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Naloxone Alters Alcohol Drinking Induced in the Rat by Tetrahydropapaveroline (THP) Infused ICV

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MYERS, R. D. AND E. C. CRITCHER. *Naloxone alters alcohol drinking induced in the rat by tetrahydropapaveroline (THP) infused ICV.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 827–836, 1982.—In male rats of the Sprague-Dawley or Long-Evans strain, intracerebroventricular (ICV) cannulae were implanted permanently using stereotaxic techniques. Tetrahydropapaveroline (THP) was infused ICV for up to 14 days either chronically around the clock or acutely once per day in doses previously found to induce an abnormally high intake of alcohol. During these periods, alcohol preference for individual rats was determined by a self-selection procedure in which the concentration of alcohol was increased from 3 to 30% on each day of a 12-day interval. Those rats which displayed a substantial increase in their intake of alcohol were selected for naloxone treatment and subsequently assigned a fixed concentration of alcohol at which maximum consumption occurred. Naloxone was administered subcutaneously two to six times per day for three consecutive days in total daily doses ranging from 1.5 to 3.0 mg/kg. Each rat served as its own control and was given 0.9% saline isovolumetrically according to the same temporal schedule. Naloxone generally suppressed alcohol intake in animals by 20% to 45%, but the reduction in drinking depended upon the injection regimen as well as the prior level of alcohol consumption. In "high drinking" rats, the mean alcohol intake of 6.2 g/kg/day was reduced to 3.7 g/kg/day by naloxone whereas in "low drinkers" the mean intake of 3.5 g/kg/day was suppressed to 2.8 g/kg/day by the opiate antagonist. These results further support the suggestion of a possible opiate receptor link in the pathogenesis and maintenance of aberrant drinking of alcohol, the mechanism of which may involve the endogenous action of an amine-aldehyde condensation product in the brain.

Opiate receptor in rat brain Naloxone and alcohol drinking Tetrahydropapaveroline (THP)
Opiate antagonist and agonist Alcohol self-selection Intracerebroventricular THP Drinking of alcohol
Amine-aldehyde condensation product Tetrahydroisoquinoline alkaloid

IN spite of the clinical dissimilarities between the addiction to ethyl alcohol and an opiate compound, the possibility nevertheless exists of a biological commonality in the molecular mechanisms responsible for these two disease states [4, 13, 42, 43]. In fact, opiate receptors in the brain conceivably may constitute one of the functional neurochemical links underlying the process of alcohol addiction [7].

The impetus for this viewpoint is founded on several intriguing pieces of experimental evidence [37]. To illustrate, acetaldehyde, the first intermediary by-product of the metabolism of alcohol, can condense with dopamine in the body to form a tetrahydroisoquinoline, salsolinol. One amine-aldehyde condensation product, tetrahydropapaveroline (THP), is the biological precursor to morphine. When infused repeatedly into the cerebral ventricle of the rat or monkey, THP and other amine-aldehyde condensation products including salsolinol can cause the animal to drink abnormal amounts of ethyl alcohol [38, 39, 41] and in some cases produce signs of withdrawal and physical dependence [31,46].

Additional evidence includes the fact that the opiate receptor antagonist, naloxone, can attenuate withdrawal-like signs in the rodent that is physically dependent on alcohol [6,24] or even retard the development of physical dependence on alcohol [5]. Further, alcohol can reduce naloxone's hyperalgesic effect to a noxious stimulus [3]. In this connection, THP and carboxy-salsolinol, when administered to the rat, can act in themselves as analgesic agents [14,18] with naloxone antagonizing the analgesia produced by carboxy-salsolinol [28].

A major question now centers on the possible relationship between endogenous opiate receptors and the volitional drinking of alcohol. In preliminary studies, a single injection of an opiate antagonist reportedly caused a variable change in voluntary drinking of alcohol albeit in volumes of questionable pharmacological efficacy [22,44]. Thus, the purpose of this investigation was to determine whether an antagonist of CNS opiate receptors would alter the pattern of self-selection of alcohol in an animal that drinks significant amounts of the fluid following repeated infusions of THP into its cerebral ventricle.

In these experiments, the effect on the self-selection of alcohol of repeated daily injections of the opiate antagonist, naloxone, was examined in terms of different temporal inter-

vals and different doses of the drug. Two strains, Sprague-Dawley and Long-Evans, were used because of the inherent differences in their basic pattern of alcohol preference [30]. Also, the effect of naloxone on non-THP treated animals selected for their alcohol drinking characteristics was determined.

METHOD

Male rats weighing between 300 and 520 g and derived from the Sprague-Dawley (n=19) and Long-Evans (n=16) strains were housed individually in stainless steel cages. The colony room was maintained on a 12-hr light-dark cycle and kept at an ambient temperature of 21–23°C. Water and Wayne Lab Blox, either pelleted or powdered, were available ad lib to each animal with measures of respective intakes taken at the same time on each day.

Surgery

Each rat was anesthetized with 30–40 mg/kg sodium pentobarbital and then its head was placed in a stereotaxic instrument. Following procedures described earlier [35], either a 23 ga stainless steel guide tube for acute injections, or a 20 ga cannula fitted within a Khavari swivel for chronic infusions, was implanted so that the tip rested 0.5 mm dorsal to the lateral ventricle. The stereotaxic coordinates were based on those of de Groot [12] as follows: AP+6.0; LAT 1.0–1.5; 3.0 mm below the dura mater. After anchor screws were inserted in the calvarium, cranioplastic cement was packed around the screws and either the guide tube or swivel base to hold the respective device firmly in place.

