Pharmacology Biochemistry & Behavior, Vol. 35, pp. 385–390. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Naloxone Attenuates Voluntary Ethanol Intake in Rats Selectively Bred for High Ethanol Preference

J. C. FROEHLICH, J. HARTS, L. LUMENG AND T.-K. LI

Departments of Medicine, Physiology/Biophysics and Biochemistry
Indiana University School of Medicine
and The Veterans Administration Medical Center, Indianapolis, IN 46223

Received 10 October 1988

FROEHLICH, J. C., J. HARTS, L. LUMENG AND T.-K. LI. *Naloxone attenuates voluntary ethanol intake in rats selectively bred for high ethanol preference*. PHARMACOL BIOCHEM BEHAV **35**(2) 385–390, 1990.—The effect of naloxone on voluntary ethanol intake was examined in rats which were selectively bred for oral ethanol preference (High Alcohol Drinking or HAD line). Rats of the HAD line were treated with naloxone in doses of 0.05–18.0 mg/kg b.wt. before access to water alone or to a free-choice between a 10% (v/v) ethanol solution and water. Naloxone suppressed water intake when water was presented as the sole source of fluid. In contrast, naloxone produced a dose-dependent decrease in ethanol consumption, without altering water intake, when rats were given a free-choice between the ethanol solution and water. Selective suppression of ethanol consumption by naloxone was not attributable to changes in blood ethanol concentrations or ethanol elimination rates following naloxone treatment. It appears that although naloxone may attenuate the positively reinforcing properties of both ethanol and water, ethanol drinking is a subset of consummatory behaviors that is particularly sensitive to opioid receptor blockade. The results suggest that activation of the endogenous opioid system may be an important mechanism which serves to maintain continued ethanol drinking.

Voluntary ethanol consumption Genetic selection for differences in ethanol preference Naloxone Opioids

MUCH evidence indicates that endogenous opioid systems are involved in controlling a variety of consummatory behaviors. This conclusion is based, in part, on the demonstration that opioid receptor antagonists alter both food and water intake. Naloxone is a potent nonselective opioid receptor antagonist which acts primarily on the CNS to block the action of endogenous opioids. Naloxone reduces water intake in nondeprived rats when administered prior to nocturnal drinking episodes, as well as water intake which has been artificially induced by fluid deprivation or the administration of hypertonic saline (3, 5–7). In rats which are both food and water deprived, naloxone has a more pronounced suppressive effect on water than on food intake (11).

Ethanol drinking can be viewed as a special type of consummatory behavior. Most animals, other than man, will not voluntarily consume ethanol in quantities sufficient to produce tolerance and dependence which characterize human alcoholism. Ethanol drinking can be enhanced by incorporating ethanol into sweetened solutions and palatable liquid diets, by intermittent and gradual introduction of increasing concentrations of ethanol, or by presenting an ethanol solution as the sole source of fluid. Some of these procedures have been used to determine whether blocking the action of endogenous opioids alters ethanol consumption. When ethanol consumption is forced by presenting an ethanol solution as the sole source of fluid every other day, acute administration of low doses of naloxone (1.0 mg/kg) decreases ethanol consumption without significantly altering water intake on alternate days (22). When ethanol consumption is enhanced in fluid-deprived rats by providing a choice between sweetened ethanol and water, acute administration of higher doses of naloxone (3.0–10.0 mg/kg) suppresses consumption of both ethanol and water (2,26). Chronic administration of naloxone (2.5 mg/kg/day), prior to a limited daily choice between sweetened ethanol and water, produces sustained suppression of ethanol intake, but only transient suppression of water intake (16). It is clear from these studies that naloxone can suppress ethanol drinking when ethanol is presented in a sweetened solution or as the sole source of fluid, and that naloxone may preferentially suppress ethanol or water intake as a function of dose of naloxone administered. Whether naloxone preferentially suppresses the free-choice drinking of unsweetened ethanol has not been determined.

