Antagonist effects on responding for 12% ethanol: time course of action. MDL injected 15 min prior to testing reduced responding for ethanol. This injection time was based on literature procedures. To establish that this was the most optimum time for C57 mice, a time course following injection of the 7.5 mg/kg dose was determined. Thus, MDL (7.5 mg/kg) or saline was injected IP, and the animals were tested as above at 15, 30, and 60 min following injections during weeks 22–24, one test per week. A similar time course was completed using the 20-mg/kg dose of naltrindole.

Phase 5: Effects of MDL 72222 on operant responding for ethanol or water during postprandial tests (high thirst, low hunger motivation) with free access to the alternative fluid. These tests were given to determine if the reduction in responding associated with MDL was selective for ethanol reward. During these tests the mice were switched to testing under higher thirst motivation (i.e., immediately after 1 h access to food with no water available). During this test procedure, the water bottles were removed from the cages 1 h prior to the operant session, and the mice were given their daily food allotment. Water was available at all other times including during the test. During the tests, the animal had access to the fluid either through the fountain as described above, or alternatively, from a centrifuge tube attached to a sipper tube that extended through the operant chamber wall. Thus, fluid was available either without the instrumental response contingency or as reward for lever responding. After ensuring that the mice would drink water from the sipper tube, responding for 12% ethanol was determined on three successive days with the water tube absent, present, and absent (ABA design). This procedure was repeated with the animal responding for water with a tube containing 12% ethanol either present or absent. Finally, the effects of MDL (7.5 mg/kg) or saline injections given 15 min prior to these tests was assessed.

Results

Operant responding for ethanol reward during postprandial tests: response out–put and BEL for 12% EtOH delivered on an FR8 schedule. Twelve percent ethanol delivered according to an FR8 schedule during postprandial tests when mice were thirsty maintained high response outputs and high consumption levels. The number of responses and BEL produced during a 15-min session are summarized in Table 1. Summaries are provided for all mice originally assigned to each antagonist drug group as well as the mice from each group finally selected for testing antagonist effects. The selected groups consisted of mice selected on the basis of making at least two reinforced ratio runs for 12% ethanol reward during the preprandial tests given prior to antagonist evaluation, and on their preference for the 12% ethanol solution rather than lower concentrations. Responses during the 15-min session provided 10–15 reward opportunities and 1.2–1.8 ml of 12% EtOH for the different groups. Although amounts of ethanol delivered to the fountain could be established, because of technical problems with determining the amounts of unconsumed ethanol during the session we could not determine ethanol intake in these experiments. The mice did, however, consume sufficient amounts of ethanol to become highly intoxicated, and some were nearly unconscious at the end of a 15-min test session. BEL for the different groups ranged from 115–208 mg%, with the highest levels noted for mice selected for assessing the effects of antagonists.

Operant responding for ethanol reward during prepradial tests: concentration curves. Concentration curves during the preprandial tests for all mice assigned to the naltrindole (A) and MDL (C) groups (n = 12/group) are shown in the upper

TABLE 1

LEVER RESPONSES FOR 12% EtOH AND BLOOD ETHANOL LEVELS (BEL) [MEAN ± SE] DURING 15-MIN POSTPRANDIAL TESTS

	Naltrindole			MDL 72222		
	Responses	BEL (mg%)	(N)	Responses	BEL (mg%)	(n)
Total group	98 ± 14	115 ± 23	(12)	114 ± 11	183 ± 23	(11)
Selected mice	120 ± 8	156 ± 22	(6)	127 ± 3	208 ± 28	(8)

graphs of Fig. 1. Repeated-measures analyses of variance (ANOVA) of these data provide statistical support for the systematic increase in responses with increasing ethanol concentration for both groups of mice [Naltrindole, $F(4, 44) = 11.621$, $p < 0.001$; MDL, $F(4, 40) = 7.788$, $p < 0.001$]. For both groups, responses for 6 and 12% ethanol were greater than responses for water reward ($p < 0.01$, Duncan's multiple *t*-test), and responding for water on the day prior to and after the ethanol tests were not different ($p > 0.10$).

