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Ethanol-reinforced behaviour in the rat: effects of naltrexone

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Abstract

It has been repeatedly reported that endogenous opioid pathways play an important role in ethanol drinking behaviour. In line with these findings, a non-selective opioid receptor antagonist, naltrexone, seems to reduce relapse rates in detoxified alcoholics. The aim of the present study was to evaluate the effects of naltrexone on (i) ethanol self-administration; (ii) extinction of responding for ethanol; (iii) reinstatement of ethanol-seeking induced by non-contingent presentations of ethanol-associated stimuli. Male Wistar rats were trained to lever-press for 8% ethanol in an operant procedure where ethanol was introduced in the presence of sucrose. The selectivity of naltrexone's actions was assessed by studying its effects on water-reinforced behaviour in separate control experiments. Acute injections of naltrexone (1 or 3 mg/kg) did not alter ethanol self-administration. Repeated treatment with naltrexone (3 mg/kg, before three consecutive self-administration sessions) progressively reduced ethanol intake. In the extinction procedure, acute administration of 3 mg/kg naltrexone suppressed responding previously reinforced with ethanol. Similarly, naltrexone (1–3 mg/kg) potently and dose-dependently inhibited reinstatement of ethanol-seeking produced by non-contingent deliveries of the liquid dipper filled with 8% ethanol. In the control experiments, lower doses of naltrexone (1–3 mg/kg) did not exert any effect on either reinforced or non-reinforced (extinction) lever-pressing for water. These results indicate that: (i) subchronic treatment with naltrexone leads to progressive reduction of ethanol self-administration; (ii) single doses of naltrexone may increase extinction and attenuate cue-induced reinstatement of ethanol-reinforced behaviour.

Keywords: Ethanol self-administration; Extinction; Reinstatement; Relapse; Opioid receptor; Naltrexone; (Rat)

1. Introduction

A large body of evidence indicates that various rewarding stimuli, including ethanol, may enhance the activity of the endogenous opioid system (for review, see Van Ree et al., 1994; Gianoulakis, 1996; Herz, 1997). Thus, it has been hypothesised that ethanol reward is mediated, at least in part, by the increase in the opioid activity. In line with this notion, non-selective opioid receptor antagonists (e.g., naloxone or naltrexone) attenuated ethanol consumption in laboratory animals tested in both limited and continuous access paradigms (Myers et al., 1986; Volpicelli et al., 1986; Iso and Brush, 1991; Myers and Lankford, 1996; for review, see Herz, 1997). Moreover, naltrexone decreased ethanol intake and promoted abstinence (i.e., prevented relapse to heavy drinking) in early problem drinkers and alcohol addicts, respectively (O'Malley et al., 1992; Volpicelli et al., 1992; Kranzler et al., 1997). The above findings have led to the suggestion that administration of opioid antagonists prior to drinking episodes may attenuate at least some rewarding effects of ethanol, thus producing extinction of ethanol drinking behaviour (Hyytiä and Sinclair, 1993; Spanagel and Zieglgänsberger, 1997; Sinclair, 1998).

Naltrexone has been also reported to block the desire ('craving') to consume alcohol in detoxified alcohol addicts (Volpicelli et al., 1992). In social drinkers, naltrexone increased latency to sip the first and the second drink in the cocktail bar situation. Thus, naltrexone might diminish urge to drink elicited by alcohol-associated cues (Davidson et al., 1996). Concluding, it is possible that the drug attenuates both the unconditioned and conditioned reinforcing effects of ethanol.

Conditioned aspects of drug reinforcement may be studied in the extinction procedure (Markou et al., 1993; Piasecki et al., 1998). Operant behaviour in the extinction procedure is thought to be initiated and maintained by

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drug-related conditioned stimuli (for review, see Bouton and Swartzentruber, 1991). This procedure provides several measures of the incentive-motivational effects of drug-associated stimuli by assessing the persistence of operant behaviour, e.g., lever-pressing, in the absence of primary drug reinforcement. Extinction sessions are identical to self-administration sessions except that drug is not available after completion of the operant response requirement. The total number of responses during a session is typically used to assess the intensity of 'experimental craving' (Markou et al., 1993; Piasecki et al., 1998).