Chronic Infusion Procedure

A 27 ga infusion needle attached by PE tubing to a calibrated 1.0 ml syringe was positioned within the guide cannula of the Khavari swivel so that its tip rested in the lumen of the lateral ventricle [35]. A custom-built infusion pump, designed to hold 12 syringes, was used to infuse either an artificial CSF [34] or THP, both delivered in a volume of 2.0–4.0 μl per infusion [38]. THP was dissolved in an artificial CSF containing 0.1 mg/ml of ascorbic acid which was used as an antioxidant [38]. An infusion of 60 sec duration was given every 30 min throughout the 24-hr treatment period. Each 1.0 μl volume of the solution of the amine-aldehyde metabolite contained 0.01–0.1 μg of the hydrobromide salt (Hoffmann-La Roche). The intraventricular infusions were given over 24 hr every day, as described previously [31], beginning one day prior to and on all days during the 12-day alcohol preference test.

Acute Infusion Procedure

A 27 ga infusion needle attached to a calibrated length of PE 20 tubing was filled with either the artificial CSF [34] or a solution of THP solubilized in the CSF medium. The needle was positioned in the guide cannula so that its tip rested within the lateral ventricle. Then, the THP solution was infused by gravity [39] either bilaterally or unilaterally in a volume of 5.0 μl or 10.0 μl, respectively. Each 10.0 μl of infusate contained 0.1 to 10.0 μg of the THP hydrobromide (Hoffmann-La Roche). An infusion of THP or the control vehicle was made 3 times a day for 2 days prior to the preference test and then once daily during the alcohol test sequence.

Alcohol Preference Sequence

The three-bottle, two-choice method was used to test alcohol preference [40]. Three 100 ml Kimax tubes were affixed equidistantly apart on the front of each cage: one contained an alcohol solution, one contained water and the third was empty and served as a "dummy." On the first day, 3% alcohol was offered with water. Thereafter, on each day the concentration of alcohol was increased so that a range of concentrations from 3% to 30% was offered to the rat in the presence of water.

Based on the results of the pre-injection preference screen, animals which drank substantial amounts of alcohol were offered water and a single, fixed concentration of alcohol. For some animals a second, fixed concentration of alcohol was placed in the "dummy" tube. The two fixed solutions of alcohol, one low and one high (e.g., 8% and 16% concentration) were chosen so that a potential change in alcohol preference due to a differential effect of the drug treatment could be determined. The opiate antagonist was administered to THP-treated rats which maintained an alcohol intake of 2.0 g/kg/day or greater. However, some animals which rejected alcohol even at low concentrations following a THP infusion sequence were given naloxone in order to test for the possible enhancement of alcohol preference. In addition, six non-THP-infused rats of the Long-Evans strain, which maintained higher than typical intakes of alcohol of over 2.0 g/kg/day throughout the 3–30% sequence, were also treated with naloxone according to the same procedure.

Preparation and Injection of Drug Solutions

Each drug was prepared freshly in 0.9% sterilized physiological saline and kept frozen until used. Naloxone (Endo) solutions were injected in doses of 0.5 or 1.0 mg/kg. The twice daily injections of the antagonist were given at 8:00 a.m. and 8:00 p.m. Injection times for the three-times-a-day regimen were 8:00 a.m., 4:00 p.m. and 12:00 p.m. When an injection of the drug was given six times a day, it was administered every four hours beginning at 8:00 a.m. For control tests, 0.9% saline was injected.

Four days of basal alcohol and water intakes were obtained for each rat prior to the three-day sequence of naloxone treatment. Then, the alcohol preference test was continued for an additional four days or longer after the drug was discontinued.

RESULTS

The results of the present experiments show that in the THP-infused rat, naloxone generally suppresses the volitional drinking of ethyl alcohol. At the same time, the ingestion of food or water over the 24-hr interval is not significantly affected. The magnitude, duration and other aspects of the change in alcohol self-selection produced by the opiate receptor antagonist depend primarily on two factors: (1) the dose and frequency of the drug's administration, and (2) the prior level of alcohol intake of the individual animal.

The potency of naloxone's effect in attenuating the self-selection of alcohol did not differ between the Long-Evans and the Sprague-Dawley strains, $t(33)=0.77$; $p>0.05$. Although the mean alcohol intake of 5.2 g/kg for the Long-Evans group prior to treatment exceeded that of the Sprague-Dawley rats of 4.4 g/kg, the percent of overall suppression for each group during naloxone treatment was virtually the same, i.e., 30%. During the four-day period follow-

TABLE 1

EFFECTS OF THREE ALTERNATIVE DOSAGE REGIMENS OF NALOXONE ON ALCOHOL INTAKE IN TERMS OF g/kg/day AND THE RATIO OF ALCOHOL TO WATER EXPRESSED AS THE PROPORTION OF ALCOHOL TO TOTAL FLUID CONSUMED

		Alcohol Intake	
Dose of Naloxone	Period	g/kg	Proportion
0.5 mg/kg	Pre	5.6 ± 0.7	0.55 ± 0.07
3XD n=4	Nal	3.4 ± 0.4 (↓39%)	0.44 ± 0.12 (↓20%)
	Post	4.9 ± 0.7	0.56 ± 0.13
1.0 mg/kg	Pre	6.0 ± 0.2	0.96 ± 0.01
3XD n=2	Nal	4.8 ± 0.8 (↓20%)	0.90 ± 0.03 (↓6%)
	Post	6.1 ± 0.3	0.96 ± 0.00
0.5 mg/kg	Pre	7.2 ± 0.2	0.95 ± 0.01
6XD n=2	Nal	4.0 ± 0.5 (↓44%)	0.81 ± 0.05 (↓15%)
	Post	5.0 ± 0.8	0.72 ± 0.10

The Periods consisted of a 4-day baseline (Pre), 3 days of drug treatment (Nal) and 4 days after naloxone administration ended (Post). Percent reduction (↓) of alcohol intake during naloxone is denoted in parentheses. Values expressed are means ± standard errors.

ing naloxone treatment, the intake of alcohol of the Long-Evans rats returned to 97% of the pre-naloxone baseline level and of the Sprague-Dawley animals to 88%. Since there were also no differences between strains in food and water intakes or in body weight in response to the naloxone treatment, the results for both strains were combined for further analyses.