The lower graphs (B and D) summarize data for the mice selected to evaluate the effects of the antagonists on responding for ethanol reward, as noted above. Although the overall response output was greater for the smaller selected groups than for the respective larger groups from which they were selected, the pattern was similar with increased responding for ethanol reward as concentration increased [Naltrindole, $F(4, 20) = 16.427$, $p < 0.001$; MDL, $F(4, 28) = 8.071$, $p < 0.001$]. For these smaller groups, responses for all ethanol concentrations was greater than for water reward (Duncan's multiple *t*-tests, $p < 0.01$), and responding for water reward after the ethanol reward tests did not differ from responses prior to the tests. Total responses for 12% ethanol by mice assigned to the naltrindole group were 91 for all 12 mice and 138 for the six selected high-responding mice. Similar values for mice assigned to the MDL group were 94 and 118. These numbers of responses on the FR8 schedule produced, respectively, 11 and 17 reward periods for the low- and high-responding mice of the naltrindole group, and 12 and 15 rewards for low and high responders of the MDL group. These response levels provided 1.4 and 2.0 ml of 12% ethanol solution over the test session for the two groups of naltrindole mice, and 1.4 and 1.8 ml for the two groups of mice in the MDL group.

Antagonist effects on operant responding for ethanol reward: dose–response tests. Dose–response plots for the effects of the antagonists on responding for 12% ethanol are shown in Fig. 2 (A—naltrindole; B—MDL), and suggest that ethanol reward is attenuated by MDL, but not by naltrindole except at an extremely high dose. Each of the drug doses was given two to four times over a 5-week period, and the average responses generated under each dose was used for analysis. Response output for water reward is included in each graph for comparison. MDL produced a systematic decrease in responding for ethanol reward, $F(6, 42) = 2.672$, $p < 0.02$, with significantly lower responding after the 10 mg/kg dose in comparison to vehicle ($p < 0.05$, Duncan's multiple *t*-test). Responding for ethanol, however, continued to be greater than for the water reward, suggesting that motor deficit was not a likely mechanism for the reduction. In contrast, responding for ethanol reward was not influenced by naltrindole, $F(7, 35) = 0.549$, until mice were given a dose that completely eliminated responding (i.e., 30 mg/kg). The time course of drug action was evaluated at doses of 20 mg/kg for naltrindole and at 7.5 mg/kg for MDL. Naltrindole produced no systematic change in response across time, whereas the attenuation of ethanol reward by MDL was absent by 60 min after injection [See Fig. 3; dose × time: $F(2, 14) = 4.698$, $p < 0.027$].

MDL 72222 effects on operant responding for ethanol or water reward during postprandial (high thirst, low hunger motivation) with free access to the alternative fluid. Mice increased responses for both ethanol or water rewards when tested under the high-thirst motivation associated with postprandial tests. For example, responding increased from ~30 responses during preprandial tests to ~110 responses during postprandial tests for water rewards from ~120 to ~220 for 12% ethanol. These changes were despite ethanol being freely available when responding for water, and water being freely available when responding for ethanol. The effect of MDL on response output under these conditions is shown in Fig. 4. A 2(ethanol/water) × 2(vehicle/MDL) ANOVA on these data indicated a significant interaction, $F(1, 7) = 27.572$, $p < 0.002$. When responding for the ethanol reward, MDL reduced response output by ~90% in comparison to a 45% reduction

