Contribution of the Opioid System to Alcohol Aversion and Alcohol Drinking Behavior


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ABSTRACT

The effect of blocking delta opioid receptors on alcohol aversion was examined in female alcohol-preferring (P) rats using a conditioned taste aversion (CTA) paradigm. In experiment 1, alcohol naive P rats were given i.p injections of 0.5, 1.0 or 1.5 g alcohol/kg BW or saline, paired with consumption of a banana-flavored solution during 5 conditioning trials. Alcohol in a dose of 0.5 g/kg was not aversive while the two higher doses (1.0 and 1.5 g/kg) were both aversive in the CTA paradigm. In experiment 2, the effect of the selective delta opioid receptor antagonist, naltrindole (NTI), on alcohol aversion was examined. Rats were pretreated with NTI in doses of 2.5, 5.0, 10.0 or 20.0 mg/kg before conditioning using the nonaversive dose of alcohol from Experiment 1. As in experiment 1, the 0.5 g/kg dose of alcohol did not produce a CTA. Administration of NTI alone in doses of 2.5, 5.0 or 10.0 mg/kg did not produce a CTA. However, when the nonaversive dose of alcohol (0.5 g/kg) was combined with NTI in a dose of either 5.0 or 10.0 mg/kg, an aversion to alcohol was seen. The highest dose of NTI (20 mg/kg) produced a CTA when given either alone and in combination with alcohol. The results indicate that blocking the action of opioid peptides at the delta opioid receptor can make a nonaversive dose of alcohol aversive which suggests that opioid peptides, acting via the delta opioid receptor, play an important role in regulating alcohol aversion.

The effects of alcohol are biphasic, rewarding during the rising portion of the BAC curve, and aversive or dysphoric during the falling portion of the curve (Pohorecky, 1977; Risinger and Cunningham 1992). A genetic propensity toward high alcohol drinking is determined by multiple genes that regulate many predisposing neurobiological factors (Froehlich, 1995; Froehlich and Wand, 1997). One of these factors may be increased sensitivity to, or lower threshold for, alcohol’s reinforcing effects that occur during the rising portion of the BAC curve (Froehlich and Li, 1993; 1994; Krishnan-Sarin et al., 1998; Li et al., 1998). Another factor may be decreased sensitivity to, or higher threshold for, alcohol’s aversive effects that occur during the falling portion of the BAC curve. Activation of the endogenous opioid system, as a consequence of alcohol ingestion, may influence both of these processes (Froehlich and Wand, 1996).

Endorphin and enkephalin peptides induce feelings of well-being, euphoria and pleasantness (Belluzzi and Stein, 1977; Bozarth and Wise, 1984) and function as positive reinforcers in a variety of behavioral paradigms (Goeders et al., 1984; Almaric et al., 1987; Shippenberg et al., 1992). We have previously postulated that alcohol-induced activation of the endogenous opioid system during the rising portion of the BAC curve enhances the hedonic value of alcohol and contribute to the positively reinforcing properties of the drug (Froehlich et al., 1991; Krishnan-Sarin et al., 1995a, 1995b; Froehlich, 1997). This may serve to support continued drinking during a single drinking bout and to increase the probability of subsequent drinking. Repeated bouts of alcohol drinking characterize rodents selectively bred for alcohol preference (P line), but not rodents bred for alcohol nonpreference (NP line) (Files et al., 1992). We now postulate that alcohol-induced activation of the endogenous opioid system during the falling portion of the BAC curve also contributes to drinking by counteracting some of the aversive consequences of alcohol ingestion, such as dysphoria or malaise, that can begin when blood alcohol levels are falling and extend long after blood alcohol levels have reached zero. In humans, a session of intoxication may be followed by malaise, nausea, headache and hyperirritability which are commonly referred to as “hangover” and are clearly aversive. Whether something akin to “hangover” occurs in the rat after alcohol exposure is less clear but rebound hyperthermia, hyperactivity and increased vocalization during handling have been reported at 16–24 hr after a single intraperitoneal (i.p.) in-
jectio of alcohol (2.5 g/kg) and these aftereffects of acute intoxication have been described as hangover signs (Sinclair and Gustafsson, 1987; Sinclair and Tiara, 1988).

