ELSEVIER

Suppression of Alcohol Preference in High Alcohol Drinking Rats

Efficacy of Amperozide versus Naltrexone

R. D. Myers, Ph.D., and Miles F. Lankford

The selectively bred high alcohol drinking (HAD) line of rat is considered as a potential model of one type of alcoholism. The purpose of the present experiments was to compare the efficacy of two drugs on the volitional drinking of the HAD rats: the 5-HT_{2A} receptor antagonist, amperozide, and a nonselective antagonist of opiate receptors, naltrexone. To determine the pattern of alcohol drinking of the HAD rats, a standard preference test was used in which water was offered with alcohol increased in concentrations from 3% to 30% over 11 days. The maximally preferred concentration of alcohol of each rat was offered for 4 days and ranged from 7% to 20% with a mean intake of 6.9 g/kg per day. Initially, 1.0 mg/kg amperozide, 2.5 mg/kg naltrexone, or the saline vehicle were injected twice daily for 4 days at 1600 and 2200 hours. Secondly, 2.0 mg/kg amperozide, 5.0 mg/kg naltrexone, or the saline vehicle were administered also for 4 days. After the drug sequences, alcohol preference tests continued for another 4 days. Whereas the saline vehicle was without effect on drinking, the administration of either drug caused a significant dose-dependent reduction in the daily intake of alcohol by the HAD rats in terms of absolute g/kg and proportion of alcohol to water consumed. A comparison of the drinking response to the higher doses of the two drugs showed that amperozide was more efficacious in suppressing alcohol intake than naltrexone. Neither amperozide nor naltrexone exerted any significant effects on food and water intakes or on body weight. These results support the concept of a functional link in the brain between the serotonergic and opioidergic systems postulated to underlie, in part, the aberrant drinking of alcohol. A marked dissociation between the temporal patterns of drinking after naltrexone and amperozide treatment suggests that the opiate receptors mediate the immediate reinforcing effects of alcohol, whereas the more vegetative phenomena underlying addictive properties of alcohol are regulated by 5-HT_{2A} receptors postsynaptic to serotonergic neurons. Finally, the inhibitory actions of both drugs imply that multiple receptor mechanisms within the mesolimbic and other systems in the brain underpin the addictive liability to alcohol. ***[Neuropsychopharmacology 14:139–149, 1996]***

KEY WORDS: *Alcohol preference; Drinking; HAD rats; Ethanol; Opiate receptors; Naltrexone; Serotonin; Amperozide; 5-HT_{2A} receptor antagonist; Genetics; Alcogene*

Recently it was shown that the antagonism of the 5-HT_{2A} receptor subtype in the brain can suppress the volitional intake of alcohol in the rat. To illustrate, amperozide attenuates significantly the intake of maximally preferred concentrations of alcohol in the Sprague Dawley rat induced to drink alcohol by cyanamide treatment (Myers et al. 1992). Amperozide also exerts the same suppressant effect on alcohol drinking in the genetic line of alcohol preferring P rats (Myers et al. 1993a). Further, the inhibitory effect on drinking behavior caused by the sustained delivery of amperozide by osmotic minipump can persist for up to 5 months after discontinuation of the drug (Myers et al. 1993b). Inter-

From the Departments of Pharmacology and Psychiatric Medicine, School of Medicine, East Carolina University, Greenville, North Carolina.

Address correspondence to: R. D. Myers, Ph.D., Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858.

Received February 10, 1995; revised April 27, 1995; accepted May 3, 1995.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/96/$15.00
SSDI 0893-133X(95)00081-N

estingly, treatment with amperozide for 3 days significantly reduces the oral consumption of a cocaine solution in rats (McMillen et al. 1993). A most significant finding is absence of adverse side effects in the rat, because amperozide neither impairs the intakes of food or water nor alters body weight (Myers et al. 1992) and is without effects on rotarod performance or the neuroleptic ptosis test (Gustafsson and Christensson 1990). This unique action of amperozide thus differs from that of other serotonergic drugs, such as p-chlorophenylalanine (pCPA), sertraline, buspirone (Myers 1985; Myers and Quarfordt 1991; Privette et al. 1988), and other compounds unrelated to 5-HT, which interfere with caloric regulation and other functions (Myers 1994). Amperozide also is a potential antipsychotic and antidepressant drug (Axelsson et al. 1991; Björk et al. 1992), which enhances the release of dopamine from mesolimbic neurons *in vivo* (Grenhoff et al. 1990; Kimura et al. 1993; Yamamoto and Meltzer 1992). The effect of amperozide on alcohol preference implicates further the role of serotonergic neurons in the brain proposed historically to underlie aberrant drinking behavior (Myers and Melchior 1977).

Another neurochemical link in the central mechanisms of alcoholism is thought to involve the in vivo formation of a family of aldehyde adducts such as a tetrahydro-isoquinoline (TIQ) or β-carboline (Collins 1985; Davis and Walsh 1970; Myers 1978, 1980, 1985). These aldehyde adducts are synthesized in brain tissue, after prolonged exposure to alcohol, by the condensation reaction between an endogenous amine and an aldehyde (Melchior and Collins 1982). One of the TIQ derivatives, tetrahydropapaveroline (THP), is the biological precursor to morphine in the opium poppy. When THP is infused intracerebroventricularly (ICV) in the rat or monkey, or directly into specific structures in the mesolimbic system of the rat, abnormally high drinking of alcohol occurs spontaneously (Melchior and Myers 1977; Myers and Privette 1989). As a consequence, the concept evolved that opiate receptors are involved in the pathologic development of abnormal drinking, secondarily to the formation of the aldehyde adduct. Support for this viewpoint is provided by the fact that opiate receptor antagonists assuage the preference for alcohol in different species. For example, naloxone and the longer acting compound, naltrexone, significantly reduce the intake of alcohol (Critcher et al. 1983; Myers et al. 1986); however, this effect depends on both the frequency and dose of their administration and the concentration of alcohol offered to the test animal (Froehlich et al. 1990; Hubbell et al. 1991; Myers and Critcher 1982; Volpicelli et al. 1986).

The present study was undertaken to compare the efficacy of amperozide and naltrexone on the intake of alcohol in the selectively bred high alcohol drinking (HAD) rat. Initially it was found that naloxone administered daily in a dose of 20 mg/kg to genetic drinkers of the Long Evans strain failed to lower their alcohol intake (Myers and Critcher 1982). However, in other strains of rat it was found that naloxone can induce a dose-dependent decline in the intake of different concentrations of alcohol (Myers and Critcher 1982; Froehlich et al., 1990; Volpicelli et al. 1986). In the present experiments, the maximally preferred concentration of alcohol was first determined individually for each HAD rat. Then either one of two doses of the 5-HT_{2A} or opiate receptor antagonist was administered to the HAD rats when alcohol was freely available. After treatment, intakes of preferred solutions of alcohol were recorded to ascertain the subsequent effects of each drug on the patterns of drinking.

METHODS

Naive male 30-day-old HAD rats from the original N/Nih heterogeneous strain (Li and Lumeng 1984; Spuhler and Dietrich 1984) were obtained from the Indiana University Alcohol Research Center. On arrival, the rats (n = 17) were quarantined until treatment with ivermectin for pinworms was completed. At 60 days of age, 10 mg/kg cyanamide were administered subcutaneously twice daily to the rats for 3 days to sustain their preference for alcohol (Barwick and Myers 1992; Myers and Critcher 1982). At 90 days of age, each rat was housed in an individual wire mesh cage at an ambient temperature of 22°C to 24°C and on a 12-hour illumination cycle with lights on at 0630 hours. Water and Purina NIH rodent chow were provided *ad lib*, and food and fluid intakes as well as body weights were recorded daily at 0730–0830 hours.

