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Effect of Mu Opioid Receptor Blockade on Alcohol Intake in Rats Bred for High Alcohol Drinking

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KRISHNAN-SARIN, S., G. S. WAND, X.-W. LI, T.-K. LI, P. S. PORTOGHESE AND J. C. FROEHLICH. *Effect of mu opioid receptor blockade on alcohol intake in rats bred for high alcohol drinking.* PHARMACOL BIOCHEM BEHAV **59**(3) 627–635, 1998.—Beta-funaltrexamine (beta-FNA), a selective mu opioid receptor antagonist, when administered in doses of 10.0, 15.0, and 20.0 mg/kg b.wt., decreased alcohol but not water intake in a dose-dependent manner in rats selectively bred for high alcohol intake (HAD line). Beta-FNA also suppressed the intake of a saccharin solution containing alcohol without altering the intake of a similar solution without alcohol. The results suggest that beta-FNA may prove useful as a pharmacotherapeutic agent for the treatment of alcohol dependence. In a second study, pituitary beta-endorphin gene expression (proopiomelanocortin or POMC messinger ribonucleic acid—mRNA) was compared in another pair of rat lines selectively bred for high or low alcohol intake (alcohol-preferring or P and alcohol-nonpreferring or NP lines). A repeated alcohol challenge (1.0 g/kg b.wt./day, IP for 4 days) produced a greater increase in POMC mRNA in the anterior and neurointermediate lobes of the pituitary of P rats compared with NP rats. The results suggest that a genetic predisposition toward high alcohol drinking may be associated with increased responsiveness of the opioid system to alcohol. © 1998 Elsevier Science Inc.

Genetic selection Rats Alcohol drinking Opioid receptors Opioid antagonists Beta-FNA mRNA Pituitary gland Alcohol preference

A major advance in the field of alcohol research has been the recognition that alcoholism is, in part, genetically determined (7,12,14,36). This finding has raised questions regarding the identity of the biological processes and pathways that, when inherited, confer increased risk for the development of alcoholism. One approach to identifying biological mechanisms that may contribute to alcohol drinking is to analyze how organisms with an identifiable genetic predisposition toward alcohol drinking differ from organisms without genetic risk (27).

The results of preclinical investigations suggest that several neurotransmitters/modulators including serotonin, dopamine, GABA, endorphin, and enkephalins may be involved in mediating alcohol drinking behavior (2,18,19). Agents that alter functional activity within these systems hold promise as potential pharmacotherapeutic agents for the treatment of alcohol dependence.

We have previously postulated that alcohol-induced activation of the endogenous opioid system is part of a neurobiological mechanism that contributes to high alcohol drinking behavior (20,21,26,28). Given that both beta-endorphin and the enkephalins function as positive reinforcers (18,68–70), alcohol-induced activation of either the endorphin or enkephalin system might be expected to enhance the reinforcing efficacy of alcohol and increase alcohol intake. Conversely, blocking the actions of these opioid peptides might be expected to reduce the reinforcing properties of alcohol and attenuate alcohol drinking. Three types of opioid receptors, mu, delta, and kappa, have been identified and found to differ

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both functionally (6,53) and in their binding characteristics (8–10,49). Beta-endorphin and the enkephalins bind to both mu and delta receptors (62). Naloxone and naltrexone are "nonselective" opioid receptor antagonists that bind to all three opioid receptor types as a function of dose administered (8,9,10). These nonselective opioid receptor antagonists have been shown to reduce alcohol self-administration in rodents (24,37,64,79) and monkeys (3,56) under a variety of experimental conditions. The results of these preclinical studies with nonselective opioid antagonists led to two clinical trials on the effects of naltrexone on alcohol drinking in outpatient alcoholics (57,74). Both studies found that naltrexone decreased mean number of drinking days per week, frequency of relapse, alcohol-induced euphoria or subjective "high," and desire to drink or alcohol craving. In 1994 naltrexone (Trexan or Revia) received FDA approval as a pharmacotherapeutic agent for the treatment of alcohol dependence.

Although the ability of nonselective opioid antagonists to reduce the intake of alcohol is likely related to their action at particular opioid receptor types, the role of each of the three opioid receptor types in mediating alcohol drinking compared with the intake of other ingesta is not well understood (24,29,42,43). Delineating the role of specific opioid receptor types in mediating alcohol drinking is critical for the future design of pharmacotherapeutic agents for the treatment of alcohol dependence. The relative importance of the three opioid receptor types in mediating alcohol drinking behavior can be determined by elucidating the effects of selective opioid antagonists on alcohol drinking. We recently reported that the opioid antagonist naltrindole (NTI), which blocks both the $delta_1$ and $delta_2$ opioid receptor subtypes, reduced the intake of a sweet solution containing alcohol as well as a sweet solution without alcohol when both solutions were presented concurrently to rats selectively bred for high alcohol intake (42). In contrast, the opioid antagonist naltriben, which is selective for the $delta_2$ opioid receptor subtype, suppressed the intake of a sweet solution containing alcohol without altering intake of a similar solution without alcohol (43). These results suggest that the $delta_2$ opioid receptor may be important in mediating the intake of alcohol compared with the intake of other ingesta. The present study extends these findings by examining whether beta-funaltrexamine (beta-FNA), a selective mu opioid receptor antagonist, is capable of suppressing alcohol intake, compared with the intake of a sweet solution, in rats selectively bred for high alcohol drinking (HAD line).

