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BRIEF COMMUNICATION

The Effect of Naloxone on Intragastric Ethanol Self-Administration[1]

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SINDEN, J. D., P. MARFAING-JALLAT AND J. LE MAGNEN. *The effect of naloxone on intragastric ethanol self-administration*. PHARMACOL BIOCHEM BEHAV **19**(6) 1045–1048, 1983.—The acute effect of 1.25 and 2.50 mg/kg naloxone was tested in a group of male Wistar rats readily self-administering 10% w/v ethanol intragastically following 12 days of forced ethanol intoxication. Compared to saline pretreatment, naloxone did not alter 24 hr intakes of food, water or ethanol. However, both does strongly and significantly inhibited lever pressing for ethanol during 2 hr following pretreatment. The results indicate that naloxones's inhibition of ethanol intake does have a transient postabsorptive component, although this component is unlikely to be specific to ethanol.

Intragastric self-administration Ethanol Naloxone Rat

RECENT clinical and experimental reports have suggested that the opiate antagonists, including naloxone, may suppress various aspects of the neuropharmacological activity of ethanol. In humans, naloxone has been shown to block the coma resulting from high doses of ethanol [11] and the sensory-motor impairments produced by low doses of ethanol [10]. Naloxone administered during forced treatment with ethanol has been shown to reduce subsequent withdrawal signs and symptoms in rats and mice [3,4]. However, other studies have failed to demonstrate an interaction between the acute actions of the opiate antagonists and ethanol's behavioural effects [19, 20, 28]. Recent human studies have indicated that such an interaction may only occur in individuals with a particular genetic susceptibility, the chlorpropramide-alcohol flushing response [9,11]. Recent data have also indicated that opiate antagonist-ethanol interactions appear to vary according to the inbred strain of mice employed [12] and thus may not clearly manifest themselves when heterogeneous strains of animals are used.

In contrast, a role for opiate antagonists in the inhibition of voluntary intake of ethanol seems to be more clearly established. Repeated injections of naloxone and naltrexone have been recently shown to block the elevated ad lib consumption of ethanol in rats treated with tetrahydroisoquinoline and tetrahydropapaveroline [9,21], suggesting a link between central "morphine-like alkaloids" and ethanol reinforcement. Naloxone has been shown to reduce the oral consumption of ethanol in both naive rats and rats rendered behaviourally dependent on ethanol by prior forced ethanol administration [17]. A difficulty in interpreting these findings, however, is the fact that the opiate antagonists have a potent antidipsogenic action [5, 8, 23, 24] and recent evidence suggests that this action may in large part be due to effects on preabsorptive or incentive mechanisms rather than feedback from postabsorptive events. For example, the addition of a sweet flavour to ingested solutions heightens the antidipsogenic action of naloxone [15] and an equally antidipsogenic effect of naloxone has been observed in rats whether or not the postabsorptive effects of sucrose solutions are eliminated by stomach fistulas [25]. Marfaing-Jallat *et al.* [17] showed that inhibition of ethanol intake by naloxone was greater than the corresponding inhibition of water intake. This was interpreted as supporting the hypothesis that the opiate antagonists act non-specifically to heighten the aversiveness of oral cues of non-preferred solutions (e.g., ethanol for naive rats) and to reduce the rewarding value of the oral cues of preferred solutions (e.g., ethanol for dependent rats) [14]. A role for naloxone in spe-

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cifically altering ethanol's postabsorptive rewarding neuropharmacological effect could not be demonstrated.

Some evidence, however, does suggest that the opiate antagonists may disrupt the rewarding value of ethanol for laboratory animals independently of the oral cues which may signal a subsequent postabsorptive reinforcement. Naloxone, for example, blocks the excitatory effect of ethanol on lateral hypothalamic self-stimulation without altering self-stimulation by itself [16]. Further, chronic naltrexone pretreatment inhibits intravenous ethanol self-administration in the monkey [1]. These authors showed that 15 days of naltrexone produces a dose-dependent inhibition which resembles an extinction pattern, a slight increase in responding for the first five days of naltrexone pretreatment followed by a large inhibition.