Alcohol Preference Tests

The pattern of preference for alcohol versus water was determined individually for each HAD rat using a standard three-bottle test procedure (Quarfordt et al. 1991). One tube contained a v/v solution of alcohol in tap water, a second tube served as a blank, and the third tube was filled with tap water. The drinking tubes were rotated daily on a semirandomized schedule to prevent the development of a position habit (Myers 1978). An 11-day preference test was carried out in which the concentration of alcohol was raised on each morning as follows: 3%, 4%, 5%, 7%, 9%, 11%, 13%, 15%, 20%, 25%, and 30%. After completion of the test sequence, each rat was offered water and a solution of its maximally preferred concentration of alcohol, as based on the 3% to 30% test. The individual test solution was the highest concentration at which the largest volume of alcohol was consumed prior to a downward shift below the

50% level in the proportion of alcohol to the total fluid consumed (Lankford et al. 1991).

Experimental Design

Each of the series of tests of alcohol drinking was carried out over a 12-day interval; a period of 6 to 10 days separated each series. After the HAD rats drank their preferred concentration for 4 consecutive days, the animals were divided randomly into treatment groups. A counterbalanced experimental design was used so that the rats were given either one or both doses of the drugs as well as the saline control vehicle. The rats were divided into three groups and initially given one of two doses of amperozide or naltrexone as well as the saline control vehicle. The groups were rotated in both the second and third series, so that rats in each group received the opposite drug or saline. In all test sequences, an injection of either amperozide (Pharmacia Therapeutics AB, Lund, Sweden), naltrexone (Sigma, St. Louis, MO), or the saline vehicle was given subcutaneously at 1600 and 2200 hours for 4 consecutive days. The preferred alcohol concentration and water were available continuously, and, following the set of injections, consumption of alcohol was recorded for an additional 4 days. During the 6- to 10-day interval between each 12-day test sequence, only water was available as the fluid source.

The number of animals in each treatment group was as follows: 1.0 mg/kg amperozide (n = 9); 2.0 mg/kg amperozide (n = 10); 2.5 mg naltrexone (n = 9); 5.0 mg naltrexone (n = 9); and the saline control vehicle (n = 11). The doses of each drug were selected on the basis of: (1) their efficacy on alcohol drinking (Critcher and Myers 1983; Myers et al. 1992); and (2) the fact that doses higher than 5.0 mg/kg suppress normal feeding and drinking in the rat (e.g., Kirkham and Blundell 1987; Cooper and Turkish 1989). The schedule of drug administration corresponded to the periods prior to onset and mid portion of the waking cycle. Only those rats that consumed greater than 5.0 g/kg per day of absolute alcohol prior to each test of a given dose of either drug were included in a respective test sequence.

Data Analyses

The means and standard errors were calculated for all groups in terms of both the daily g/kg per day intake of alcohol and the proportion of alcohol to water during the pretest as well as during the injection sequences and the posttest period. Further, the means and standard errors were analyzed in terms of the amount of food and water consumed as well as body weight under each test condition. Analyses of variance were performed using the Instat software program (GraphPad, San Diego, CA) to compare each value obtained under successive test conditions. An F test with a p value of $<.05$ was considered to be statistically significant.

RESULTS

The maximally preferred concentration of alcohol of this group of HAD rats ranged between 7% and 20%. The mean intake of alcohol of the animals was 6.9 ± 0.9 g/kg per day whereas the proportion of alcohol to total fluid averaged 0.77 ± 0.05 per day, both of which values corresponded to those of a previous study (Lankford and Myers 1994). After amperozide or naltrexone was

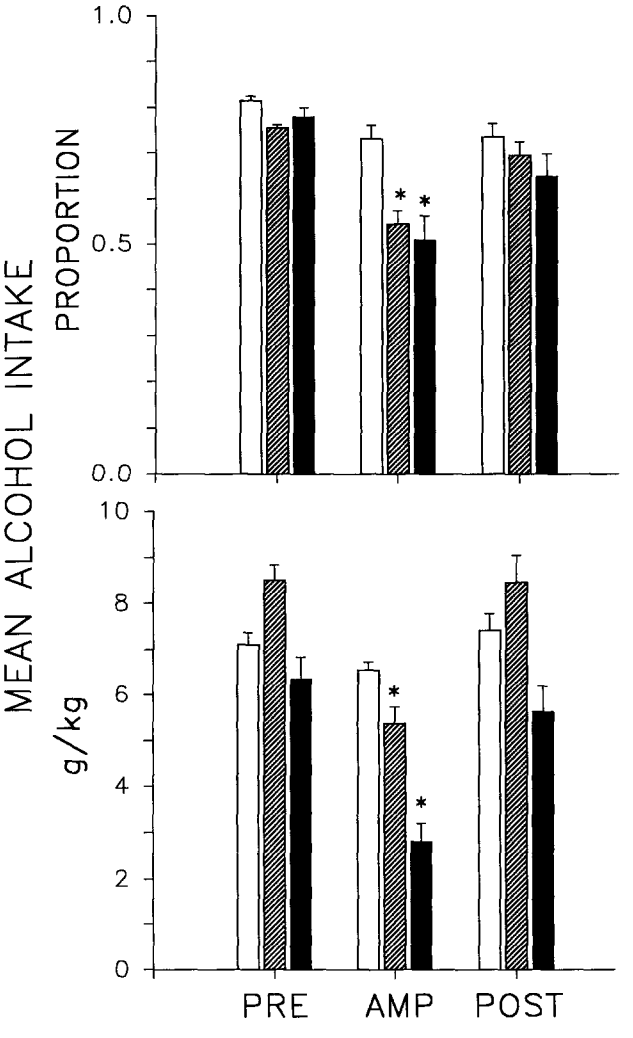

Figure 1. Mean ± SE composite intakes of alcohol in HAD rats in terms of the proportion of alcohol to total fluid (*upper panel*) and absolute g/kg per day (*lower panel*) 4 days before (PRE), during 4 days of subcutaneous injections (INJECT) b.i.d. of saline vehicle (*open bar*) (n = 11) or amperozide (AMP) in a dose of 1.0 mg/kg (*diagonal lined bar*) (n = 9) or 2.0 mg/kg (*solid bar*) (n = 10), and 4 days after (POST) treatment.

given subcutaneously for 4 days in low or high doses, the voluntary intake of each maximally preferred solution of alcohol was attenuated significantly in the HAD rats.