Dose Response Analysis

When 2.0 mg/kg of naloxone were administered in two equal doses, a 29% reduction in alcohol intake occurred, as shown in Fig. 1 (BOTTOM). The mean baseline alcohol consumption of 4.8 g/kg/day prior to treatment declined significantly to 3.5 g/kg during the days of naloxone injections, $t(34)=5.35; p<0.01$. A total daily naloxone dose of 1.5 mg/kg divided equally in three 0.5 mg/kg injections produced a 39% reduction in alcohol ingestion. As shown in Table 1, 3.0 mg/kg/day of naloxone injected in three doses suppressed alcohol drinking by 20%, whereas the same 3.0 mg/kg/day injected in six doses produced a 44% decline.

The overall effect of naloxone on the proportion of alcohol to total fluid consumption was somewhat less pronounced than on the rats' absolute intake of alcohol in terms of g/kg. As shown in Fig. 1 (TOP), the average proportional intake of the THP-induced alcohol drinking animals was 0.74 before naloxone treatment. During the days when naloxone was injected in a dose of 2.0 mg/kg/day, the proportional intake declined to 0.66, as depicted in Fig. 1 (TOP). As presented in Table 1, naloxone produced a similar effect in rats which received injections either three or six times a day.

The amount of alcohol consumed during control tests in rats given isovolumetric injections of saline twice daily increased significantly from 4.8 to 5.3 g/kg, $t(22)= 2.77; p<0.02$.

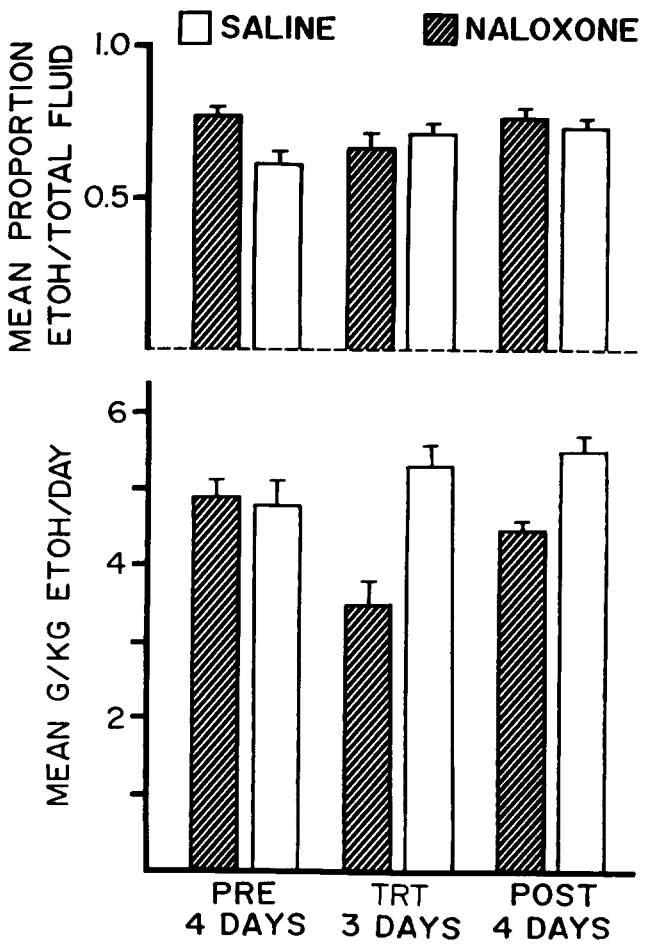

FIG. 1. Effect of saline (n=23) or 2.0 mg/kg/day naloxone (n=35) on the mean±S.E. intake of alcohol in terms of the ratio of alcohol to water (TOP), expressed as the proportion of alcohol to total fluid consumed, and g/kg/day (BOTTOM). A 4-day baseline (PRE) preceded 3 days of twice daily injections of the naloxone or saline (TRT), followed by 4 days of no injection (POST).

This is illustrated in Fig. 1 for both proportion and g/kg values and is presumably due to the alcohol acclimation effect described previously [51].

Analysis of "High" Versus "Low" Alcohol Intakes

As a result of previous observations with drugs which alter alcohol self-selection [40], further analyses of the drinking data of the THP-treated animals were undertaken. The mean baseline intakes which equalled or exceeded 5.0 g/kg/day were categorized as "high" whereas mean intakes of less than 5.0 g/kg/day were classified as "low." The group means for these "high" and "low" intake categories were 6.2 g/kg/day and 3.5 g/kg/day, respectively.