An animal model that is thought to address mechanisms of relapse to drug-seeking is the reinstatement paradigm (Self and Nestler, 1998). In this paradigm, reinstatement to drug-seeking after extinction is primed by non-contingent drug injections, application of stress or presentation of drug-associated cues (for review, see Carroll and Comer, 1996). Using the reinstatement paradigm, Chiamulera et al. (1995) have shown resumption of ethanol-seeking in rats receiving small amounts of ethanol solution contingent upon lever-pressing. Lê et al. (1998) have found that footshock stress and, to a lesser extent, i.p. injections of ethanol reinstated lever-pressing after prolonged extinction. Both groups have used between-session reinstatement procedure with long-term extinction (4–10 daily sessions) preceding delivery of the priming stimuli. Recently, using a within-session procedure, we have shown that non-contingent presentations of ethanol-associated stimuli produced reliable reinstatement of ethanol-seeking after extinction (Bienkowski et al., in press).

The aim of the present study was to evaluate the effects of naltrexone on both unconditioned and conditioned aspects of ethanol reinforcement. First, the effects of naltrexone on alcohol self-administration were assessed. Second, the effects of naltrexone on ethanol-motivated responding in the extinction procedure were evaluated. The actions of naltrexone were also examined in the reinstatement paradigm where ethanol-seeking was primed by ethanol-associated stimuli. To our knowledge, this is the first study in which naltrexone was tested in the extinction and reinstatement procedure in rats learned to respond for ethanol.

The doses of naltrexone used in the present study were selected on the basis of previous experiments (Volpicelli et al., 1986; Hill and Kiefer, 1997). To address the problem of the selectivity of naltrexone's actions, separate control experiments were conducted (Piasecki et al., 1998). In the control experiments, the effects of the drug on water reinforcement were studied.

2. Materials and methods

2.1. Subjects

Male Wistar rats (360–400 g at the beginning of the study) were housed two per standard plastic cage ($25 \times 40 \times 20$ cm, $W \times L \times H$). The animals were supplied by a licensed breeder (HZL, Warsaw, Poland) 14 days before the start of water or ethanol self-administration experiments. The rats were kept under standard laboratory conditions at 22 ± 1°C, 60% humidity and a 12-h light–dark cycle (light on at 0600 h). Food (Bacutil, Poland) was always available ad libitum. Treatment of the rats in the present study was in a full accordance with the ethical standards laid down in respective European and Polish regulations.

2.2. Apparatus

Operant responding for ethanol or water (oral self-administration) was tested in commercially available chambers (Coulbourn Instruments, Allentown, PA, USA). The chambers (for detail, see Bienkowski et al., 1997b) consisted of test cages enclosed within sound-attenuating cubicles with fans for ventilation and background white noise. A white house light was centred near the top of the front of the cage. The start of experimental sessions was signalled by turning the house light on. The cage was also equipped with two response levers separated by a liquid delivery system (the liquid dipper; Coulbourn). Only one lever ('active' lever) activated the dipper. Presses on the other lever ('inactive' lever) were recorded but not reinforced. During a self-administration session, the liquid delivery system presented ethanol in a 0.1-ml portion for 5 s. The availability of reinforcer was signalled by a brief audible click and a small white light (4 W) located inside the liquid dipper hole. Programming of every sessions as well as data recording made use of the L2T2 Software package (Coulbourn) running on an IBM-compatible PC.

2.3. Water-reinforced behaviour—control experiments

2.3.1. Water self-administration

To facilitate lever-pressing for water, the rats ($n = 8$) were deprived of water throughout the course of the experiment. Their access to tap water in the home cages was limited to 2 h/day and started $\sim$30 min after the end of an operant session. The subjects were trained to lever-press on a fixed ratio 1 (FR1) schedule of water reinforcement (0.1 ml/response) in the 30-min daily sessions. All rats learned to self-administer water within the first 4 days of the experiment. After additional six to seven sessions, water intake stabilised and test sessions with naltrexone were initiated. Naltrexone (1, 3 or 10 mg/kg, i.p.) or its vehicle was administered, in a balanced order, 30 min before the start of the test session in randomly selected groups of six to eight subjects. In order to be tested in each subsequent test session, the rat had to show stable ($\pm 20\%$) responding for at least three consecutive drug-free sessions.