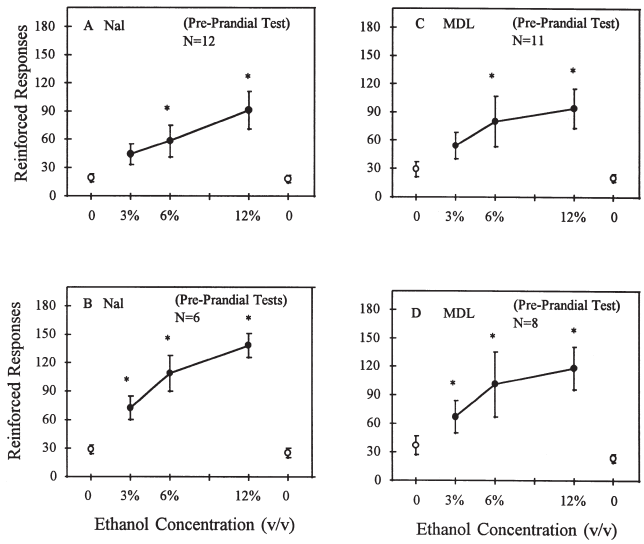

FIG. 1. Reinforced responses (FR8) for various concentrations of ethanol reward for mice assigned to naltrindole (A and B) and MDL (C and D) antagonist groups. The upper graphs summarize data for all mice originally assigned to the groups, whereas the lower graphs summarize data for all mice from each group that reliably made sufficient responses to have access to 12% ethanol at least four times per 15-min session. Responses increased with increasing ethanol concentrations, an effect that was stronger for the smaller groups of mice used to assess the antagonist effects on ethanol reward.

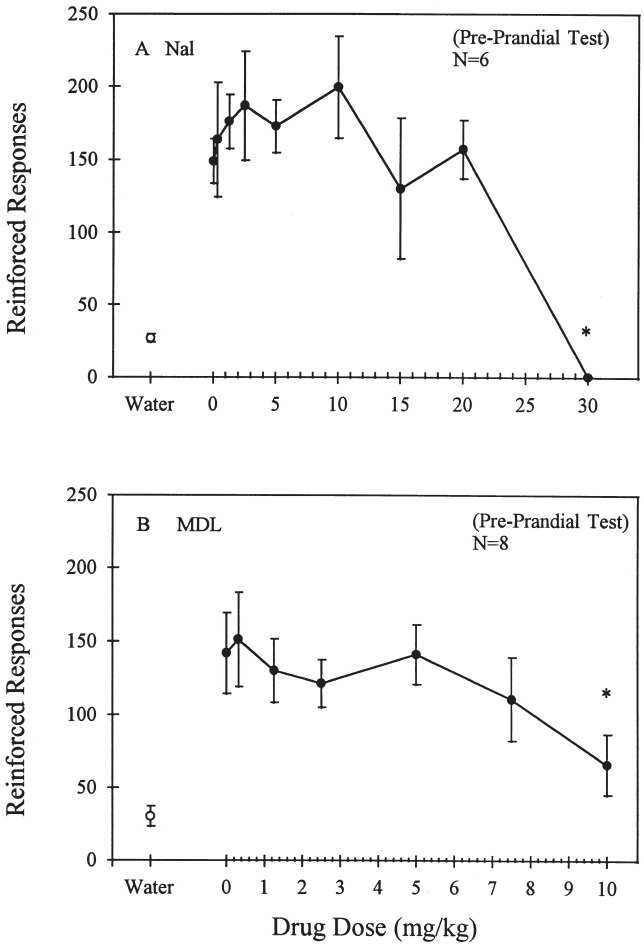

FIG. 2. Effects of naltrindole (A) and MDL (B) on responding for 12% ethanol delivered on an FR8 schedule. The open symbol reflects responses made for water reward as a comparison.

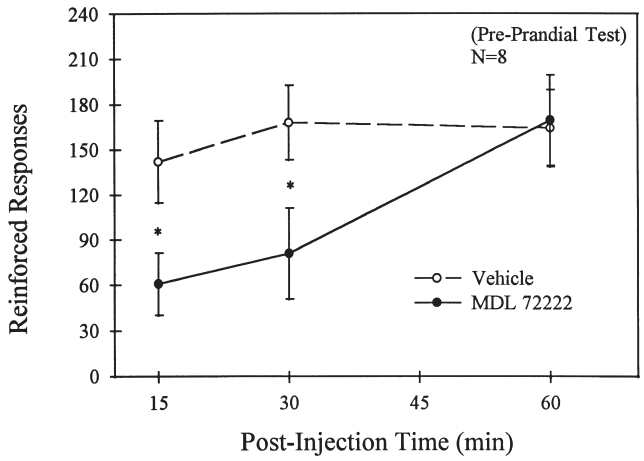

FIG. 3. Time course for MDL (7.5 mg/kg) effects on responding for 12% ethanol reward. The attenuating effects of the drug decline with increasing time after injection.

when responding for water. Resolution of the interaction indicates that responding was greater for the ethanol than for the water reward when mice were injected with vehicle, $t(7) = 3.717$, $p < 0.01$, but not following injections of MDL, $t(7) = 1.377$, $p = 0.210$. Thus, the 5-HT_3 antagonist appeared to attenuate ethanol reward.