The efficacy of naloxone in reducing THP-induced alcohol preference was significantly greater in those rats which maintained the higher mean intake of alcohol. As portrayed in Fig. 2 (BOTTOM), 2.0 mg/kg naloxone reduced by 40% the g/kg intake of alcohol from an average of 6.2 g/kg/day

TABLE 2

EFFECT OF DIFFERENT DOSAGE REGIMENS OF NALOXONE (Nal) AND OF SALINE (Sal) ON FOOD AND WATER INTAKES AND ON BODY WEIGHTS

	Food (g)			Water (ml)			Body Weight (g)		
Dose	Pre	TRT	Post	Pre	TRT	Post	Pre	TRT	Post
1.0 mg/kg Nal 2XD n=35	24 ± 0.4	22 ± 0.5	24 ± 0.5	12 ± 1.2	14 ± 1.8	12 ± 1.6	474 ± 5	473 ± 7	475 ± 6
0.5 mg/kg Nal 3XD n=4	23 ± 2.6	24 ± 1.3	20 ± 2.5	17 ± 3.9	20 ± 5.8	16 ± 4.9	428 ± 16	428 ± 17	431 ± 16
1.0 mg/kg Nal 3XD n=2	19 ± 0.5	16 ± 1.2	17 ± 1.7	2 ± 0.3	4 ± 0.8	2 ± 0.1	458 ± 6	459 ± 5	454 ± 5
0.5 mg/kg Nal 6XD n=2	22 ± 1.2	15 ± 1.3	23 ± 0.8	3 ± 0.7	6 ± 1.6	13 ± 4.9	497 ± 7	495 ± 8	491 ± 8
Sal 2XD n=21	23 ± 0.7	23 ± 0.8	22 ± 0.5	15 ± 1.2	12 ± 1.4	10 ± 0.9	455 ± 5	463 ± 5	470 ± 5

Values expressed are means ± standard errors for the 4-day baseline (Pre), 3 days of injections (TRT), and 4 days following injections (Post).

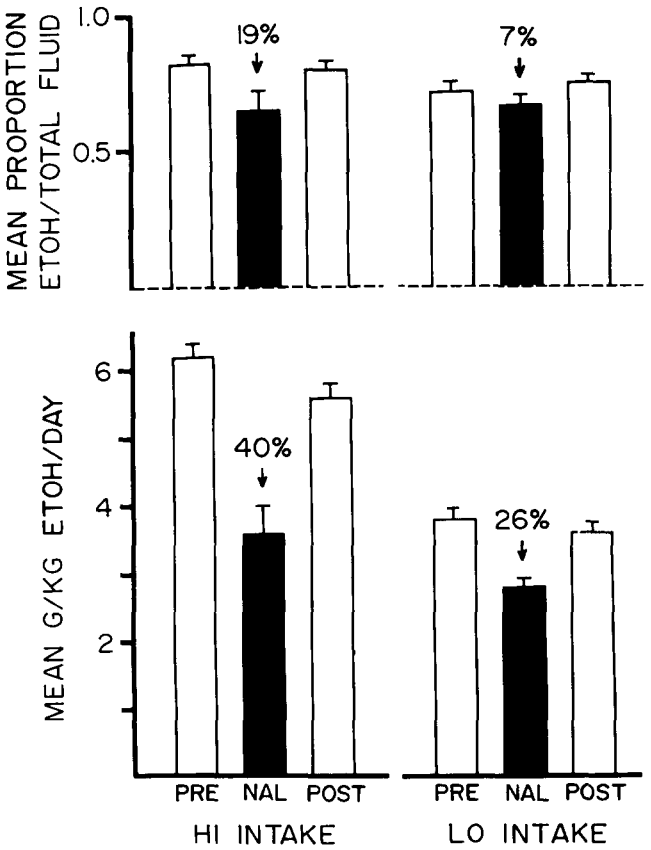

FIG. 2. Comparison of effect of 2.0 mg/kg/day naloxone on "high" (HI) (n=14) versus "low" (LO) (n=21) alcohol intakes in terms of mean (±S.E.) ratio of alcohol to water (TOP) and g/kg/day (BOTTOM). Percent reduction of alcohol intake during naloxone is denoted (↓). Treatment regimen as in Fig. 1.

prior to treatment of 3.7 g/kg/day during injections, $t(13)=6.11$; $p<0.01$. On the other hand, in rats which maintained a lower pre-injection alcohol intake of 3.5 g/kg/day, the same dose of naloxone reduced their consumption by 26% to 2.8 g/kg. This reduction, however, was nevertheless statistically significant, $t(20)=2.51$; $p<0.025$. Although there were distinct individual responses to naloxone treatment, a Spearman Rho between the initial level of alcohol intake and the percent of its suppression revealed a significant correlation coefficient of +0.37 ($p<0.05$). The effect of naloxone on the mean proportional intake of the "high drinkers" (Fig. 2 TOP) was less pronounced than on the absolute intake in g/kg.

Although 2.0 mg/kg/day naloxone produced a 45% reduction in the intake of alcohol of the "high drinking" Sprague-Dawley rats, as opposed to a 38% reduction in the Long-Evans "high drinkers," there were essentially no strain differences among "low drinkers."

Effect of Naloxone on Food and Water Intakes

Generally, the amount of food consumed as measured at 24-hr intervals was relatively unaffected in the rats treated with naloxone. The sole exception to this finding occurred when 0.5 mg/kg naloxone was administered six times a day. In this case, the amount of food ingested was reduced 29%, by more than 6.0 g/day, a finding which corresponds to earlier studies [9, 23, 25]. Conversely, the intake of water, as presented in Table 2, tended to rise during treatment with the opiate antagonist, which would be expected in view of the reciprocal relationship between water and alcohol drinking. Little or no variation occurred in the body weights of the rats following the administration of naloxone (Table 2). Within the saline control tests, essentially no changes in food intake occurred although water consumption did decrease slightly.