2.3.2. Extinction procedure

The effects of naltrexone were also studied in the extinction procedure. The extinction sessions were identical to the training/test sessions except that no water was delivered after the rats responded on the 'active' lever. The liquid delivery system was off and the stimuli associated with dipper presentation were absent. Naltrexone (1 or 3 mg/kg) or its vehicle was injected as described above.

2.4. Ethanol-reinforced behaviour

2.4.1. Ethanol self-administration

The rats ($n = 16$) were trained to respond to ethanol according to the Samson's sucrose-fading procedure (Samson, 1986; Files et al., 1997) with some minor modifications (for details, see Piasecki et al., 1998). The animals were deprived of water for 22 h/day during the first 4 days of training and shaped to lever-press for 10% sucrose solution on a fixed ratio (FR1) schedule of reinforcement. As soon as lever-pressing was established, water started to be freely available in the home cages. All training sessions were 30 min long and one session was given each day. Starting on day 5, the animals received 2% ethanol–10% sucrose. Then, over the next 10–14 sessions, ethanol concentrations were gradually increased (from 2 to 8%, v/v) and sucrose concentrations were decreased (from 10 to 0%). The rats were allowed to stabilise their 8% ethanol consumption for at least 45 days.

In the experiment with acute injections of naltrexone, the rats were injected, in a balanced order, with the drug (1 or 3 mg/kg, i.p.) or its vehicle 30 min before the start of the test session. Each dose was tested in randomly selected group of six to eight rats.

In the experiment with subchronic naltrexone administration, the rats were randomly assigned to one of two experimental groups ($n = 8$ rats/group). The subjects in the control group received saline injection 30 min before each of nine consecutive sessions. The naltrexone-treated animals were administered with saline before the sessions 1–3 and 7–9, and with 3 mg/kg naltrexone before the sessions 4–6.

In order to be tested in the subsequent test session the subject had to show stable ($\pm 20\%$) responding for at least three consecutive drug-free sessions.

2.4.2. Extinction procedure

The effects of acute naltrexone treatment on lever-pressing for ethanol were also studied in the extinction procedure. The extinction sessions were identical to the training/test sessions except that no ethanol was delivered after the rats responded on the 'active' lever. The liquid delivery system was off and the stimuli associated with each ethanol delivery were absent. Naltrexone (1 or 3 mg/kg) or its vehicle was injected as described above.

2.4.3. Reinstatement procedure

A within-session design was used to study reinstatement to ethanol-seeking after extinction. Importantly, we have previously shown that extinction of ethanol-reinforced responding is relatively rapid and that almost no lever-pressing occurs in the last 10 min of the 30-min extinction session (Bienkowski et al., 1999). The reinstatement sessions lasted 30 min. The animals were first allowed to lever-press in extinction for 20 min. The liquid delivery system was off during this period. Then, within the next 6–8 min, an ethanol-associated stimulus complex was repeatedly delivered (15×7.5 s) according to a random time 15 s schedule (RT15 s). The stimulus complex included brief audible click associated with the activation of the liquid dipper and illumination of the light located inside the dipper hole. The dipper cup was filled with 8%