Our laboratory has developed, through selective breeding, two lines of rats which differ in voluntary ethanol consumption. The high alcohol drinking (HAD) line and the low alcohol drinking (LAD) line were derived from a heterogenous foundation stock of N/Nih rats (20). Each generation of rats was tested for ethanol preference during four weeks of free-choice between an ethanol

[1]Requests for reprints should be addressed to J. C. Froehlich, Department of Medicine, Emerson Hall 421, Indiana University School of Medicine, 545 Barnhill Drive, Indianapolis, IN 46223.

solution (10% v/v) and water with food available ad lib. Selective breeding for differences in ethanol consumption produced a clear divergence in ethanol intake between the lines by the second generation of selection. By the third generation of selection, 51% of the rats of the HAD line consumed in excess of 5 g ethanol/kg b.wt./day, while 60% of the rats of the LAD line consumed less than 1.5 g ethanol/kg b.wt./day.

In a preliminary study we reported that naloxone, in doses of 1.0 and 3.0 mg/kg b.wt., decreased ethanol consumption without altering water intake when rats of the HAD and LAD lines were given a free-choice between a 10% (v/v) ethanol solution and water (13). The present study extends these findings and investigates whether naloxone, in doses which antagonize different opioid receptor subtypes, attenuates ethanol and/or water consumption by rats of the high alcohol-drinking line.

METHOD

Subjects

Twenty-six male rats from the HAD line weighing 250–450 g were used. All rats were from the third generation of selective breeding for high voluntary ethanol consumption. The animals were housed individually under controlled temperature and lighting conditions (lights on from 0700–1900 hr) with standard laboratory food pellets available ad lib throughout the experiments.

Drugs

Naloxone hydrochloride (Sigma) was dissolved in 0.9% NaCl and injected IP in doses of 0.05–18.0 mg/kg b.wt. in a volume of 1.0 ml/kg b.wt. Saline was injected IP in a volume of 1.0 ml/kg b.wt.

Design and Procedure

Ethanol preference testing. All rats were tested for oral ethanol preference using the procedures and criteria which have routinely been used in the selection of the high alcohol drinking (HAD) and the alcohol-preferring (P) lines (21). Briefly, rats received four days of access to a 10% (v/v) ethanol solution followed by four weeks of free-choice between the ethanol solution and water with food freely available. Ethanol consumption was calculated for each rat during the four-week free-choice period and was expressed as 1) grams of ethanol/kg b.wt./day, 2) ml of 10% ethanol/day and 3) ratio of ml 10% ethanol/ml water. The criteria for selection of the HAD rats were the same as those previously reported for the selection of the P rats (21). To qualify as a high alcohol drinker, a rat must consume in excess of 5.0 g ethanol/kg b.wt./day or more than 20.0 ml of 10% ethanol/day, and must demonstrate a 2:1 preference ratio of ethanol to water.

All of the rats used in the present study met the criteria for designation as high alcohol drinkers and consumed an average of 35 ml of the 10% (v/v) ethanol solution per day (7.6 g ethanol/kg b.wt./day) during ethanol preference testing. Following ethanol preference testing, rats were given ad lib access to food with water available as the sole source of fluid for six weeks prior to initiation of the experiments.

Experiment 1. After six weeks of ad lib access to food and water, fluid access was restricted to a daily two-hour period of free-choice between a 10% (v/v) ethanol solution and water, presented in two 100 ml Richter tubes, with food freely available. Rats were weighed and the position of the tubes containing ethanol was alternated daily. The amount of ethanol and water consumed was recorded daily at the end of the two-hour fluid access period. Limiting fluid access to 2 hours a day did not significantly alter fluid intake. Rats receiving access to fluids for 2 hours daily consumed as much fluid during the 2-hour interval as they ha consumed when fluids were available ad lib and they continued t gain weight at the same rate (4 grams/week) as age-matched rat living under similar conditions with food and water freely avail able (4–5 grams/week).

