

Oral Ethanol-Reinforced Responding in Rhesus Monkeys: Effects of Opioid Antagonists Selective for the μ -, κ -, or δ -Receptor

Keith L. Williams and James H. Woods

To determine the mechanism by which naltrexone (NTX) reduces oral ethanol-reinforced responding, opioid antagonists that show μ -, κ -, or δ -selectivity were evaluated. Rhesus monkeys ($n = 6$) were given opportunities to respond and receive ethanol (1% or 2%) or water during daily 3-hr drinking sessions. Before some drinking sessions, the monkeys received intramuscular injections of saline or the following drugs: the μ -selective irreversible antagonist clocinnamox (CCAM), the κ -selective long-lasting antagonist nor-binaltorphimine (nor-BNI), or the δ -selective antagonist naltrindole. Also, NTX was administered along with either CCAM or nor-BNI. When given alone, CCAM (0.1 mg/kg) had no effect on ethanol-reinforced responding. When NTX (0.32 mg/kg) was given with CCAM, responding maintained by ethanol was decreased. Nor-BNI (3 mg/kg) reduced ethanol-reinforced responding only on the day of injection. On subsequent days, when other studies report continued κ -antagonism, responding maintained by ethanol returned to control levels. Also, NTX (0.32 mg/kg), administered in the presence of nor-BNI, was still able to reduce ethanol-reinforced responding. Naltrindole failed to alter responding maintained by ethanol. Because selective antagonism at the μ -, κ -, or δ -receptor did not reduce ethanol-reinforced responding, NTX's ability to reduce ethanol consumption may not be mediated by these previously characterized opioid receptors. NTX may exert its effects through an uncharacterized opioid binding site or through a nonopioid mechanism.

Key Words: Alcohol, Naltrexone, Drinking, Opioids, Receptors.

THE OPIOID antagonist, naltrexone (NTX), has been effective in reducing craving and relapse rates in clinical studies with alcohol-dependent patients.^{1,2} In one of the studies, patients who failed to remain abstinent reported that the subjective "high" produced by alcohol consumption was reduced while on NTX.³ One hypothesis suggests that alcohol consumption increases activation of the endogenous opioid system.^{4,5} Thus, NTX may produce its clinical effects by blocking this ethanol-induced opioid activity.

Opioid antagonists reduce ethanol consumption in a selective and dose-dependent manner. In a previous study,⁶ we found that ethanol-reinforced responding was reduced dose-dependently by NTX. An antagonist similar to NTX, naloxone, selectively reduced ethanol consumption in High

Alcohol Drinking rats when water was also available.⁷ In that study, low doses were effective (0.075 and 0.1 mg/kg), but higher doses (1 to 18 mg/kg) were even moreso. Evidence suggests that, at very low doses, naloxone selectively occupies μ -receptors, while at larger doses, naloxone also occupies δ - and κ -receptors.⁸⁻¹⁰ Thus, the reductions in alcohol drinking may be related to naloxone's binding properties at μ -receptors.

Some research suggests that it may be possible to differentiate the opioid receptor subtype through which naloxone or NTX reduces alcohol consumption. The δ -antagonist, ICI 174,864, was shown to reduce alcohol consumption selectively in rats when alcohol was available concurrently with water.¹¹ The δ -antagonist, naltrindole, had similar effects.¹² However, one study demonstrated that ICI 174,864 did not affect alcohol drinking, whereas CTOP, the μ -antagonist, reduced alcohol, without affecting water or food intake.¹³ The μ -antagonist, β -funaltrexamine, has also been reported to reduce alcohol consumption selectively on the day of injection, as well as on the day after the antagonist injection.¹⁴ This finding is in agreement with data that show β -funaltrexamine to be a long-duration irreversible antagonist at the μ -receptor.¹⁵ Together, these studies suggest that the δ - or μ -receptor may be involved in the ethanol consumption-decreasing effects of naloxone or NTX.