In order to examine the effects of acute naloxone administration on the postabsorptive reinforcing effects of ethanol in dependent rats using the same route of administration as oral intake, the present experiment employs an intragastric ethanol self-administration paradigm. Previous research has shown that forced intoxication by the intragastric route will produce subsequent high levels of oral ethanol consumption in rats [17,18]. We have recently shown that this forced intoxication procedure will produce subsequent behavioural dependence on ethanol in rats lever pressing for intragastric ethanol ([29], Sinden *et al.* in preparation). The results demonstrate that the postabsorptive effects of ethanol intake are modified by naloxone pretreatment.

METHOD

Subjects

The animals were six male rats of the Wistar strain (IFFA-CREDO, France) weighing 363±19 g at the time of the experiment. All rats had ad lib access to standard laboratory chow (Pietrement) in powder form and tap water and were maintained on a 12 hr light/12 hr dark cycle (6 hr–18 hr day) and a constant room temperature (22±1°C). The home cages were 43 cm high, 25 cm diameter clear acrylic cylinders with perforated stainless steel floors. The rats were implanted with a chronic intragastric catheter under Nembutal anesthesia (45 mg/kg) according to the techniques described previously [22]. Briefly, a 0.765 mm i.d. silastic tube terminated by a more rigid polyethylene tube was passed through the lower stomach (fundus) wall and tied in place. The catheter was then passed under the skin and connected at the top of the head to a metal tube by means of skull screws and dental acrylic.

Forced Intoxication Procedure

Following six days of recovery from surgery, the rats were connected via polyethylene tubing and swivel joints to motor-driven syringe infusion pumps (60 ml syringe, 0.5 rpm motor). For 12 consecutive days, all rats were chronically intoxicated in their home cages with periodic and automatically-programmed intragastric infusions. From 10 hr to 2 hr they received five evenly-spaced pluses of 2 g/kg ethanol prepared from 95% ethanol diluted in physiological saline (3.36 ml solution per infusion). A gap of 8 hr each morning without infusion allowed the rats to eat and drink unaffected by the disturbance due to the acute effects of ethanol. The animals' intoxication state was monitored regularly and occasional infusions were interrupted to avoid overdose.

Experimental Procedure

At the cessation of the forced ethanol infusion period, 12 hr after the final infusion, an operant lever was introduced into the home cage 45° to the left of the food cup. The rats were neither pretrained nor shaped to press the lever. Each depression of the lever delivered 0.25 ml of 20% w/v ethanol in 0.9%saline solution over an approximately 3 sec infusion period via the polyethylene tubing and syringe pump (60 ml syringe, 6 rpm motor). If a further lever press occurred during the 3 sec self-infusion period, it was neither counted nor did it lead to another infusion. The rats had access to their familiar food, water and to the operant lever 24 hr per day for the duration of the experiment. A multichannel event recorder (Esterline Angus) monitored all self-administrations during the nocturnal phase. At 17.30 hr each day, body weight, total ethanol, food and water intake were recorded and catheters cleared with 0.5 ml saline.

Following three days of self-administration at 20%, the ethanol concentration was reduced to 10% w/v for two further training days. The experiment proper which followed immediately consisted of three days of 0.9% saline or naloxone (1.25 and 2.50 mg/kg) IP pretreatment preceded by a no-pretreatment baseline day to monitor response stability throughout the experiment. Naloxone HCl (Endo Laboratories, U.S.A.) was dissolved in physiological saline and injected in a volume of 1 ml/kg body weight. The order of presentation of the two doses of naloxone and saline was randomized for the six rats and pretreatments were injected 5–10 min before the beginning of the dark cycle (18 hr). As a final experimental condition, saline was substituted for the ethanol in the syringe infusion pumps.