Amperozide Treatment

During the administration of 1.0 mg/kg and 2.0 mg/kg amperozide given twice daily, the overall mean proportion of alcohol to total fluid consumed by the HAD rats was reduced in a dose dependent manner, $F(2,119) = 13.12$, $p < .01$. Further, both doses of amperozide similarly attenuated the overall mean daily intake of absolute g/kg of the maximally preferred alcohol concentration, $F(2,119) = 26.99$, $p < .01$. As portrayed in Figure 1 (*upper panel*), the 1.0 mg/kg, and 2.0 mg/kg doses of amperozide attenuated the mean daily proportional intakes of alcohol to total fluid consumed prior to treatment from 0.76 ± 0.01 to 0.54 ± 0.03, $F(1,71) = 32.4$, $p < .01$, and from 0.78 ± 0.03 to 0.51 ± 0.05, $F(1,79) = 19.6$ $p < .01$, respectively. The lower and higher doses of amperozide also reduced the absolute intakes of alcohol (Figure 1, *lower panel*) from a mean of 8.5 ± 0.3 to 5.4 ± 0.4 g/kg per day, $F(1,71) = 36.9$, $p < .01$, and from 6.3 ± 0.2 to 2.9 ± 0.4 g/kg per day, $F(1,79) = 39.8$, $p < .01$, respectively.

As shown in Table 1, the mean daily volume of alcohol consumed by the HAD rats declined during treatment with 1.0 mg/kg amperozide from 37.1 ± 1.5 ml to 21.5 ± 1.9 ml per day, $F(1,71) = 34.6$, $p < .01$. The total fluid values also declined from 47.4 ± 1.9 ml to 36.5 ± 1.7 ml per day $F(1,71) = 18.2$, $p < .01$. The 2.0 mg/kg dose of amperozide decreased the total ml of alcohol from 28.9 ± 2.0 ml to 13.5 ± 1.6 ml per day $F(1,79) = 36.4$, $p < .01$, and total fluid intake from 37.6 ± 1.3 ml to 26.0 ± 1.3 ml per day, $F(1,79) = 38.9$, $p < .01$. Table 1 shows also that the decline in alcohol consumption following the lower and higher doses of amperozide paralleled the intakes of water, which rose from 11.6 ± 1.1 ml to 16.6 ± 1.2 ml per day and 8.2 ± 1.3 to 12.0 ± 1.6 ml per day, respectively. Neither dose of amperozide nor the saline control vehicle had significant effects on body weight or daily food intakes of the HAD rats.

Naltrexone Treatment

The mean daily intake of alcohol of the HAD rats decreased significantly during injections of both the 2.5 mg/kg and 5.0 mg/kg doses of naltrexone in terms of both the proportional values, $F(2,115) = 14.89$, $p < .01$, and absolute g/kg intakes $F(2,115) = 11.90$, $p < .01$. As portrayed in Figure 2 (*upper panel*), 2.5 mg/kg naltrexone did not reduce the mean proportional intake of

Table 1. Mean ± SE Daily Body Weight (Gm), Intakes of Food (Gm), Water (ml), Alcohol Solution (ml), and Total Fluid (ml) of HAD Rats During Treatment with Amperozide or Naltrexone (bid) in the Presence of Preferred Alcohol versus Water

	Body Weight	Food Intake	Water Intake	ETOH Intake	Total Fluid
Pre	497.0 ± 6.9	20.6 ± 1.2	11.6 ± 1.1	37.1 ± 1.5	47.4 ± 1.9
AMP 1.0 mg	493.0 ± 6.6	21.0 ± 0.9	16.6 ± 1.2[a]	21.5 ± 1.9[a]	36.5 ± 1.7[a]
Post	495.6 ± 6.5	19.7 ± 1.1	14.6 ± 1.1	36.2 ± 2.1	48.3 ± 2.1
($n = 9$)					
Pre	454.5 ± 7.0	18.9 ± 0.9	8.22 ± 1.3	28.9 ± 2.0[a]	37.6 ± 1.3
AMP 2.0 mg	451.0 ± 7.2	19.4 ± 0.9	12.0 ± 1.6	13.5 ± 1.6[a]	26.0 ± 1.3[a]
Post	451.7 ± 7.7	21.8 ± 1.0	12.4 ± 1.5	25.6 ± 2.5	38.0 ± 2.3
($n = 10$)					
Pre	466.7 ± 10.3	21.5 ± 1.3	9.8 ± 1.4	30.1 ± 1.9	39.9 ± 2.1
NLTX 2.5 mg	459.5 ± 9.4	18.7 ± 0.9	10.2 ± 1.3	24.1 ± 1.7[b]	34.3 ± 1.6[b]
Post	458.0 ± 9.9	19.5 ± 1.3	11.2 ± 1.4	32.5 ± 2.5	43.7 ± 2.0
($n = 9$)					
Pre	459.7 ± 7.9	19.3 ± 0.7	13.8 ± 2.4	31.8 ± 2.5	40.7 ± 2.1
NLTX 5.0 mg	456.3 ± 8.1	18.0 ± 1.2	10.6 ± 1.9	24.5 ± 2.3[b]	32.9 ± 1.8[b]
Post	456.7 ± 4.6	19.2 ± 0.7	9.6 ± 1.1	30.5 ± 2.4	40.2 ± 2.0
($n = 9$)					
Pre	461.8 ± 7.0	19.4 ± 0.6	8.5 ± 1.2	34.5 ± 1.8	43.5 ± 2.0
Saline	462.2 ± 7.1	21.3 ± 1.0	11.8 ± 1.5	32.3 ± 2.2	43.5 ± 2.2
Post	463.3 ± 7.6	20.1 ± 0.82	10.9 ± 1.3	34.9 ± 2.5	45.7 ± 2.6
($n = 11$)					

[a] $p < .01$.
[b] $p < .05$.

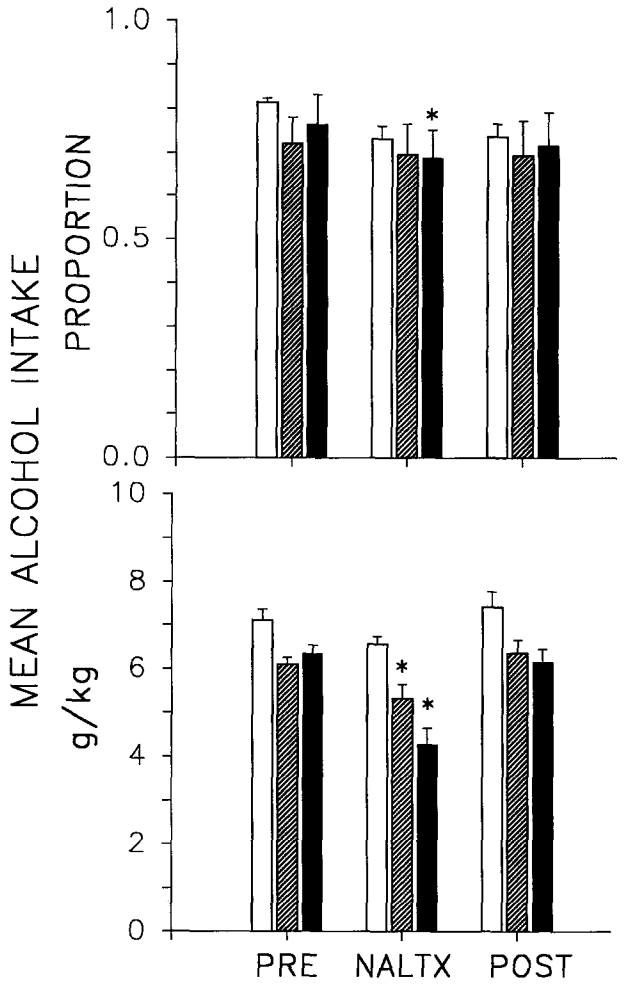

Figure 2. Mean ± SE composite intakes of alcohol in HAD rats in terms of the proportion of alcohol to total fluid (*upper panel*) and absolute g/kg per day (*lower panel*) 4 days before (PRE), during 4 days of subcutaneous injections (INJECT) b.i.d. of saline vehicle (*open bars*) (n = 11) or naltrexone (NALTREX) in a dose of 2.5 mg/kg (*diagonal lined bar*) (n = 9) or 5.0 mg/kg (*solid bars*) (n = 9), and 4 days after (POST) treatment.