We postulate that an elevated threshold for, or reduced sensitivity to, the aversive effects of alcohol may contribute, in part, to high alcohol drinking in rats selectively bred for alcohol preference (P line). This view is supported by our prior finding that P rats have a higher alcohol aversion threshold than do NP rats as measured in a conditioned taste aversion (CTA) test (Froehlich et al., 1988). We further postulate that this line difference in alcohol aversion may be due to differences in sensitivity of the opioid system to the postigestive effects of alcohol. Specifically, the aversive effects of alcohol, experienced after an initial exposure to an intoxicating dose of alcohol, may be attenuated by alcohol-induced activation of the opioid system in P rats. This view is supported by our recent finding that an intragastric (i.g.) infusion of alcohol (2.5 g/kg BW) elevates genetic message for beta-endorphin synthesis (POMC mRNA levels) in the pituitary of P but not NP rats at 8 hr after onset of the infusion, at a time when blood alcohol content (BAC) has returned to near preinfusion levels (Froehlich, 1995). While dysphoric symptoms may arise after initial exposure to an intoxicating dose of alcohol in both P and NP rats, greater alcohol-induced activation of the opioid system in P rats may serve to attenuate dysphoria and subsequent aversion to alcohol and contribute to high alcohol drinking in this rat line. If so, one might expect that blocking the action of endogenous opioid peptides, by pretreatment with an opioid receptor antagonist, would lower the alcohol aversion threshold in P rats. One approach to measuring alcohol aversion threshold is to use a CTA test which involves pairing the consumption of a novel and detectable taste solution with the administration of alcohol. Aversion to alcohol is indicated by a reduction of intake of the taste solution in subsequent exposures (Froehlich et al., 1988). A CTA test was used in the present study to examine whether administration of the selective delta opioid receptor antagonist, naltiridone (NTI), would lower the alcohol aversion threshold in P rats.

Methods

Subjects. Alcohol-naive female rats that were selectively bred for alcohol preference (P line) served as subjects. The P rat line, from which these subjects were taken, was derived from a foundation stock of outbred Wistar rats. Rats of the P line are selected for breeding based on the results of a 4-week oral alcohol preference test (Li et al., 1981; Lumeng et al., 1977). Each generation of rats is given alcohol as the sole source of fluid for 4 days followed by 4 weeks of ad libitum free-choice between a 10% (v/v) alcohol solution and water with food freely available. Alcohol intake is calculated for each rat during the four weeks of preference testing and alcohol intake is expressed as g alcohol/kg BW/day. Rats selected for breeding in each generation are those that consume more than 5 g alcohol/kg BW/day and demonstrate a greater than 2:1 preference ratio of alcohol to water. Rats of the P line represent an animal model that is useful for examinations of the biological mechanisms underlying alcohol drinking behavior.

The P rats which served as subjects in the present studies were taken from generations 39 to 41 of selection for alcohol preference but were not tested for alcohol preference, and hence were alcohol-naive. All rats from the 39 to 41 generations of selection that did not serve as subjects in the present study were tested for alcohol preference. Rats from the 39th generation of selection consumed an average of 7.1 g alcohol/kg BW/day ± 0.23 (S.E.) with a range of 3.7–11.87 g/kg BW. Rats from the 40th generation consumed an average of 7.0 g alcohol/kg BW/day ± 0.63 (S.E.) with a range of 2.6–13.7 g/kg BW. Rats from the 41st generation consumed an average of 6.9 g alcohol/kg BW/day ± 0.31 (S.E.) with a range of 2.0–11.2 g/kg BW. All rats were housed individually in a temperature and humidity controlled room with a 12 hr light/dark cycle (lights on at 0700 hrs) with food and water available ad libitum except as dictated by the experimental design.

Drugs. Naltiridone hydrochloride or NTI (17-cyclopromethyl-6,7-dehyro-4,5a-epoxy-3, 14-dihydroxy-6,7,2',3'-indolomorphinan) is a nonpeptide, highly potent antagonist with high affinity and selectivity for delta opioid receptors (Portoghese et al., 1988; 1990). Both beta-endorphin and the enkephalins, but not dynorphin, evidence a high affinity for the delta opioid receptor subtype (Kosterlitz and Paterson, 1985; Raynor et al., 1994). NTI has a prolonged duration of action owing to its resistance to enzymatic degradation by peptidases (Portoghese et al., 1988; 1990; Sofuoglu et al., 1991).

