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Naloxone, but not Tyr-MIF-1, Reduces Volitional Ethanol Drinking in Rats: Correlation With Degree of Spontaneous Preference

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PULVIRENTI, L. AND A. J. KASTIN. *Naloxone, but not Tyr-MIF-1, reduces volitional ethanol drinking in rats: Correlation with degree of spontaneous preference.* PHARMACOL BIOCHEM BEHAV 31(1) 129–134, 1988.—The possible relationship between the actions of ethanol and opiates led us to examine the effect of opiate antagonists on ethanol intake in rats with a free choice of water. Naloxone (NAL) significantly reduced intake of ethanol. This effect was much greater in "high-preferring" (ethanol/total fluid intake >60%) than in "low-preferring" (ethanol/total fluid intake <30%) rats. Furthermore, a correlation was found between the degree of spontaneous preference (ethanol/total fluid intake ratio) and the reduction of ethanol drinking by NAL. Sensitivity to NAL increased with increased preference for ethanol. Neither Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) nor MIF-1 (Pro-Leu-Gly-NH_2) caused a significant modification of ethanol intake. This study shows that NAL can reduce volitional ethanol intake in rats and provides further evidence that Tyr-MIF-1 does not always act like NAL.

Tyr-MIF-1 Naloxone Ethanol Opiate Drinking

THE possibility of a common mechanism for ethanol- and opiate-seeking behavior has been studied by several investigators in the past few years. The brain opiate system might even consitute one of the neurochemical substrates involved in the process of alcohol addiction (4). It has been shown that the opiate antagonist naloxone (NAL) can modulate tolerance to ethanol and physical dependence (5,6), as well as self-administration of ethanol (2). Conversely, ethanol can reduce NAL-induced hyperalgesia to a noxious stimulus (3).

Acetaldehyde, the first product of ethanol metabolism, can condense with monoamines in the body to form a tetrahydroisoquinoline (TIQ) (23). This amine-aldehyde condensation product not only can stimulate opiate receptors in the brain (11) but also can induce excessive alcohol drinking (24), an effect modulated by morphine and NAL (11,25). Since TIQ inhibits contraction of the guinea pig ileum (19), an effect mediated by mu opiate receptors (20), and its action on intake of ethanol is modulated by morphine and NAL, two drugs acting mainly on mu receptors (20), it is conceivable that this interaction between opiates and ethanol depends at least in part on activation of mu receptors. Opiate antagonists have been shown to reduce fluid intake in a variety of paradigms (8,12). It has been proposed that drinking behavior is maintained primarily by the rewarding value of the fluid (28). Opiate antagonists decrease the intake of highly palatable solutions and the preference for saccharin in a free-choice condition (10,27). NAL has been reported to reduce preference for alcohol, even when ethanol was mixed with saccharin or quinine (13).

The antiopiate effects of MIF-1 (Pro-Leu-Gly-NH_2) and Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) have led to the concept of a system of endogenous antiopiates (16). These peptides antagonize morphine- and stress-induced analgesia (14,17) as well as other opiate-mediated behaviors such as aggression and defeat-induced food intake (30). In a previous study of fluid consumption, MIF-1, like NAL, was shown to inhibit intake of sucrose solutions (27). Tyr-MIF-1 was not tested in that study but is able to displace mu receptor ligands from their binding sites (31). The present investigation attempted to determine whether Tyr-MIF-1 and NAL could modulate

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TABLE 1

MEAN (±SEM) INTAKE (ml) OF ETHANOL AND WATER AFTER Tyr-MIF-1

	Tyr-MIF-1 (mg/kg)			
	0 (diluent)	0.01	0.1	1
A				
Ethanol	10.6 ± 1.1	10.5 ± 1.3	11.0 ± 1.3	11.6 ± 1.6
Water	16.1 ± 1.0	17.0 ± 1.3	17.0 ± 1.4	14.4 ± 1.5
Ratio (%)	38.3 ± 4.3	38.0 ± 4.6	39.4 ± 4.7	45.5 ± 5.2
B				
Ethanol	19.3 ± 1.6	16.0 ± 2.8	19.9 ± 1.1	21.8 ± 1.0
Water	6.3 ± 1.1	10.7 ± 2.2	7.1 ± 1.0	4.8 ± 1.3
Ratio (%)	74.8 ± 3.9	58.7 ± 8.6	73.8 ± 6.0	83.6 ± 6.0
C				
Ethanol	5.2 ± 1.6	5.4 ± 2.1	5.6 ± 1.8	4.9 ± 1.2
Water	24.4 ± 2.0	22.0 ± 2.7	22.0 ± 2.4	21.3 ± 3.2
Ratio (%)	18.2 ± 6.3	19.0 ± 6.9	20.5 ± 6.7	19.7 ± 6.9