alcohol to total fluid significantly from the pretreatment level of 0.72 ± 0.02 to 0.69 ± 0.03. However, 5.0 mg/kg naltrexone attenuated the mean proportional values (Figure 2, *upper panel*) significantly from 0.76 ± 0.01 to 0.68 ± 0.03, $F(1,71) \pm 5.99$, $p < .05$. As shown in Figure 2 (*lower panel*), both the 2.5 mg/kg and 5.0 mg/kg doses of naltrexone suppressed the mean g/kg intakes of alcohol significantly from 6.1 ± 0.16 g/kg per day to 5.3 ± 0.33 g/kg per day, $F(1,71) = 5.2$, $p < .05$ and from 6.3 ± 0.2 to 4.3 ± 0.4 g/kg per day $F(1,71) = 21.4$, $p < .01$, respectively.

Injections of 2.5 mg/kg and 5.0 mg/kg of naltrexone decreased the daily volume of alcohol ingested by the HAD rats (Table 1) from 30.1 ± 1.9 to 24.1 ± 1.7 ml per day, $F(1,71) = 5.49$, $p < .05$ and from 31.8 ± 2.5 ml to 24.5 ± 2.3 ml per day $F(1,71) = 4.62$, $p < .05$, respectively. The total fluid ingested also declined during the administration of the lower and higher doses of naltrexone from 39.9 ± 2.1 to 34.3 ± 1.6, $F(1,71) = 4.67$, $p < .05$ and from 40.7 ± 2.0 ml to 32.9 ± 2.1 ml per day $F(1,71) = 8.24$, $p < .05$, respectively. However, these doses of naltrexone or the control saline vehicle did not alter body weight nor intakes of food significantly (Table 1).

Temporal Patterns Of Drinking: Amperozide versus Naltrexone

The shift in the daily patterns of drinking of the maximally preferred concentrations of alcohol in HAD rats treated for 4 days with either amperozide or naltrexone varied in terms of their effects as well as duration. As shown in Figure 3 (*upper panel*), the proportional intakes of alcohol per day differed significantly during injections of saline, 1.0 mg/kg amperozide and 2.5 mg/kg naltrexone $F(2,115) = 9.50$, $p < .01$. Although the drug-induced decline in drinking was paralleled by a similar but not significant fall in the daily g/kg consumed (Figure 3, *lower panel*), alcohol drinking rebounded to pretreatment levels upon discontinuation of the drugs.

As presented in Figure 4, the shift in the daily consumption of alcohol on the first and following days of injections of the higher doses of amperozide and naltrexone was significant in terms of both mean proportional values (Figure 4, *upper panel*), $F(2,119) = 8.27$, $p < .01$, and g/kg intakes (Figure 4, *lower panel*), $F(2,119) = 26.36$, $p < .01$. Further, on the second through fourth days of administration of 2.0 mg/kg amperozide, both the mean daily proportional intakes (Figure 4, *upper panel*) and absolute g/kg of alcohol consumed (Figure 4, *lower panel*) continued to be suppressed. Conversely, the mean daily proportional and g/kg values of alcohol ingested during the injections of 5.0 mg/kg naltrexone rebounded on the second day and then rose on the following days. The differences between amperozide and naltrexone given to the HAD rats also were significant in terms of the daily proportional intakes, $F(1,75) = 8.02$, $p < .01$, and g/kg consumed, $F(1,75) = 6.77$, $p < .05$. The daily intakes in g/kg alcohol consumed during amperozide and naltrexone injections also were significantly different from that of the saline controls $F(1,83) = 43.2$, $p < .01$, and $F(1,79) = 32.2$, $p < .01$, respectively).

Percent Decline in Drinking

As presented in Figure 5, amperozide injected in the HAD rats at a dose of 1.0 mg/kg reduced the mean percent proportional values of alcohol consumed by 29.2% as well as the mean percent g/kg intake of absolute alcohol by 37.0%. The 2.5 mg/kg dose of naltrexone suppressed the mean proportional intake by 4.0% and

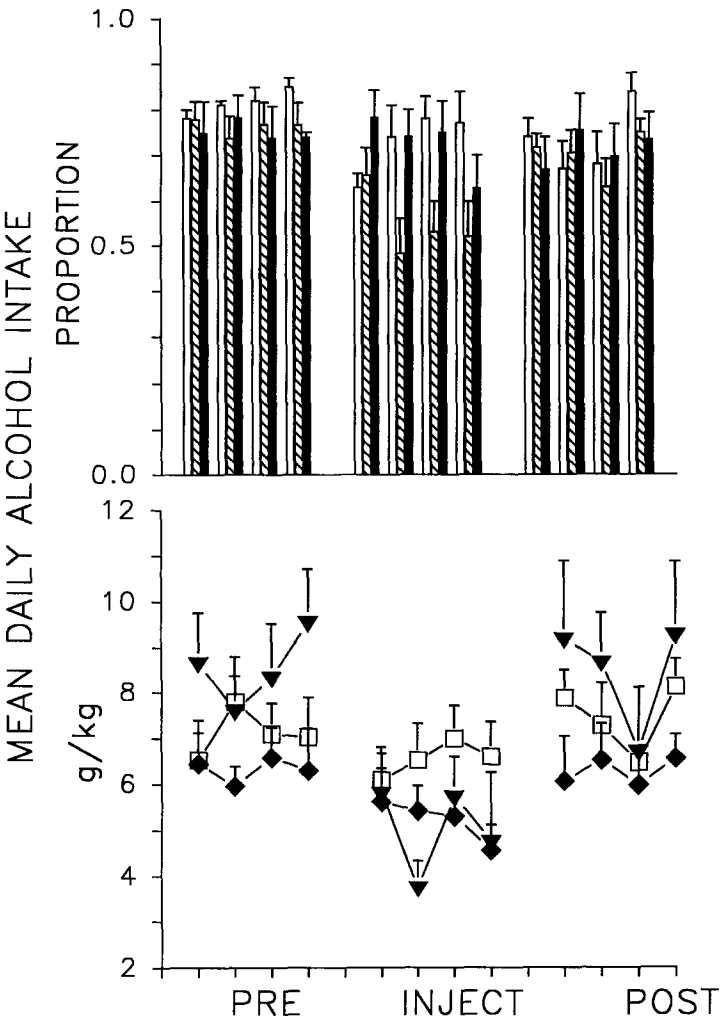

Figure 3. Comparison of mean ± SE daily intakes of preferred concentrations of alcohol in HAD rats in terms of the proportion of alcohol to total fluid (*upper panel*: □ saline, ▨ AMP 1.0, ■ NAL 2.5) and absolute g/kg per day (*lower panel*: □ saline, ▼ AMP 1.0, ◆ NAL 2.5) for 4 control days before (PRE), during 4 days of subcutaneous injections (INJECT) b.i.d. of saline vehicle (n = 11), 1.0 mg/kg amperozide (AMP) (n = 9) or 2.5 mg/kg naltrexone (NALTREX) (n = 9), and for 4 days after (POST) treatment.

the absolute g/kg intake of alcohol by 13.1% (Figure 5). When the dose of amperozide was doubled to 2.0 mg/kg, the mean proportional intake of the HAD animals fell by 34.6%, whereas the mean g/kg intake declined by 56.3%. However, naltrexone reduced the mean proportion of alcohol to total fluid intake by 10.5% and the mean g/kg intake of alcohol by 32.6% (Figure 5).