Data Analysis

The time course of naloxone's effects was examined by measuring the number of lever presses for ethanol within 2-hr blocks of the nocturnal phase. This provides a useful reference for comparison with previous reports of naloxone's effects on the time-course of ad lib food and water intake in rats [8]. In order to reduce inter-subject variability in the data analysis, a Rats × Pretreatment × 2 hr Blocks analysis of covariance was performed, with the previous day's (no treatment) responding in each 2 hr block used as the baseline covariate [31].

RESULTS AND DISCUSSION

Following forced intoxication, all rats showed signs of physical dependence, particularly hyperactivity and hyperreactivity. The daily self-administration for the three days of 20% w/v ethanol is 10.2±1.2, 7.5±0.8 and 7.8±1.4 g/kg respectively. The rats quickly learn to adjust their self-administration to the change in concentration of ethanol to 10% w/v, self-administering 5.4±0.6 and 7.7±1.3 g/kg respectively on the following two days. An examination of the baseline daily ethanol intakes in Table 1 indicates that this level of ethanol intake does not significantly change throughout the experiment. Nor is there large variability in baseline lever pressing when broken down into 2 hr periods during the night phase (Fig. 1).

The increase in ethanol self-administration activity observed following the change in concentration of ethanol self-administered from 20% to 10% has been shown in other experiments not to occur in rats submitted to prior forced administration of an isocaloric glucose solution ([29], Sinden

TABLE 1

MEAN ± SEM DAILY 10% W/V ETHANOL (g/kg), WATER (ml) AND FOOD (g) INTAKES FOR THE THREE BASELINE (NO PRETREATMENT) DAYS AND FOLLOWING PRETREATMENT WITH 0.9% SALINE AND TWO DOSES OF NALOXONE (NX)

	Condition					
	baseline	saline	baseline	NX 1.25 mg/kg	baseline	NX 2.50 mg/kg
Ethanol	8.5 ±1.3	7.8 ±2.6	6.9 ±1.4	7.2 ±3.5	7.4 ±0.9	8.7 ±2.9
Water	18.2 ±5.0	16.8 ±5.3	28.7 ±5.6	26.5 ±3.6	25.8 ±4.1	25.4 ±2.2
Food	19.0 ±2.9	20.2 ±1.3	22.2 ±3.1	22.8 ±3.0	24.2 ±1.6	22.2 ±1.5

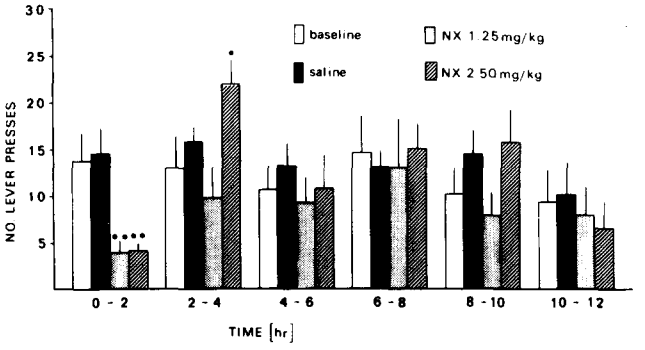

FIG. 1. Mean±SEM lever presses for 10% w/v ethanol during two-hr periods in the nocturnal phase for the three baseline (no pretreatment) days and following pretreatment with 0.9% saline and two doses of naloxone (NX). Compared with saline: $*p<0.05$, $**p<0.01$, Dunnett comparisons from the Analysis of Covariance.