Recent evidence, in both rodents and humans, suggests that a genetic predisposition toward alcohol drinking may be associated with increased responsiveness of the opioid system to alcohol. For instance, we have recently reported that a single alcohol challenge produced a larger increase in beta-endorphin gene expression (POMC mRNA) in the anterior and neurointermediate lobes of the pituitary of rats selectively bred for alcohol preference (P line) compared with those bred for alcohol nonpreference (NP line) (21). Using a similar design, we have also found that a single alcohol challenge increases enkephalin gene expression (PPENK mRNA) in the nucleus accumbens of P but not NP rats (48). Gianoulakis and colleagues have reported that in vitro perfusion of alcohol produces a much larger and more prolonged release of beta-endorphin from the hypothalamus of alcohol-preferring C57BL/6 mice compared with alcohol-nonpreferring DBA/2 mice (17). In humans, beta-endorphin responses to alcohol differ in subjects with and without a family history of alcoholism. Alcohol induces a significant increase in plasma beta-endorphin content in subjects at high risk, but not in those at low risk, for the future development of alcoholism (33). Taken together, these results suggest that genetic differences in alcohol drinking may be due, in part, to differences in responsiveness of the opioid system to alcohol. The present study extends these investigations to include a comparison of the effect of a repeated alcohol challenge on beta-endorphin gene expression (POMC mRNA) in the pituitary of rats from the alcohol-preferring (P) line and the alcohol-nonpreferring (NP) line.

EXPERIMENT 1

Method

Subjects. Sixteen male rats, weighing 450–550 g, from the 13th generation of the selectively bred high alcohol-drinking (HAD) line served as subjects. The rats were tested for alcohol preference using the procedures and criteria that are routinely used in the selection of the alcohol-preferring (P and HAD) and alcohol-nonpreferring (NP and LAD) lines (45,46,50,51). Briefly, alcohol preference was determined during 4 weeks of 24-h access to a free-choice between a 10% (v/v) alcohol solution and water with food freely available. Fluids were presented in calibrated glass Richter tubes that were read to the nearest 0.5 ml. The position of the Richter tubes containing alcohol and water was alternated daily to minimize the effect of a possible positional preference. Daily alcohol and water intakes were calculated for each rat. To qualify as a high alcohol drinker (HAD line) rats had to consume in excess of 5.0 g alcohol/kg b.wt./day and demonstrate a 2:1 preference ratio of alcohol to water. All rats in Experiment 1 qualified as high alcohol drinkers. Rats were housed individually in standard steel laboratory cages in a temperature controlled room maintained on a 12-h reverse light/dark cycle (lights off at 1015 h and on at 2215 h). Rats were weighed daily during the 4 weeks of alcohol preference testing and during each week of antagonist administration, and were handled during each weighing to minimize stress associated with handling on the day of antagonist administration.

Drugs. Beta-funaltrexamine (beta-FNA, M.W. 491), an alkylated derivative of naltrexone, is a nonpeptide opioid antagonist with high affinity and selectivity for the mu opioid receptor and does not demonstrate agonist activity at mu or delta opioid receptors (77,78). Beta-FNA has an initial kappa agonist effect that lasts for 2–3 h, which is followed by a pure mu antagonist effect that can last for 2–4 days, as evidenced by inhibition of morphine-induced analgesia (54,60,61,71,78). To isolate the mu antagonist action, beta-FNA was administered SC 16 h prior to the daily 2-h period of free-choice between alcohol and water. The route of administration and doses tested are similar to those previously used to antagonize the antinociceptive activity of mu receptor selective agonists (78).

Beta-FNA, as a hydrochloride salt, was dissolved in distilled water and brought up to volume with 0.09% NaCl to produce a final concentration of 2.0 mg/1.0 ml of solution (2:1, saline to water). Beta-FNA was injected SC in doses of 5.0, 10.0, 15.0, or 20.0 mg/kg b.wt. Rats in the control group were injected SC with an equal volume of saline (0.09%). When the volume of drug or saline exceeded 3.0 ml per rat, the volume was divided in half and injected SC in two locations on the dorsal surface of the neck.

