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Naloxone Decreases Ethanol Consumption Within a Free Choice Paradigm in Rats

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SANDI, C., J. BORRELL AND C. GUAZA. *Naloxone decreases ethanol consumption within a free choice paradigm in rats.* PHARMACOL BIOCHEM BEHAV **29**(1) 39–43, 1988.—The effect of subcutaneous naloxone administration on the consumption of a weak ethanol solution in rats on the three consecutive days (testing days) was investigated using a behavioral paradigm which includes a first forced ethanol exposure (conditioning day) followed by a two-bottle ethanol/water choice procedure. Besides reducing fluid intake, naloxone treatment prior to forced ethanol exposure interferes with the acquisiton of ethanol preference. Post-conditioning naloxone administration fails to affect ethanol preference. Administration of naloxone prior to the first testing session induces a reduction on total fluid intake, at the day of treatment; a decrease on ethanol preference throughout the three consecutive testing days is also observed with the higher dose of the antagonist (5 mg/kg). An involvement of endogenous opioids in ethanol consumption is suggested through the modulation of alcohol reinforcement or the affective quality of the gustatory cue.

Naloxone Opioids Ethanol consumption Rats

THE putative role of endogenous opioid peptides in the physiological modulation of ingestive behaviour has received considerable attention in recent years. A large number of studies indicate that opiate antagonists inhibit food and/or fluid intake [3, 6, 13] in deprived as well as non-deprived rats [2, 9, 24]. Oral palatability factors might be related to the supressive effectiveness of opiate antagonists on ingestive behavior, and it has been suggested that flavor can modulate the antidipsogenic effect of naloxone [14]. Lynch and Libby [15] reported that naloxone suppresses the intake of highly preferred saccharin solutions in food-deprived as well as in sated rats. Rockwood and Reid [18] also claimed the existence of oral palatability factors for opiate antagonist effects, since naloxone was able to reduce sugar-water intake in rats drinking with open gastric fistulas. However, there is some support for naloxone-induced taste aversion [12, 25, 27] as a consequence of interference with ingestive processes by virtue of the drug's aversive effects.

There is ample evidence for the existence of several relationships between ethanol and opioid effects [1]. Beside reports suggesting biochemical and behavioral mechanisms in common, there are some studies indicating that opiates may affect voluntary ethanol consumption [11, 20, 22].

Rats consume little alcohol in a spontaneous choice situation [5,17]. However, when a weak ethanol solution is offered as the only drinking liquid prior to the free choice period, alcohol consumption is considerably increased and taste preference to the drug is developed [5,8].

The aim of the present study was to investigate whether blockage of opioid receptors by naloxone administration may influence the intake of a weak alcohol solution in rats. The following experiments were designed to determine: whether naloxone administered (1) shortly before the initial forced ethanol presentation (pre-conditioning); (2) immediately after the first forced ethanol exposure (post-conditioning); (3) shortly before the first testing session (pre-retention), could affect voluntary free choice ethanol consumption.

METHOD

Subjects

The subjects were 170 adult male Wistar rats (CIB, Spain) weighing 200–250 g at the beginning of the experiment. They were housed in group cages (4–5 animals per cage) and maintained under light (7:00–19:00 hr) and temperature (22°C) controlled conditions. Food and water were available ad lib in the home cages. After one week of adaptation to the drinking bottles, rats were weighed and individually housed. Water was removed from the cages 72 hours before the first experimental session. Subsequently, on the five consecutive days of experimental procedure, rats were maintained on a 24 hour water deprivation schedule with fluids only available during the period of drinking sessions.

