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# Alcohol drinking is reduced by a $\mu_1$- but not by a $\delta$-opioid receptor antagonist in alcohol-preferring rats

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**Abstract**

To assess the roles of opioid receptor subtypes in voluntary alcohol drinking, alcohol-preferring AA (Alko, Alcohol) rats, non-deprived of food or water, were used in a paradigm where access to 10% alcohol solution was limited to 1–4-h sessions on every 2nd working day. The $\delta$-opioid receptor antagonist naltrindole (1–5 mg/kg i.p. 15 min before the session) had no effect on alcohol drinking, while it attenuated the $\delta$-opioid receptor agonist [D-Pen$^2$,D-Pen$^5$]enkephalin-induced locomotor stimulation. The $\mu_1$-opioid receptor antagonist naloxonazine (1–15 mg/kg i.p. 20 h before the session), at the largest dose, decreased alcohol drinking. It also decreased food intake. When naltrindole (1 mg/kg) and naloxonazine (15 mg/kg) were given prior to 3 consecutive sessions, the former had no effects at any session. Naloxonazine decreased alcohol consumption only in the 1st session, although the reduction of daily water intake became stronger during repeated administration. 4 days after the last drug administration, naloxonazine-treated animals consumed alcohol nearly twice as much as in the control session before any drug treatment. These data suggest that $\delta$-opioid receptors are not involved in the regulation of alcohol drinking in AA rats. $\mu_1$-Opioid receptors may be involved in alcohol drinking, although the data suggest that even their prolonged blockade alone is insufficient to induce a sustained decrease in alcohol drinking.

*Keywords:* Alcohol drinking; Food intake; $\delta$-Opioid receptor; $\mu$-Opioid receptor; Reinforcement; (Selected rat line)

## 1. Introduction

Agents acting on central opioid receptors affect consummatory behaviour, suggesting that brain opioid mechanisms participate in the regulation of feeding and drinking (for review, see Cooper and Kirkham, 1993). Opioid receptor blockade decreases eating and drinking, whereas stimulation of these receptors with low doses of agonists increases ingestive behaviour. There is some evidence that opioid receptor antagonists are especially potent in reducing the intake of preferred or palatable food (Cooper and Kirkham, 1993). Since central opioid systems are important in the control of motivational and reward processes (Di Chiara and North, 1992; Shippenberg, 1993), opioids may, at least partly, mediate the reward induced by eating and drinking.

Non-selective opioid receptor antagonists reduce alcohol intake effectively in experimental animals (Altshuler et al., 1980; Sinden et al., 1983; Samson and Doyle, 1985; Sinclair, 1990; Hyytiä, 1993; Wegelius et al., 1994). As with preferred food, it is possible that reduction of alcohol drinking occurs at lower antagonist doses than e.g. reduction of water intake (Samson and Doyle, 1985; Froehlich et al., 1991). Opioid receptor antagonist-induced reduction of alcohol consumption may be related to findings that alcohol activates brain opioid mechanisms, i.e. increases the release and turnover of opioid peptides (Gianoulakis, 1989). These findings suggest that alcohol's reinforcing effects may be due to its ability to stimulate endogenous opioid mechanisms. Thus, reduction of alcohol drinking by opioid receptor blockade might be due to elimination of alcohol-induced reinforcement (Sinclair, 1990), which could explain the findings of recent clinical studies where chronic treatment with non-selective opioid receptor antagonists decreased the alcohol consumption and relapse rates of alcoholics (Volpicelli et al., 1992; O'Malley et al., 1992).

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Several recent studies with experimental animals have attempted to identify the opioid receptor subtype(s) involved in alcohol drinking. Since agonists of $\mu$- and $\delta$-opioid receptor subtypes, but not those of $\kappa$-opioid receptors, are reinforcing in experimental animals (Shippenberg, 1993), the research has focused on the former two opioid receptor subtypes.