A=all rats (n=30); B="high-preferring" rats (n=7); C="low-preferring" rats (n=5). Ratio values represent mean (±SEM) of ratio for each rat.

volitional drinking of ethanol in a free-choice paradigm and whether the effect of these compounds was different in high- and low-ethanol perferring rats.

METHOD

Male albino rats (Zivic-Miller, Allison Park, PA) weighing 180–220 g at the beginning of the experiment were housed individually under constant temperature (22°C) and 12-hour light-dark cycle (lights on between 7.00 a.m.–7.00 p.m.) with food freely available. Water and ethanol (3% v/v solution) were available for only 2 hours daily (11 a.m.–1.00 p.m.) in two separate bottles. This experimental design was chosen to encompass the time expected for the maximal effect of the peptides in drinking paradigms. The position of each bottle was reversed every day (10) to minimize preference for side. Ethanol and water intake was measured every day by weight until a constant level of total fluid consumption and ethanol/total consumption ratio was reached for each rat. A rat was defined as "high-preferring" if its ratio was always above 60%, "low-preferring" if below 30%.

Thirty rats were used for each experiment. For the first experiment, involving only Tyr-MIF-1, each rat was injected intraperitoneally (IP) with diluent (0.9% NaCl, 0.01 M acetic acid) every other day and, on alternate days, with a single injection of each dose of peptide (0, 0.01, 0.1 and 1 mg/kg) dissolved in diluent. To minimize any carry-over effect, the peptide was administered from the lowest to the highest dose; an identical procedure was followed for NAL (1, 2 and 4 mg/kg, IP), generously provided by Endo Labs. In the last experiment, the most effective dose of NAL (2 mg/kg) was compared with the same dose of Tyr-MIF-1 and MIF-1 in a counterbalanced design. All coded solutions were administered immediately before presentation of the fluid.

Intake of ethanol and water was measured in individual animals every day. Ethanol/total intake ratios (%) were then calculated for each rat. The ratio under basal conditions (% after diluent) for each rat was correlated with the change in ratio observed after treatment (% after NAL minus % after diluent). Statistical evaluations were performed by ANOVA for repeated measures followed by Duncan's test for multiple comparisons. In addition, a drug × preference analysis was performed (basal ethanol preference vs. ethanol intake after drug treatment). Regression analysis was used to evaluate correlation between effect of drug and degree of preference.

RESULTS

Tyr-MIF-1, at all doses tested, failed to significantly modify intake of ethanol or water and did not significantly change preference in "high-" or "low-preferring" rats (Table 1). Also, there was no correlation between the ratios under basal condition (% after diluent) and the ratios after Tyr-MIF-1 (% after Tyr-MIF-1–% after diluent) (Fig. 1).

NAL decreased overall intake of ethanol, $F(3,75)=11.73$, $p<0.01$ (Table 2). Further analysis revealed that this effect occurred mainly in the subgroup of "high-preferring" rats, $F(3,18)=3.58$, $p<0.05$. As with the total group, "high-preferring" rats showed a significant decrease in the intake of ethanol at each dose of NAL. The percent change in preference ratio after NAL was −26.5%, −38.1%, and −22.3% for the doses of 1, 2, and 4 mg/kg respectively. These changes in ratio did not reach statistical significance ($p=0.08$ by ANOVA). While ethanol intake decreased in "high-preferring" rats, water intake was significantly reduced, $F(3,9)=8.41$, $p<0.01$, in the "low-preferring" rats. A significant ($p<0.001$) correlation between basal ratios and degree of inhibition induced by NAL was also evident (Fig. 2). The drug × preference analysis showed a significant correlation between the degree of basal ethanol intake and effect of NAL, $F(3,57)=5.14$; $p<0.01$.