Twenty-six rats from the HAD line comprised the subjec population. Once every two weeks, baseline ethanol and wate consumption was calculated for each rat over the prior fou consecutive days to even out minor daily fluctuations in ethano and water intake and the rats were ranked in descending order i terms of average daily ethanol consumption. Sixteen to 20 rat from the HAD subject population were assigned to the naloxone o saline groups in a manner which ensured that the two groups di not differ in baseline ethanol or water intake prior to treatment Specifically, the top two ethanol drinkers were randomly assigned one to the naloxone and the other to the saline treatment group followed by the next two highest drinkers, likewise randoml assigned, etc. Thus, the naloxone and saline treatment group were matched on ethanol intake prior to administration of eac naloxone dose. Ethanol and water intakes are not independen variables as evidenced by the fact that rats which drink larg amounts of ethanol drink small amounts of water. Therefore, b rank ordering and counterbalancing the treatment groups based o ethanol intake, we effectively counterbalanced the groups on wate intake as well. This design, which constitutes a new random dra of subjects out of the same population sample every two weeks allowed us to make maximal use of these genetically selecte animals which are of limited availability.

One of eight doses of naloxone was administered to rats of th HAD line once every two weeks. The order of presentation o naloxone doses was random and each rat received 2–5 doses o naloxone during the course of the experiment. Given that naloxon has a relatively short half-life, and that administration of each dos of naloxone was separated by a 2-week interval, it is extremel unlikely that the response to a given dose of naloxone wa influenced by prior naloxone treatment.

On treatment days, a single dose of naloxone or an equa volume of saline was injected IP 30 minutes prior to the dail two-hour free-choice between the ethanol solution and water Fluid consumption was recorded at the end of the two-hour fluid access period. The effect of naloxone on ethanol and wate consumption was determined by comparing mean intakes of eac fluid by the naloxone- and saline-treated groups on the day o naloxone administration.

Experiment 2. Following completion of Experiment 1, 16 rat from the HAD line continued to receive daily two-hour access t fluid with food freely available. However, during the fluid acces period, water was presented as the sole source of fluid. Rats were weighed, and water consumption was recorded daily for 20 weeks. Every 2 weeks, starting at week 6, rats of the HAD line were divided into matched pairs based on average water intake during the prior five days and one rat of each matched pair was assigned to the naloxone treatment group and the other to the saline treatment group as described in Experiment One. The rats were given a single dose of naloxone IP, or an equal volume of saline, 30 minutes before the two hours of access to water. Wate consumption was recorded at the end of the two-hour interval. Eight doses of naloxone were administered to the rats in a random sequence, one dose every two weeks. The effect of naloxone o water consumption was determined by comparing mean wate intake by the naloxone- and saline-treated groups on the day o naloxone administration.

Effect of naloxone on blood ethanol elimination. The effect o naloxone on blood ethanol levels and ethanol elimination rates wa determined in 20 additional HAD rats from the third generation o selective breeding. All rats were given a free-choice between a