In high-alcohol drinking HAD rats, a $\delta$-opioid receptor antagonist *N,N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI 174,864 i.p.) reduces alcohol drinking (Froehlich et al., 1991). The involvement of $\delta$-opioid receptors was confirmed in C57BL/6 mice with systemic naltrindole, a non-peptide $\delta$-opioid receptor antagonist (Takemori and Portoghese, 1992), which reduced alcohol drinking while a $\mu$-opioid receptor antagonist $\beta$-funaltrexamine was ineffective (Lê et al., 1993). In contrast, ICI 174,864 (i.c.v.) failed to affect alcohol drinking in alcohol-preferring AA (Alko, Alcohol) rats (Hyytiä, 1993) whereas the non-selective antagonist naloxone (Sinclair, 1990; Wegelius et al., 1994) and a $\mu$-opioid receptor antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-$NH_2$ (CTOP i.c.v.; Hyytiä, 1993), reduced it.

Owing to the conflicting data from AA rats and other rodent lines, our first goal in the present study was to examine whether alcohol drinking by AA rats could be reduced with the $\delta$-opioid receptor antagonist naltrindole. Naltrindole is a selective and more potent $\delta$-opioid receptor antagonist than ICI 174,864 (Takemori and Portoghese, 1992); it can be given systemically and it is devoid of neurotoxic properties of ICI 174,864 (Long et al., 1988). Since $\mu$-opioid receptors have subtypes (Pasternak and Wood, 1986), the second goal of our study was to explore the role of these subtypes in alcohol drinking by investigating the effects of a $\mu_1$-opioid receptor selective antagonist naloxonazine on alcohol drinking behaviour in AA rats.

## 2. Materials and methods

### 2.1. Determination of alcohol consumption

55 male, adult AA rats from generations $F_{65}$ and $F_{68}$ were used (Eriksson, 1968). At the age of 3 months, the rats were removed from group cages to individual stainless steel wire-mesh cages. The animals were maintained on a 12:12-h dark:light cycle (lights on at 06:00 h), at an ambient temperature of $20 \pm 2$°C and a relative air humidity of $50 \pm 5$%. The animals received powdered rodent food (Ewos R3, Södertälje, Sweden) and tap water ad libitum except as noted below. All animal experiments were approved by the Institutional Animal Care and Use Committee at Alko-Group.

A modification (Wegelius et al., 1994) of the limited access paradigm described by Sinclair et al. (1992) was used. First, the animals underwent forced alcohol drinking for 1 week, 10% (by volume) ethanol in tapwater being the only fluid available. During the next 3 weeks both water and alcohol were available, after which the access to alcohol was restricted to 4 h/day 3 × a week, i.e. on every 2nd working day. At the beginning of each session, the rats were weighed and their food jars were changed to newly weighed ones. Alcohol consumption was recorded 1 h after access to alcohol and at the end of the 4-h session. During the experiments, 24-h water and food consumptions were recorded. Food intake was also recorded at the end of each 4-h session, but water intake was not, since the non-deprived rats drink only negligible amounts of water when trained to drink alcohol solution during the sessions.

When the rats had achieved a steady level of alcohol drinking, they were given an i.p. injection of saline (1 ml/kg body weight) 15 min before access to alcohol. For the next session, the animals, divided into four equal groups according to their 1-h alcohol drinking in the saline session, received naltrindole (Research Biochemicals, Natick, MA) in doses of 1, 3 or 5 mg/kg (in 5% gum arabic, 1 ml/kg i.p.), or vehicle, 15 min before access to alcohol. After a 2-week washout period with continued drinking sessions without injections, the animals were again divided into four groups according to their 1-h alcohol drinking after saline injection. 20 h before the next session, the animals were given naloxonazine (Research Biochemicals) in doses of 1, 7.5 or 15 mg/kg (in 5% gum arabic, 1 ml/kg i.p.), or vehicle, and 15 min before the session, a saline injection (1 ml/kg i.p.).

Another group of AA rats were trained to drink alcohol by using the same paradigm except that alcohol was given only for 1 h on on every 2nd working day. After the basal alcohol consumption was achieved, the rats were divided into three equal groups which then received either vehicle (5% gum arabic), naltrindole (1 mg/kg) or naloxonazine (15 mg/kg) before 3 consecutive sessions with the same time schedules for the opioid antagonists as in the first experiment. In addition, alcohol drinking was recorded in 1 posttreatment session after saline injection.