In the last experiment, NAL again significantly decreased intake of ethanol in all rats taken together, $F(3,84)=6.11$, $p<0.01$, and in "high-preferring" rats, $F(3,18)=21.35$, $p<0.01$. Neither Tyr-MIF-1 nor MIF-1 tested at the same dose (2 mg/kg) influenced ethanol intake. Water intake, however, was reduced in "low-preferring" rats, $F(3,30)=9.86$, $p<0.01$, by all three compounds (Table 3). A drug × preference analysis revealed, as in the other experiment, a significant interaction between the two factors, $F(3,54)=4.00$, $p<0.05$.

DISCUSSION

This study shows that Tyr-MIF-1 did not modify volitional intake of ethanol at any of the doses tested. NAL, however, decreased it significantly. The effect of NAL was most evident in "high-preferring" rats and there was a correlation between the percentage of inhibition and the degree of preference under basal conditions.

An interaction between ethanol and the endogenous opiate system has been demonstrated in several studies. TIQs are formed during the metabolism of ethanol under physiological conditions (23) and they possess opiate-like actions (11, 21, 24, 25). Other evidence of an ethanol-opiate interaction comes from studies dealing with a genetic predisposition towards alcohol drinking. Mice with a genetically higher content of brain enkephalin seemed to show less preference for ethanol (7). Also, clinical studies in alcoholics revealed a marked reduction in beta-endorphin levels in the CSF (18). The correlation between ethanol preference and

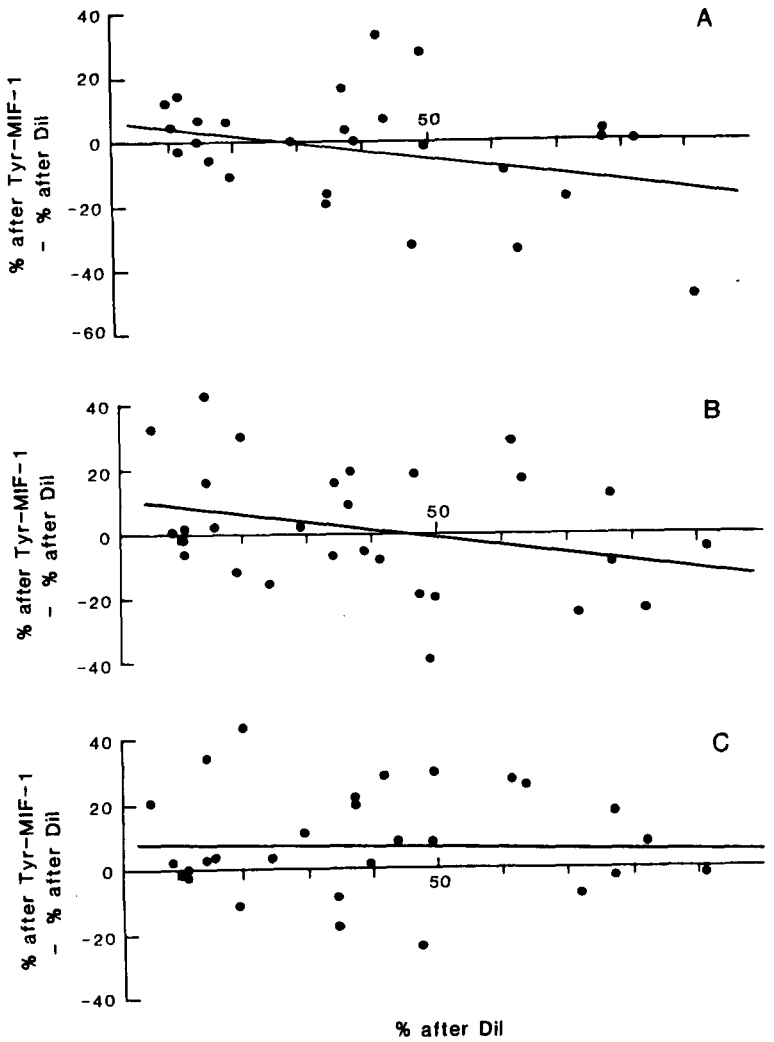

FIG. 1. Correlation between the ratio of ethanol/total intake under basal conditions (% after diluent) and after Tyr-MIF-1 (% after Tyr-MIF-1–% after diluent). (A) 0.01 mg/kg: r=−0.35, NS; (B) 0.1 mg/kg: r=−0.34, NS; (C) 1 mg/kg: r=−0.46, NS.

the inhibitory effect of NAL showed by the present study supports the hypothesis of a link between preference for ethanol and the opiate system; a similar effect of NAL has been reported in rats treated with TIQs (25).