EXPERIMENT II: ANTAGONIST EFFECTS ON ETHANOL DISCRIMINATION

Method

Subjects. Male and female, 12 each per antagonist, experimentally naive adult C57 mice (3 months old; 20–25 g) were individually housed under conditions described above. Each mouse had unlimited access to water. Their food intake was reduced to maintain 80% of their ad lib weight, with the daily food allotment provided immediately after testing, which occurred between 0800–1200 h. The experimental protocols included in this study were approved by the IACUC, Medical University of South Carolina (AR #1441), 11/17/98, and comply with the Public Health Policy on Humane Care and Use of Laboratory Animals and all applicable provisions of the Animal Welfare Act.

Apparatus. Six operant chambers enclosed in sound- and light-controlled environmental chambers were used. The operant chambers (16 × 16 × 11.4 cm) were constructed of gray Plexiglas, with stainless steel grid floors. Food pellets (20 mg A/I Rodent Pellets, Noyes Co., Lancaster, NH) were provided in a food tray located at floor level behind a 1.9 × 2.5-cm opening in the center of one wall. Light was provided by miniature bulbs (GTE 18-19) located directly above the food tray. Response levers (Model SRL-003, BRS/LVE, Laurel, MD) were located 4 cm to each side of the food tray and 3 cm above the floor. Levers were set so that a force of 8 g dead weight activated a microswitch and defined a response. The units were interfaced via LabLinc (Coulbourn Instruments, Lehigh Valley, PA) with a PC computer. Software derived in this laboratory determined reinforcement schedules, appropriate lever for reinforcement, and recorded responses.

Procedure. The experiment proceeded according to several phases as previously described (30–32) and detailed be-

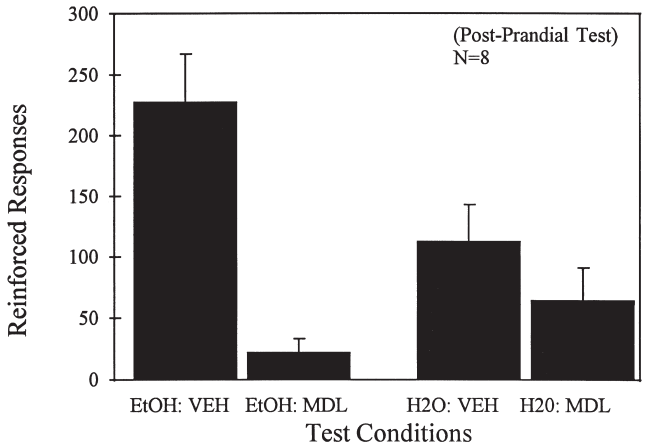

FIG. 4. Responses for 12% ethanol (left bars) or water (right bars) following injections of saline or MDL (7.5 mg/kg). Water was freely available during the 12% ethanol reward tests, and 12% ethanol was freely available during the water reward tests. MDL significantly reduced responding for the ethanol but not the water reward.

low. Briefly, the animals were maintained at 80 ± 3% of their ad lib feeding body weight throughout the experiment, and were tested 15 min per day, 5 days per week. Ethanol discrimination acquisition began after the animals had acquired a lever response for food reward and had stable responding on a FR20 reinforcement schedule. Discrimination training continued until the animal met our criterion and generalization tests were then conducted to establish dose–response functions. After these tests, the effects of various doses of the antagonists on ethanol discrimination were determined.