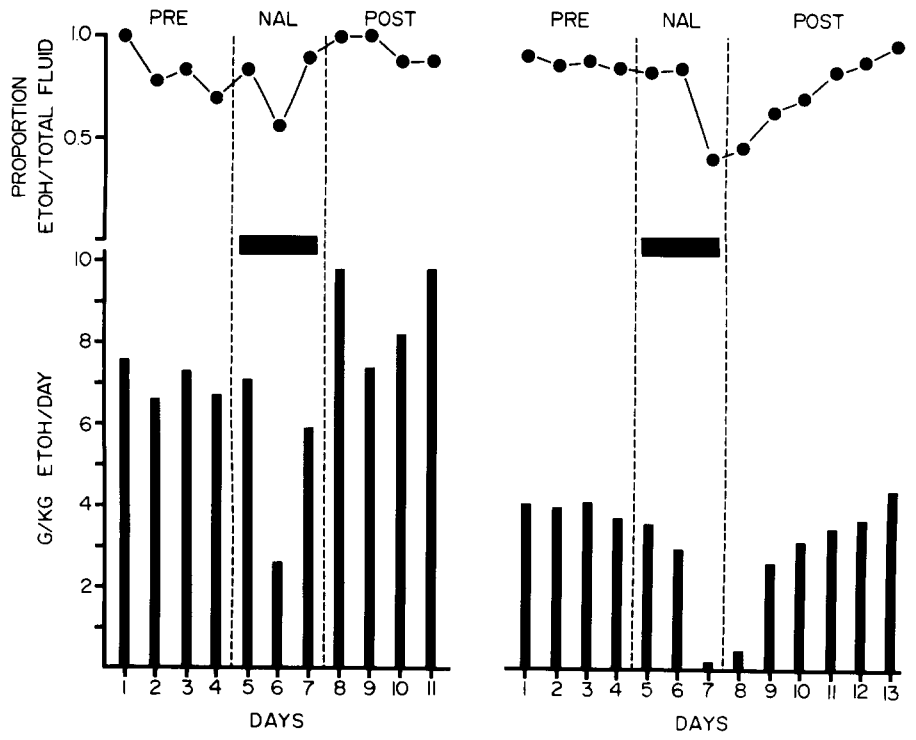

FIG. 3. Short term effect of 2.0 mg/kg/day naloxone on alcohol intake of two individual animals in terms of the ratio of alcohol to water (TOP) and g/kg/day (BOTTOM). Values are based on the combined intake of two concentrations of alcohol offered to each rat (10% and 20%, LEFT; 7% and 14%, RIGHT). Treatment regimen as in Fig. 1.

Individual Responses to Naloxone

Even though naloxone generally suppressed alcohol consumption, the patterns of alcohol drinking observed in response to the drug often differed. In some animals naloxone produced its maximal effect on alcohol intake only on one day of the three-day injection period. To illustrate, the g/kg intake of alcohol of a representative rat, as shown in Fig. 3 (LEFT), declined on the second day of the injection series by 70% below the 4-day pre-naloxone level. However, a rebound increase in alcohol consumption above the baseline (Fig. 3 LEFT) occurred during the preference sequence following the naloxone injections. Usually this rebound enhancement was characteristic of those rats which exhibited the sharpest decline in alcohol drinking during the treatment with the opiate antagonist.

As shown in Fig. 3 (RIGHT), naloxone given to another rat significantly reduced alcohol intake only on the third day of injection, as reflected by g/kg and proportional measures. This reduction continued for one day following the naloxone injections (Fig. 3 RIGHT). Thereafter, the amount of alcohol consumed returned to the pre-injection baseline level within six days.

A longer-lasting reduction in alcohol preference was produced by naloxone in certain rats. As illustrated in Fig. 4 (LEFT), the g/kg alcohol ingested by an individual rat did not return to the pre-injection level of 8.0 g/kg until the eighth day following the last injection of naloxone. However, the proportional intake of 1.00, reflecting complete preference for alcohol, recovered within only four days after the naloxone injections had ended. This distinct response to the opiate antagonist is notable in that alcohol consumption was totally blocked on the first day of naloxone injection.

An even more protracted suppression of alcohol intake was observed in other animals. As portrayed in Fig. 4 (RIGHT), the rat's intake of alcohol declined from approximately 4.0 g/kg to 1.0 g/kg and, thereafter, stabilized at the 1.0–2.0 g/kg level. The proportion values likewise remained low.

Naloxone's differential effect on the intake of alcohol depended also on the specific fixed concentrations of alcohol offered to the rat. Figure 5 (LEFT) shows that when a lower concentration of alcohol such as 8% was selected over a higher, i.e., 16%, prior to naloxone, a declining amount of the lower concentration was taken during naloxone treatment (Rat A). Following the naloxone injections (POST), the intake of the lower concentration (8% alcohol) continued to decrease, whereas the ingestion of the higher concentration (16%) rose above its previous baseline. This pattern persisted well past the injection period. Conversely, as illustrated in Fig. 5 (RIGHT), approximately one-fourth of the total intake of another rat (Rat B) prior to naloxone administration consisted of the higher concentration (16%). Following the injections of the opiate antagonist, the lower concentration (8%) alone accounted for the total daily amount of alcohol consumed. Both Rats A and B were Sprague-Dawley animals, and thus the effect of naloxone cannot be attributed to the strain.