Procedure

As the two-bottle procedure has been reported to be a

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TABLE 1

WATER, ETHANOL AND TOTAL DAILY FLUID CONSUMPTION (ml/100 g BODY) ON RATS SUBJECTED TO SALINE OR NALOXONE TREATMENT 15 MIN BEFORE FORCED ETHANOL EXPOSURE AT THE CONDITIONING DAY

		Conditioning Day	Retention Days								
			1			2			3		
	Number of Animals	Total Intake (ethanol)	Water Intake	Ethanol Intake	Total Intake	Water Intake	Ethanol Intake	Total Intake	Water Intake	Ethanol Intake	Total Intake
Saline	(18)	5.54 ± 0.25	2.73 ± 0.38	3.42 ± 0.39	6.16 ± 0.25	2.61 ± 0.42	4.27 ± 0.41	6.88 ± 0.28	3.11 ± 0.48	4.30 ± 0.40	7.42 ± 0.27
Naloxone (1 mg/kg)	(10)	3.29† ± 0.13	3.16* ± 0.37	2.03† ± 0.51	5.19† ± 0.43	4.70* ± 0.41	1.37† ± 0.25	6.07† ± 0.39	4.01* ± 0.62	2.58† ± 0.56	6.64† ± 0.25
Naloxone (5 mg/kg)	(9)	2.57† ± 0.16	3.10* ± 0.52	2.32† ± 0.72	5.42† ± 0.14	3.84* ± 0.39	1.96† ± 0.54	5.81† ± 0.26	4.29* ± 0.48	2.59† ± 0.53	6.88† ± 0.29

Results are the means ± SEM. *$p<0.05$ and †$p<0.005$ vs. corresponding saline group from Tukey's multiple comparison test.

sensitive test for taste preference evaluations [7], this choice procedure was used. Our previous experiments [8] showed that rats forced to drink a weak ethanol solution (2.5%) develop a stable baseline on alcohol preference in consecutive days, while concentration of 4% or 7% failed to develop ethanol preference in subsequent testing. This alcohol session is termed "conditioning session"—in terms of operant behavior—since rats without this session do not develop a preference for alcohol. Drinking sessions were of 15 minutes' duration and fluid consumption was measured after those periods of time. The experiments started with one day of habituation when the animals were allowed access to tap water in the two bottles. The following day (conditioning day) the two bottles were filled with a 2.5% ethanol solution. On the three subsequent days (retention days) the animals were presented with a two bottle-choice between 2.5% ethanol solution and tap water.

Treatments

Naloxone hydrochloride (Dupont Pharmaceuticals, Switzerland) was used throughout the experiment. The drug (in doses of 1 and 5 mg/kg) was freshly prepared by dissolving it in physiological saline and was injected subcutaneously (SC) in a volume of 0.5 ml/rat. Control animals received the same volume of saline as a placebo.

Statistics

Fluid intake measures were corrected in relation to animal body weight. Ethanol preference is expressed by the index: EP=[Ethanol intake/(Ethanol+Water intake)]×100. Data were statistically analyzed using one or two way analysis of variance with repeated measures. For statistical analysis the preference scores for each subject were subjected to arcsin transformation in order to satisfy the assumptions of the analysis of variance before carrying out the ANOVA. This transformation is adjusted for the lack of normality of percentage or proportional data [23]. A Posteriori Tukey's multiple comparison tests were carried out when ANOVAs revealed significant effects.

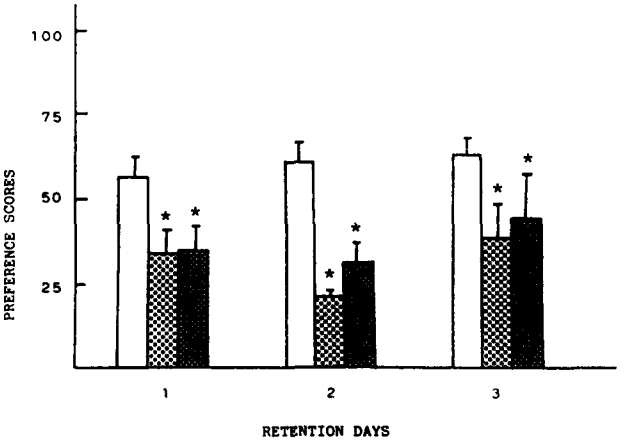

FIG. 1. Effect of administration of naloxone 15 minutes before the conditioning session on ethanol preference throughout the three consecutive retention testing days. Results show ethanol preference scores of control (clear box), 1 mg/kg (black-dot box) and 5 mg/kg (white-dot box) naloxone-treated rats. Variance bars indicate the standard deviation. *$p<0.001$.