### 2.2. Determination of locomotor activity

Ten AA rats from generation $F_{71}$ were stereotaxically implanted (under halothane anaesthesia) with a 23-G guide cannula into the lateral ventricle as described earlier (Hyytiä, 1993). The coordinates of the cannula (relative to bregma) were: A, −0.9; L, −1.5; V, −3.5 (Paxinos and Watson, 1982). A dummy cannula, cut to the same length as the guide cannula, was inserted into to the guide cannula between injections. The 30-G injection cannula extended 1 mm beyond the tip of the guide cannula. After surgery, animals were placed into individual cages, accustomed to handling and injection procedures and allowed to recover at least for 10 days before locomotor activity determinations.

The horizontal locomotor activity of the animals were registered in transparent 25 × 42 × 15 cm plastic cages by

means of computer controlled photocells. On the day preceding the 1st experimental day, the animals were preadapted to the locomotor activity cages for 120 min. On 2 experimental days, after 40-min habituation periods all animals were given 5 $\mu$l of saline i.c.v. The solution was given with a 25-$\mu$l microsyringe by means of a infusion pump over period of 60 s and the injection cannula was left in place for 30 s to allow complete solution delivery. Immediately after the injection, the animals were placed into the activity cages and the locomotor activity of the animals was registered for 60 min. Thereafter, half of the animals were given 5 mg/kg of naltrindole (i.p.) and the rest were given vehicle. 15 min later all animals were given an i.c.v. injection of $\delta$-opioid receptor agonist [D-Pen$^2$,D-Pen$^5$]enkephalin (DPDPE; Bachem Feinchemicalien, Budendorf, Switzerland) at the dose of 10 $\mu$g in a volume of 5 $\mu$l/animal and the locomotor activity of the animals was registered for 60 min. The experiment was conducted as a cross-over study, i.e. on the next day the experiment was repeated, and the animals that were given naltrindole on the 1st experimental day were given vehicle on the 2nd day and vice versa.

### 2.3. Statistics

The drug effects on food and alcohol intakes were statistically evaluated by one-way analyses of variance (ANOVA) followed by the Tukey compromise posthoc test when appropriate (Super-Anova software package by Abacus Concepts, Berkeley, CA). The effects of repeated antagonist administrations on alcohol intake and those of naltrindole on locomotor activity were tested with ANOVA for repeated measures, followed by Student's $t$-test as a posthoc test. The paired $t$-test was used to compare the alcohol drinking values of pre- and posttreatment sessions within treatment groups.