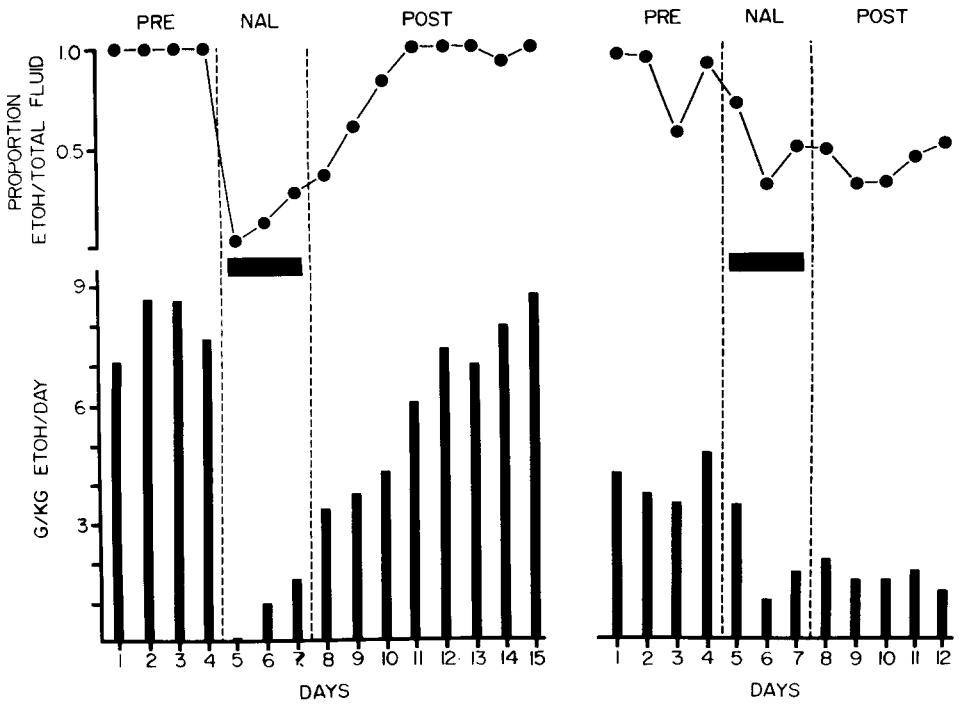

FIG. 4. Prolonged effect of 2.0 mg/kg/day naloxone on alcohol intake of two individual animals in terms of the ratio of alcohol to water (TOP) and g/kg/day (BOTTOM). Values are based on the intake of a single concentration (7%) offered to one rat (RIGHT) and the combined intake of two concentrations offered to the other (8% and 16%, LEFT).

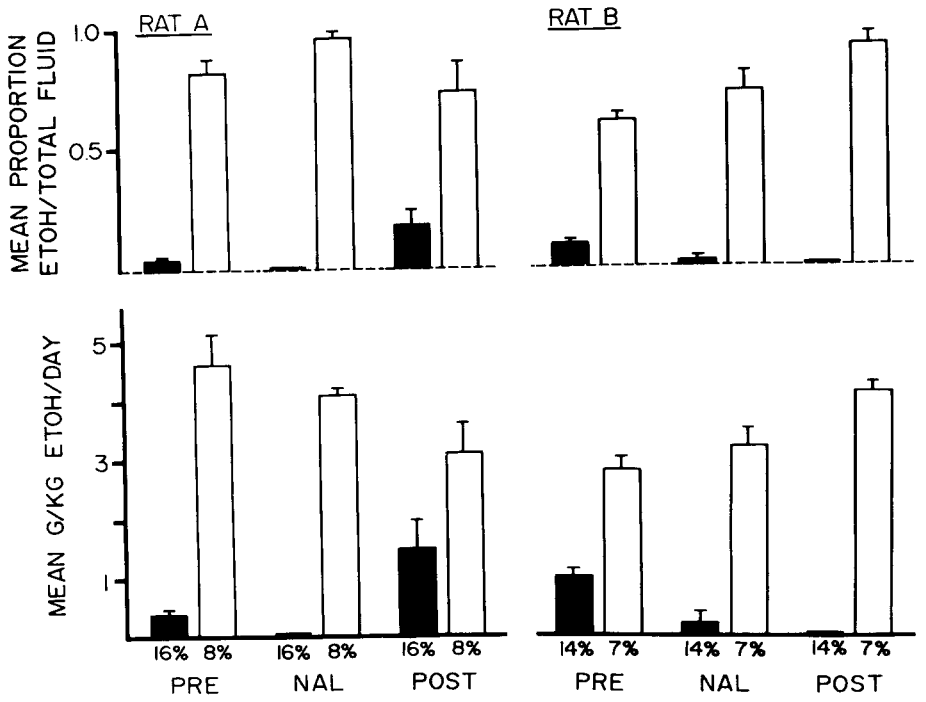

FIG. 5. Effect of 2.0 mg/kg/day naloxone on differential alcohol intake of two rats in terms of the ratio of alcohol to water (TOP) and g/kg/day (BOTTOM), expressed as mean±S.E. Rat A was offered 8% and 16% alcohol simultaneously; Rat B was offered 7% and 14%. Treatment regimen as in Fig. 1. Solid bars represent higher concentration; open bars, the lower concentrations.