It appears from our results that the suppressive effect of NAL on ethanol drinking is more pronounced at higher than at lower levels of preference under conditions of spontaneous, nonpharmacologically-induced drinking. The inhibitory capacities of NAL on opiate receptors depend to a certain extent upon the level of the agonist present at the receptor area of the nervous tissue (29). Other neurotransmitters may also be involved. Serotonin and dopamine were reduced in various brain areas of inbred alcohol-preferring as compared with nonpreferring lines of rats (22), and ethanol preference in rats can be reversed by destruction of noradrenergic but not dopaminergic terminals in the brain (9). PCPA, a serotonin synthesis inhibitor, affected high- more than low-ethanol intake (26). Excessive volitional drinking in rats and possibly humans, therefore, may involve a complex interaction among several neurochemical mechanisms.

Ethanol is a rewarding drug that can be self-administered by animals, but the neurophysiological basis for this behavior has been a matter of debate. There is some evidence, however, that opiate receptors may be involved in the positive reinforcement of ethanol (2). It is possible, therefore, that the inhibitory effect of NAL on volitional drinking may be, at least in part, due to an impairment of the rewarding properties that ethanol exerts in certain rats.

The choice between different palatable solutions does not seem to be inolved in the effect of NAL on ethanol. Although the preference for sweet solutions over water was decreased by NAL (10), the effect of NAL on ethanol intake was unaf-

TABLE 2

MEAN (±SEM) INTAKE (ml) OF ETHANOL AND WATER FOR TWO HOURS AFTER NAL

	NAL (mg/kg)			
	0 (diluent)	1	2	4
A				
Ethanol	14.0 ± 1.5	8.8 ± 1.0*	7.8 ± 1.0*	9.1 ± 1.1*
Water	15.9 ± 1.8	14.6 ± 1.2	17.1 ± 1.5	14.3 ± 1.0
Ratio (%)	48.5 ± 5.4	38.6 ± 4.4	32.3 ± 3.9	38.0 ± 4.0
B				
Ethanol	22.4 ± 1.6	14.4 ± 2.2*	13.2 ± 2.6*	15.7 ± 2.3*
Water	5.7 ± 1.3	11.9 ± 2.6	16.1 ± 4.8	8.9 ± 1.2
Ratio (%)	80.1 ± 4.2	58.9 ± 8.1	49.6 ± 10.6	62.3 ± 6.3
C				
Ethanol	6.8 ± 1.3	5.3 ± 1.2	4.3 ± 1.0	4.1 ± 1.1
Water	24.6 ± 0.5	15.5 ± 2.4*	17.5 ± 2.5*	18.7 ± 1.6*
Ratio (%)	21.3 ± 3.3	26.2 ± 7.6	19.2 ± 6.4	18.2 ± 5.6

A=all rats (n=30); B="high-preferring" rats (n=7); C="low-preferring" rats (n=5).Ratio values represent mean (±SEM) of ratio for each rat. *$p<0.05$.

TABLE 3

MEAN (±SEM) INTAKE (ml) OF ETHANOL AND WATER AFTER NAL, Tyr-MIF-1, AND MIF-1 (2 mg/kg IP)

	Tyr-MIF-1	MIF-1	NAL
	0 (diluent)	2 mg/kg	2 mg/kg	2 mg/kg
A				
Ethanol	13.4 ± 1.6	13.1 ± 1.5	12.5 ± 1.4	10.0 ± 1.1†
Water	19.4 ± 1.4	17.8 ± 1.0	16.9 ± 1.1	14.3 ± 1.0
Ratio (%)	41.1 ± 5.0	43.4 ± 8.0	41.1 ± 4.5	40.3 ± 4.0
B				
Ethanol	24.1 ± 1.2	22.4 ± 1.2	20.8 ± 1.3	15.8 ± 1.0†
Water	8.2 ± 1.0	8.5 ± 1.3	9.0 ± 1.4	10.8 ± 1.6
Ratio (%)	75.5 ± 3.1	74.2 ± 2.5	74.2 ± 2.5	69.8 ± 2.9
C				
Ethanol	6.1 ± 1.4	8.8 ± 1.8	6.6 ± 1.2	5.6 ± 1.7
Water	28.1 ± 1.3	22.6 ± 1.3*	21.7 ± 1.5*	17.0 ± 1.6†
Ratio (%)	16.5 ± 3.6	27.5 ± 5.5	22.0 ± 3.5	25.0 ± 5.1

A=all rats (n=30); B="high-preferring" rats (n=8); C="low-preferring" rats (n=11). Ratio values represent mean (±SEM) of ratio for each rat. *$p<0.05$, †$p<0.01$.

fected by the palatability of the solutions (13), and NAL did not alter the discriminative property of alcohol in a two-lever task (1).