NTI (MW = 450.6) was dissolved in saline containing enough NaOH to solubilize the compound and the pH of the solution was adjusted to 6.9–7.2 by titrating with 0.1 N HCl before i.p. injection. The solution containing NTI was heated in order to get the drug into solution and to keep it in solution. NTI was administered in doses of 2.5 mg/2.5 ml NaCl/kg BW, 5.0 mg/5.0 ml NaCl/kg BW, 10 mg/5.0 ml NaCl/kg BW or 20.0 mg/10 ml NaCl/kg BW.

Experiment 1: Establishing a dose-response curve for aversion to alcohol. Prior to initiation of experiment 1, a concentration of a banana-flavored solution that was both detectable and neutral was identified. Two separate groups of alcohol-naive, female P rats from the 39th generation of selection for alcohol preference served as subjects (n = 9/group). Rats were given a 24-hr free-choice between water and one of two different concentrations of a banana-flavored solution (0.5% or 0.025%) for 5 to 7 days. Of the 9 rats given access to water and the 0.5% banana-flavored solution, 7 rats drank more water than banana solution, 1 rat drank more banana-flavored solution than water and 1 rat consumed equal amounts of water and banana. Hence, the 0.5% banana-flavored solution was not neutral. In contrast, of the 9 rats given access to water and the 0.025% banana-flavored solution, 5 rats drank more water than banana, 3 rats drank more banana than water and 1 rat consumed equal amounts of water and banana. Consequently, the 0.025% concentration of banana-flavored solution was considered “neutral” in terms of overall taste preference. In order to ensure that the banana-flavored solution was detectable to every rat, the position of the bottle containing banana-flavored solution was alternated daily which forced the rats to switch drinking sides daily in order to “track” either banana or water. All rats tracked one solution or the other every day except the 1 rat which consumed equal volumes of water and the banana-flavored solution. Therefore, the 0.025% concentration of banana solution was considered detectable and neutral and was used as the CS in the CTA conditioning trials.

A dose-response curve for alcohol aversion in P rats was established using a CTA paradigm. Establishing a CTA to alcohol involves presenting conditioning trials over several days, each of which consists of pairing consumption of a neutral yet distinctive flavor, such as banana, with administration of alcohol or vehicle. During post-conditioning rats are given a free-choice between the flavor and water and aversion to alcohol is inferred from a decrease in intake of the flavored solution.

Thirty-six female P rats from the 39th generation of selection for alcohol preference served as subjects. All rats weighed 250 to 300 g at the start of the experiment. During preconditioning, access to water was limited to 20 min/day for 8 days until water intake stabilized. Water was presented in one of two 100 ml calibrated drinking tubes with the other tube empty. The positions of the two tubes were rotated daily to control for the effects of a potential side preference. On the last day of preconditioning, rats were counterbalanced on water intake over the 8 days of preconditioning, and were...
assigned to one of four groups (0.5, 1.0, 1.5 g/kg EtOH or saline control; n = 9/group) in a manner which ensured that the groups did not differ in average daily water intake.

During conditioning, access to banana-flavored solution was substituted for access to water during the 20 min/day fluid access period. Five conditioning trials were given, one every other day, during which access to the banana solution (McCormick's; 0.025% v/v) was paired with an intraperitoneal (i.p.) injection of one of three doses of alcohol (0.5 g/10 ml/kg, 1.0 g/10 ml/kg or 1.5 g/15 ml/kg BW) or an equal volume of saline (control). Alcohol was administered 30 min after termination of access to the banana solution. The volume of alcohol injected i.p. was altered as a function of dose so that the concentration injected would not exceed 15% (v/v) in order to minimize tissue irritation at the site of injection (Barry and Walgren, 1968; Linakis and Cunningham, 1979). On conditioning days, the banana solution was presented in one calibrated drinking tube, with the other tube empty, and the two tubes were rotated on each conditioning day. On intervening days, water alone was presented for 20 min per day in one of two calibrated drinking tubes, with the other tube empty, in the absence of injections. In the CTA paradigm, development of aversion to alcohol is reflected by a decrease in consumption of the flavored solution which is paired with alcohol. Given that aversion can develop during conditioning, access to water on intervening days is necessary to prevent dehydration. Consumption of the banana-flavored solution and water were recorded on conditioning and intervening days respectively. On conditioning days, all rats were weighed and injected with saline (0.8 ml/100 g BW) before onset of the conditioning trial in order to mimic the conditions required for drug administration in experiment 2.

During postconditioning rats were presented with a choice between water in one drinking tube and banana solution (0.025%) in the other tube for 20 min/day without injections. One postconditioning trial was given per day for 10 consecutive days and the positions of the tubes containing water and banana solution were rotated daily. Intake of water and banana solution was recorded daily, during both conditioning and postconditioning, and rats were weighed daily.