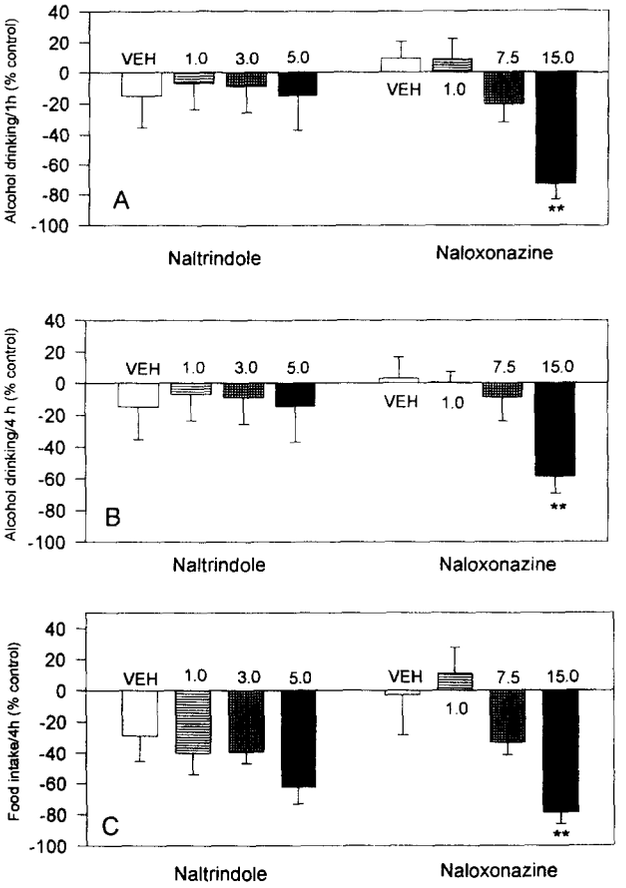


Fig. 1. Effects of various doses of naltrindole and naloxonazine (mg/kg i.p., as indicated by the numbers at the bars) or vehicle (VEH, 5% gum arabic) on (A) 1-h alcohol intake, (B) 4-h alcohol intake, and (C) 4-h food intake in alcohol-preferring AA rats ($n = 6$–8 animals/dose). The bars represent mean percentage changes (±S.E.M.) from the values in the control session where the animals were given saline injection (1 ml/kg i.p.) 15 min before access to alcohol. Naltrindole was given 15 min and naloxonazine 20 h before the 4-h sessions. The control 1-h alcohol, 4-h alcohol and 4-h food intakes averaged 0.73 g/kg, 1.12 g/kg and 11.2 g/kg, respectively. Significance of difference from the vehicle-treated group (Tukey compromise posthoc test after one-way ANOVA): ** $P < 0.01$.

## 3. Results

### 3.1. Dose dependence of alcohol drinking responses to naltrindole and naloxonazine

Naltrindole (1–5 mg/kg) had no effect on either 1- or 4-h alcohol drinking [$F(3,25) = 0.05$, $P = 0.99$ and $F(3,25) = 0.01$, $P < 0.96$, respectively] (Fig. 1). In addition, naltrindole did not alter 4-h food intake during alcohol drinking [$F(3,23) = 1.42$, $P = 0.26$] nor 24-h food and water intake [$F(3,25) = 1.57$, $P = 0.22$ and $F(3,24) = 0.38$, $P = 0.77$, respectively] (Table 1). Naloxonazine, especially at the dose of 15 mg/kg, reduced both 1- and 4-h alcohol drinking [$F(3,27) = 11.1$, $P < 0.001$ and $F(3,27) = 5.8$, $P < 0.01$, respectively] (Fig. 1). However, 4-h food intake during the drinking session was reduced in a similar manner [$F(3,26) = 6.8$, $P < 0.01$]. ANOVA showed a significant difference between doses also in daily food intake measured 20 and 48 h after the drug treatment [$F(3,27) = 89.5$, $P < 0.001$ and $F(3,27) = 16.5$, $P < 0.001$, respectively] (Table 1). The dose of 15 mg/kg of naloxonazine, but not lower doses, reduced food intake during the 2nd posttreatment day. Daily water intake was also decreased by naloxonazine during the first 20 h after injection [$F(3,27) = 5.4$, $P < 0.01$], but not during the next 24 h. Posthoc comparison revealed that the decrease in water intake only occurred with the intermediate naloxonazine dose of 7.5 mg/kg.

### 3.2. Effects of repeated injections of naltrindole and naloxonazine

When given repeatedly, naltrindole had no effect on 1-h alcohol drinking, 24-h water drinking or the body weight

of rats (Fig. 2). In contrast, ANOVA showed a significant difference in 1-h alcohol drinking between the vehicle and naloxonazine groups [treatment × session interaction: $F(4,52) = 3.9$, $P < 0.01$]. Naloxonazine reduced 1-h alcohol intake by 50% in the 1st session ($P < 0.05$, Student's $t$-test), but no longer in the 2nd and 3rd sessions. In the posttreatment session, naloxonazine-treated animals consumed alcohol almost twice as much as in the control session ($P < 0.01$, paired $t$-test). Posttreatment alcohol drinking in vehicle and naltrindole groups did not differ