The μ -selective irreversible antagonist, clocinnamox (CCAM), can be used to discriminate between opioid agonist effects at the μ -receptor and the δ - or κ -receptor.¹⁶ In food-reinforced rhesus monkeys, CCAM (0.1 mg/kg) antagonized the rate-suppressant effects of μ -agonists but not δ - or κ -agonists.¹⁷ In the warm-water tail withdrawal antinociception assay performed in rhesus monkeys, CCAM (0.1 mg/kg) both reduced the potency of and suppressed the maximum antinociceptive effect of the potent μ -agonist, alfentanil.¹⁸ In that study, the number of μ -receptors available to interact with alfentanil was decreased by CCAM, and full recovery was observed 2 to 4 weeks later. In mice and rats, CCAM blocked both the analgesic effects and binding of μ -receptor agonists for days to weeks.^{19,20} These studies indicate that CCAM is a long-lasting and irreversible opioid antagonist with selectivity for the μ -receptor in mice, rats, and monkeys.

Other selective antagonists can be useful for discriminating effects at the κ - or δ -receptor. For example, nor-

From the Department of Pharmacology (J.H.W.) and Psychology (K.L.W., J.H.W.), University of Michigan, Ann Arbor, Michigan.

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Reprint requests: Keith L. Williams, Ph.D., 1301 MSRB III, Department of Psychology, University of Michigan, Ann Arbor, MI 48109-0632.

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binaltorphimine (nor-BNI) is a κ -selective antagonist with a long duration of action.²¹ One study,²² using the warm-water tail withdrawal assay in monkeys, found nor-BNI (3.2 mg/kg) to reduce the potency of κ -selective agonists, but not agonists selective for other opioid receptors. Similar to CCAM, nor-BNI's effect persisted for several (17 to 35) days. Another useful research tool, naltrindole (NTI), is an opioid antagonist selective for the δ -receptor. Using rhesus monkeys responding for food, Negus et al.²³ examined whether NTI could antagonize the reduced responding caused by various opioid agonists. They showed that NTI (1 and 3.2 mg/kg) completely reversed the rate-decreasing effect produced by the δ -agonist BW373U86. NTI (3.2 mg/kg) had no effect when rates of food-maintained responding were suppressed by μ - and κ -agonists. These studies provide evidence for the receptor-selective nature of nor-BNI and NTI for the κ - and δ -receptors, respectively, in rhesus monkeys.

The purpose of this study was to use μ -, κ -, or δ -selective opioid antagonists to determine the mechanism by which NTX reduces ethanol-reinforced responding in rhesus monkeys. Because CCAM and nor-BNI antagonist effects continued for a long time in the antinociceptive assays in monkeys, these drugs should be effective in reducing ethanol-reinforced responding for several days if the μ - and/or κ -receptor is involved in ethanol-reinforced responding. In addition, if NTX is acting through μ - or κ -receptors to suppress ethanol-maintained responding, NTX's potency to reduce consumption may be reduced by prior administration of CCAM or nor-BNI.²⁴ Using these selective antagonists alone and in conjunction with NTX may help to determine the opioid receptor through which NTX produces its alcohol consumption-decreasing effects.

MATERIALS AND METHODS

Subjects

Subjects were 6 adult male rhesus monkeys (*Macaca mulatta*; weighing 6.3 to 9.9 kg) maintained at ~80% of their free-feeding weights. In these experiments, the "Guide for the Care and Use of Laboratory Animals" (NIH publication, vol. 25, no. 28, revised 1996) was followed.

Apparatus

The animal housing room was on a 12-hr light/dark cycle (lights on at 0700 hr, lights off at 1900 hr). The monkeys were housed in individual cages measuring 64 cm \times 72 cm \times 85 cm high. A fluid-delivery panel, similar to that used in other studies,^{6,25} was attached to one wall of each cage during daily sessions. Holes were cut in the cage wall so that two brass spouts on the fluid-delivery panel protruded into the cage 50 cm from the floor. A stimulus light that could be illuminated red or green was located 3 cm above each spout. The drinking solutions were contained in 1000 ml plastic bottles attached to the back of the panel. Plastic tubing connected each bottle to the spout valve. The fluid containers were elevated so that the liquid was gravity-fed to the spout valve and delivery was controlled by a solenoid switch. Contact with either spout closed an electrical circuit (drinkometer), and a response was recorded. The stimulus light above the spout flashed the appropriate color (green or red, depending on the fluid available) when contact was made with the spout. When the reinforcement schedule was satisfied, the solenoid was activated, and 0.5 ml of fluid was

delivered. Solutions were measured after the session using graduated cylinders to confirm delivery amounts. The experiments were controlled and data recorded using IBM PCjr microcomputers located in a room adjacent to the housing room.