At the conclusion of experiment 1, all rats were maintained in their home cages and were provided with food and water ad libitum for 6 weeks. A subset of 24 rats were then randomly selected to serve as subjects in an additional study which was designed to determine the BAC that was produced by each of the three doses of alcohol used in experiment 1. In order to reestablish the fluid access conditions used in experiment 1, rats were deprived of food and water for 15 hr and were then given access to water for 20 min immediately before receiving an injection of alcohol in one of the three doses used in experiment 1 (0.5, 1.0 and 1.5 g/kg, i.p.).

Blood samples (50–100 μl) were collected from the tail of each rat into heparinized capillary tubes at 15, 30, 60 and 180 min after alcohol injection. Blood was centrifuged and the plasma stored at −20°C until assayed for plasma alcohol concentration by high performance liquid chromatography. Plasma alcohol content was analyzed by direct injection of 1.0 μl of plasma into a Hewlett-Packard 5730A gas chromatograph equipped with a flame ionization detector and a 3380A integrator. The glass columns were packed with Poropack Q (80/100 mesh) and the oven temperature was 150°C. Isopropanol was used as the internal standard.

**Experiment 2: Effect of NTI on conditioned taste aversion to alcohol.** Experiment 2 was designed to determine whether blocking delta opioid receptors would serve to make alcohol more aversive and hence shift the alcohol aversion threshold to the left. Seventy-two alcohol-naïve female P rats from the 40–41st generations of selection for alcohol preference served as subjects. All rats weighed 250–300 g at the start of the experiment. The dose of alcohol that was found to be nonaversive in experiment 1, 0.5 g/kg, was paired with consumption of the banana-flavored solution (0.025%) in separate groups of P rats. Rats were pretreated with either saline or one of 4 doses of the delta opioid receptor antagonist, NTI. As in experiment 1, aversion to alcohol was inferred from a decrease in intake of the banana-flavored solution.

During preconditioning, the same paradigm was used as in the preconditioning phase of experiment 1. On the last day of preconditioning, the rats were assigned to groups and were pretreated with either saline or one of four doses of NTI (2.5, 5.0, 10.0 and 20.0 mg/kg) before injection of the nonaversive dose of alcohol (0.05 g/kg BW) or an equal amount of saline. Rats were counterbalanced and assigned to groups (n = 8/group) based on their average daily water consumption (20 min/day) during the 8 days of preconditioning.

During conditioning, 5 conditioning trials were given, one every other day. On conditioning days, all rats were given 20 min access to banana-flavored solution (McCormick's, 0.025%) in one drinking tube, with the other tube empty. Ten minutes after termination of access to the banana-flavored solution, rats received an i.p. injection of one of four doses of NTI (2.5 mg/2.5 ml/kg, 5.0 mg/5.0 ml/kg, 10.0 mg/5 ml/kg or 20.0 mg/10 ml/kg BW) or an equal volume of saline, which was followed, 20 min later, by an injection of the nonaversive dose of alcohol (0.5 g/10 ml/kg BW i.p.) or an equal volume of saline. We have previously reported that NTI is physiologically active within 20 min after i.p. administration in rats (Krishnan-Sarin et al., 1995a). As in experiment 1, water was presented alone for 20 min per day in one of two calibrated drinking tubes, with the other tube empty, on intervening days in the absence of injections in order to prevent potential dehydration. The position of the tubes containing banana solution and water were rotated daily during conditioning and fluid intake and body weight were recorded daily.

During postconditioning, the rats were presented with a choice between water and the 0.025% banana solution for 20 min a day without injections. One postconditioning trial was given per day for 10 consecutive days, and the positions of the tubes containing water and banana solution were rotated daily. Intake of water and banana solution was recorded daily, during both conditioning and postconditioning, and rats were weighed daily.

At the conclusion of experiment 2, all rats were maintained in their home cages and were provided with food and water ad libitum for 4 weeks. A subset of 12 rats were randomly selected to serve as subjects in an additional study which was designed to determine the effect of NTI on BAC. In order to reestablish the fluid access conditions used in experiment 2, rats were deprived of food and water for 15 hr and were then given access to water for 20 min followed, 10 min later, by an injection of NTI (10 mg/5 ml/kg BW i.p.) or an equal volume of saline which, in turn, was followed 20 min later by an injection of alcohol (0.05/10 ml/kg BW, i.p.). Blood samples were collected and alcohol concentration in plasma was analyzed as described in experiment 1.