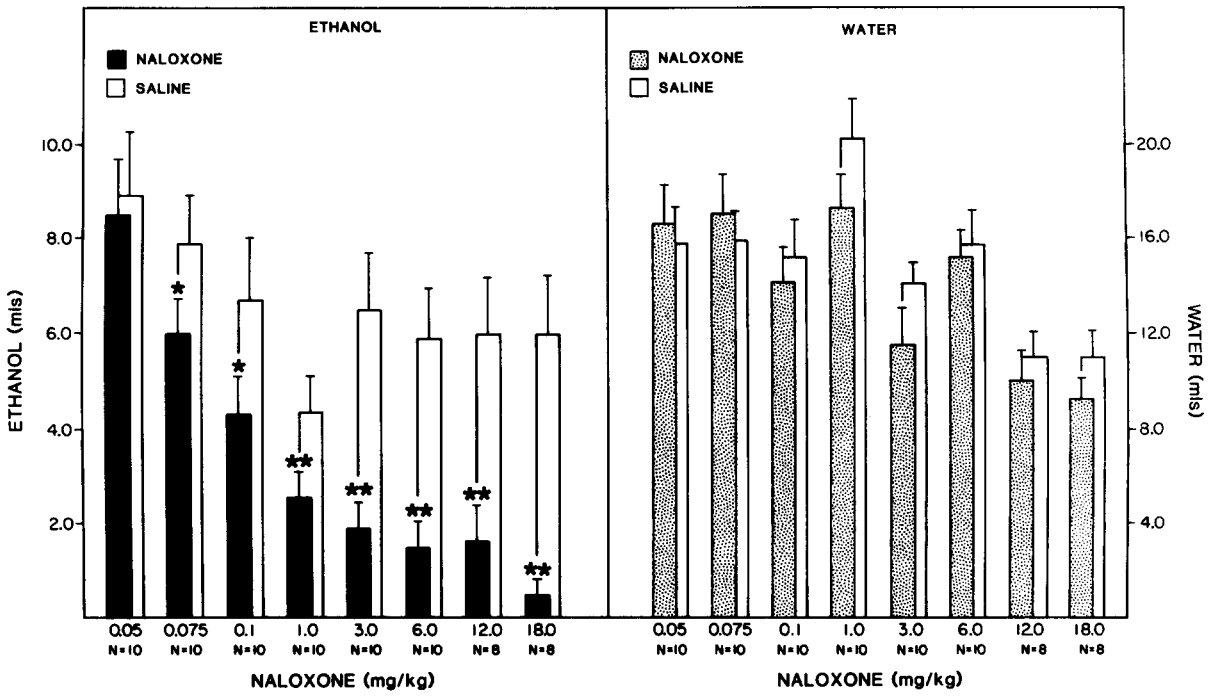

FIG. 1. Effect of naloxone or saline on ethanol and water intake by rats of the HAD line given a free-choice between ethanol (10% v/v) and water. Each bar represents the mean (±S.E.) and N indicates the number of rats per group. Asterisks indicate significant differences between the naloxone- and saline-treated groups (*$p<0.05$; **$p<0.01$). Different scales are used on the left and right ordinates.

ethanol solution (10% v/v) and water for two hours per day (0900–1100 hours) with food freely available for 22 days. Immediately following the 2-hour fluid access period on day 22, food was removed and rats were surgically implanted with a transesophageal catheter for the intragastric delivery of ethanol (27). At 0850 hours on day 23, rats were randomly assigned to the naloxone or saline treatment groups and were injected IP with 18.0 mg/kg b.wt. of naloxone or an equal volume of saline. Thirty minutes following naloxone or saline administration, rats received an IG infusion of 0.3 grams ethanol/kg b.wt. in a 10% (v/v) solution. Blood samples (0.1 ml) were collected from the retro-orbital sinus in heparinized capillary tubes at 5, 15, 30, 60 and 120 minutes following the onset of the ethanol infusion. Blood was centrifuged and the plasma stored at −20°C until assayed for plasma ethanol concentration by direct injection of 1.0 μl of plasma into a Hewlett-Packard 5730A gas chromatograph equipped with a flame ionization detector and 3380A integrator. The glass columns were packed with Poropak Q (80/100 mesh) and the oven temperature was 150°C. Isopropanol was used as the internal standard.

Data Analysis

Within-line comparisons of the effects of naloxone vs. saline on ethanol or water intake were made using paired *t*-tests. The protocol for Experiments 1 and 2 involved rank ordering and assigning rats to the naloxone or saline treatment groups in a manner which produced groups that did not differ in ethanol or water intake prior to drug or saline administration. Given that the groups were not formed by random assignment of animals, but rather by assignment of pairs of animals, one to each group, a basic assumption required for use of an unpaired *t*-test was not met. This counterbalanced design, which eliminates group differences in the variables of interest, namely ethanol and water consumption, prior to naloxone or saline administration, serves to reduce the degree of potential variance between the groups. This type of counterbalanced design dictates the use of a paired *t*-test for comparison of group means (29). Analysis of some of the preliminary data revealed that paired *t*-tests were more sensitive for detecting group differences than was a repeated measures analysis of variance on independent groups.

In order to determine whether naloxone administration resulted in prolonged suppression of ethanol consumption or significant weight loss, within-subject comparisons of ethanol consumption were made on the day before vs. the day after naloxone administration at each naloxone dose, and weight comparisons were made on the day of naloxone administration (prior to injection) vs. the day after naloxone administration at the two highest naloxone doses (12.0 and 18.0 mg/kg). All within-subject comparisons were made using paired *t*-tests.

Comparisons of blood ethanol levels in HAD rats treated with naloxone or saline were made using unpaired *t*-tests.