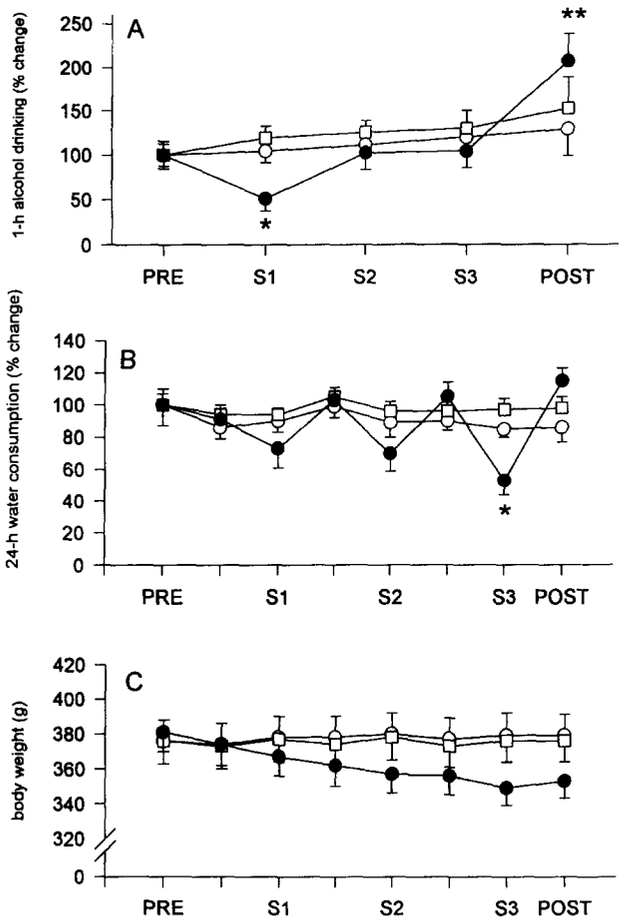


Fig. 2. The effect of repeated treatment with naltrindole (○, 1 mg/kg i.p.), naloxonazine (●, 15 mg/kg i.p.) or vehicle (□, 5% gum arabic, 1 ml/kg i.p.) on (A) 1-h alcohol intake, (B) 24-h water intake and (C) body weight of alcohol-preferring AA rats ($n = 7$-8 animals/treatment). The animals had three alcohol drinking sessions/week [on Monday (S1), Wednesday (S2) and Friday (S3)]. Naltrindole was given 15 min and naloxonazine 20 h before each alcohol drinking session. Water intake and body weight were also recorded on days between the sessions. The data on alcohol drinking and water intake are presented as mean percentage changes (±S.E.M.) from the control session (PRE) where the animals were given saline (1 ml/kg i.p.) 15 min before access to alcohol. Alcohol, water and food intakes were also measured in 1 posttreatment session (POST, 3 or 4 days after the last naltrindole or naloxonazine treatment, respectively), with saline injections being given prior to the alcohol drinking session. The control 1-h alcohol and 24-h water intakes (PRE) averaged 0.61 g/kg and 68 ml/kg, respectively. Significance of difference from the vehicle-treated group (Student's $t$-test after ANOVA for repeated measures): $^{*}$ $P < 0.05$. Significance of difference from the control value (PRE) of the treatment group (paired $t$-test): $^{**}$ $P < 0.01$.

Table 1
Effects of various doses of naloxonazine, naltrindole and vehicle on 24-h food and water intake in alcohol-preferring AA rats

| Treatment (mg/kg i.p.) | Daily food intake | | Daily water intake | |
|---|---|---|---|---|
| | 20 h | 48 h | 20 h | 48 h |
| Vehicle | 99± 6 | 102±4 | 97± 7 | 100± 7 |
| Naloxonazine (1) | 93± 3 | 108±4 | 101± 9 | 101± 6 |
| Naloxonazine (7.5) | 69± 6 [b] | 100±4 | 56± 6 [a] | 109± 6 |
| Naloxonazine (15) | 8± 1 [b] | 60±8 [b] | 102±14 | 101±10 |
| Vehicle | 101±11 | – | 122±18 | – |
| Naltrindole (1) | 102± 4 | – | 117±10 | – |
| Naltrindole (3) | 109± 3 | – | 119± 9 | – |
| Naltrindole (5) | 95± 5 | – | 102±14 | – |

The data, recorded at 20 and 48 h after injections, are presented as mean percent (±S.E.M., $n = 6$–8) of the control level measured after saline injection (1 ml/kg i.p.). The control food and water intakes averaged 50±1 g/kg and 60±4 ml/kg, respectively. [a] $P < 0.05$, [b] $P < 0.01$, in comparison with the corresponding vehicle-treated group (Tukey compromise test).

significantly from pretreatment levels ($P = 0.32$ and $P = 0.20$ in vehicle- and naltrindole-treated groups, respectively). Naloxonazine also decreased 24-h water intake slightly after each injection [treatment × session interaction: $F(7,91) = 4.13$, $P < 0.01$]. However, compared with vehicle-treated animals, this effect reached statistical significance only after the third injection ($P < 0.05$, Student's $t$-test). The 24-h water intake in the posttreatment session tended to be higher in naloxonazine-treated than in vehicle-treated animals ($P = 0.07$). The body weights of naloxonazine-treated animals decreased by 8% during the experiment [$F(7,91) = 29.7$, $P < 0.01$].