Procedure

Experimental sessions were conducted each day. Each session lasted 3 hr, during which the animal could respond and obtain either ethanol or concurrently available water. Ethanol was available under the green stimulus light, and water was available under the red stimulus light. The monkeys were reinforced with 0.5 ml of fluid for every four mouth contacts on the spout (reinforcement schedule = fixed-ratio 4). The reinforcement schedule on each of the two spouts operated concurrently and independently such that the responses on one spout did not alter the number of responses required on the opposite spout. Water was always available during the session from one of the spouts. The animals were fed after the session.

The opioid antagonists were administered while the monkeys had access to ethanol concentrations that maintained the greatest amount of responding. Three monkeys were responding and receiving 1% ethanol, and three were responding and receiving 2% ethanol. Due to the similarity, these data were pooled to obtain the average fluid deliveries and ethanol intake (g/kg).

Injections of saline, NTX, nor-BNI, and NTI were each given 30 min before sessions during which ethanol was available concurrently with water. CCAM was given 4 hr before these sessions. CCAM (0.1 mg/kg) was given to each monkey four times, each separated by at least 2 weeks. Saline was given 3.5 hr after the first and third CCAM pretreatments, and NTX (0.32 mg/kg) was given 3.5 hr after the second and fourth CCAM pretreatments. Nor-BNI (3 mg/kg) was given twice to each monkey, each injection separated by ~3 weeks. Six days after the first administration of nor-BNI, NTX (0.32 mg/kg) was administered along with nor-BNI. NTI (1 and 3.2 mg/kg) was given twice to each animal separated by 5 to 8 days. Saline pretreatments were also given frequently before and after administration of nor-BNI, NTI, or NTX. For three monkeys, the antagonist order was CCAM, nor-BNI, then NTI. The other monkeys were tested first with NTI, CCAM, then NTI. Approximately 1.5 to 2 months of noninjection baseline and saline-pretreated sessions intervened between the different antagonist pretreatments.

Data Analysis

Each monkey's average data (fluid deliveries, intake in g/kg, and fluid deliveries expressed as percent of noninjection baseline) were used to calculate the mean and standard error of the mean for the group of monkeys. Data are presented as the mean and standard error of the mean of the group data ($n = 6$ monkeys).

The fluid delivery data for the CCAM and nor-BNI pretreatments were analyzed together using a two-way repeated measures analysis of variance (RM ANOVA) testing for an effect of treatment, as well as an interaction of solution and treatment. The fluid-delivery data for the NTI pretreatments were analyzed in a similar manner. A one-way RM ANOVA was used to analyze the ethanol intake data for the CCAM and nor-BNI pretreatments. Ethanol intake for the NTI pretreatments was analyzed in a similar manner. For data expressed as a percent of noninjection baseline control, a one-way RM ANOVA on the ranks (Friedman test) was applied to the CCAM, nor-BNI, and NTI data. NTX data were from a previous study with different subjects,⁶ and thus were not compared statistically to the CCAM, nor-BNI, or NTI data. However, NTX data were previously analyzed using a one-way RM ANOVA. When significant differences were detected in any of the aforementioned tests, a post-hoc Student-Newman-Keuls test (SNK) was applied. For the results of all statistical analyses, significance refers to $p < 0.05$.