RESULTS

Experiment 1

Ethanol consumption by rats of the HAD line varied across weeks as illustrated in Fig. 1 (saline-injected controls). Matching the subjects on ethanol and water intake prior to treatment, and assigning one subject from each matched pair to the naloxone group and the other to the saline group, served to control for variance in ethanol intake over the course of the experiment.

Naloxone, in doses of 0.075–18.0 mg/kg, suppressed voluntary ethanol consumption, without altering water intake, by rats of the HAD line given a free-choice between ethanol and water (Fig. 1). Selective suppression of ethanol intake by naloxone resulted in decreased ethanol preference ratios (Fig. 2). The effects of

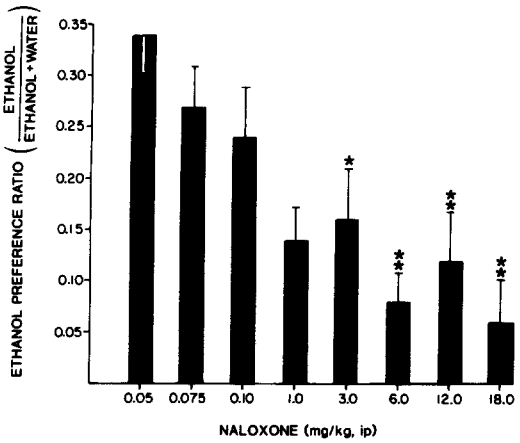

FIG. 2. Effect of naloxone on mean ethanol preference ratio in rats of the HAD line given a free-choice between ethanol (10% v/v) and water. Asterisks indicate significant differences between the naloxone- and saline-treated groups (*$p<0.05$; **$p<0.01$).

naloxone on ethanol intake and preference ratio were dose-dependent. Ethanol intake returned to preinjection baseline levels on the day following naloxone administration at 7 of the 8 doses tested. Administration of naloxone, even at the two highest doses (12.0 and 18.0 mg/kg), did not result in significant weight loss on the day following naloxone administration.

Saline injections did not alter either ethanol or water intake by rats of the HAD line when compared with 5-day ethanol and water preinjection baseline levels of consumption.

Experiment 2

Naloxone, in doses of 1.0–18.0 mg/kg, suppressed water intake by rats of the HAD line given access to water alone (Fig. 3).

Blood Ethanol Concentrations

Even the highest dose of naloxone used in Experiments 1 and 2 (18.0 mg/kg) did not alter blood ethanol levels or ethanol

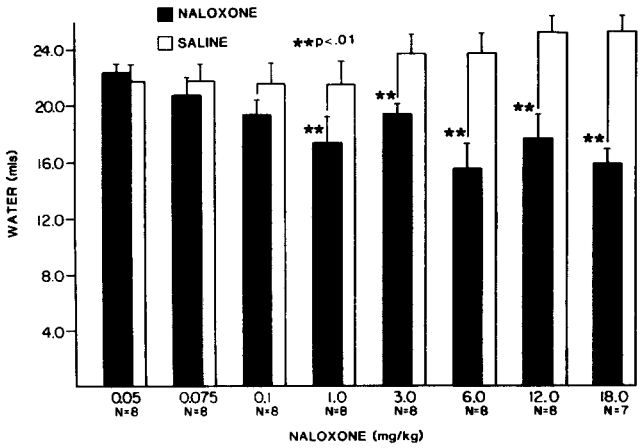

FIG. 3. Effect of naloxone or saline on water intake by rats of the HAD line given water as the sole source of fluid. Each bar represents the mean (±S.E.) and N indicates the number of rats per group. Asterisks indicate significant differences between the naloxone- and saline-treated groups (**$p<0.01$).

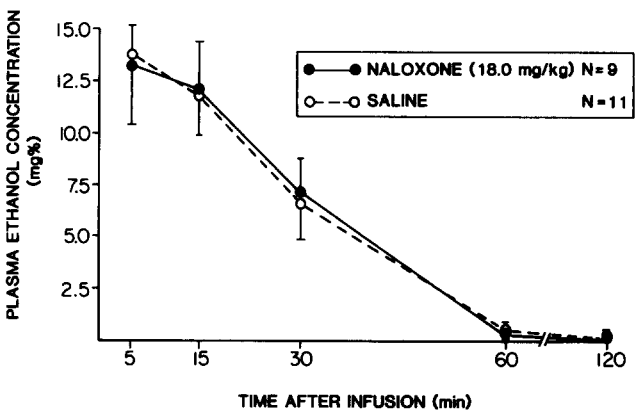

FIG. 4. Plasma ethanol concentrations in rats of the HAD line following an intragastric infusion of ethanol (0.3 g/kg b.wt.).

elimination rates in rats of the HAD line when compared with saline-injected controls (Fig. 4).