Lever response acquisition. Lever response acquisition began after 2 days of habituation to the operant chambers. On the first day, both response levers were covered and two food pellets were available in the food tray. On the second day, two pellets were initially available in the tray, and a food pellet was dispensed every 2 min. On day 3, both response levers were available, and food pellets were initially placed in the food tray and on top of each lever (two each). In addition, food pellets were delivered for each response on either lever (FR1). FR1 training was continued for eight sessions (4 days with both levers available, and 4 days with lever availability alternated). During this time the number of responses on each lever was equalized across the different mice. After 30 responses on each lever the reinforcement schedule was increased to FR5 for 4 days (two sessions per lever), and then to an FR20 schedule.

Ethanol discrimination acquisition training. Animals were injected IP with either ethanol (1.0 g/kg) or vehicle (distilled water) 5 min prior to testing. Drug and vehicle injections were given according to a schedule requiring that the animal receive no more than 2 successive days of vehicle or ethanol, and that a total of five vehicle and five ethanol injections be given for each 10 days of training. The levers designated as vehicle or ethanol were counterbalanced across the subjects. The number of responses on each lever was recorded daily and a discrimination index (DI) was calculated (DI = the number of responses on the current lever/total responses made prior to the delivery of the first reinforcement). Three consecutive days with a DI of 85% was set as a criterion for ethanol discrimination.

Generalization testing. After attaining the ethanol discrimination criterion, the animals were given a series of generalization tests to establish a dose–response function of ethanol discrimination. The tests were 2 min in length, with no reinforcement given for responses on either lever. The number of responses on each lever was recorded, and the percentage of responses on the drug lever was calculated for each test. After each generalization test, the animal was placed on daily ethanol discrimination training sessions until criterion responding was again achieved. Ethanol discrimination dose–response generalization tests began 5 min after injections of 1.0, 0.75, 0.50, or 0.25-g/kg doses of ethanol.

Antagonist effects on ethanol discrimination. A series of generalization tests were completed to determine dose–response and time course functions for naltrindole and MDL effects on ethanol discrimination. The antagonists were prepared as described previously, and were administered IP at doses of 0, 1.25, 2.50, 5.0, 10.0, and 15.0 mg/kg. Within each antagonist group, animals received each dose one to three times. For the dose–response tests, antagonists were injected 15 min prior to testing and 10 min prior to the training dose of ethanol (1.0 g/kg). After completing the dose–response testing, the time course of drug action was assessed for both antagonists (15 mg/kg for both drugs at 15, 30, and 60 min postinjection).

Results

The ethanol discrimination criterion (3 successive days of 85% correct responding) was acquired in (mean ± SEM) 30 ± 3 and 34 ± 3 sessions for mice assigned, respectively, to naltrindole and MDL testing. The results of ethanol dose–response generalization tests and the effects of increasing doses of antagonist on discrimination for the two groups are summarized in Fig. 5. Ethanol discrimination declined with decreasing ethanol dose for both groups [Naltrindole (A): $F(4, 40) = 35.743$, $p < 0.001$; MDL 72222 (D): $F(4, 44) = 48.597$, $p < 0.001$], indicating some specificity for the cue produced by the 1.0 g/kg ethanol injection.

Figure 5 summarizes the effects of the different doses of naltrindole and MDL on ethanol discrimination (B and E) and on total responses (C and F) during discrimination tests. Naltrindole had no significant systematic influence on ethanol discrimination across the doses tested, $F(5, 50) = 1.641$, $p > 0.10$, although total response output was reduced at the higher doses, $F(5, 50) = 4.451$, $p < 0.01$. MDL tended to reduce ethanol discrimination, $F(5, 55) = 2.271$, $p = 0.059$; however, the data were quite variable and the effect was not systematic across the doses tested. The drug, however, was behaviorally active producing a systematic reduction in responding with increasing dose, $F(5, 55) = 5.866$, $p < 0.001$.

Analyses of the time course data generated by mice injected with the 20-mg/kg dose of naltrindole indicated no systematic effect across time on either ethanol discrimination or total response output. Thus, the 20 mg/kg dose of naltrindole reduced lever responding for up to 1 h postinjection. Although the effects of MDL on ethanol discrimination did not change across time, the ~48% reduction of total response output produced by the drug at 15 min postinjection declined to ~32% by 60 min postinjection. Because of the extreme variability of these data, they were analyzed with a Friedman's ANOVA by ranks, which provided statistical support for a change across time in total response out put ($\chi^2 = 12.40$, $p = 0.037$), but not ethanol discrimination ($\chi^2 = 6.191$, $p = 0.088$).