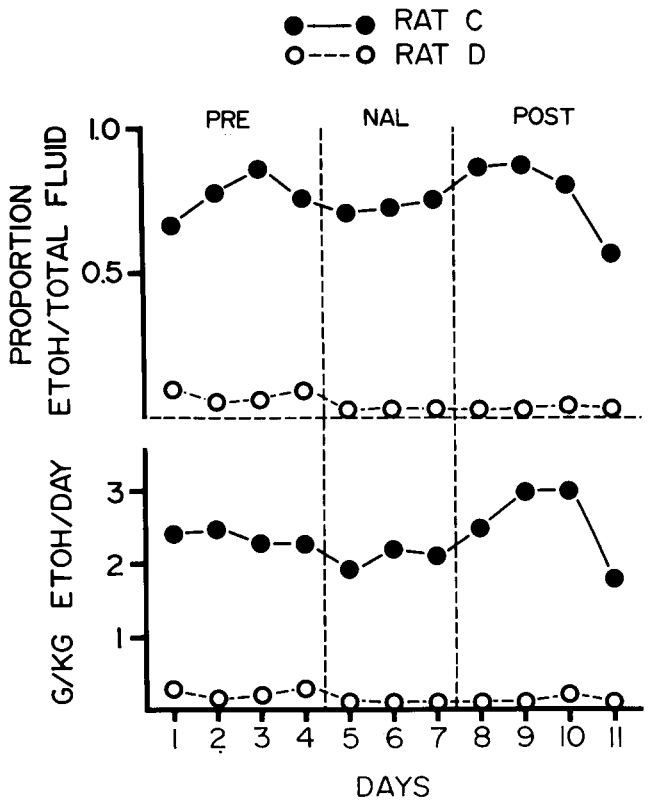

FIG. 6. Effect of 2.0 mg/kg/day naloxone on alcohol intake of a "low drinking" rat (C) and a non-drinking rat (D). Values are based on the combined intake of two alcohol concentrations (3% and 6%) offered to each rat. Intake measures and treatment regimen as in Fig. 1.

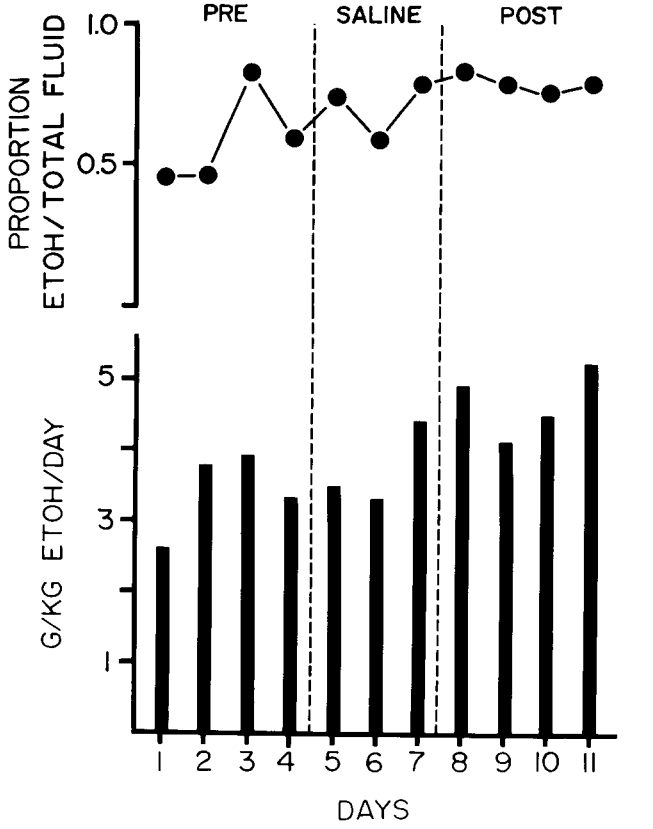

FIG. 7. Effect of 0.9% saline on alcohol intake of a representative rat. Values are based on the combined intake of two alcohol concentrations (7% and 14%). Intake measures and treatment regimen as in Fig. 1.

The lack of effect of naloxone on both "low drinking" (2.0–3.0 g/kg/day) and non-drinking (<2.0 g/kg) animals is illustrated in Fig. 6 for two representative animals, Rats C and D, respectively. Of special note is the fact that no enhancement of alcohol drinking occurred during the period following naloxone treatment in the rat which rejected alcohol even at concentrations of only 3% or 6%. In this rat, as in all others, the saline control injections also failed to reduce alcohol preference. Figure 7 portrays the effect of saline on proportion and absolute intake measures in a representative rat which drank approximately 3.0 to 5.0 g/kg/day of alcohol.

Genetic Drinkers

Long-Evans control rats were identified in our animal colony which drank voluntarily a substantial amount of alcohol in low concentrations but did not receive intracerebroventricular THP. As presented in Table 3, when treated with 2.0 mg/kg naloxone daily, they lowered their alcohol consumption by only 13%, which was not statistically significant, $t(5)=1.17; p>0.05$. Neither proportional alcohol intake nor food and water intakes or body weight changed during the course of naloxone injections.

TABLE 3

EFFECT OF 2.0 mg/kg/day NALOXONE ON ALCOHOL INTAKE OF NON-THP-TREATED RATS (n=6) IN TERMS OF g/kg AND THE RATIO OF ALCOHOL TO WATER, EXPRESSED AS THE PROPORTION OF ALCOHOL TO TOTAL FLUID CONSUMED

	Alcohol Intake				
Period	g/kg	Proportion	Food (g)	Water (ml)	Body Weight (g)
Pre	4.5 ± 0.5	0.75 ± 0.10	24 ± 0.9	8.6 ± 5.0	591 ± 17
Nal	3.8 ± 0.3	0.75 ± 0.08	23 ± 0.7	10.3 ± 3.8	589 ± 18
Post	4.2 ± 0.6	0.72 ± 0.08	25 ± 1.0	12.0 ± 4.0	590 ± 19

Measures of food and water intakes and body weights are presented in respective columns. Values expressed as means ± standard errors. Periods as in Table 1.

DISCUSSION

The finding that naloxone generally exerts a suppressant effect on voluntary alcohol drinking rather than augments alcohol preference was not anticipated. The reason for this is that in previous reports morphine was found to attenuate alcohol intake in the rodent [22, 44, 47], whereas naloxone or naltrexone purportedly caused a slight enhancement in the drinking of alcohol [44]. Thus, based on these preliminary findings, the antagonism of the opiate receptors by naloxone would be expected to elevate the consumption of alcohol. In the present study, however, the experimental conditions differed from previous ones as follows: (1) intracerebroventricular THP was repeatedly administered to our rats; (2) the test animals voluntarily drank considerable amounts of alcohol; and (3) the opiate antagonists were given repeatedly over time and in different doses.