Experimental Design

Phase 1. Following 4 weeks of alcohol preference testing rats from the HAD line were given free access to food and

scheduled access to a free choice between a 10% (v/v) alcohol solution and water for 8 h per day during the dark portion of the light/dark cycle for 2 weeks. The period of daily access to water and alcohol was then shortened to 2 h per day during the dark portion of the light–dark cycle (1015–1215 h) for 4 weeks prior to administration of beta-FNA. Alcohol and water intakes were recorded daily at the end of the 2-h fluid access period. We have previously demonstrated that rats receiving access to fluids for 2 h daily consume as much fluid during the 2-h interval as they do when fluids are available ad lib, and they continue to gain weight at the same rate as age-matched rats living under similar conditions with food and water freely available (24).

Rats were weighed daily for 4 days preceding, and for 4 days following, administration of each dose of beta-FNA, and were weighed twice weekly at all other times. On the day of antagonist treatment, one of four doses of beta-FNA (5.0, 10.0, 15.0, or 20.0 mg/kg b.wt.), or an equal volume of saline, was administered SC 16 h prior to the daily 2-h fluid access period. At least 5 weeks separated administration of each dose of beta-FNA, during which time rats were maintained with free access to food and scheduled access to water and a 10% (v/v) alcohol solution for 2 h per day.

Prior to administration of each dose of beta-FNA, average daily alcohol and water intake was calculated for each rat over 4 consecutive days prior to the day of drug treatment. Rats were ranked in descending order based on their average daily alcohol intake (ml/day) over this 4-day period and assigned to the drug- or saline-treatment groups based on alcohol intake as previously described (24,29,42,43). This assignment procedure produced two groups of rats that were matched on alcohol and water intake prior to administration of antagonist or saline. Alcohol and water intake during the 2-h fluid access period was monitored daily throughout the experiment to ensure that consumption of alcohol and water returned to stable baseline levels following treatment with each dose of beta-FNA.

Phase 2. Following completion of phase 1, the HAD rats were maintained with free access to food and were presented with scheduled access to a sweetened solution without alcohol (saccharin, 0.45 g/liter of water) in one tube and a sweetened solution containing alcohol (10% alcohol v/v in water containing 0.45 g saccharin per liter) in another tube, presented concurrently, for 2 h each day (1015–1215 h). Consumption of the sweetened solution without alcohol and the sweetened solution with alcohol was recorded daily at the end of the 2-h fluid access period. The rats were maintained on this fluid access schedule for 6 weeks until the daily intake of the two sweetened solutions (with and without alcohol) was stable and relatively equal. Average daily intake of the two sweet solutions (with and without alcohol) was calculated for each rat over 4 consecutive days prior to the day of beta-FNA administration. Rats were assigned to the beta-FNA- or saline-treatment groups based on their average daily intake of the sweet solution containing alcohol as previously described.

Beta-FNA, in a dose of 20 mg/10 ml/kg b.wt., or an equal volume of saline, was administered SC 16 h prior to the 2-h fluid access period. Intake of each of the two sweetened solutions (with and without alcohol) was recorded at the end of the daily 2-h fluid access period (1015–1215 h) on the day of antagonist administration and on days 1–4 preceding and following administration.

Data analysis. Comparisons of the effects of beta-FNA vs. saline on intake of alcohol, water, or sweetened solutions in rats of the HAD line were made using paired *t*-tests. The use of a counterbalanced design for assigning animals to groups prior to treatment serves to eliminate group differences in the variables of interest, namely alcohol and water intake. This reduction in the degree of potential variance between groups dictates the use of a paired *t*-test for comparison of group means (80). It has been shown that assigning animals to groups in matched pairs results in greater sensitivity of statistical tests than is achieved using repeated measures analysis of variance on independent groups (24). One-tailed significance levels were used for comparisons of the effect of beta-FNA on alcohol intake because previous results predicted that the opioid antagonist would produce a unidirectional change (suppression) in alcohol intake. Two-tailed significance levels were used for comparisons of the effect of beta-FNA on water intake because no prediction could be made regarding the direction of change to be expected.

EXPERIMENT 2

Method

Subjects. Due to limited availability of rats from the HAD and LAD lines, 11 male rats from the alcohol-preferring (P) line and 8 male rats from the alcohol-nonpreferring (NP) line served as subjects. All rats weighed 300–440 g, were from the 34th generation of the selectively bred alcohol-preferring (P) and alcohol-nonpreferring (NP) lines, and were not tested for alcohol preference, and hence, were alcohol naive. All other rats from the 34th generation of selection were tested for alcohol preference and those from the P line consumed an average of 5.2 g alcohol/kg b.wt./day while those from the NP line consumed an average of 0.5 g alcohol/kg b.wt./day. It should be noted that similar criteria are used in the derivation of the HAD/LAD and the P/NP lines [for review see (47)]. All rats were housed in a quiet environment and were handled and weighed daily for 2 weeks prior to initiation of alcohol or saline injections to minimize the stress associated with handling on injection days.

Experimental design. Rats were given IP injections of alcohol in a dose of 1.0 g/kg b.wt., or an equal volume of saline, once a day for 4 consecutive days. Alcohol was administered in a concentration of 10% (v/v) to minimize tissue irritation at the site of injection, which can be seen when concentrations above 12% (v/v) are used (5). Rats were decapitated within 15 s of handling at 2 h following the fourth alcohol or saline injection, and the anterior and neurointermediate (NIL) lobes of the pituitary were dissected, frozen in liquid nitrogen and stored at −70°C until assayed for POMC mRNA content by Northern blot.