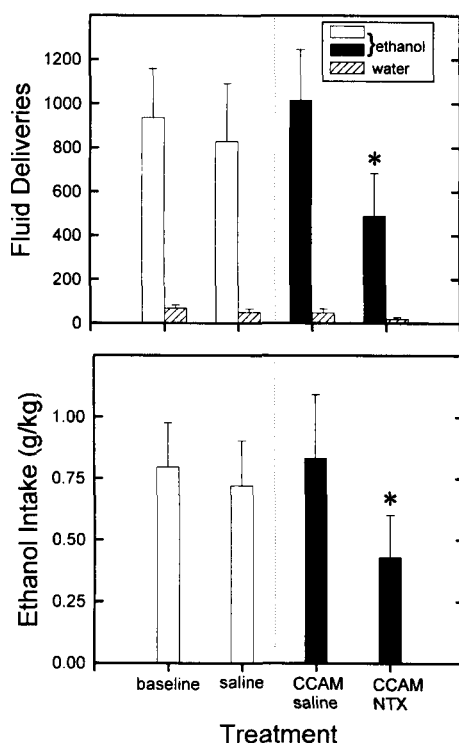


Fig. 1. (Top panel) Average number of fluid deliveries of ethanol (open and solid bars) and concurrently available water (lined bars) after different pretreatments: noninjection baseline, saline, 0.1 mg/kg of CCAM, and 0.1 mg/kg of CCAM followed by 0.32 mg/kg of NTX. (Bottom panel) Average ethanol intake (g/kg) after the same pretreatments. Bars represent the average with the standard error ($n = 6$ monkeys; 3 consuming 1% ethanol and 3 consuming 2% ethanol). * Significant difference ($p < 0.05$) from saline.

Drugs

Ethanol solutions were prepared by mixing the appropriate volumes of 95% w/v ethanol and tap water. NTI (provided by K. Rice, NIH), CCAM (provided by J. Lewis, Bristol University, UK), nor-BNI (provided by H. Mosberg, University of Michigan), and NTX (provided by the National Institute on Drug Abuse Research Technology Branch) were dissolved in sterile water.

RESULTS

Over all three experiments, the ethanol solution maintained a greater number of fluid deliveries than did concurrently available water. During the first part of the experiment (CCAM administration), the average number of ethanol fluid deliveries for noninjection baseline was 934 (± 223), whereas the average after saline pretreatment was 828 (± 262). The average number of water deliveries during noninjection baseline was 67 (± 15), whereas the average after saline pretreatment conditions was 48 (± 17). These averages remained similar throughout the different antagonist pretreatments.

When CCAM (0.1 mg/kg) was administered followed by saline injections, ethanol fluid deliveries and ethanol intake (g/kg) were similar to those shown during noninjection and saline injection controls (Fig. 1). When NTX (0.32 mg/kg) was given after CCAM, the number of ethanol fluid deliveries was reduced, compared with that after saline injection

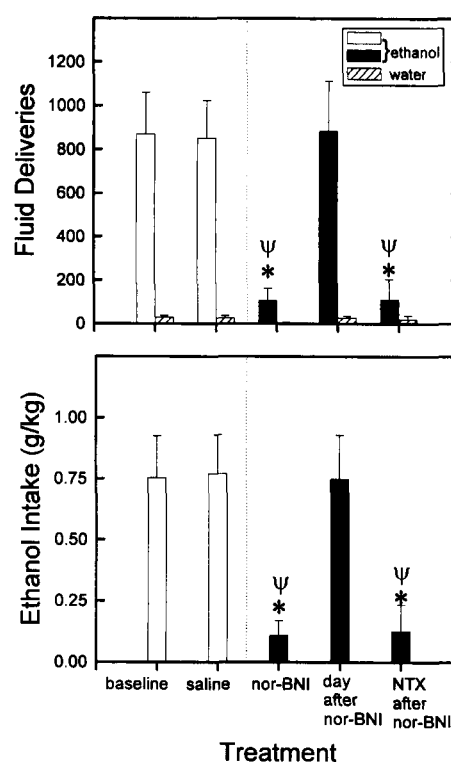


Fig. 2. (Top panel) Average number of fluid deliveries of ethanol (open and solid bars) and concurrently available water (lined bars) after different pretreatments: noninjection baseline, saline, 3 mg/kg of nor-BNI, the day after nor-BNI, and 0.32 mg/kg of NTX given 6 days after nor-BNI. (Bottom panel) Average ethanol intake (g/kg) after the same pretreatments. Bars represent the average with the standard error ($n = 6$ monkeys; 3 consuming 1% ethanol and 3 consuming 2% ethanol). * Significant difference ($p < 0.05$) from saline. ψ Significant difference from CCAM with NTX (in Fig. 1).

[$F(7,35) = 12.1$; SNK $q = 5.50$], and ethanol intake (g/kg) was also reduced [$F(7,35) = 14.6$; SNK $q = 3.67$]. On the days subsequent to testing with CCAM followed by saline or CCAM followed by NTX, the ethanol fluid deliveries and ethanol intake (g/kg) were no different from noninjection and saline controls. Thus, CCAM alone had no effect on ethanol-reinforced responding, whereas NTX in the presence of CCAM decreased ethanol deliveries and intake on the day of administration.