DISCUSSION

Food-deprived C57 mice lever responded for ethanol delivered on a FR8 schedule of reinforcement with responses increasing as ethanol concentrations increased from 3–12%. When tested postprandially, responding and consumption were sufficient to produce BELs of 115–208 mg%, depending on the particular group assignment and whether or not the mice were categorized as "good ethanol responders." The responses maintained by the FR8 schedule and the BEL in the present experiments are similar to previous reports for C57 mice from our laboratory (32,34) as well as another laboratory (9).

MDL reduced responding by C57 mice for 12% ethanol at doses that had no influence on responding for water reward as previously reported for Long–Evans rats injected with another 5-HT_3 antagonist, ICS 205-930 (20). The results of these studies differ from reports that 5-HT_3 antagonists have little effect on ethanol consumption by rats when its access is limited (24,28). The different outcome of these two types of experiment suggests that 5-HT_3 antagonists might reduce the incentive, but not the unconditional rewarding properties of ethanol. The reduction in operant responding for ethanol reward produced by the 5-HT_3 antagonists, however, is consistent with the reductions in voluntary consumption of continuously available ethanol reported following injections of za-

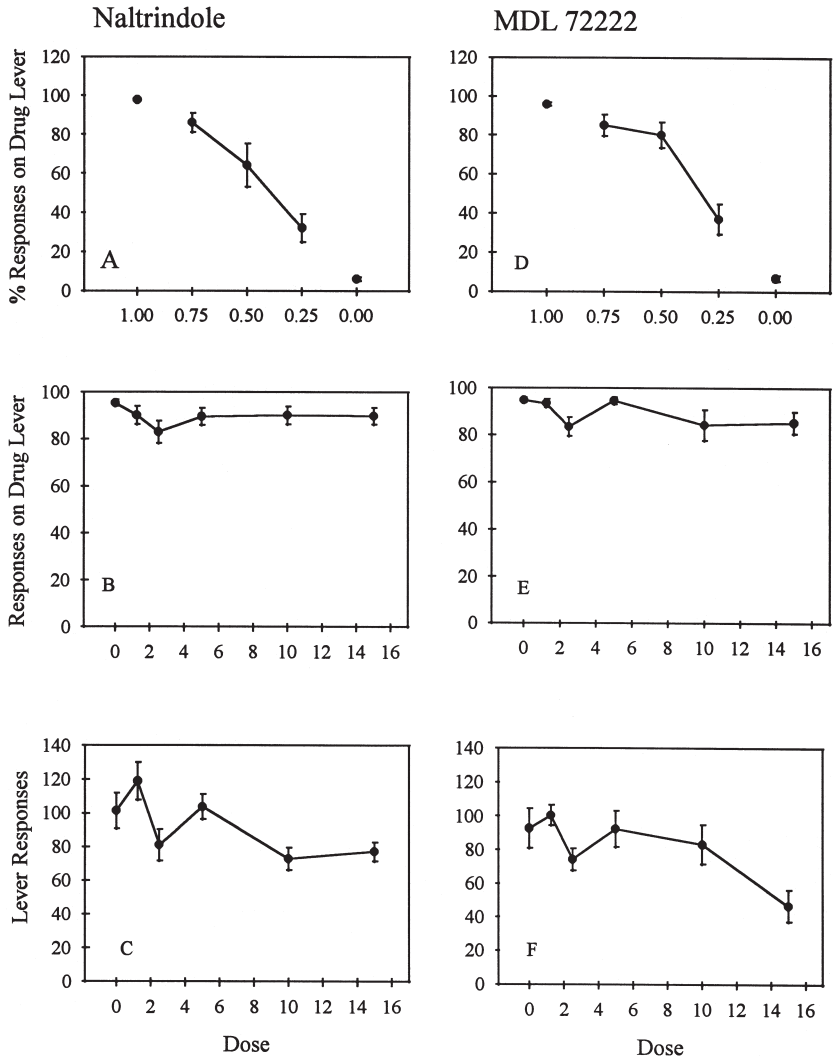

FIG. 5. Ethanol discrimination dose–response generalization curves for mice trained to discriminate a 1.0-g/kg dose of ethanol that were assigned to the naltrindole (A) and the MDL (D) evaluation groups. The interoceptive disciminative cue associated with the 1.0-g/kg dose of ethanol declined with declining dose for both groups of mice. The effects of naltrindole (B and C) and MDL (E and F) on ethanol discrimination (middle graphs) and on response output (lower graphs) during ethanol discrimination generalization tests. MDL, but not naltrindole, significantly attenuated the ethanol cue. Both drugs reduced response output at the higher doses.

copride (18), MDL (13), and odansetron (46) in rats. Although the limited access procedure appears to be less sensitive to the effects of 5-HT_3 antagonists on ethanol consumption in rats (24,28), one report (46) indicates that ondansetron reduced the consumption of ethanol by C57 mice in limited-access tests. Thus, for the ethanol-preferring C57 mouse, 5-HT_3 antagonists appear to attenuate behavior directed toward obtaining ethanol reward, the incentive for ethanol, as well as its consumption, or unconditional reward value.

The literature, based almost exclusively on rats (3,13,24, 28,45), indicates that 5-HT_3 antagonists attenuate ethanol consumption at lower doses when its availability is relatively unlimited (i.e., free-access paradigms) rather than time limited (limited-access paradigms) (28). For example, MDL at 1 mg/kg reduced ethanol consumption of P rats by approximately 50% when measured over a 24-h period, whereas doses up to 3 mg/kg injected 60 min prior to a limited-access (4 h) test had no effect on consumption. Further, the doses of ICS 205-930 required to reduce responding for ethanol reward by rats (20) and the doses of MDL required for mice in the current study ethanol were higher than those required to reduce consumption.