DISCUSSION

We have recently hypothesized that the reinforcing properties of ethanol, which serve to sustain continued and repeated bouts of ethanol drinking, may be due, in part, to ethanol-induced activation of the endogenous opioid system (15). Specifically, ethanol drinking may result in the release of endogenous opioid peptides which serve to reinforce and maintain subsequent ethanol drinking. If this hypothesis is correct, opioid receptor antagonists which block the action of endogenous opioids might be expected to decrease voluntary ethanol drinking.

In the present study, administration of naloxone, a potent opioid receptor antagonist, reduced ethanol, but not water consumption when rats which normally consume large quantities of ethanol (HAD line) were given a free-choice between ethanol and water for 2 hours a day. These results support the concept, proposed by Hubbell *et al.* (16), that naloxone may selectively suppress ethanol consumption in paradigms that provide the animal with an alternative opportunity to meet fluid needs.

Naloxone suppressed water consumption when rats of the HAD line were given access to water alone. These results are in good agreement with previous reports that naloxone suppresses water consumption when water is presented as the sole source of fluid (1,7).

It appears that naloxone can suppress water intake when water is presented alone, but that naloxone selectively suppresses ethanol consumption, without altering water intake, when rats are presented with a 2-hour free-choice between ethanol and water. These results suggest that although naloxone may attenuate the positively reinforcing properties of both ethanol and water, ethanol drinking is a subset of consummatory behaviors that is particularly sensitive to opioid receptor blockade.

Naloxone suppressed oral ethanol intake in a dose-dependent manner which may suggest that specific opioid receptor subtypes are involved in mediating ethanol drinking. Naloxone is a potent opioid receptor antagonist that acts primarily on the CNS and binds differentially to mu, delta and kappa receptors as a function of dose administered. Low doses of naloxone (below 1.0 mg/kg) result in the selective occupation of mu receptors which have a very high affinity for naloxone (8–10), while doses of naloxone above 1.0 mg/kg result in the occupation of delta opioid receptors (8, 17, 18). In contrast, 20–30 times more naloxone is needed to

antagonize actions at kappa receptors than is necessary to antagonize actions at mu receptors (8,17). In the current study, low doses of naloxone (below 1.0 mg/kg), which preferentially block mu receptors, produced a slight suppression of ethanol drinking, but stronger suppression of drinking was seen following administration of higher doses of naloxone which occupy both mu and delta receptors. Further work will be needed to determine the relative importance of different opioid receptor subtypes in mediating the initiation and maintenance of ethanol drinking.

The restricted fluid access paradigm used in the present study requires that rats consume enough water to meet their 24-hour metabolic needs in a 2-hour period. Therefore, it is not surprising that even rats with high ethanol preference (HAD line) drank more water than ethanol during the restricted access period. We have previously noted that under identical conditions of fluid restriction, rats of the HAD line still consumed 3 to 5 times more ethanol than rats of the LAD line (13).