RESULTS

Experiment 1

Effect of naloxone administration 15 min prior to forced ethanol exposure (conditioning session).

The data about fluid intake measures are shown in Table 1. One-way analysis of variance showed a significant effect of naloxone treatment upon fluid consumption on the day of treatment, when only ethanol was available, $F(2,34)=48.96$, $p<0.001$; administration of the antagonist reduces ethanol intake significantly and in a dose-response relationship. Two-way ANOVA of the data obtained for the three consecutive retention days revealed a significant effect of naloxone treatment upon total fluid intake, $F(8,102)=5.58$, $p<0.001$. Naloxone treated rats consume significantly less fluid than controls, $F(2,102)=7.69$, $p<0.001$. Analyzing

TABLE 2

TOTAL DAILY FLUID CONSUMPTION (ml/100 g BODY) (vol.) AND ETHANOL PREFERENCE SCORES (EP%) ON RATS SUBJECTED TO SALINE OR NALOXONE ADMINISTRATION IMMEDIATELY AFTER THE DRINKING SESSION AT THE CONDITIONING DAY

	Number of Animals	Conditioning Day	Retention Days 1		2		3			
		Vol.	Vol.	EP%	Vol.	EP%	Vol.	EP%	Days Effects	Treatment Effects
Saline	(18)	6.23 ± 0.41	6.56 ± 0.37	62.4 ± 11.1	7.73 ± 0.35	74.1 ± 7.5	7.43 ± 0.49	68.4 ± 12.3	Fvol.(2.102)= 6.55, $p<0.005$	Fvol.(1.102)= 0.07, n.s.
Naloxone (1 mg/kg)	(18)	5.64 ± 0.29	6.85 ± 0.33	53.0 ± 12.0	7.55 ± 0.30	71.0 ± 8.0	7.88 ± 0.32	64.3 ± 8.7	Fep%(2.102)= 2.14, n.s.	Fep%(1.102)= 0.87, n.s.
Saline	(10)	5.58 ± 0.49	6.79 ± 0.17	59.5 ± 23.7	7.30 ± 0.18	70.8 ± 8.0	7.21 ± 0.29	51.8 ± 12.4	Fvol.(2.54)= 3.44, $p<0.05$	Fvol.(1.54)= 2.54, n.s.
Naloxone (5 mg/kg)	(10)	5.51 ± 0.30	6.01 ± 0.48	65.6 ± 14.9	6.93 ± 0.37	60.6 ± 10.2	7.10 ± 0.34	58.2 ± 18.1	Fep%(2.54)= 0.41, n.s.	Fep%(1.54)= 0.004, n.s.

Values represent the means ± SEM (vol.) and the means ± SDM (EP%).

separately ethanol and water ingestion values, a significant reduction in ethanol intake, $F(2,102)=17.43$, $p<0.001$, is observed, whereas water consumption is significantly increased, $F(2,102)=5.60$, $p<0.005$.

ANOVA of the data corresponding to ethanol preference scores (Fig. 1) showed a significant reduction in ethanol preference by naloxone treatment, $F(2,102)=13.13$, $p<0.001$, through the three consecutive retention testing days studied.

Experiment 2

Effect of naloxone administration immediately after forced ethanol exposure (conditioning session).

Table 2 shows the data corresponding to both total fluid intake and ethanol preference scores. A significant increase in total fluid consumption throughout the testing days is observed, although there are not significant differences between control and naloxone-treated groups in daily total fluid intake. Naloxone, when administered immediately after conditioning, failed to affect significantly water or ethanol intake—and therefore ethanol preference—in the following testing days.

Experiment 3

Effect of naloxone administration 15 min before the first retention testing day.