It appears that ethanol reward obtained under more defined and trained conditions requires higher 5-HT_3 antagonists doses to be disrupted. As previously noted (20,46), ethanol consummatory behavior during free access may be more easily disrupted because it is relatively unregulated and requires minimal training with little opportunity to become associated with other stimuli. In comparison, under limited access conditions, including operant experiments, the con-

summatory response for ethanol occurs during similar times of the day, under the same environmental conditions, and over months rather than weeks of training. During this training phase, ethanol's interoceptive effects can become conditioned to a variety of additional interoceptive cues as well as exteroceptive stimuli, which in turn, contribute toward the instrumental response to obtain ethanol reward. The variety of contributing reinforcing stimuli as well as the well-trained habitual behavioral response may contribute to the higher doses of the 5-HT_3 antagonist required for its attenuation. Despite the relatively higher doses of the 5-HT_3 antagonist required, the reduction in responding for ethanol reward was noted at doses that did not reduce responding for water reward. Further, the 7.5-mg/kg dose of MDL in our experiment had little influence on responding for food reward in our ethanol discrimination experiment. Thus, the 5-HT_3 antagonist appears to have some selectivity for ethanol over water and food reward.

In contrast to the 5-HT_3 antagonist, the δ-opioid antagonist, naltrindole, had little effect on ethanol reward at doses that were not debilitating (30.0 mg/kg). This result contrasts with reports that naltrindole reduced the consumption of ethanol by C57 mice (27), HAD rats (16), and P rats (25). The fact that naltrindole did not reduce responding for ethanol reward despite reports that it reduced ethanol consumption (16,25,27) again suggests the possibility that different mechanisms mediate the consummatory and instrumental responses for ethanol as noted above for MDL. Consistent with this interpretation is a report that doses of naltrexone and naloxone that reduced the consumption of food and water had little influence on lever responding for food or water reward (39). An additional report suggests that the consummatory and appetitive (instrumental) behavior for ethanol are likely under separate (but perhaps overlapping) control mechanisms (38), an interpretation consistent with recent reports from our laboratory (32,33). Because food restriction increases the rewarding properties of abused drugs (5), it is possible that testing mice in a food-deprived state contributed the lack of a naltrindole effect in our study. It should be noted, however, that in agreement with our experiment, naltrindole did not reduce responding for ethanol by nonfood-deprived rhesus monkeys (50), and did not reduce ethanol consumption by nonethanol-preferring Wistar rats (44). Thus, a general conclusion that the δ-opioid receptor systems mediate ethanol reward appears to be premature.