The magnitude of the decline in alcohol preference from the 4-day predrug baseline, as a result of repeated injections of amperozide, was greater proportionally than that produced by naltrexone. An analysis of the mean decrease in the g/kg intakes of alcohol during treatment with 1.0 mg/kg amperozide compared to the 2.5 mg/kg dose of naltrexone was significant $F(1,143) = 136.1$, $p < .01$. Similarly, the higher dose of amperozide produced a significantly greater mean reduction from baseline in g/kg intake than the higher dose of naltrexone $F(1,151) = 56.9$, $p < .01$. Likewise, the mean deviation from baseline of the proportion of alcohol to total fluid intake in the HAD rats given amperozide at 1.0 and 2.0 mg/kg versus that after naltrexone at 2.5 and 5.0 mg/kg was statistically significant $F(1,143) = 29.2$, $p < .01$ and $F(1,151) = 104.5$, $p < .01$, respectively.

Individual Responses to Amperozide and Naltrexone

To evaluate the effects of amperozide and naltrexone on individual HAD rats, the percent change from the 4-day predrug baseline in g/kg and proportion of alcohol to total fluid consumed was calculated for the 4 days of injections and 4 days postdrug. As presented in Figure 6, the percent reduction in the proportional and absolute g/kg intakes of alcohol during the administration of amperozide and naltrexone essentially was dose related. After the injections (POST) of 1.0 mg/kg amperozide, the decline in the proportion and g/kg measures of alcohol drinking persisted in 6 of 9 HAD rats. After treatment (POST) with the higher dose of amperozide, the percent change in proportion and g/kg consumption of alcohol persisted in six of nine rats and in seven of nine rats, respectively.

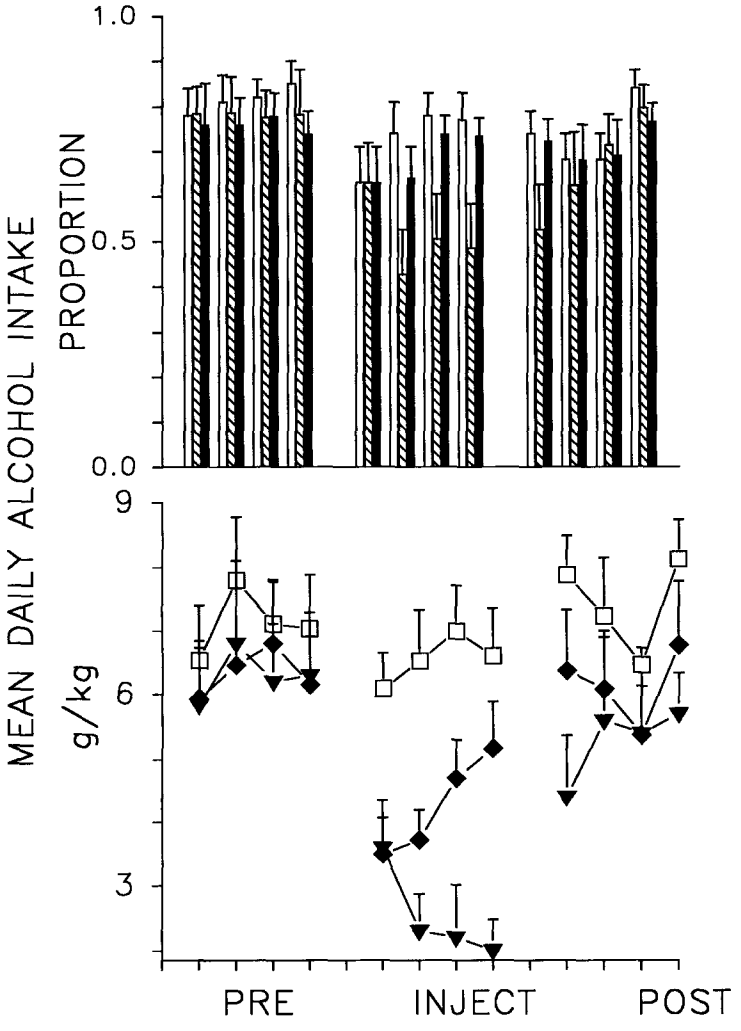

Figure 4. Comparison of mean ± SE daily intakes of preferred concentrations of alcohol in HAD rats in terms of the proportions of alcohol to total fluid (*upper panel:* □ saline, ▨ AMP 2.0, ■ NAL 5.0) and absolute g/kg per day (*lower panel:* □ saline, ▼ AMP 2.0, ◆ NAL 5.0) for 4 control days before (PRE), during 4 days of subcutaneous injections (INJECT) b.i.d. of saline vehicle (n = 11), 2.0 mg/kg amperozide (AMP) (n = 10) or 5.0 mg/kg naltrexone (NALTREX) (n = 9), and for 4 days after (POST) treatment.

The drinking response to the injections of the 2.0 mg/kg dose of naltrexone was somewhat more variable than that after treatment with 1.0 mg/kg amperozide, particularly in the intake measures after treatment (POST). However, after the injections with the higher dose of amperozide, the percent change in proportion and g/kg consumption of alcohol persisted in five of eight rats and in four of eight HAD rats, respectively.

DISCUSSION

The present results clearly demonstrate that amperozide, a 5-HT_2 receptor antagonist, and naltrexone, an opiate receptor antagonist, are efficacious in attenuating the apparent drive, reinforcing quality or addictive property of alcohol in the selectively bred, HAD rat. The daily administration of either compound at both lower and higher doses causes a significant, dose-dependent decline in alcohol consumption with little or no adverse side-effects. However, a comparison of the differential intakes of alcohol in response to the higher doses of these drugs revealed that amperozide is more efficacious in suppressing drinking in the HAD rats than naltrexone. Moreover, during the period after the administration of the drugs, the inhibitory effect of amperozide on drinking persists in many of the animals. Thus, these findings are not in accord with an earlier viewpoint that the opiate system in the brain plays a more prominent role than other transmitters in the mechanisms underlying the consumption of alcohol (Hubbell et al. 1991). As the experimental procedures for the administration of both amperozide and naltrexone were similar to those described in previous studies (Critcher et al. 1983; Myers et al. 1992), a methodologic variable would not seem to account for the differences in their potency in reducing alcohol drinking.