Isolation and quantification of RNA. The anterior and neurointermediate lobes of the pituitary were homogenized in a solution of 4 M guanidine thiocyanate, 0.7% beta-mercaptoethanol, 30 mM sodium citrate, and centrifuged. Total RNA was isolated as described by Chomczynski and Sacchi (11) and estimated by measurement of UV absorption at 260 nm. Total RNA from samples was fractionated by electrophoresis on 1.5% agarose–6% formaldehyde denaturing gels in 20 mM MOPS [3-(n-Morpholino)propanesulfonic acid], 5 mM sodium acetate, and 1 mM EDTA buffer, pH 7.0, and transferred to Nitrocellulose Plus filters by capillary blotting with 20 × SSC (3.0 M NaCl and 0.3 M sodium citrate, pH 7.0). Filters were fixed by baking for 2 h at 80°C under vacuum. Filters were prehybridized for 4 h at 42°C in 5 × SSC, 4 × Denhardt's (50 × Denhardt's is 1% Ficoll-400, 1% polyvinylpyrrolidine, and 1% BSA in water), 50% deionized formamide, 20 mM sodium phosphate (pH 6.8), 0.1% sodium dodecyl sulfate (SDS), and 0.1% mg/ml denatured salmon sperm DNA. Filters were

then hybridized for 18 h at 4°C with the denatured nick-translated (New England Nuclear nick translation kit), ^{32}P-labeled POMC cDNA probe (73) (10^6 cpm/ml; SA, 0.2–1.0 × 10^8 cpm/mg cDNA) in the prehybridization buffer. The POMC probe (a gift from Drs. Richard Mains and Betty Eipper, Johns Hopkins University) was a 1000 base pair cDNA encoding all exons of the rat POMC sequence. Filters were washed at room temperature for 30 min in 2 × SSC–0.1% SDS, followed by four 30-min washes in 0.1 × SSC–0.1% SDS at 50°C and exposed to x-ray film with intensifying screens at −70°C. For molecular weight determination, 2 μg of a RNA ladder was fractionated in an adjacent lane and stained with acridine orange.

To correct for variations in the amount of total RNA in the samples, or in the amount transferred to the filters, all blots were stripped by boiling in 0.1 × SSC–1% SDS for 20 min and rehybridized with frog 18s cDNA, which is constitutively expressed in many tissues and is not under hormonal or ethanol regulation.

The integrated optical densities of the individual bands on the autoradiograms were determined using a LOATS RAS-1000 Image Analysis system. For each sample, data for POMC mRNA were normalized to the amount of 18s present on the blot, the mean of the saline control group within each line was assigned a value of 100%, and the treatment group within each line was calculated relative to it and expressed as a percentage of control.

Data analysis. Comparisons of group means were made using unpaired Student's *t*-tests. One-tailed significance levels were used based on previous results indicating a unidirectional prediction of either an alcohol-induced enhancement or no change in opioid activity.

RESULTS

Experiment 1

When access to alcohol and water was scheduled to 2 h per day, rats of the HAD line consumed equal amounts of the alcohol solution and water during the 2-h fluid access period. This is due to the fact that blood alcohol concentration (BAC) is the major factor that limits alcohol intake in rats selectively bred for high alcohol drinking, with cessation of drinking occurring when BAC reaches 45–77 mg% (55). When access to alcohol and water is scheduled to 2 h per day, rats normally consume enough alcohol in a single drinking episode to raise BACs to 45–77 mg% then cease drinking alcohol and spend the duration of the fluid access period alternately eating food and drinking water. Scheduled fluid access paradigms require that rats consume enough water to meet their 24-h metabolic needs in a 2-h period. Therefore, it is not surprising that even rats with high alcohol preference (HAD line) drink as much water as alcohol during the scheduled access period. We have previously noted that under identical conditions of fluid access, rats of the HAD line still consume three to five times more alcohol than do rats selectively bred for low alcohol preference (LAD line) (22).

Beta-FNA produced a dose-dependent suppression of alcohol intake with maximal suppression seen at the highest dose tested (20.0 mg/kg). In a dose of 5.0 mg/kg b.wt., beta-FNA did not alter alcohol or water intake (Fig. 1A). At 10.0 mg/kg b.wt., beta-FNA significantly suppressed alcohol intake by 23%, which was accompanied by a compensatory increase in water intake (Fig. 1B). Increasing the dose of beta-FNA to 15.0 mg/kg b.wt. significantly attenuated alcohol intake by 35% without altering water intake (Fig. 1C). Neither alcohol nor water intake was altered on any of the days following treatment with beta-FNA in doses of 5.0, 10.0, or 15.0 mg/kg b.wt. (data not shown).