**Data analysis.** In order to determine which doses of alcohol were aversive in experiment 1, comparisons were made between alcohol vs saline for each of 3 doses of alcohol (0.5, 1.0 and 1.5 g/kg BW) using separate two-way repeated measures analysis of variance (ANOVA) in the conditioning and postconditioning phases of the experiment. Specifically, three separate two-way, repeated measures ANOVAs, one for each dose of alcohol vs saline, were used to analyze the data from the conditioning phase of the study. An additional three separate two-way, repeated measures ANOVAs, one for each dose of alcohol vs saline, were used to analyze the data from the postconditioning phase of the study. In order to determine whether combining different doses of NTI with a nonaversive dose of alcohol makes alcohol aversive, the following comparisons were made using separate two-way repeated measures ANOVAs for conditioning: alcohol (0.5 g/kg BW) vs. NTI for each of 4 doses of NTI (2.5, 5.0, 10.0 and 20.0 mg/kg BW); alcohol (0.5 g/kg BW) vs. NTI + alcohol for each of 4 doses of NTI (2.5, 5.0, 10.0 and 20.0 mg/kg BW); NTI vs. NTI + alcohol for each of 4 doses of NTI (2.5, 5.0, 10.0 and 20.0 mg/kg BW). The same analyses were used to analyze the data from the postconditioning phase.

In order to determine the blood alcohol concentration (BAC) produced by various doses of alcohol, BACs were analyzed at each time.
point using a one-way ANOVA. Post-hoc comparisons of the differences between the means at a given time point, for each of the alcohol doses, were made using Bonferroni’s $t$ tests for all pairwise multiple comparisons. Blood alcohol elimination rates were calculated from the slope of the falling portion of the BAC curve using linear regression analyses for each rat and comparisons of alcohol elimination rate between groups were made using an unpaired student’s $t$ test. Area under the BAC curve was calculated using AUC version 1.1, and comparisons of area under the curve between groups were made using unpaired students $t$ tests.

**Results**

**Experiment 1: Establishing a dose-response curve for aversion to alcohol.** Figure 1 illustrates intake of the banana solution during conditioning and postconditioning in experiment 1. During conditioning, there were no differences in intake of the banana solution between the groups receiving alcohol in doses of 0.5, 1.0 or 1.5 g/kg BW compared to those receiving saline. Alcohol in a dose of 0.5g/kg was not aversive to P rats as evidence by the fact this dose, when compared with saline, did not suppress intake of the banana solution during postconditioning. In contrast, alcohol in a dose of 1.0 g./kg BW was aversive as evidence by the fact that this dose, when compared with saline, significantly suppressed intake of the banana solution during postconditioning. F(1,16)=39.184, $P < .001$. The highest dose of alcohol tested, 1.5 g/kg BW, produced the strongest aversion in comparison with saline, F(1,16)=166.87, $P < .001$.

Total fluid intake (banana-flavored solution + water) did not differ when the various treatment groups (0.5, 1.0 and 1.5 g/kg BW) were compared to each other or to the saline control group.

**BAC.** Analysis of the blood alcohol dose-response curves showed that BAC was dose-dependent and that peak blood alcohol levels were achieved at 30 min after administration of each of the three doses of alcohol (fig. 2). There was a significant difference between the blood alcohol concentrations produced by each of the three alcohol doses (0.05, 1.0 and 1.5 g/15 ml/kg BW i.p.) at all time points: 15 min, F(2,21)=18.85, $P < .001$; 30 min, F(2,21)=58.46, $P < .001$; 60 min, F(2,21)=67.08, $P < .001$; and 180 min, F(2,21)=92.22, $P < .001$. Post-hoc comparisons demonstrated that the BAC was significantly lower after administration of the 0.5 g/kg BW compared with the 1.0 and 1.5 g/kg doses at all time points. BAC levels were lower in the 1.0 g/kg group compared with the 1.5 g/kg group at 30, 60 and 180 min. BAC levels returned to base line by 180 min after administration of alcohol in a dose of 0.5 g/kg but BAC was still elevated above base line at 180 min after administration of the other two alcohol doses (1.0 and 1.5 g/kg BW). Area under the BAC curve after administration of the 0.5 g/kg dose of alcohol was significantly lower than that produced by 1.0 g/kg alcohol, t(14)=−16.58, $P < .001$ or 1.5 g/kg alcohol, t(14)=−11.70, $P < .001$. Area under the curve differed between the two highest alco-
hol doses (1.0 and 1.5 g/kg) as well t(14) = -5.15, P < .001. Alcohol elimination rate, based on the slope of the linear regression analysis, did not differ when the groups receiving alcohol (0.5, 1.0 and 1.5 g/kg BW) were compared.