The day by day pattern of alcohol drinking during the administration of naltrexone and amperozide revealed a clearcut dissociation between the effects of the antagonism of opiate and 5-HT_2 receptors, respectively. The immediate suppressive action of naltrexone on drink-

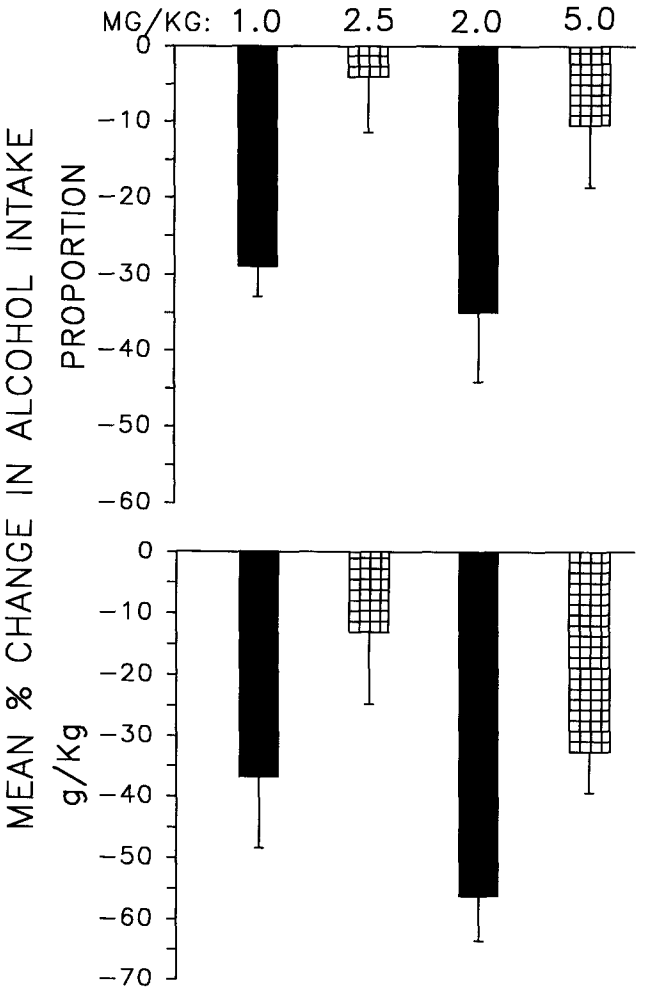

Figure 5. Mean ± SE composite percent decline from baseline in intake of alcohol in HAD rats in terms of the proportion of alcohol to total fluid (*upper panel*) and absolute g/kg per day (*lower panel*) during 4 days of subcutaneous injections b.i.d. of amperozide (AMP) (*solid bar*) in doses of 1.0 and 2.0 mg/kg or naltrexone (NALTX) (*grid bar*) in doses of either 2.5 or 5.0 mg/kg and 4 days after (POST) treatment. Each percent value represents the mean of 4 days during the respective injection series.

ing began to subside rapidly in that the preference for alcohol returned to the predrug level even during its administration. This phenomenon coincides with the nature of the attenuation of alcohol drinking by naloxone in the rat (Myers and Critcher 1982) and by naltrexone in the monkey (Myers et al. 1986). The rapid action of naltrexone is clearly apparent also in the monkey in that alcohol drinking is diminished within 2 hours after the opiate antagonist is administered (Kornet et al. 1991). In addition, naltrexone causes a dose-dependent suppression of drinking in Wistar rats trained to drink 12% alcohol when the solution is made available for only 1 hour on each day (Linseman 1989). Taken together, these findings suggest that naltrexone exerts an immediate, short-term effect on the reinforcing properties of alcohol and are in accord with the earlier proposal that opiate receptors in the brain underlie, in part, the reinforcing action of alcohol (Critcher et al. 1983; Myers, 1989). In relation to this, alcoholic patients report diminished euphoric effects when they drink alcohol during a "lapse" phase in their treatment with naltrexone, in contrast to control patients who continue to experience the alcohol induced euphoria when they are taking the placebo (Volpicelli et al. 1992). Clinically, this immediate action of naltrexone on the euphoric characteristics of alcohol apparently serves to reduce the urge or drive to drink (O'Brien 1994). These subjective reports correspond to the reduction in alcohol drinking observed experimentally in the present study during the first 24-hour period of naltrexone treatment.

In terms of the mechanism of action of naltrexone in attenuating volitional drinking, the delta opiate receptor subtype may be responsible for the suppressant effect on alcohol intake as based on experiments in the C57BL/6 mouse (Le et al. 1993). Alternatively, naltrexone administered intraperitoneally reverses alcohol induced extracellular release of dopamine and homovanillic acid in a dose-dependent manner, without affecting the release or metabolism of serotonin (Benjamin et al. 1993). However, mixed effects of the opiate antagonists on alcohol intake also have been reported. For example, naltrexone fails to attenuate cadmium-induced alcohol preference (Nation et al. 1990), and the drug induces both an increase or decrease in alcohol drinking in rats that showed high and low shock avoidance behavior, respectively (Iso and Brach 1991). Interestingly, naloxone was found to reduce alcohol intake in the rat induced to drink alcohol after the central administration of the dopamine-dopaldehyde adduct, THP (Myers and Critcher 1982). Other studies revealed that naloxone also attenuates alcohol selection in the HAD rat (Froehlich et al. 1990). The possibility also cannot be excluded that naltrexone may enhance the aversive taste of alcohol in the rat, thus making the fluid less palatable and possibly negatively reinforcing to the animal (Miceli et al. 1979).

In terms of the protracted inhibitory effect of amperozide on alcohol drinking, its action is clearly differentiated from that of naltrexone in that the action of this 5-HT_{2A} receptor antagonist is even greater with its continued administration. Previously, it was demonstrated that amperozide delivered chronically produces a sustained reduction in alcohol intake of the rat long after its termination (Myers et al. 1993a). Similarly, several studies show that a tryptophan hydroxylase inhibitor such as pCPA also exerts a prolonged action on drinking after the treatment with the drug is terminated (Myers and Melchior 1977). A mixed 5-HT_{1A}/5-HT_{2A} agonist/antagonist, FG5893, also has the same sort of prolonged inhibitory effect on drinking (Singh et al. 1993). Because