In a dose of 20.0 mg/kg b.wt., beta-FNA significantly suppressed alcohol consumption by 65%, without altering water intake, on the day of antagonist treatment (Fig. 1D). Alcohol intake remained suppressed (36%) on day 1 following beta-FNA administration and a trend towards suppression (15%, $p < 0.06$) was still evident on day 2 following treatment with no suppression seen on day 3 following treatment (Fig. 2). Although food intake was not directly measured, no significant differences in body weight were found when weights of individual rats were compared prior to, during, and following beta-FNA treatment in any dose tested. Additionally, the weight of animals in the antagonist-treated groups did not differ from the weight of those in the saline-treated groups on the day prior to, day of, or day following antagonist treatment.

In phase 2, HAD rats consumed roughly equivalent amounts of the two saccharin solutions, with and without al-

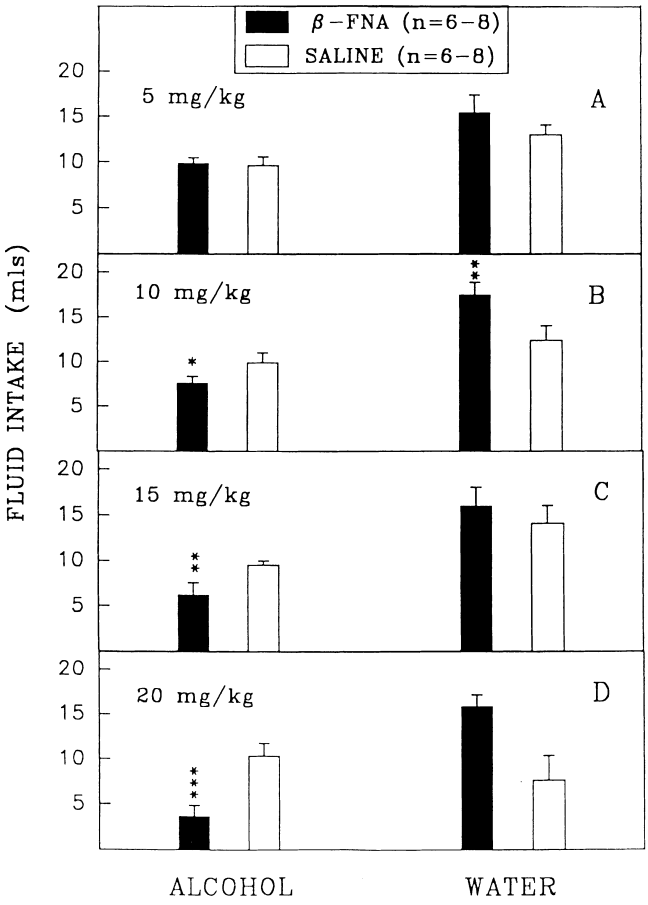

FIG. 1. Effect of beta-FNA, in doses of 5.0, 10.0, 15.0, and 20.0 mg/kg b.wt. SC, on alcohol and water intake in rats of the HAD line. Rats were given ad lib access to food and scheduled access to alcohol (10% v/v) and water, presented concurrently, for 2 h daily. Each bar represents the mean (±SE). *n* indicates the number of rats per group and the range (n = 6–8) reflects the fact that one or two rats in one or more groups dislodged a tube containing either alcohol or water, which prevented the acquisition of drinking scores on the day of drug treatment. Asterisks indicate significant differences between the beta-FNA- and saline-treated groups (*$p < 0.05$, **$p < 0.01$, ***$p <$ 0.001; paired *t*-test).

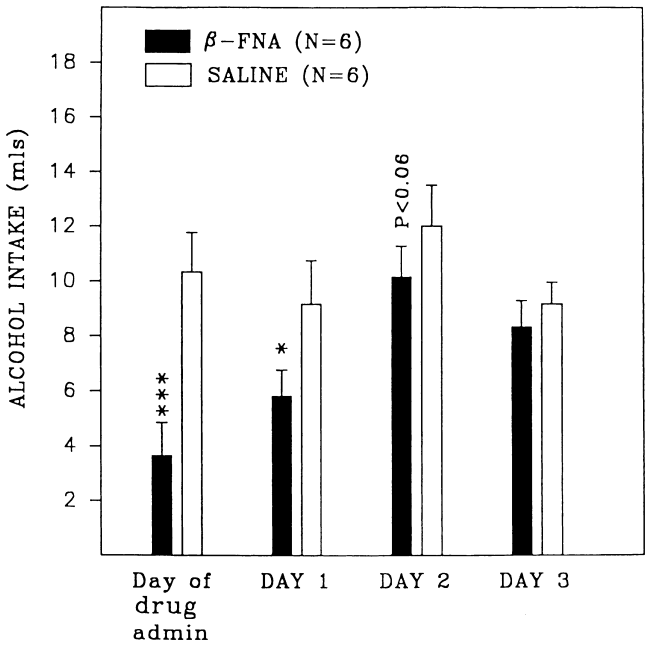

FIG. 2. Duration of action of beta-FNA, in a dose of 20.0 mg/kg b.wt., in suppressing alcohol intake in rats of the HAD line. Rats were given ad lib access to food and scheduled access to alcohol (10% v/v) and water for 2 h daily. Each bar represents the mean (±SE) and *n* indicates the number of rats per group. Asterisks indicate significant differences between the beta-FNA- and saline-treated groups (*$p < 0.05$, ***$p < 0.001$; paired *t*-test).