Given that naloxone can produce conditioned place and taste aversions (12, 19, 23, 24, 28), one may question whether the decrease in ethanol intake seen in Experiment 1 reflects the development of naloxone-induced conditioned taste aversion to ethanol. This is unlikely for several reasons. First, conditioned taste aversion is not normally detected on the first conditioning trial. The initial pairing of the conditioned stimulus (CS) and the unconditioned stimulus (UCS) begins to establish the association between the CS and the UCS. The development of conditioned taste aversion is indexed by a decrease in consumption of a substance paired with an aversive UCS and this decrease is detected on trials subsequent to the first conditioning trial (14). In the experiments described here, naloxone suppressed ethanol consumption during the 2-hour free-choice between ethanol and water only on the day that naloxone was administered which would represent the first conditioning trial. Ethanol consumption returned to baseline, or exceeded baseline, on the first day and on all subsequent days following naloxone administration. These findings indicate that conditioned aversion to ethanol did not develop. Given that ethanol consumption was not suppressed during the 2-week intervals separating each naloxone injection, it is not likely that suppression of ethanol consumption on the day of naloxone administration reflects the development of a taste aversion to ethanol resulting from a previous naloxone treatment. Second, the eight doses of naloxone, each of which was administered 2 weeks apart, were administered randomly rather than in an ascending or descending order. Therefore, the dose-dependent decrease in ethanol intake observed was not due to a cumulative increase in strength of aversion to ethanol as a function of increasing naloxone dosage. Third, naloxone-treated rats given a choice between ethanol and water displayed normal bouts of water drinking and food consumption following naloxone administration, and no significant weight loss was detected on the day following naloxone treatment. These results indicate that naloxone-induced suppression of ethanol consumption cannot be attributed to the development of naloxone-induced taste aversion or malaise.

It has recently been reported that naloxone may facilitate gastrointestinal absorption of ethanol which may result in higher blood ethanol levels following ethanol ingestion (4). We have previously noted that oral ethanol intake is limited by the blood ethanol level resulting from individual drinking bouts (25). Therefore, it could be postulated that suppression of ethanol intake by naloxone in the present study was due to an increase in ethanol absorption rates following ethanol ingestion in naloxone-treated rats. However, even at the highest dose tested (18.0 mg/kg), naloxone did not alter blood ethanol concentrations or ethanol elimination rates. Blood ethanol levels were identical in naloxone- and saline-treated rats of the HAD line following an IG infusion of ethanol in an amount similar to that voluntarily consumed by HAD rats treated with 18.0 mg of naloxone.

In summary, the results provide further evidence that activation of the endogenous opioid system may be necessary for the continuation of voluntary ethanol drinking. The selective suppression of ethanol, but not water intake by naloxone may indicate that endogenous opioid peptides play a more important role in maintaining ethanol drinking than in maintaining other types of consummatory behaviors.

ACKNOWLEDGEMENTS

We thank Dr. James Norton for statistical consultation and M. Zweifel for technical assistance. The research was supported, in part, by grants AA03243, AA07611 and AA08312 from the PHS.

REFERENCES

1. Akkok, F.; Manha, N. A.; Czirr, S. A.; Reid, L. D. Naloxone persistently modifies water-intake. Pharmacol. Biochem. Behav. 29:331–334; 1988.
2. Beaman, C. M.; Hunter, G. A.; Dunn, L. L.; Reid, L. D. Opioids, benzodiazepines and intake of ethanol. Alcohol 1:39–42; 1984.
3. Bellinger, L. L.; Williams, F. E. Suppressive effects of naloxone on food and water intake in vagotomized rats. Physiol. Behav. 30: 273–278; 1983.
4. Benitez, M.; Boada, J.; Diaz, E.; Feria, M.; Prunell, M. Naloxone-induced increase in blood and brain ethanol concentrations in rats. Pharmacol. Res. Commun. 19:723–729; 1987.
5. Brown, D. R.; Blank, M. S.; Holtzman, S. G. Suppression by naloxone of water intake induced by deprivation and hypertonic saline in intact and hypophysectomized rats. Life Sci. 26:1535–1542; 1980.
6. Brown, D. R.; Holtzman, S. G. Suppression of deprivation-induced food and water intake in rats and mice by naloxone. Pharmacol. Biochem. Behav. 11:567–573; 1979.
7. Brown, D. R.; Holtzman, S. G. Suppression of drinking by naloxone in the rat: A further characterization. Eur. J. Pharmacol. 69:331–340; 1981.
8. Chang, K. J.; Cuatrecasas, P. Heterogeneity and properties of opiate receptors. Fed. Proc. 40:2729–2734; 1981.
9. Chang, K. J.; Hazum, E.; Killian, A.; Cuatrecasas, P. Interactions of ligands with morphine and enkephalin receptors are differentially affected by guanine nucleotide. Mol. Pharmacol. 20:1–7; 1981.
10. Childers, S. R.; Creese, I.; Snowman, A. M.; Snyder, S. H. Opiate receptor binding affected differentially by opiates and opioid peptides. Eur. J. Pharmacol. 55:11–18; 1979.
11. Cooper, S. J. Naloxone: Effects on food and water consumption in the non-deprived and deprived rat. Psychopharmacology (Berlin) 71:1–6; 1980.
12. Dymshitz, J.; Lieblich, I. Opiate reinforcement and naloxone aversion, as revealed by place preference paradigm, in two strains of rats. Psychopharmacology (Berlin) 92:473–477; 1987.
13. Froehlich, J. C.; Harts, J.; Lumeng, L.; Li, T.-K. Naloxone attenuation of voluntary alcohol consumption. In: Lindros, K. O.; Ylikahari, R.; Kiianmaa, K., eds. Advances in biomedical alcohol research. New York: Pergamon Press; 1987:333–337.
14. Froehlich, J. C.; Harts, J.; Lumeng, L.; Li, T.-K. Differences in response to the aversive properties of ethanol in rats selectively bred for oral ethanol preference. Pharmacol. Biochem. Behav. 31:215–222; 1988.
15. Froehlich, J. C.; Harts, J.; Lumeng, L.; Li, T.-K. Enkephalinergic involvement in voluntary ethanol consumption. Excerpta Med. Int. Congr. Series 805:235–238; 1988.
16. Hubbell, C. L.; Czirr, S. A.; Hunter, G. A.; Beaman, C. M.; LeCann, N. C.; Reid, L. D. Consumption of ethanol solution is potentiated by morphine and attenuated by naloxone persistently across repeated daily administrations. Alcohol 3:39–54; 1986.
17. Leander, D. J. A kappa opioid effect: increased urination in the rat. J.