Experiment 2: Effect of NTI on conditioned taste aversion to alcohol. As illustrated in figure 3, NTI in a dose of 2.5 mg/kg, when administered either alone or in combination with a nonaversive dose of alcohol, did not alter intake of the banana solution or total fluid intake during conditioning or postconditioning when compared with intake in the group receiving alcohol alone. Hence, this dose of NTI (2.5 mg/kg) is not aversive when administered alone or when combined with a nonaversive dose of alcohol.

As illustrated in figure 4, NTI in a dose of 5.0 mg/kg BW, was not aversive when administered alone as evidenced by the fact that this dose did not alter intake of the banana solution when compared with intake in the group receiving a nonaversive dose of alcohol alone during either conditioning [F (1,13)=3.60, P = .08] or postconditioning [F(1,13)=0.15, P = .91]. In contrast, combining the nonaversive dose of NTI (5.0 mg/kg) with the nonaversive dose of alcohol (0.5 g/kg) resulted in the formation of a significant aversion to alcohol as evidenced by a reduction in intake of the banana-flavored solution during postconditioning when the group receiving NTI + alcohol was compared with the group receiving alcohol alone, F(1,13)=14.56, P < .002 or the group receiving NTI alone F(1,14)=5.64, P < .032 (fig. 4). Aversion to alcohol in the group receiving NTI and alcohol was first evident during the conditioning phase when intake in this group was compared with intake in the group receiving NTI without alcohol, F(1,14)=7.90, P < .014. Total fluid intake (banana-flavored solution + water) did not differ when the treatment group (NTI + alcohol) was compared to the NTI alone or alcohol alone control groups.

Fig. 3. Intake of banana-flavored solution in P rats receiving alcohol (0.5g/kg, i.p.) paired with banana solution, or NTI (2.5 mg/kg, i.p.) + saline paired with banana solution, or NTI (2.5 mg/kg, i.p.) + alcohol (0.5g/kg, i.p.) paired with banana solution, during conditioning (panel 1). Intake of banana-flavored solution in P rats when banana solution is presented concurrently with water during postconditioning in the absence of drug treatment (panel 2). Each point represents the mean ± S.E.M. and N indicates the number of rats per group.

Fig. 4. Intake of banana-flavored solution in P rats receiving alcohol (0.5g/kg, i.p.) paired with banana solution, or NTI (5.0 mg/kg, i.p.) + saline paired with banana solution, or NTI (5.0 mg/kg, i.p.) + alcohol (0.5g/kg, i.p.) paired with banana solution, during conditioning (panel 1). Intake of banana-flavored solution in P rats when banana solution is presented concurrently with water during postconditioning in the absence of drug treatment (panel 2). Each point represents the mean ± S.E.M. and N indicates the number of rats per group.
As illustrated in figure 5, NTI in a dose of 10.0 mg/kg BW, was not aversive when administered alone as evidenced by the fact that this dose did not alter intake of the banana solution when compared with intake in the group receiving alcohol alone during either conditioning [*F*(1,13) = 5.32, *P* = .58], or postconditioning, [*F*(1,13) = 1.05, *P* = .32]. However, as was seen with the 5.0 mg/kg dose of NTI, when a dose of 10.0 mg/kg of NTI was combined with the nonaversive dose of alcohol (0.5 g/kg), a significant aversion to alcohol was apparent as evidenced by a reduction in intake of the banana-flavored solution during postconditioning when the group receiving NTI and alcohol was compared with the group receiving alcohol alone, *F*(1,13) = 6.06, *P* < .029 (fig. 5). Total fluid intake (banana-flavored solution + water) did not differ when the treatment group (NTI + alcohol) was compared to the NTI alone or alcohol alone control groups.

As illustrated in figure 6, the highest dose of NTI tested, 20 mg/kg BW, was aversive when administered either alone, *F*(1,13) = 7.29, *P* < .018 or in combination with the nonaversive dose of alcohol (0.5 mg/kg), *F*(1,13) = 8.23, *P* < .015 during postconditioning. Total fluid intake (banana-flavored solution + water) did not differ when the treatment group (NTI + alcohol) was compared to the NTI alone or alcohol alone control groups.