If the mechanism of THP's action in inducing and sustaining alcohol drinking [36] involves the condensation product's occupation of opiate receptor sites, then one could envisage that an opiate antagonist would inhibit the rat's alcohol drinking response. Recently, it was demonstrated that the binding properties of opiate receptors of both salsolinol and THP were comparable to those of an enkephalin in terms of both dose and IC-50 values [14]. Moreover, each of these aldehyde conjugates inhibits the stero-specific binding of naloxone to opiate receptors in the brain of the rat [49]. These same amine-aldehyde metabolites exert an analgesic effect when they are injected intracerebroventricularly, in very high doses, and this effect is blocked by naloxone [14]. Coupled with the fact that naloxone is a competitive inhibitor of opiate receptor activity, which depends to some degree upon the level of agonist in the receptor area of nerve tissue [45], this set of findings could explain why naloxone would exert a greater suppressive effect on the drinking of alcohol at a higher level than at a lower level. Interestingly, another drug, pCPA, which inhibits serotonin synthesis, also exerts a greater suppressant effect on high alcohol intake as compared to low [40].

Since THP itself possesses an analgesic property which is antagonized by naloxone [14], the activity of this condensation product may involve an opiate receptor mechanism of which several types have been postulated [26,50]. With regard to the precise classification of the receptor involved, μ-receptors have a 10-fold and 30-fold greater affinity for naloxone than κ- and δ-receptors, respectively [20]. Since naloxone partially suppresses the alcohol drinking response induced by THP, the cellular action of THP may involve its uptake and occupation by κ- or μ-receptors. In this connection, when alcohol is added to membrane fractions of δ-receptors, enkephalin binding is reduced at the same time that naloxone binding is unaffected [21]. Thus, δ-receptors may not necessarily be involved in the effect of naloxone seen in our experiments. The viewpoint that THP-induced alcohol drinking is mediated by a mechanism which indirectly involves the μ opiate receptor would instead seem to be more likely.

One would expect that the limited duration of action of naloxone, because of its short half-life, would preclude any long term inhibition of alcohol self-selection even though this did occur in many "high drinking" animals given naloxone. However, THP could act pharmacologically in the CNS to trigger the new synthesis of THP within the brain by an autocatalytic mechanism [36]. That is, if the further synthesis of THP would in turn depend on the additional consumption of alcohol, and this drinking itself is antagonized over the short term by naloxone, then a longer term effect of naloxone would be brought about by the consequent interruption in the chain of metabolic events. Should the newly formed THP occupy opiate receptor sites which are blocked by the opiate antagonist, then naloxone could exert its action on alcohol drinking which is longer than the antagonists's own half-life. Since THP is degraded rapidly *in vivo* [32], the time interval during which alcohol intake and the subsequent formation of THP are attenuated would be determined by the dose and frequency of administration of the opiate antagonist.

Pharmacological interactions between opiate receptor antagonists, agonists and ethyl alcohol also exist [19]. Since lever-pressing for a drug infused intravenously continues when alcohol is substituted for morphine [48], both drugs may possess similar reinforcing properties. Naloxone attenuates heightened locomotor activity [33] or self-stimulation responding [27] produced by a low dose of alcohol. In addition, an opiate antagonist reportedly influences the rewarding property of alcohol, since the rate of operant responding for intravenous alcohol may be reduced in the rhesus monkey by naltrexone [2]. Naloxone apparently does not alter the discriminative property of alcohol in a two-lever task [1].

One alternative explanation for our observations is that naloxone reduces alcohol intake by interfering with the caloric requirements of the animal. That an opiate antagonist suppresses both food and water intakes is well known. However, in nearly all previous studies, an experimental condition of deprivation was imposed with food or water available for only a short period. In general, when naloxone is injected parenterally in doses of 0.1 to 10.0 mg/kg the intake of food and/or water is either effectively reduced or blocked in the rat [10, 11, 23, 25], mouse [9] and cat [15]. Water intake is similarly suppressed in the rat by naltrexone [17]. Although the conclusion has been reached, therefore, that an opiate mechanism is involved in the mediation of the ingestive response, such a deduction may not be entirely valid because of the recently reported non-specific effects of opiate antagonists [45]. As indicated in Table 2 of the RESULTS, no appreciable shifts in food and water intakes occur following naloxone administration when the rat is given free access to both substances around the clock. Coinciding with this result is the finding that a single injection of naloxone fails to alter the intake of food when the drug is given to a non-food-deprived rat during the dark-cycle interval of maximal feeding [8].

Although the reason why naloxone does not always suppress alcohol drinking in all animals is not entirely clear, one factor to be considered is certainly the time of day when the drug is injected during the period of alcohol drinking. In the rat, which drinks and feeds nocturnally, diurnal variations in pain sensitivity have been noted which are exacerbated by naloxone; this suggests the involvement of an endogenous opioid mechanism [29]. Because the hyperalgesic effect of an opiate antagonist also depends on the time of day of its administration [16], and since alcohol drinking occurs mainly at night, naloxone given repeatedly throughout 24 hrs would presumably attenuate alcohol intake more effectively than a once or twice daily treatment regimen. In fact, this more complete blockade of alcohol intake was indeed observed in the animal given the drug six times a day.

Finally, since naloxone possesses other non-specific properties [45], it is conceivable that the antagonism of

THP-induced drinking is due to a mechanism other than that of opiate receptor blockade. In this context, current questions that now arise clearly concern the differential effect on THP-induced drinking of the longer-lasting antagonist, naltrexone, as well as the opiate agonist, morphine. Experiments to elucidate these issues are presently underway in this laboratory.

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