When compared with saline pretreatment, nor-BNI (3 mg/kg) significantly reduced the number of ethanol fluid deliveries [$F(7,35) = 12.1$; SNK $q = 10.50$] and ethanol intake (g/kg) [$F(7,35) = 14.6$; SNK $q = 8.30$] (as shown in Fig. 2). However, on the days after nor-BNI, the ethanol fluid deliveries and intake (g/kg) returned to levels similar to those during noninjection baseline or after saline pretreatment. When NTX (0.32 mg/kg) was given 6 days after nor-BNI, both the ethanol fluid deliveries and intake (g/kg) were reduced (SNK $q = 10.45$ and 8.07). Ethanol fluid deliveries and intake (g/kg) were unaffected on the days after NTX administration. Both nor-BNI alone and NTX given 6 days after nor-BNI reduced ethanol fluid deliveries below that of CCAM followed by NTX (SNK $q = 5.40$ and 5.34). This effect was also observed for ethanol intake (g/kg) (SNK $q = 4.02$ and 3.80). Thus, nor-BNI had an

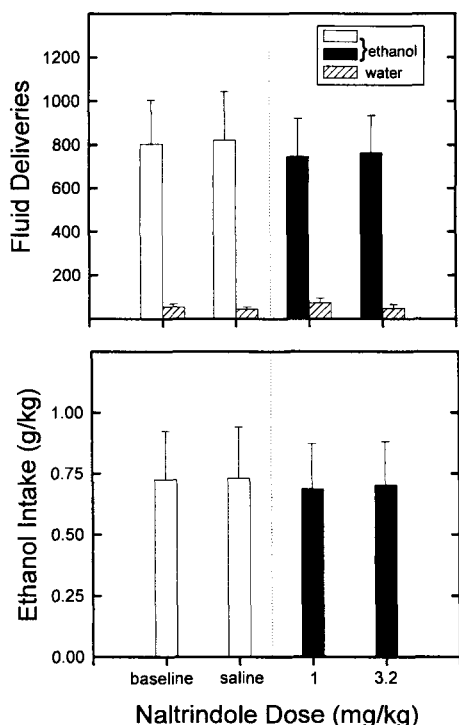


Fig. 3. (Top panel) Average number of fluid deliveries of ethanol (open and solid bars) and concurrently available water (lined bars) after different pretreatments: noninjection baseline, saline, 1 mg/kg of NTI, and 3.2 mg/kg of NTI. (Bottom panel) Average ethanol intake (g/kg) after the same pretreatments. Bars represent the average with the standard error ($n = 6$ monkeys; 3 consuming 1% ethanol and 3 consuming 2% ethanol).

effect only on the day of injection and NTX after nor-BNI reduced ethanol-reinforced responding. Also, nor-BNI alone and NTX after nor-BNI showed the greatest potency in reducing ethanol-reinforced responding.

NTI (1 and 3.2 mg/kg), shown in Fig. 3, failed to reduce ethanol or water fluid deliveries [$F(3,15) = 1.35; p < 0.30$]. Consequently, ethanol intake (g/kg) was unaffected as well [$F(3,15) = 0.36; p < 0.78$].

To make a better comparison across antagonist pretreatments, the ethanol fluid deliveries were converted to a percent of noninjection baseline (Fig. 4). For comparison, the effects on NTX alone are also shown.⁶ From this previous study, we found NTX (0.32 mg/kg) alone had a significant effect. When NTX was given after CCAM administration, ethanol fluid deliveries were reduced, compared with sessions in which saline followed CCAM administration [$\chi^2(9) = 33.4; SNK q = 7.55$]. Compared with saline pretreatment, ethanol fluid deliveries were decreased when either nor-BNI alone or nor-BNI with NTX was given before the session [SNK $q = 7.10$ and $q = 6.66$, respectively]. Also, nor-BNI alone and nor-BNI with NTX significantly reduced ethanol fluid deliveries more than the pretreatment of CCAM followed by NTX [SNK $q = 4.91$ and $q = 4.70$, respectively]. Saline pretreatment during each antagonist pretreatment phase did not alter ethanol fluid deliveries.