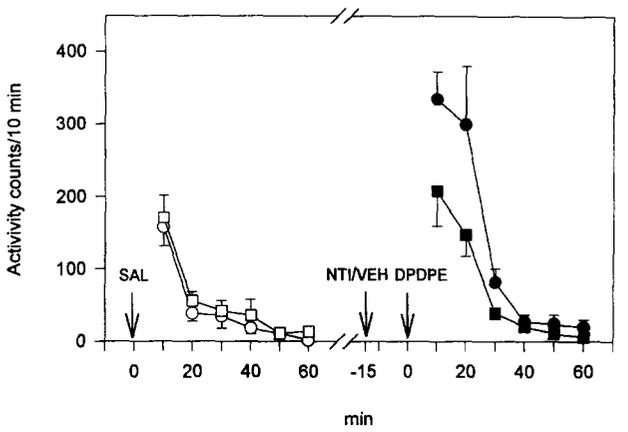


Fig. 3. Effect of saline (i.c.v., open symbols) and DPDPE (i.c.v., closed symbols) on horizontal locomotor activity in the AA rats after pretreatment with naltrindole (NTI, 5 mg/kg i.p.) or vehicle (VEH, 1 ml/kg i.p.). The rats were first habituated to the activity cages for 40 min, after which they were given saline (5 μl, i.c.v.) and their locomotor activity was registered for 60 min (○, vehicle group; □, naltrindole group). Immediately after that (−15 min) the animals were given either 5 mg/kg of naltrindole (i.p.) or vehicle. 15 min later (0-point), all animals were given an injection of δ-opioid receptor agonist DPDPE (i.c.v., 10 μg/animal) and their locomotor activity was registered for 60 min (●, vehicle group; ■, naltrindole group).

### 3.3. Effect of naltrindole on locomotor stimulation induced by δ-opioid receptor agonist

The i.c.v. infusion of δ-opioid receptor agonist DPDPE induced locomotor stimulation that lasted at least for 30 min (Fig. 3). Pretreatment with systemic naltrindole (5 mg/kg i.p.) significantly attenuated DPDPE-induced stimulation [naltrindole vs. vehicle treatment effect: $F(1,18) = 5.81$, $P < 0.05$].

## 4. Discussion

Receptor-binding studies suggest that δ-opioid receptors may be more sensitive to actions of alcohol than are other opioid receptor subtypes (Hiller et al., 1981; Hynes et al., 1983; Pfeiffer et al., 1981; Charness, 1989). In addition, focally applied naltrindole has been shown to antagonize alcohol-induced dopamine release in the nucleus accumbens of rats (Acquas et al., 1993). As dopaminergic mechanisms in the nucleus accumbens are believed to be critically involved in the rewarding effects of various drugs of abuse, including alcohol (Koob, 1992), δ-opioid receptors are expected to participate in alcohol reinforcement. However, our present results and those of Hyytiä (1993) indicate that blockade of δ-opioid receptors does not affect alcohol drinking in the alcohol-preferring AA rats. Single injection of the δ-opioid receptor antagonist naltrindole at doses up to 5 mg/kg failed to decrease alcohol consumption in these rats. Similarly, no change in alcohol drinking was seen when naltrindole (1 mg/kg) was given repeatedly in 3 consecutive sessions.

In contrast, 5 mg/kg naltrindole decreased the locomotor activity induced by i.c.v. δ-opioid receptor agonist DPDPE, indicating that naltrindole is able to block the δ-opioid receptor stimulation in AA rats. The doses of naltrindole used in the present study have been shown to attenuate swim-stress-induced analgesia in the rat, a response that appears to be at least partly mediated by δ-opioid receptors (Hart et al., 1983; Kitchen and Pinker, 1990). At doses higher than those used here, naltrindole may express agonistic activity (Jackson et al., 1989). Therefore, the doses of naltrindole that we used were high enough to attenuate any possible component of alcohol drinking by AA rats mediated by δ-opioid receptors and still low enough not to cause any any opioid receptor stimulation.