ANOVA of the data (Table 3) showed a significant effect of naloxone treatment on total fluid intake (NX 1 mg/kg: $F(5,105))=25.59$, $p<0.001$; NX 5 mg/kg: $F(5,114)=66.30$, $p<0.001$) due to a reduction in total fluid consumption on the day of treatment with the drug (first retention day). Analyzing separately ethanol and water ingestion values, it is clear that on the day of treatment with naloxone the consumption of both fluids is reduced, although only the effect of the antagonist upon ethanol intake reaches a statistically significant level. Administration of 5 mg/kg of naloxone significantly reduces ethanol preference (Fig. 2). However, animals never developed ethanol aversion, since the preference scores were at a level of 50%. At the second and third day of testing, water consumption in animals treated with 5 mg/kg of naloxone is significantly increased and therefore ethanol preference is reduced.

DISCUSSION

The results of the present study show that under the schedule which was used, ethanol consumption can be modified by naloxone administration, indicating that the endogenous opioid system might influence the acquisition and maintenance of preference to alcohol. Systemic administration of naloxone before forced ethanol exposure at the conditioning drinking session significantly reduces alcohol intake in a dose-related relationship. Furthermore, this preconditioning treatment decreases later ethanol preference evaluated on the three consecutive testing days.

The fact that naloxone reduces fluid intake when administered before drinking session under a deprivation schedule agrees with results of previous studies [2,24]. It is interesting to note that in our study the suppressant effects of naloxone on fluid intake remain throughout the three consecutive testing days. It must be noted, however, that the reduction in total fluid intake induced by pre-conditioning injection of naloxone is primarily due to a reduction in alcohol ingestion which is simultaneous to a slight increase in the amount of water consumed in the choice paradigm; therefore, a reduction of ethanol preference under these conditions is observed.

Oral palatability factors may play a significant role in the modulation of the antidipsogenic properties of naloxone [14]. Lynch and Libby [15] reported that naloxone suppresses the

TABLE 3

WATER, ETHANOL AND TOTAL DAILY FLUID CONSUMPTION (ml/100 g BODY) ON RATS SUBJECTED TO SALINE OR NALOXONE TREATMENT 15 MIN PRIOR TO THE FIRST RETENTION DAY

		Conditioning Day	Retention Days								
			1			2			3		
	Number of Animals	Total Intake (ethanol)	Water Intake	Ethanol Intake	Total Intake	Water Intake	Ethanol Intake	Total Intake	Water Intake	Ethanol Intake	Total Intake
Saline	(19)	4.80 ± 0.26	2.08 ± 0.40	3.44 ± 0.47	5.52 ± 0.25	2.60 ± 0.37	3.69 ± 0.35	6.25 ± 0.27	2.27 ± 0.46	4.47 ± 0.53	6.74 ± 0.24
Naloxone (1 mg/kg)	(18)	5.52 ± 0.30	1.22 ± 0.18	2.26* ± 0.17	3.46† ± 0.20	2.60 ± 0.48	3.79 ± 0.45	6.39 ± 0.32	1.64 ± 0.33	5.60 ± 0.37	7.24 ± 0.30
Saline	(20)	4.81 ± 0.21	2.07 ± 0.37	3.95 ± 0.39	6.02 ± 0.16	1.77 ± 0.37	4.54 ± 0.38	6.31 ± 0.19	2.25 ± 0.42	4.45 ± 0.45	6.70 ± 0.18
Naloxone (5 mg/kg)	(20)	5.73 ± 0.27	1.82 ± 0.19	1.58† ± 0.14	3.40† ± 0.12	3.17* ± 0.48	3.27 ± 0.42	6.44 ± 0.15	3.17* ± 0.44	3.99 ± 0.39	7.16 ± 0.17

Results are the means ± SEM. *$p<0.05$ and †$p<0.005$ vs. corresponding saline group from a Tukey's multiple comparison test.