**Effect of NTI on BAC.** As illustrated in figure 7, combining NTI (10.0 mg/kg) with alcohol (0.5g/kg BW i.p.) did not alter peak BAC, area under the BAC curve or rate of alcohol elimination when compared with a group receiving alcohol alone.

**Discussion**

We have previously shown that rats selectively bred for alcohol-preference (P line) show an increased responsiveness
of the opioid system to alcohol in comparison to rats bred for alcohol-nonpreference (Li et al., 1998; Froehlich and Wand, 1996; Froehlich, 1997). Other investigators have also noted a positive association between alcohol preference and increased sensitivity of the opioid system to alcohol in mice (De Waele et al., 1992). In addition, increased responsiveness of the opioid system to alcohol has been reported in humans at high risk for the future development of alcoholism compared with those at low risk (Gianoulakis et al., 1996). We postulate that alcohol-induced activation of the opioid system serves to attenuate the aversive properties of alcohol ingestion and increase the probability of subsequent drinking in rats of the P line. If this view is correct, blocking the action of opioid peptides, via administration of an opioid receptor antagonist, would be expected to lower the alcohol aversion threshold in P rats. The present study examined the effects of the selective delta opioid receptor antagonist, NTI, on alcohol aversion threshold. A dose-response curve for alcohol aversion was established in female P rats in experiment 1. Alcohol, in doses of 1.0 and 1.5 g/kg BW, was aversive to P rats while a dose of 0.5 g/kg BW was not. These results confirm prior reports that alcohol in a dose of 0.5 g/kg BW is not aversive to P rats while higher doses, in excess of 1.0 g/kg BW, are aversive (Froehlich et al., 1988; Stewart et al., 1991). A nonaversive dose of alcohol (0.5 g/kg BW) is the optimal dose to use in combination with an opioid receptor antagonist in order to investigate whether blocking the action of opioid peptides makes alcohol more aversive.

In experiment 2, the effect of the selective delta opioid receptor antagonist NTI on alcohol aversion was examined. The lowest dose of NTI (2.5 mg/kg BW) was not aversive when administered alone or when administered in combination with the nonaversive dose of alcohol (0.5 g/kg BW). In contrast, higher doses of NTI (5.0 or 10.0 mg/kg BW) were not aversive when administered alone, but when combined with alcohol, both doses converted a nonaversive dose of alcohol into an aversive dose in P rats. The highest dose of NTI tested (20.0 mg/kg) was aversive when administered either alone or in combination with alcohol. It appears that pretreating P rats with a nonaversive dose of NTI, before administration of a nonaversive dose of alcohol, shifted the alcohol aversion threshold to the left. These results suggest that the endogenous ligands with high affinity for the delta opioid receptor subtype, namely beta-endorphin and the enkephalins, normally play a role in regulating alcohol aversion threshold. It should be noted that the doses of NTI used in the present study are similar to those that we have previously found to be effective in decreasing alcohol drinking in rats of the P line (Krishnan-Sarin et al., 1995). It should also be noted that the NTI-induced alteration of aversion threshold seen in P rats in the present study was not due to an NTI-induced change in alcohol pharmacokinetics since blood alcohol concentration at peak BAC, rate of alcohol disappearance and area under the BAC curve did not differ in rats pretreated with NTI vs those pretreated with saline. It is possible that the decrease in intake of the banana-flavored solution seen in rats receiving nonaversive doses of NTI and alcohol in combination may have resulted from a summation of the effects of alcohol and NTI since neither of the drugs were aversive when administered alone in the doses used in the combination, yet both of the drugs were aversive when administered alone at higher doses. However, summation of the effects of alcohol and NTI is not likely in the present study. Although the alcohol dose used in the combination (0.5 g/kg) was probably just below threshold since this dose produced no aversion but doubling the dose to 1.0 g/kg produced aversion, the 5.0 mg/kg dose of NTI used in the combination was not near threshold since doubling the dose to 10.0 g/kg still did not produce aversion when administered alone. Hence the decrease in intake of the banana-flavored solution seen in rats treated with NTI and alcohol in combination is probably not due to summation of subthreshold effects of alcohol and NTI.