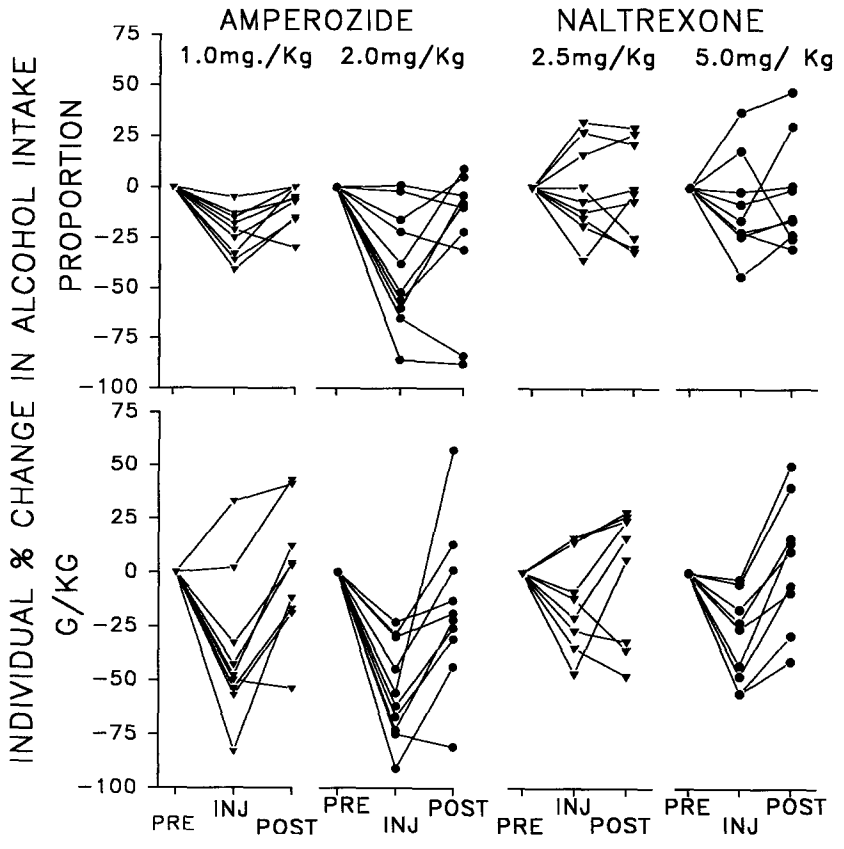

Figure 6. Individual percent change in alcohol intakes of each rat from the zero baseline level (PRE) in terms of the proportion of alcohol to total fluid (*upper panel*) and absolute g/kg per day (*lower panel*). Each percent value represents the individual mean obtained during 4 days of subcutaneous injections (INJ) b.i.d. of amperozide in doses of 1.0 and 2.0 mg/kg or naltrexone in doses of either 2.5 or 5.0 mg/kg and 4 days after (POST) treatment.

HAD rats used in the present study are known to possess a deficiency in the level of cerebral serotonin (Gongwer et al. 1989), serotonergic pathways in the brain may be particularly susceptible to their perturbation by the blockade of 5-HT_{2A} receptors in relation to the addictive nature of alcohol. Overall, these obserations would imply, therefore, that serotonergic neurons underlie the cerebral mechanisms responsible for the addictive component of alcohol drinking, which would thus appear to be mediated, at least in part, by the 5-HT_{2A} subtype of receptor (Myers 1994).

Amperozide possesses multiple pharmacologic properties apart from its high affinity binding to 5-HT_{2A} receptors (Grenhoff et al. 1990): an enhancement in the release of dopamine from neurons in the mesolimbic system (Eriksson and Christensson 1990; Grenhoff et al. 1990; Yamamoto and Meltzer 1992) and clinical efficacy as a potential antipsychotic and antidepressant drug (Axelsson et al. 1991, Björk et al. 1992). Previously amperozide was shown to suppress alcohol intake in rats induced to drink by cyanamide (McMillen et al. 1994; Myers et al. 1992) as well as in the selectively bred line of alcohol preferring P rat, but not in the nonalcohol preferring NP rat (Myers et al. 1993b). Further, the oral consumption of cocaine in cocaine-addicted rats was reduced by up to 47% after treatment with amperozide (McMillen et al. 1993).

In conclusion, the present results indicate that in light of previous reports, naltrexone attenuates significantly the self-selection of alcohol by its action on endogenous opiate receptors in the mesolimbic system involved in the rewarding effects of alcohol. The similar magnitude in the suppression of drinking caused by amperozide likewise supports the view that serotonin receptors localized also within mesolimbic neurons participate simultaneously in the phenomenon of excessive alcohol drinking not only in the HAD rat but in other strains as well. Differences in the amount of 5-HT in the brain could comprise one explanation for the prolonged effect of amperozide, and the somewhat greater efficacy than naltrexone in reducing alcohol drinking behavior. However, the dissociation between the temporal effect of naltrexone and amperozide treatment over 4 days suggests that the immediate reinforcing effect of alcohol is regulated transiently by the opiate class of receptors, whereas the more vegetative phenomena associated with the addictive attributes of alcohol are mediated by 5-HT_2 receptors on serotonergic neurons or postsynaptic to them. However, this dissociation between the actions of amperozide and naltrexone may be applicable only to individuals genetically predisposed to drink alcohol abnormally, rather than to those who use or abuse alcohol intermittently as an anxiolytic agent or for other reasons.

ACKNOWLEDGMENTS

This research was supported in part by Grant AA-04200-13 from the National Institute on Alcohol Abuse and Alcoholism to R.D.M. The authors thank Dr. Anders Björk of Pharmacia Therapeutics AB for the kind gift of amperozide and are grateful to Professor B.A. McMillen for his helpful critique of the manuscript.

REFERENCES

Axelsson R, Nilsson A, Christensson E, Björk A. (1991): Effects of amperozide in schizophrenia: An open study of a potent 5-HT_2 receptor antagonist. Psychopharmacol 104:287–292

Barwick VS, Myers RD (1992): Age-dependent development of ethanol drinking in rats after inhibition of aldehyde dehydrogenase. Alcohol 9:501–507

Benjamin D, Grant ER, Pohorecky LA (1993): Naltrexone reverses ethanol-induced dopamine release in the nucleus accumbens in awake, freely moving rats. Brain Res 621:137–140

Björk A, Bergman I, Gustavsson G (1992): Amperozide in the treatment of schizophrenic patients. A preliminary report. In Meltzer HY (ed), Novel Antipsychotic Drugs. New York, Raven, pp 47–57

Brewerton TD, Murphy DL, Jimerson DC (1994): Test meal responses following m-chlorophenylpiperazine and L-tryptophan in bulimics and controls. Neuropsychopharmacology 11:63–71

Collins MA (ed), (1985): Aldehyde Adducts in Alcoholism. New York, Alan Liss

Cooper SJ, Turkish S (1989): Effects of naltrexone on food preference and concurrent behavioral responses in food-deprived rats. Pharmacol Biochem Behav 33:17–20

Critcher EC, Lin CI, Patel J, Myers RD (1983): Attenuation of alcohol drinking in tetrahydroisoquinoline-treated rats by morphine and naltrexone. Pharmacol Biochem Behav 18:225–229

Davis V, Walsh M (1970): Alcohol, amines and alkaloids: A possible biochemical basis for alcohol addiction. Science 167:1,005–1,007

Eriksson E, Christensson E (1990): The effect of amperozide on uptake and release of [^{3}H]-dopamine in vitro from perfused rat striatal and limbic brain areas. Pharmacol Toxicol. 66 (Suppl) 1:45–48

Froehlich JC, Harts J, Lumeng L, Li TK (1990): Naloxone attenuates voluntary ethanol intake in rats selectively bred for high ethanol preference. Pharmacol Biochem Behav 35:395–390

Gongwer MA, Murphy JM, McBride WJ, Lumeng L, and Li TK (1989): Regional brain contents of serotonin, dopamine and their metabolites in the selectively bred high- and low-alcohol drinking lines of rats. Alcohol 6:317–320

Grenhoff J, Tung CS, Ugedo L, Svensson T (1990): Effects of amperozide, a putative antipsychotic drug, on rat midbrain dopamine neurons recorded *in vivo*. Pharmacol Toxicol 66(Suppl) 1:29–33

Gustafsson B, Christensson E (1990): Amperozide and emotional behavior. Pharmacol Toxicol 66(Suppl)1:34–39

Hubbell CL, Marglin SH, Spitalnic SJ, Abelson ML, Wild KD, Reid LD (1991): Opioidergic, serotonergic, and dopaminergic manipulations and rats' intake of a sweetened alcoholic beverage. Alcohol 8:355–367

Iso H, Brush RF (1991): Opposite effects of naltrexone on ETOH intake by Syracuse high and low avoidance rats. Alcohol 8:443–448