It is interesting to note that although naltrindole failed to significantly alter responding for ethanol reward in this study, under identical experimental conditions, the general opioid antagonist, naltrexone, attenuated operant responding for ethanol reward by C57 mice (34). Thus, the general opioid antagonist was more effective than the more specific δ-opioid receptor antagonist in attenuating ethanol reward, which is consistent with the report for rhesus monkeys (50). In combination, these experiments suggest that the hypothesis (16,25, 27) indicating that naltrexone attenuates ethanol reward by its action on δ-opioid receptors may be restricted to ethanol consumption rather than its general rewarding properties. In addition, the predominant role of δ-opioid receptors in mediating naltrexone effects on ethanol reward may be further restricted to ethanol-preferring rodents, because naltrexone effectively reduced ethanol consumption by nonpreferring rats, whereas naltrindole did not (44).

Neither antagonist systematically influenced the discriminative cue properties of injected ethanol for C57 mice, although one dose of MDL attenuated the cue, as was previously reported for pigeons (18). The MDL effect was not systematic, and is difficult to interpret. Certainly, the 10–12% reduction does not compare with the reductions observed for pigeons injected with either MDL (5.6 and 10.0 mg/kg) or ICS 205-930 9 (0.1–3.0 mg/kg) (18). In our study on C57 mice, ethanol discrimination was unaffected at an MDL dose (15 mg/kg) that substantially reduced response rates, indicating that the drug was biologically active. Further, reducing the salience of the ethanol cue by testing at a later time after ethanol injections did not reveal drug effects; however, the 60-min time delay may have exceeded the effective time range for MDL in mice. This interpretation is consistent with the reduced drug effect on total response output during the discrimination tests at later postinjection times, as well as its inability to attenuate ethanol reward by 60 min after injection. Thus, several doses of MDL were unable to alter the ability of C57 to discriminate the cues associated with injected ethanol at several postinjection times. The absence of a substantial literature on this topic suggests the possibility that the 5-HT_3 antagonists do not reduce ethanol's interoceptive discriminative effects in C57 mice or other rodents. However, additional experiments using additional training doses and/or testing doses would be necessary to rule out this possibility.

In summary, the present experiments established that the 5-HT_3 antagonist, MDL, attenuated lever responding by food deprived C57 mice for 12% ethanol rewards at a dose that did not influence responding for water reward. This result in combination with a report that ethanol consumption by C57 mice is reduced by ondansetron suggests that the 5-HT_3 system, perhaps by its action on the dopamine reward system (47), is involved in mediating behavior directed toward obtaining ethanol reward as well as ethanol consumption. These results are also consistent with the potential effectiveness of the 5-HT_3 antagonists for reducing the impact of ethanol or ethanol conditioned cues (i.e., the operant environment) for alcohol abuse treatment procedures. The effects of the 5-HT_3 antagonist in the present study compare with our previously reported effects of naltrexone on C57 mice (34), which indicated that the opioid systems also contribute to ethanol reward. The effects of both MDL and naltrexone on ethanol discrimination were slight in comparison to their effects on reward. In contrast to the attenuation of ethanol reward by the 5-HT_3 antagonist and the general opioid antagonist, naltrindole, a specific δ-opioid receptor antagonist, was without effect on ethanol reward, which is consistent with recent reports for rats (44) and monkeys (50). In combination, these results suggest that more general opioid antagonists may be more effective than more specific ones in attenuating ethanol reward, which is in agreement with a recent article suggesting that more general acting compounds may be more efficacious for the treatment of alcohol abuse (49). The 5-HT_3 antagonist also slightly reduced the discriminative cue for ethanol in C57 mice in the present study, as previously reported for pigeons (18); however, the effect was very slight. Thus, it appears that the rewarding and discriminative effects of ethanol are not mediated by identical neural mechanisms, an effect previously observed for naltrexone (34).

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