A genetic difference in alcohol aversion, which may be mediated via the endogenous opioid system, has also been noted in mice by Cunningham and colleagues (Broadbent et al., 1996). Neither alcohol alone, nor naloxone alone, produced a CTA to alcohol in the alcohol-prefering C57BL/6J mice but pretreating the C57 mice with naloxone, before alcohol, resulted in a CTA. In contrast, a comparable dose of alcohol alone was sufficient to produce a CTA in alcohol-avoiding DBA/2J mice (Broadbent et al., 1996). These results suggest that alcohol is less aversive for the alcohol-prefering C57 mice compared to the alcohol-avoiding DBA mice and that this strain difference in aversion to alcohol may be due, in part, to greater alcohol-induced activation of the opioid system in the alcohol-prefering C57 mice.

The results of the present study suggest that alcohol-induced activation of the endorphin and enkephalin systems may serve to reduce aversion to alcohol and hence increase the probability of subsequent alcohol drinking. The importance of these finding lies in the elucidation of the biological basis of motivation for alcohol drinking in humans. Several clinical studies have demonstrated that the nonselective opioid receptor antagonist, naltrexone, decreases alcohol drinking in detoxified outpatient alcoholics (O'Malley et al., 1992; Volpicelli et al., 1992). Specifically, naltrexone decreases mean number of drinking days per week, frequency of relapse, and desire to drink or subjective craving for alcohol (O'Malley et al., 1992; Volpicelli et al., 1992; O'Malley et al., 1996). The results of these clinical trials led to FDA approval

![Fig. 7. BAC in P rats pretreated with NTI (10.0 mg/kg BW, i.p.), or an equal volume of saline, before i.p. injection of alcohol in a dose of 0.5 g/kg BW. Each point represents the mean ± S.E.M. and N indicates the number of rats per group.](image-url)
in 1994 of naltrexone as a pharmacotherapeutic agent for the treatment of alcohol dependence. One of the most interesting effects of naltrexone was found in subjects who drank alcohol or “slipped” while taking naltrexone. Naltrexone was found to be effective in decreasing subsequent drinking once drinking had occurred. Relapse, or significant resumption of drinking was seen in 54% of the subjects receiving placebo but in only 23% of the subjects receiving naltrexone (Volpicielli et al., 1992). Similar results have been reported by O’Malley (O’Malley et al., 1996). The effect of naltrexone in these studies has been attributed, in part, to a naltrexone-induced decrease in the reinforcing or euphoriant effects of alcohol (Volpicielli et al., 1995; O’Malley et al., 1996). However, the results of the present study suggest that opioid antagonists, such as naltrexone, may also decrease the probability of subsequent alcohol drinking by blocking the action of opioid peptides which may normally serve to diminish the aversive effects of alcohol and protect against hangover. The results of a recent study, conducted in social drinkers, are of interest with regard to this hypothesis. Swift and colleagues investigated the effects of naltrexone vs placebo on several subjective and objective measures of alcohol intoxication in social drinkers (Swift et al., 1994). Subjects were given naltrexone (50 mg p.o.) or placebo on two different occasions, each time followed by a standard, intoxicating dose of alcohol. As a control for naltrexone effects, additional subjects received naltrexone or placebo followed by a nonintoxicating “placebo” dose of alcohol. Subjects receiving naltrexone followed by alcohol not only found that the positively reinforcing stimulant effects of alcohol were reduced, but also that the dysphoric effects were augmented after alcohol consumption. No dysphoria was reported in response to naltrexone alone or alcohol alone. These results again suggest that alcohol-induced release of endogenous opioid peptides may normally serve to attenuate the aversive properties of alcohol and hence, when the action of endogenous opioids are blocked, via co-administration of naltrexone and alcohol, the aftereffects of alcohol may be more aversive. Consistent with this interpretation, subjects in the Swift study reported that they liked the placebo-alcohol session more than the naltrexone-alcohol session. It should be noted that naltrexone did not alter alcohol pharmacokinetics or the magnitude of alcohol-induced performance deficits in this study (Swift, 1994).

The results of the present study indicate that alcohol-induced activation of the opioid system may make the post-ingestional effects of alcohol less aversive in rats selectively bred for alcohol preference. Genetic differences in sensitivity of the opioid system to alcohol may be part of a biological mechanism underlying individual differences in aversion to the post-ingestional effects of alcohol commonly referred to as “hangover.” It is interesting to speculate that increased sensitivity of the endorphin and enkephalin systems to alcohol may represent a potential biological “marker” that could be used to identify individuals at risk for the future development of alcoholism.

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