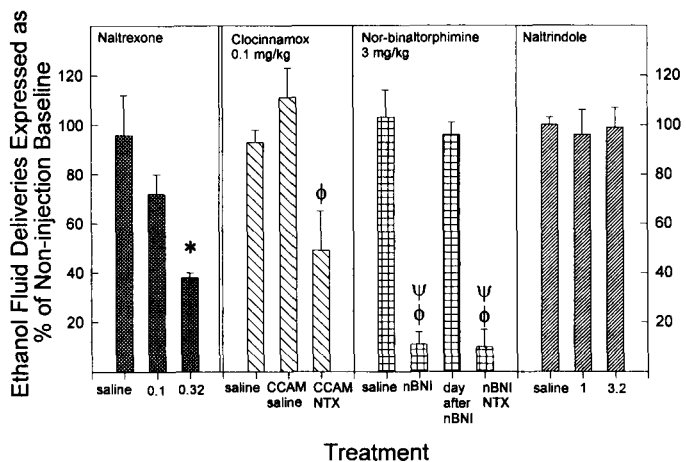


Fig. 4. Average number of fluid deliveries for ethanol, expressed as a percent of noninjection baseline control over various treatments. For comparison, the far left panel shows the effects of NTX (saline: 0.1 mg/kg and 0.32 mg/kg) from a previous study. The rest of the panels (from left to right) show the effects of NTI (saline, 1 mg/kg, and 3.2 mg/kg), CCAM (saline, 0.1 mg/kg, and 0.1 mg/kg of CCAM followed by 0.32 mg/kg of NTX), and nor-BNI (saline, 3 mg/kg, day after nor-BNI, and 0.32 mg/kg of NTX 6 days after nor-BNI). For the NTX data, bars represent the average with the standard error ($n = 5$ monkeys). The rest of the bars represent the average with the standard error ($n = 6$). On the NTX panel, asterisk indicates a significant difference from saline on that panel. On the other three panels, ϕ indicates a significant difference from all saline pretreatments (during NTI, CCAM, and nor-BNI testing), whereas ψ indicates a significant difference from CCAM with NTX.

DISCUSSION

This study used receptor-selective opioid antagonists to determine which opioid receptor subtype mediates NTX's ability to decrease ethanol-reinforced responding. The μ -antagonist, CCAM, failed to reduce ethanol-reinforced responding. Furthermore, NTX's ability to reduce ethanol-reinforced responding was maintained in the presence of CCAM. The κ -antagonist nor-BNI, caused a reduction in ethanol-reinforced responding only on the day of nor-BNI injection. Given in the presence of nor-BNI, NTX continued to reduce ethanol-reinforced responding. Lastly, we found that the δ -antagonist, NTI, had no effect on ethanol- or water-reinforced responding.

The failure of CCAM to reduce ethanol-reinforced responding suggests that μ -selective antagonism does not result in suppression of ethanol's reinforcing effects. This, in turn, indicates that NTX's antagonist activity at the μ -opioid receptor is not responsible for NTX's effect on ethanol intake. In other studies with rhesus monkeys, 0.1 mg/kg of CCAM effectively blocked the effects of μ -agonists. This CCAM dose reduced the potency of and suppressed the maximum antinociceptive effect of μ -agonist alfentanil for 7 to 14 days.¹⁸ CCAM also reduced the reinforcing potency of intravenously self-administered alfentanil for 24 hr.²⁶ In monkeys responding for food, this same dose of CCAM decreased the rate suppressing effects of μ -agonist fentanyl for at least 24 hr.²² These studies indicate that the CCAM dose used in these experiments was effectively blocking μ -opioid receptors. It is possible, however, that the CCAM dose was too small to reduce oral

ethanol-reinforced responding. The fact that doses of NTX required to suppress ethanol drinking are larger than those required to antagonize μ -opioid effects in other systems²⁷ suggests that, if NTX acts to suppress ethanol-reinforced responding or consumption through a μ -opioid mechanism, it is on less sensitive μ -opioid receptors, perhaps those that are less available to binding by CCAM.