A previous report showed that MIF-1 reduced fluid consumption when sucrose solutions were used (27). In the present study, NAL and the two peptides, when used at the highest dose (2 mg/kg IP), significantly reduced water intake only in "low-preferring" rats. It seems, therefore, that MIF-1 and NAL may show differential effects for different types of motivational incentives.

The present findings, while confirming previous reports on the inhibitory effect of NAL on alcohol drinking, provide evidence of a correlation between spontaneous preference and NAL-induced inhibition. This further supports the idea that the endogenous opiate system may be involved in the maintenance of ethanol-seeking behavior but indicates that if the endogenous anti-opiate system plays a role, it involves endogenous antiopiates other than Tyr-MIF-1.

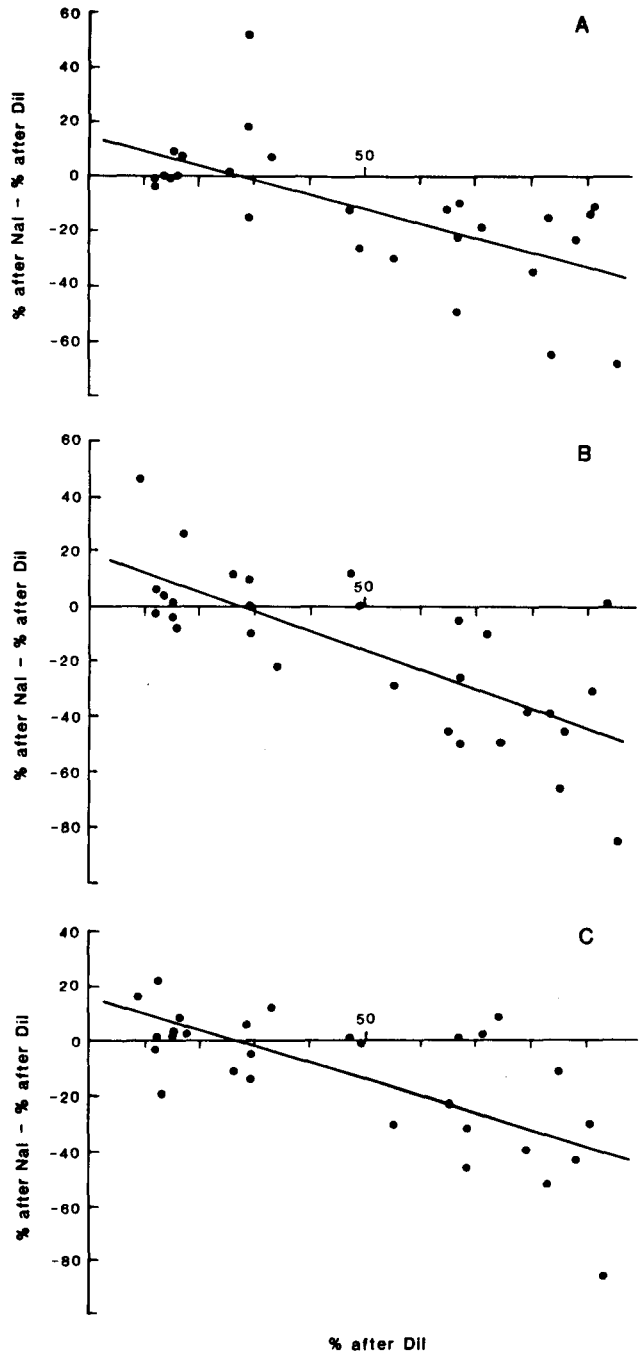

FIG. 2. Correlation between the ratio of ethanol/total intake under basal conditions (% after diluent) and after NAL (% after NAL–% after diluent). (A) 1 mg/kg: $r=-0.658$, $p<0.001$; (B) 2 mg/kg: $r=-0.742$, $p<0.001$; (C) 4 mg/kg: $r=-0.695$, $p<0.001$.

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