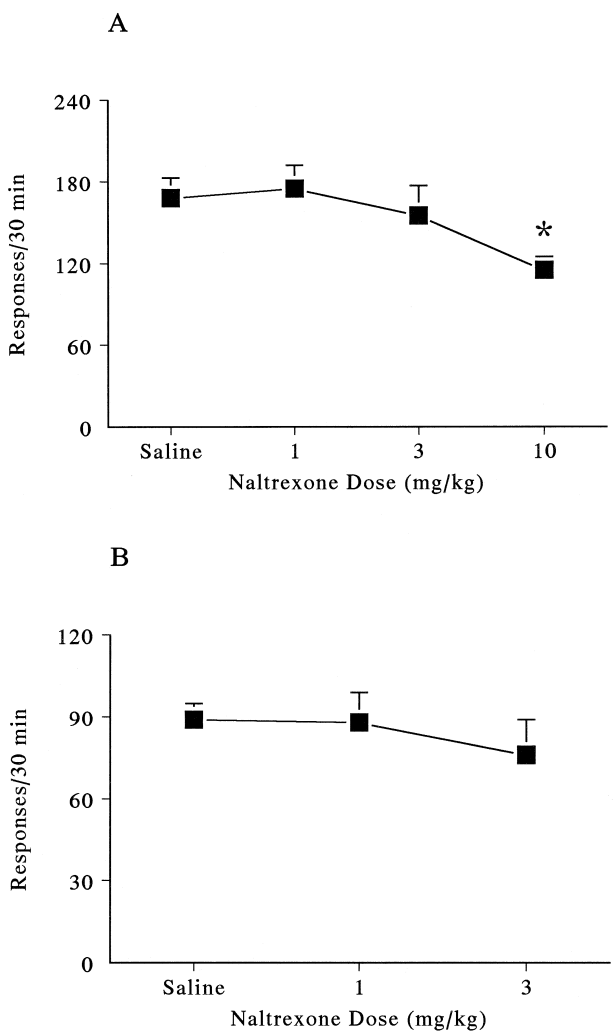

Fig. 1. Effects of acute naltrexone treatment on operant responding for water during self-administration sessions (water reinforcement present) (A) and extinction sessions (water reinforcement absent) (B). Results are expressed as the mean (with SEM) number of responses on the 'active' lever in 30-min session; $n = 6$–8 rats; $^*P < 0.05$ vs. saline-treated subjects.

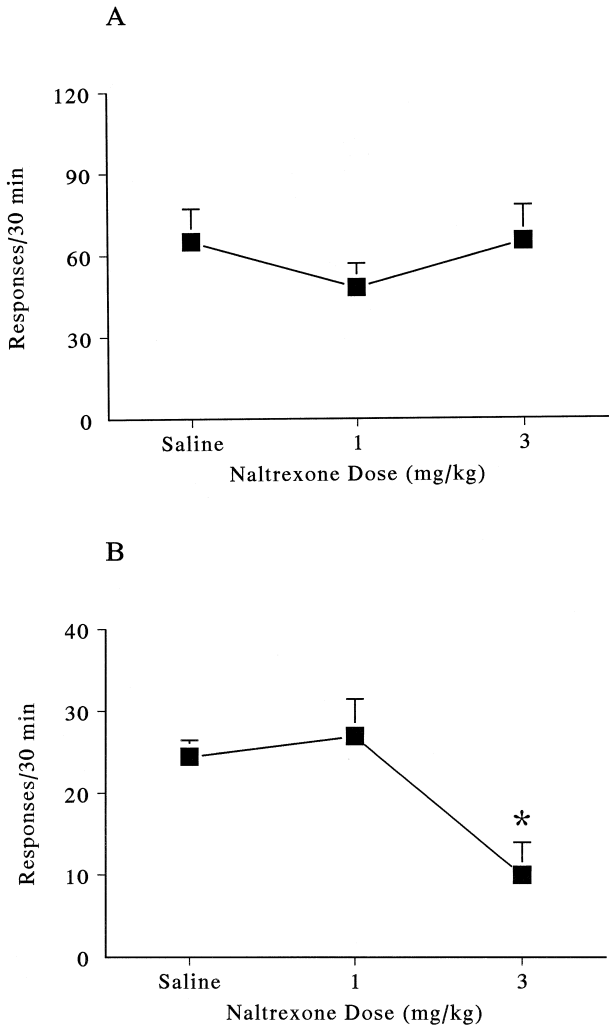

Fig. 2. Effects of acute naltrexone treatment on operant responding for ethanol during self-administration sessions (ethanol reinforcement present) (A) and extinction sessions (ethanol reinforcement absent) (B). Results are expressed as the mean (with SEM) number of responses on the 'active' lever in 30-min session; $n = 6–8$ rats; $^*P < 0.05$ vs. saline-treated subjects.

ethanol. Following the non-contingent stimulus complex presentations, the extinction conditions were maintained to the end of the session. The ethanol intake during the reinstatement session could be estimated by monitoring interruptions of a photocell located inside the dipper hole.