There is a lack of research concerning the effects of κ -antagonists on ethanol consumption. Most research has focused on the role of κ -receptors on consummatory behaviors in general.²⁸ Some studies report that nor-BNI reduces consumption of a variety of substances in rodents.^{29,30} In addition, nor-BNI antagonized κ -opioid effects for days and weeks in rhesus monkeys²² and pigeons.³¹ In the current experiment, nor-BNI (3 mg/kg) reduced ethanol-reinforced responding only on the day of injection. Because a nor-BNI blockade of κ -receptors should have been maintained for a much longer time, the reduction in ethanol-reinforced responding presumably does not reflect a κ -mediated effect. In addition, κ -agonists do not produce positive-reinforcing effects.³² This evidence suggests that activity at the κ -receptor may not be responsible for the ethanol consumption-decreasing effect of NTX.

The short-lived reduction of ethanol-reinforced responding by nor-BNI may be due to a NTX-like effect. Because nor-BNI consists of two joined NTX compounds, this NTX-like effect is not surprising. Interestingly, nor-BNI has been shown to produce a short-lived antagonism of morphine-induced analgesia in mice, followed by a delayed antagonism of κ -opioids.³³ If nor-BNI's acute antagonism of morphine is through the same mechanism as its acute suppression of ethanol-reinforced responding, then we may conclude that nor-BNI and NTX suppress ethanol-reinforced responding through a μ -antagonist mechanism. Because the μ -antagonist CCAM failed to modify ethanol-reinforced responding, CCAM, nor-BNI, and NTX may not be acting at the same type of μ -receptor.

Although some research indicates that δ -opioids mediate ethanol consumption,^{11,34} our study fails to support this evidence. In our experiment, the δ -antagonist NTI did not decrease ethanol-reinforced responding. The doses tested have been shown to antagonize the rate-suppressant effects of δ -agonist, BW373U86, in monkeys responding and receiving food reinforcement.²³ In that study, NTI produced a reduction in potency of the δ -agonist. When NTI 10 mg/kg or higher was administered alone, they found little effect on response rates. However, these large doses began to antagonize the effects of μ -agonist alfentanil. Thus, to observe the effects of selective δ -antagonism, we only tested NTI doses up to 3.2 mg/kg. Our data indicate that the δ -receptor does not mediate ethanol-reinforced responding in monkeys.

The results of this study and our previous study⁶ indicate that the effect observed with NTX on ethanol-reinforced responding in rhesus monkeys may not be entirely explained by antagonist effects at the, μ -, κ -, or δ -receptor. If ethanol increases endogenous opioid activity and NTX re-

duces ethanol reinforced-responding by blocking that opioid activity, then the ethanol/NTX interaction should display some characteristics similar to that of an opioid agonist/competitive antagonist interaction. However, we previously demonstrated that the NTX effect was not surmountable by increasing the ethanol concentration (oral self-administration) or ethanol dose (intravenous self-administration). The NTX doses that affect ethanol-reinforced responding are much higher than those needed to antagonize exogenously administered opioid effects. Furthermore, pretreatment with the μ -selective antagonist CCAM had no effect on ethanol-reinforced responding. These results do not appear congruent with the idea that NTX reduces ethanol consumption by blocking ethanol-induced μ -opioid activity.

NTX given in the presence of CCAM or nor-BNI produced some interesting results. The CCAM-induced removal of μ -receptors might be expected to affect any NTX activity through these receptors. However, in the presence of CCAM, NTX suppressed ethanol-reinforced responding. Previous experiments^{18,26} demonstrated that the apparent affinity of μ -agonists is the same before and after CCAM treatment, which indicates that the NTX apparent affinity may be unchanged as well. Thus, NTX may possess the same ability to compete for the receptor before and after CCAM treatment and therefore produce the same effect. The other interesting result was that, in the presence of nor-BNI, NTX reduced ethanol fluid deliveries to a greater extent than that observed with NTX alone (our previous study⁶) or NTX in the presence of CCAM. To produce this enhanced NTX effect, the competitive antagonist action of nor-BNI may be increasing NTX availability to the site responsible for the consumption-decreasing effect.

NTX may be producing its effects via an opioid binding site other than those that have been characterized to date. Because some, but not all, opioid antagonists reduce ethanol consumption, NTX may be exerting its effect through an uncharacterized recognition site. To characterize this proposed mechanism, it is necessary to categorize and rank-order opioid antagonists based on their potency to reduce ethanol consumption. By combining this behavioral classification with a systematic analysis of the antagonists' binding properties for this presumed "NTX recognition site," we may be able to discover if this site is distinct from the already characterized opioid binding sites.

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