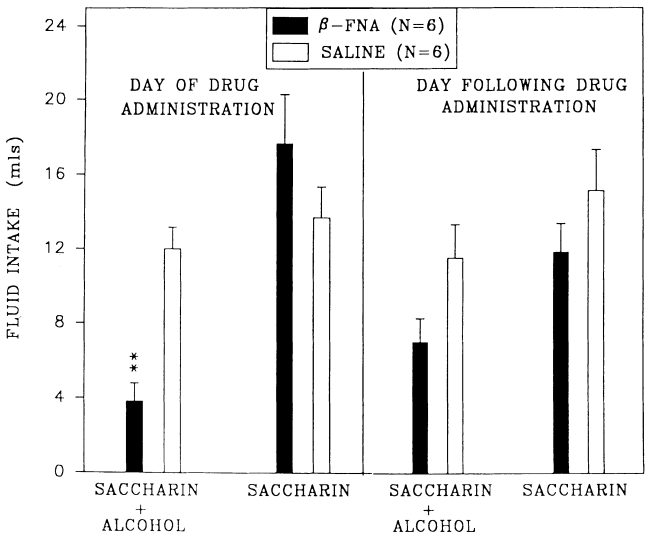

FIG. 3. Effect of beta-FNA, in a dose of 20.0 mg/kg b.wt., on intake of a sweet solution containing alcohol and a sweet solution without alcohol presented concurrently for 2 h per day with food freely available in rats of the HAD line. Each bar represents the mean (±SE) and *n* indicates the number of rats per group. Asterisks indicate significant differences between the beta-FNA- and saline-treated groups (*$p < 0.01$; paired *t*-test).

cohol, when the solutions were presented concurrently. Beta-FNA, in a dose of 20.0 mg/kg b.wt., significantly reduced intake of the saccharin solution containing alcohol by 68% without altering intake of the saccharin solution without alcohol on the day of antagonist treatment (Fig. 3). Intake of the saccharin solutions with and without alcohol was not altered on days 1–3 following beta-FNA treatment (Fig. 3 and data not shown). No change in body weight was seen as a function of antagonist treatment, which suggests that beta-FNA did not alter food intake.

Experiment 2

Repeated alcohol challenge (1.0 g/kg b.wt.), once a day for 4 days, produced an increase in POMC mRNA in the anterior pituitary of both P rats ($p < 0.001$) and NP rats ($p < 0.05$), when compared with saline-treated controls, with a much greater increase seen in P rats (Fig. 4). Repeated alcohol challenge also produced an increase in POMC mRNA in the NIL of P rats ($p < 0.001$), but did not alter POMC mRNA in the NIL of NP rats (Fig. 5).

DISCUSSION

We have previously hypothesized that alcohol-induced activation of the endogenous opioid system is part of a neural network that is functionally involved in mediating alcohol reinforcement and alcohol drinking behavior (20,21,26–28). This hypothesis is based on several lines of evidence. First, both nonselective and delta receptor selective opioid antagonists have been found to attenuate alcohol self-administration in rodents (25,42,43,52,64,79) and the nonselective opioid antagonist naltrexone reduces alcohol drinking in outpatient alcoholics (57,74). The fact that agents, such as opioid receptor antagonists, attenuate alcohol intake in both rodents and humans lends predictive validity to the use of rodent models for testing pharmacotherapeutic agents that may be of value in the treatment of alcohol dependence. Our laboratory has long been interested in identifying opioid receptor antagonists that: 1) decrease alcohol intake without altering food or water intake, 2) exhibit a long duration of action, and, 3) produce few side effects. Opioid antagonists that are selective for one opioid receptor type may be particularly useful for the treatment of alcohol dependence if they evidence increased efficacy at lower doses with fewer side effects when compared with nonselective antagonists.

The present study examined the effect of a selective mu opioid receptor antagonist (beta-FNA) on alcohol intake in rats bred for high alcohol drinking (HAD line). Beta-FNA produced a dose-dependent reduction of alcohol intake with the greatest reduction (65%) seen at the highest dose tested (20.0 mg/kg b.wt.). The attenuation of alcohol intake was not attributable to a generalized suppression of fluid intake because water consumption was not suppressed following beta-FNA treatment. Although food intake was not directly measured, beta-FNA did not alter body weight, which suggests that food intake was not affected. The results of the present study are consistent with a previous report that ICV administration of another mu receptor antagonist, CTOP, decreased alcohol drinking in another rat line selectively bred for high alcohol intake (Alko-Alcohol or AA line) (38). It is not clear why no effect of beta-FNA on alcohol intake has been seen in mice (44).