In contrast to naltrindole, the $\mu_1$-opioid receptor antagonist naloxonazine decreased both alcohol drinking and food intake in AA rats. This is consistent with previous evidence that the i.c.v. administration of the $\mu_1/\mu_2$-opioid receptor antagonist CTOP reduces alcohol drinking in these rats (Hyytiä, 1993). There were, however, also notable differences in the effects of naloxonazine and CTOP. CTOP had no effect on 24-h food and water consumption whereas naloxonazine induced a long-lasting depression of food consumption and also slightly reduced water intake. The strong effect of naloxonazine on food intake is in line with previous reports (Simone et al., 1985; Mann et al., 1988). The difference between the drugs may be due to different routes of administration and duration of action, in addition to differences in subtype selectivity. The elimination rate of i.c.v.-administered CTOP is unknown, but it has been reported that μ-opioid receptor antagonism by i.c.v. CTP (another μ-opioid receptor antagonist differing from CTOP by only one residue; Orn replaced by Lys) lasted less than 7 h in the analgesia test (Shook et al., 1987). Therefore, if duration of μ-opioid receptor blockade by CTOP resembles that of CTP, it is likely that CTOP does not affect food and water intake during the dark phase when given several hours earlier. Systemic naloxonazine blocks central $\mu_1$-opioid receptors irreversibly, thus, being able to antagonize them for well over 24 h (Ling et al., 1986). It should be noted that, during the first few hours after administration, naloxonazine may also cause a reversible blockade of $\mu_2$-opioid receptors, an effect which had likely faded away by the time of the present experimental sessions. Considering the long-lasting reduction of food consumption by naloxonazine, it is not surprising that it also decreased the intake of novel food during the alcohol drinking sessions, here interpreted as a result of $\mu_1$-opioid receptor antagonism.

The present results and those by Hyytiä (1993) might suggest that the regulation of alcohol drinking in the AA rat line is different from that in several other rodent models. In high-alcohol drinking HAD, alcohol-preferring P rats and C57BL/6 mice, for instance, alcohol drinking behaviour is sensitive to blockade of δ-opioid receptors (Froehlich et al., 1991; Lê et al., 1993; Krisnan-Sarin et al., 1995). Since, at present, we are unaware of the neurochemical and genetic factors differentiating the above animal models, it is worth commenting on some experimental differences between studies on AA and other rodent models. In the C57BL/6 mouse study (Lê et al., 1993) and the P rat study (Krisnan-Sarin et al., 1995), the effective dose of naltrindole (10 mg/kg) exceeded our dose range (up to 5 mg/kg), which makes it possible that naltrindole acted as an agonist in these studies (cf. Jackson et al., 1989). Since agonists, such as morphine, and treatments that enhance endogenous opioidergic mechanisms decrease alcohol drinking (Sinclair et al., 1973; Blum et al., 1987; Volpicelli et al., 1991; Kornet et al., 1992), possible agonism by naltrindole could explain their findings. However, the ineffectiveness of a relevant dose of the μ-opioid receptor antagonist β-funaltrexamine suggests that the μ-opioid receptors are not involved in the regulation of alcohol drinking in C57BL/6 mice.

The notion of the involvement of δ-opioid receptors in the regulation of alcohol drinking in HAD rats is based on experiments where systemic ICI 174,864 decreased alcohol drinking (Froehlich et al., 1991). However, due to the peptide structure of ICI 174,864 its access to brain may

have been limited, making it unclear whether its action was centrally or peripherally mediated. When ICI 174,864 was administered centrally to AA rats (Hyytiä, 1993), it was ineffective. Furthermore, ICI 174,864 has been reported to produce neurotoxic, non-opioid receptor-mediated effects (Long et al., 1988), inducing e.g. postural abnormalities that where observed in female AA rats after i.c.v. administration (Hyytiä, 1993). Froehlich et al. (1991) used a modification of the limited access paradigm, where, in addition to alcohol, also the period of water intake was limited to 30 min/day. Although the amount of daily water intake in this paradigm might not have been affected, 23-h water deprivation may still decrease the release of opioid peptides and/or change central opioid receptors (Blake et al., 1987). Therefore, it is possible that fluid deprivation had modified the incentive properties of the water and alcohol solutions given simultaneously, thereby enabling δ-opioid receptor blockade to selectively affect alcohol drinking in HAD rats. Further studies are obviously needed to settle these experimental shortcomings before the rodent models can be firmly differentiated.