Kimura K, Nomikos GG, Svenson TH (1993): Effects of amperozide on psychostimulant-induced hyperlocomotion and dopamine release in the nucleus accumbens. Pharmacol Biochem Behav 44:27–36

Kirkham TC, Blundell JE (1987): Effects of naloxone and naltrexone on meal patterns of freely feeding rats. Pharmacol Biochem Behav 26:515–520

Kornet M, Goosen C, Van Ree JM (1991): Effect of naltrexone on alcohol consumption during chronic alcohol drinking and after a period of imposed abstinence in free-choice drinking rhesus monkeys. Psychopharmacol 104: 367–376

Lankford MF, Myers RD (1994): Genetics of alcoholism: simultaneous presentation of a chocolate drink diminishes alcohol preference in high drinking HAD rats. Pharmacol Biochem Behav 49:417–425

Lankford MF, Roscoe AK, Pennington S, Myers RD (1991): Drinking of high concentrations of ethanol versus palatable fluids in alcohol-preferring (P) rats: Valid animal model of alcoholism. Alcohol 4:293–299

Le AD, Poulos CX, Quan B, Chow S (1993): The effects of selective blockade of delta and mu opiate receptors on ethanol consumption by C57BL/6 mice in a restricted access paradigm. Brain Res 630:330–332

Li TK, Lumeng L, Doolittle DP (1984): Alcohol preference and voluntary alcohol intakes of inbred rat strains and the national institutes of health heterogeneous stock of rats. Alcohol Clin Exp Res 8:485–486

Linseman MA (1989): Central versus peripheral mediation of opioid effects on alcohol consumption in free-feeding rats. Pharmacol Biochem Behav 33:407–413

McMillen BA, Jones EA, Hill LJ, Williams HL, Björk A, Myers RD (1993): Amperozide, a 5-HT_2 antagonist, attenuates craving for cocaine by rats. Pharmacol Biochem Behav 46:125–129

Melchior C, Collins M (1982): The route and significance of endogenous synthesis of alkaloids in animals. CRC Crital Rev Toxicol 9:313–356

Melchior CL, Myers RD (1977): Prefernce for alcohol evoked by tetrahydropapaveroline (THP) chronically infused in the cerebral ventricle of the rat. Pharmacol Biochem Behav 7:19–35

Meltzer HY, Zhang Y, Stockmeier CA (1992): Effect of amperozide on rat cortical 5-HT_2 and striatal and limbic dopamine D_2 receptor occupancy: Implications for antipsychotic action. Eur J Pharmacol 216:67–71

Miceli, D, Marfaing-Jallat P, Le Magnen J (1979): Nonspecific enhancement of ethanol-induced taste aversion by naloxone. Pharmacol Biochem Behav 11:391–394

Myers RD (1978): Psychopharmacology of alcohol. Annu Rev Pharmacol Toxicol 18:125–144

Myers RD (1980): Pharmacological effects of amine-aldehyde condensation products. In Rigter H, Crabbe J (eds), Alcohol Tolerance and Dependence. The Netherlands, Elsevier, pp 339–370

Myers RD (1985): Multiple metabolite theory, alcohol drinking and the alcogene. In Collins MA (ed), Aldehyde Adducts in Alcoholism. New York, Alan Liss, pp 201–220

Myers RD (1989): Isoquinolines, β-carbolines and alcohol drinking: Involvement of opioid and dopaminergic mechanisms. Experientia 45:436–443

Myers RD (1994): New drugs for the treatment of experimental alcoholism. Alcohol 11(6):439–451

Myers RD, Critcher EC (1982): Naloxone alters alcohol drinking induced in the rat by tetrahydropapaveroline (THP) infused ICV. Pharmacol Biochem Behav 16:827–836

Myers RD, Melchior CL (1977): Alcohol and alcoholism: Role of serotonin. In Essman WB (ed), Serotonin in Health and Disease, vol II. Physiologic Regulation and Pharmacologic Action. New York, Spectrum, pp 373–430

Myers RD, Privette TH (1989): A neuroanatomical substrate for alcohol drinking: Identification of tetrahydropapaveroline (THP)-reactive sites in the rat brain. Brain Res Bull 22(5):899–911

Myers RD, Quarfordt SD (1991): Alcohol drinking atttenuated by sertraline in rats with 6-OHDA or 5,7-DHT lesions of N. accumbens: A caloric response? Pharmacol Biochem Behav 40:923–928

Myers RD, Veale WL (1968): Alcohol preference in the rat: Reduction following depletion of brain serotonin. Science 160:1,469–1471

Myers RD, Borg S, Mossberg, R (1986): Antagonism by naltrexone of voluntary alcohol selection in the chronically drinking macaque monkey. Alcohol 3:383–388

Myers RD, Lankford M, Björk A (1992): Selective reduction by the 5-HT antagonist amperozide of alcohol preference induced in rats by systemic cyanamide. Pharmacol Biochem Behav 43:661–667

Myers RD, Lankford MF, Björk A (1993a) 5-HT_2 receptor blockade by amperozide suppresses ethanol drinking in genetically preferring (P) rats. Pharmacol Biochem & Behav 45(3):741–747

Myers RD, Lankford M, Björk A (1993b): Irreversible suppression of alcohol drinking in cyanamide-treated rats after sustained delivery of the 5-HT_2 antagonist amperozide. Alcohol 10:117–125

Nation JR, Horger BA, Pugh CD, Bratton GR, Rowe LD (1990): The effects of naltrexone on cadmium-induced increases in oral ethanol self-administration. Alcohol 7:17–20

O'Brien CP (1994): Treatment of alcoholism as a chronic disorder. Alcohol 11:433–437

Pehek EA, Meltzer HY, Yamamoto BK (1993): The atypical antipsychotic drug amperozide enhances rat cortical and striatal dopamine efflux. Eur J Pharmacol 240:107–109

Privette TH, Hornsby RL, Myers RD (1988): Buspirone alters alcohol drinking induced in rats by tetrahydropapaveroline injected into brain monoaminergic pathways. Alcohol 2:147–152

Quarfordt SD, Kalmus GW, Myers RD (1991): Ethanol drinking following 6-OHDA lesions of nucleus accumbens and tuberculum olfactorium of the rat. Alcohol 8:211–217

Singh GK, Kalmus GW, Björk AK, Myers RD (1993): Alcohol drinking in rats is attenuated by the mixed 5-HT_1 agonist/5-HT_2 antagonist FG 5893. Alcohol 10:243–248

Spuhler K, Deitrich RA (1984): Correlative analysis of ethanol-related phenotypes in rat inbred strains. Alcohol Clin Exp Res 5:480–484

Svensson L, Fahlke C, Hard E, Engel JA (1993): Involvement of the serotonergic system in ethanol intake in the rat. Alcohol 10:219–224

Volpicelli JR, Alterman AI, Hayashida M, O'Brien CP (1992): Naltrexone in the treatment of alcohol dependence. Arch Gen Psychiatry 49:876–880

Volpicelli JR, Davis MA, Olgin JE (1986): Naltrexone blocks the postshock increase of ethanol consumption. Life Sci 38:841–847

Yamamoto BK, Meltzer HY (1992): The effect of the atypical antipsychotic drug, amperozide, on carrier-mediated striatal dopamine release measured in vivo. J Pharmacol Exper Ther 263:180–185