Pharmacol. Exp. Ther. 224:89–94; 1983.
18. Leander, D. J.; Hart, J. C.; Lochner, M. A.; Hynes, M. D.; Zimmerman, D. M. Novel phenylpiperidine opioid antagonists and partial agonists: effects on fluid consumption. Eur. J. Pharmacol. 81:185–192; 1982.
19. LeBlanc, A. E.; Cappell, H. Antagonism of morphine-induced aversive conditioning by naloxone. Pharmacol. Biochem. Behav. 3:185–188; 1975.
20. Lumeng, L.; Doolittle, D. P.; Li, T.-K. New duplicate lines of rats that differ in voluntary alcohol consumption. Alcohol Alcohol. 21:A125; 1986.
21. Lumeng, L.; Hawkins, T. D.; Li, T.-K. New strains of rats with alcohol preference and nonpreference. In: Thurman, R. G.; Williamson, J. R.; Drott, H. R.; Chance, B., eds. Alcohol and aldehyde metabolizing systems. vol. 3. New York: Academic Press; 1977: 537–544.
22. Marfaing-Jallat, P.; Le Magnen, J. Decrease in ethanol consumption by naloxone in naive and dependent rats. Pharmacol. Biochem. Behav. 18:537–539; 1983.
23. Mucha, R. F.; Iversen, S. D. Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: a procedural examination. Psychopharmacology (Berlin) 82:241–247; 1984.
24. Mucha, R. F.; Walker, M. J. K. Aversive property of opioid receptor blockade in drug-naive mice. Psychopharmacology (Berlin) 93:483–488; 1987.
25. Murphy, J. M.; Gatto, G. J.; Waller, M. B.; McBride, W. J.; Lumeng, L.; Li, T.-K. Effects of scheduled access on ethanol intake by the alcohol-preferring (P) line of rats. Alcohol 3:331–336; 1986.
26. Reid, L. D.; Hunter, G. A. Morphine and naloxone modulate intake of ethanol. Alcohol 1:33–37; 1984.
27. Smith, S. G.; Werner, T. E.; Davis, W. M. Technique for intragastric delivery of solutions: Application for self-administration of morphine and alcohol by rats. Physiol. Psychol. 3:220–224; 1975.
28. Van der Kooy, D.; Phillips, A. G. Temporal analysis of naloxone attenuation of morphine-induced taste aversion. Pharmacol. Biochem. Behav. 6:637–641; 1977.
29. Winer, B. J. Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill; 1971.