Beta-FNA, in a dose of 20.0 mg/kg, displayed a long duration of action as evidenced by significant suppression of alco-

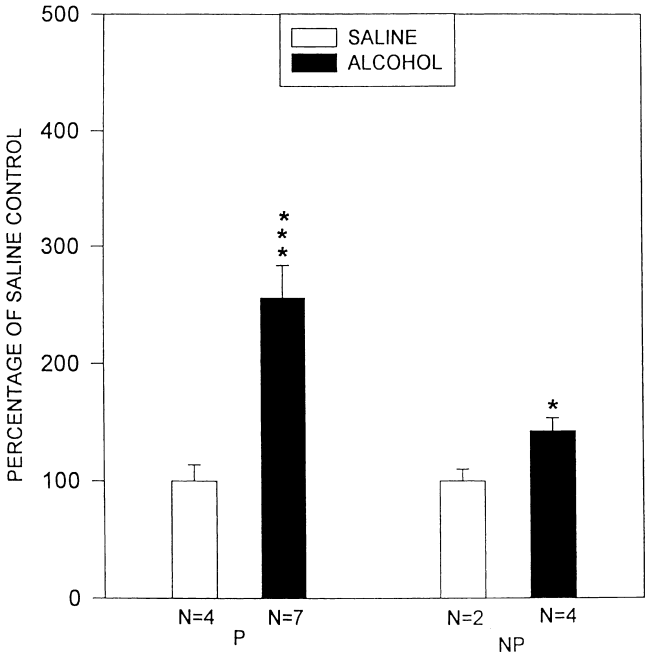

FIG. 4. POMC mRNA content in the anterior pituitary of rats from the P and NP lines following four daily injections of saline or alcohol (1.0 g/kg b.wt., IP). Each bar represents the mean (±SE) and *n* indicates the number of rats per group. RNA from two of the saline-treated NP rats degraded and could not be used. Asterisks indicate significant differences between the B-FNA- and saline-treated groups (*$p < 0.05$, ***$p < 0.001$; unpaired *t*-test).

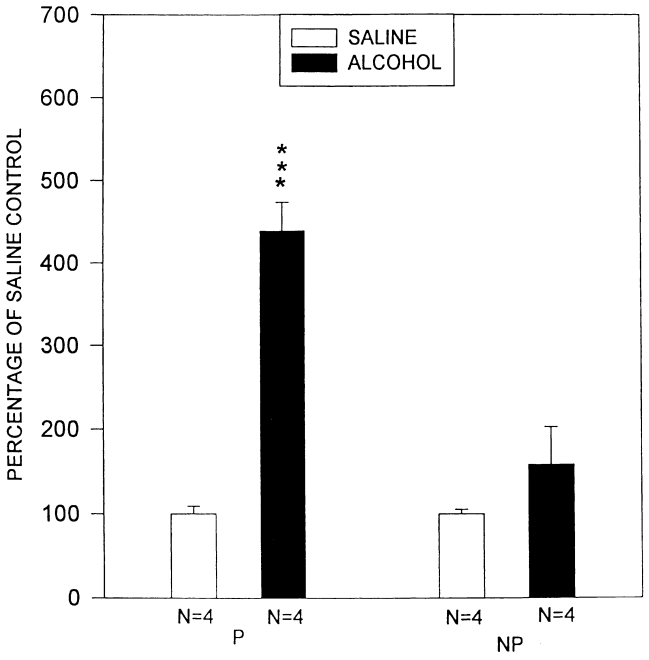

FIG. 5. POMC mRNA content in the neurointermediate lobe of the pituitary of P and NP rats following four daily injections of saline or alcohol (1.0 g/kg b.wt., IP). Each bar represents the mean (±SE) and *n* indicates the number of rats per group. Asterisks indicate significant differences between the beta-FNA- and saline-treated groups (***$p < 0.001$; unpaired *t*-test).

hol intake on day 1 following antagonist treatment. A similar long duration of action has previously been reported for beta-FNA in studies of both morphine dependence and antinociception (1,78). In the present study, beta-FNA suppressed the intake of a saccharin solution containing alcohol by 68% without altering intake of a saccharin solution without alcohol. Thus, it appears that beta-FNA is specific for alcohol, which is similar to the action of NTB, the delta$_2$ opioid receptor antagonist (43).