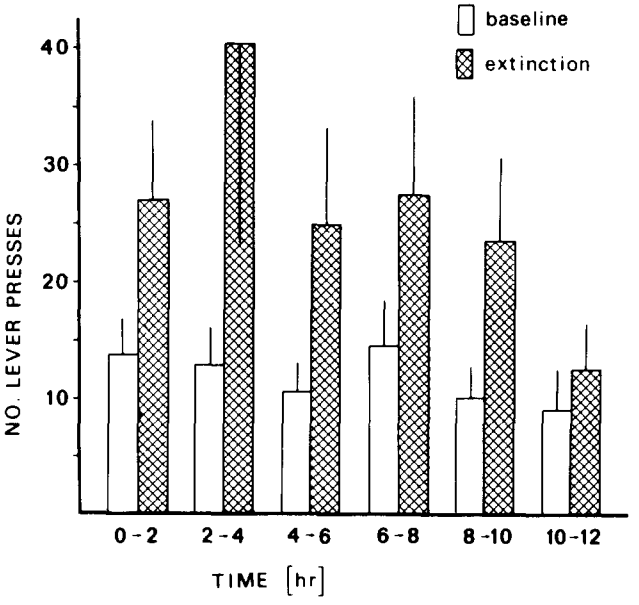

FIG. 2. Mean±SEM lever presses during two-hour periods in the nocturnal phase for the three baseline (no pretreatment) 10% w/v ethanol days and the extinction (saline substitution) day.

et al. in preparation). Together with the acute rise in self-administration following saline substitution for ethanol at the end of the experiment (Fig. 2), this suggests that the prior forced intoxication protocol produces a regulation of the daily intake of ethanol by self-administration at between 7 and 8 g/kg. The level of daily intake observed here corresponds closely to the quantities orally consumed ad lib in dependent rats previously subjected to forced ethanol inhalation [13].

Table 1 shows that a single acute dose of naloxone has no effect on ethanol, water or food intake measured over 24 hr. However, an analysis of the time course of lever pressing for ethanol during the nocturnal phase in Fig. 1 shows that naloxone pretreatment produces significant transient changes in operant activity for ethanol. From the analysis of covariance, the Pretreatment × 2 hr Blocks interaction is significant, $F(10,49)=2.416$, $p<0.05$. Post hoc Dunnett comparisons revealed that, compared to saline, lever pressing for the first 2 hr block following naloxone pretreatment is significantly and equivalently reduced for the two naloxone doses ($p<0.01$). Further, 2–4 hr after 2.50 mg/kg, but not 1.25 mg/kg, naloxone, lever pressing for ethanol is significantly increased compared to saline ($p<0.05$). No significant differences between saline and naloxone in the later blocks were found.

These data indicate that the recently observed diminution of ethanol drinking by 1.0 mg/kg naloxone [17] does not solely depend on the preabsorptive reward or aversion (incentive) value of oral cues associated with ethanol [14, 15, 25]. The present experiment shows that ethanol intake is transiently reduced by naloxone under conditions where the taste and smell of ingested ethanol are minimized.

The depression of lever pressing observed here is unlikely to represent a motor deficit as naloxone at the doses tested here does not significantly alter motor activity in rats [2,6]. Further, it has been recently shown that naloxone does not reduce FR 20 lever-pressing for an oral sweet milk reward [26].

There is little evidence for an acute rise in self-administration such as has been observed in other self-

administration paradigms following drug-induced reward blockade [1, 27, 32]. Such an increase in self-administration due to frustrative non-reward would be expected to resemble the effect of saline substitution for the ethanol in the infusion pumps (Fig. 2). Some increase in self-administration is observed 2–4 hr after 2.50 mg/kg naloxone. However, as this increase does not follow the depression of self-administration produced by 1.25 mg/kg naloxone, this most probably reflects a non-specific effect of the drug on ad lib consummatory behaviors. Cooper [8], for example, has observed rebound increases in food and water consumption following initial depressions at 2 and 5 mg/kg, but not 0.5 and 1 mg/kg, naloxone.

Overall the data indicate that the mechanisms underlying naloxone's inhibition of ethanol intake in dependent rats do have a transient postabsorptive component, but it is unlikely to be specific to the rewarding effects of ethanol. Further research is required to determine if naloxone has similar effects on self-administration of other reinforcing solutions, both intoxicating and non-intoxicating.

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