While the reducing effect of CTOP and naloxone on alcohol drinking becomes stronger in AA rats when the drug is given in repeated sessions (Sinclair, 1990; Hyytiä, 1993), naloxonazine reduced alcohol drinking only in the 1st session and was ineffective in 2 later sessions (Fig. 2). In contrast, naloxonazine's effect on 24-h water intake appeared to become stronger by repeated treatment. Also, no tolerance developed to the body weight-decreasing effect of naloxonazine during treatment. This suggests that these actions are either mediated by different mechanisms (receptor subtypes) or that alcohol drinking is still preferred even in a situation where food intake is compromised, possibly due to availability of energy from ethanol, which can be readily utilized by AA rats (Eriksson, 1973).

The enduring ability of naloxonazine to decrease water and food intake, while having a transient effect on alcohol drinking, suggests that $\mu_1$-opioid receptors might not be crucially involved in the maintenance of alcohol drinking in AA rats. Since the $\mu_1/\mu_2$-opioid receptor antagonist CTOP persistently decreases alcohol drinking in AA rats (Hyytiä, 1993), it might be the $\mu_2$-opioid receptors that are important for the regulation of alcohol consumption in these rats. This seems unlikely, however, because the elimination half-life of naloxonazine is less than 3 h (Ling et al., 1986), lowering the concentration of free naloxonazine, needed to block this receptor subtype, to a non-significant level within 20 h, i.e. before the start of the alcohol sessions. Likewise, the involvement of $\mu_2$-opioid receptor blockade in reducing 24-h water and food intakes is also unlikely since rats can compensate on a daily basis for any decrease in feeding and drinking induced by opioid receptor blockade after the drug effect has been eliminated (Jalowiec et al., 1981). Therefore, it seems likely that the decreases in 24-h intakes observed after naloxonazine treatment result from $\mu_1$-opioid receptor antagonism.

Prolonged $\mu_1$-opioid receptor blockade by naloxonazine may alter behaviour differently from repeated short-term blockades by reversible antagonists. Naloxonazine-treated animals increased their alcohol drinking above control levels in a posttreatment session after saline administration. 3-day continuous naloxone infusion, a treatment corresponding in length to our naloxonazine treatment, has been shown to produce supersensitive $\mu$-opioid receptors (Millan et al., 1988). We suggest here that the naloxonazine treatment induced supersensitivity of $\mu_1$-opioid receptors, apparently supported by the naloxonazine prevention of the increase in alcohol drinking especially during the 3rd treatment session. The rebound increase in alcohol drinking observed in our rats during the 4th posttreatment session may, thus, have been due to stronger reinforcement induced by alcohol via supersensitive $\mu_1$-opioid receptors already recovered from the blockade.

It is still possible that both $\mu_1$- and $\mu_2$-opioid receptors must be blocked in the brains of AA rats in order to achieve a persistent decrease in alcohol reinforcement. This would be somewhat surprising, however, since the $\mu_1$-opioid receptor has been proposed to be the common high-affinity binding site for endogenous opioid peptides and exogenous compounds, such as morphine, and since the affinity of endogenous ligands for the $\mu_2$-opioid receptor is significantly weaker (Pasternak and Wood, 1986). The $\mu_1$-opioid receptor site is implicated in the actions of morphine related to feeding behaviour, analgesia and reward (Simone et al., 1985; Mann et al., 1988; Piepponen et al., 1994). Therefore, if alcohol accelerates the release of endogenous opioid peptides, one would expect the $\mu_1$-opioid receptor to be their most likely site of action. Unfortunately, at present, the assessment of the relative contributions of $\mu$-opioid receptor subtypes to the regulation of alcohol drinking behaviour awaits the availability of $\mu_2$-opioid receptor-selective antagonists.

In conclusion, antagonism of δ-opioid receptors by effective systemic naltrindole doses does not affect the regulation of alcohol drinking in the alcohol-preferring AA rats. Furthermore, while the $\mu_1$-opioid receptor subtype of the AA rats may be involved in the regulation of alcohol intake, it appears that its blockade alone is insufficient to induce a sustained reduction in alcohol drinking.

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