Given that both beta-endorphin and the enkephalins function as positive reinforcers (18,68–70), alcohol-induced activation of the endorphin or enkephalin systems might be expected to enhance the hedonic value of alcohol and lead to an increase in alcohol drinking. Conversely, blocking the action of these opioid ligands might be expected to reduce the reinforcing properties of alcohol and attenuate alcohol drinking. Beta-endorphin and the enkephalins are the endogenous ligands with the highest affinity for the mu and delta opioid receptors [(41,49,62); for review, see (13)]. A recent pharmacological characterization of cloned opioid receptors indicates that the enkephalins and beta-endorphin bind with equal affinity to cloned mu and delta receptors (62). We have previously demonstrated that delta opioid receptor antagonists are capable of attenuating alcohol intake. We now report that the selective mu opioid receptor antagonist, beta-FNA, also reduces alcohol intake. Because beta-FNA exhibits a long duration of action and is capable of specifically suppressing the intake of alcohol compared with the intake of another palatable ingesta, it may prove useful in the treatment of alcohol dependence.

Acute and chronic alcohol consumption and/or treatment has been reported to increase activity in the opioid peptide system. For instance, alcohol increases the release of opioid peptides from the pituitary of both rats and humans in vivo (31,32,59,72) and from preparations of rodent hypothalamus (17,30,58,63) and pituitary in vitro (31,33,39). Alcohol also increases beta-endorphin and enkephalin gene expression in rat (21,35,48,66) and mouse (75) brain and pituitary. In the present study a repeated alcohol challenge produced an increase in POMC mRNA in the anterior and neurointermediate lobes of the pituitary of P rats. Although the results of the present study supports the results of several prior studies (21,35,48,66), previous reports on the effect of alcohol on beta-endorphin gene expression have not been entirely consistent. Although the majority of reports indicate that chronic alcohol treatment increases POMC mRNA as well as the biosynthesis of POMC and POMC-derived peptides in rat hypothalamus (4,30) and pituitary (34,35,75,76), increases have not always been found (16,65,67). This discrepancy may be due to the fact that some of the methods used for chronic alcohol administration, such as continuous exposure of rats to alcohol vapor in an inhalation chamber (16,65), produce sustained elevations of blood alcohol content (BAC) (40). This contrasts with the BAC profile that is produced by oral alcohol self-administration in rodents and humans, which is normally characterized by periodic drinking bouts with intermittent periods of relative or actual withdrawal. Alterations in opioid activity that occur when BAC is continuously elevated may differ markedly from the changes that occur in response to a repeated, intermittent, alcohol challenge.

Another line of evidence that supports the hypothesis that alcohol-induced activation of the opioid system contributes to

high alcohol drinking comes from studies that suggest that a genetic predisposition toward alcohol drinking may be associated with increased responsiveness of the opioid system to alcohol in both rodents and humans. For instance, acute alcohol treatment increases enkephalin gene expression (PPENK mRNA) in the nucleus accumbens of alcohol-preferring (P) rats but not alcohol-nonpreferring (NP) rats (48). Alcohol consumption by human subjects with a family history of alcoholism (high risk) results in increased plasma content of beta-endorphin, while no such elevations are seen in low risk subjects without a family history of alcoholism (32). The present study compared the effect of a repeated alcohol challenge on beta-endorphin gene expression (POMC mRNA) in the anterior pituitary and neurointermediate lobe (NIL) of rats selectively bred for alcohol preference (P) and nonpreference (NP). It should be noted that rats of the P and NP lines do not differ in blood alcohol concentration or alcohol elimination rate following administration of alcohol in doses equivalent to those used in the current study (23). Alcohol treatment, once a day for 4 days, produced a greater increase in POMC mRNA in the anterior pituitary and NIL of P rats compared with NP rats.

Demonstration that a disorder, such as alcoholism, has a strong genetic component raises questions regarding the nature of the physiological and neurochemical traits, which, when inherited, increase the probability of expression of that disorder. Comparisons of rats selectively bred for alcohol preference and nonpreference have resulted in the identification of a number of traits that are associated with alcohol preference and high alcohol drinking behavior. Although many alcohol-related traits may contribute to alcohol preference and high alcohol intake, it is unlikely that all of these alcohol-related traits are necessary for the expression of the alcohol drinking phenotype. It is also unlikely that any single trait is sufficient for the expression of the alcohol drinking phenotype. Instead, the presence of certain environmental variables and alcohol-related traits might serve to increase the probability of high alcohol drinking behavior. Establishing trait causality is a difficult task. The task begins with the identification of alcohol-related traits, such as enhanced responsiveness of the opioid system to alcohol, in multiple lines and strains selected for the same phenotype, namely alcohol preference or high alcohol intake. The appearance of an association between alcohol preference and the alcohol-related trait in more than one line selected for the same phenotype serves to decrease the likelihood that the observed relationship between the two traits is fortuitous (15). The results of the present study raise the possibility that greater alcohol-induced activation of the opioid system in genetically predisposed organisms might serve to enhance the reinforcing efficacy of alcohol and increase the probability of subsequent drinking. Additional studies will be needed to confirm the observed association between alcohol preference and enhanced responsivity of the opioid system to alcohol in additional rodent lines selectively bred for alcohol preference. It is interesting to speculate that enhanced responsiveness of the opioid system to alcohol may be a heritable trait that is associated with, and causally related to, high alcohol drinking behavior.

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