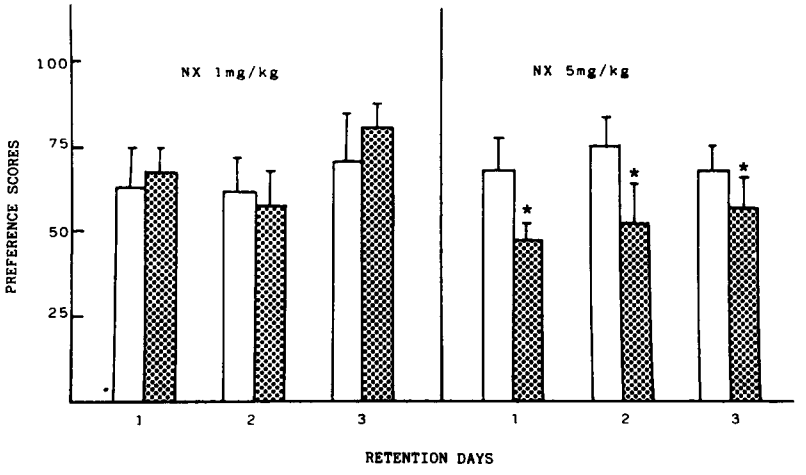

FIG. 2. Effect of administration of naloxone 15 min before the first retention testing day on ethanol preference on the same and consecutive retention testing days. Results show ethanol preference scores of control animals (clear box) and naloxone-treated rats (dotted box). Variance bars indicate the standard deviation. *$p<0.005$.

intake of highly preferred saccharin solutions in rats, suggesting that endogenous opiates can modulate the affective quality of gustatory events. In our paradigm, naloxone might suppress ethanol intake by blocking opiate receptors involved in such gustatory learning, either by interfering with the taste or by decreasing the reward value of ethanol [4]. If naloxone decreases taste acuity for ethanol, clearly it becomes less preferred and therefore its intake is reduced. It has also been reported that total caloric intake is diminished two hours after naloxone administration in doses of 0.1 and 10 mg/kg [16]. Therefore, another possibility would be that naloxone may affect ethanol intake by interfering with the caloric requirements of the animals. However, the fact that the effect of the opioid is maintained even 72 hr after its administration makes unlikely the last possibility for interpreting our results.

There is also some evidence for naloxone-induced conditioned taste aversion [12,25], influencing ingestive behavior by virtue of the drug's aversive effects. One way for discerning whether or not the effect of naloxone upon ethanol preference is linked to its aversive properties involves a comparison of its suppresive effect when administered shortly prior to drinking (pre-conditioning) with its potential to induce taste aversion when administered post-conditioning is paired with the taste. The results of the present study clearly indicate that the putative aversive sequelae of naloxone cannot account for the reduction in ethanol preference observed in a pre-conditioning regime, since when

the antagonist was administered post-conditioning it failed to affect later ethanol preference. In general, studies report CTA administered naloxone in a dose range between 5 and 10 mg/kg. Although in one study the development of CTA at low doses (3.2 mg/kg) has been reported following the consumption of a novel saccharin solution, it should be noted that aversion was evident after four trials of pairing the drug with the taste [25]. Using one trial paradigm, Rodgers *et al.* [19] also failed to find CTA to saccharin by naloxone in mice.

In our study, naloxone administration shortly before the first testing day diminished ethanol preference at the higher dose used (5 mg/kg). It is interesting to note that ethanol preference for naloxone-treated animals was in a range of approximately 50%, indicating that the antagonist, although able to eliminate the factors responsible for the maintenance of preference, fails to induce aversion to ethanol.

Several neurochemical and behavioral mechanisms have been postulated indicating specific opiate system-ethanol interactions [1]. Sinclair *et al.* [22] observed that a high dose of morphine (60 mg/kg), decreases voluntary ethanol consumption in rats. Other authors [21] found that naloxone (20 mg/kg) induces a decrease in the response to ethanol of up to 50% on a two lever concurrent for ethanol and water in rats. Although the above-mentioned authors suggested that the reinforcing properties of ethanol might be, at least in part, functioning via the endogenous opioid system, they considered unlikely a direct opiate receptor activity by ethanol or its metabolites as indicated in other studies [10,26]. Therefore it cannot be discounted that the reduction on ethanol consumption after naloxone treatment here reported may be linked to a specific ethanol-opiate interaction.

In conclusion, the present study shows that naloxone, in addition to its well-known suppressive effects on fluid intake, is able to decrease ethanol intake, over a subsequent three day period, in a two bottle choice procedure. These data indicate that endogenous opioid peptides systems may be involved in the acquisition and maintenance of ethanol preference by modulating alcohol reinforcement or the affective quality of the gustatory cue.

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