Naltrexone (1 or 3 mg/kg, i.p.) or its vehicle was administered, in a balanced order, in randomly selected groups of 6–8 subjects. In order to be tested in each subsequent test session, the subject had to show stable ($\pm 20\%$) responding for at least three consecutive drug-free sessions. As in the reinstatement session, naltrexone should have exerted its effects ~ 20 min after the start of the session (i.e., when the stimulus presentations started); the pre-treatment time was shortened to 10 min.

The above schedule of the dipper presentation has been shown to produce robust reinstatement of ethanol-seeking (Bienkowski et al., in press). To further confirm that significant priming effects were obtained in the present study, a group of control subjects received saline 10 min before the 30-min extinction session. Accordingly, these rats were presented with the sham stimuli (no stimulus presentations). The number of presses during the last 10 min of the session was treated as control behaviour induced by sham dipper deliveries.

2.5. Drugs

Ethanol solutions (v/v) were prepared daily from a 95% stock solution and tap water. Naltrexone HCl (RBI, Natick, MA, USA) was dissolved in sterile physiological saline and administered in a volume of 1 ml/kg. The doses of naltrexone referred to the salt form. All solutions were prepared immediately prior to use.

2.6. Statistics

A one- or two-way analysis of variance (ANOVA) with repeated measures where appropriate was used to compare the data. Newman–Keuls test was used for post-hoc comparisons. Student's *t*-test was employed when the data from only two groups were compared.

3. Results

3.1. Water-reinforced behaviour—control experiments

Naltrexone altered lever-pressing for water as revealed by a significant effect of Treatment [$F(4,24) = 16.52$, $P < 0.001$]. Post-hoc analysis indicated that only the highest dose of naltrexone (10 mg/kg) significantly decreased

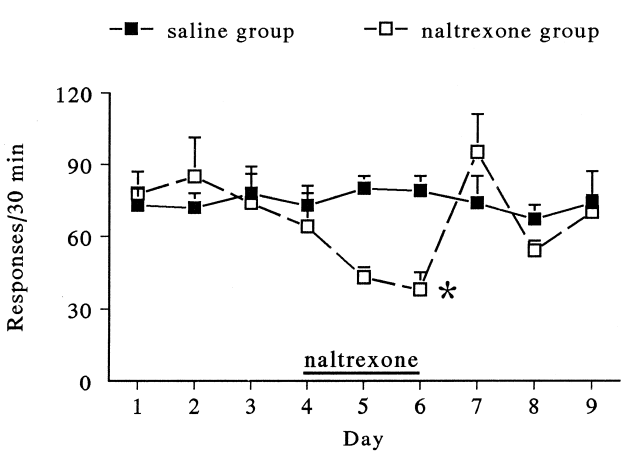

Fig. 3. Effects of repeated naltrexone treatment on ethanol self-administration. Saline group received saline before nine consecutive sessions (Days 1–9). Naltrexone group was injected with saline before sessions 1–3 and 7–9, and with 3 mg/kg naltrexone before sessions 4–6 (marked on the graph with a horizontal line). Results are expressed as the mean (with SEM) number of responses on the 'active' lever in 30-min session; $n = 8$ rats; $^*P < 0.05$ vs. saline group.

water self-administration (Fig. 1A). Operant behaviour in the extinction procedure was not affected by naltrexone (1–3 mg/kg) [$F < 1$, $P = 0.58$; Fig. 1B].

3.2. Ethanol-reinforced behaviour

The baseline number of ethanol deliveries ranged from 45 to 90 dipper deliveries/30 min, with individual ethanol intakes of 0.5–0.8 g/kg/30 min. Typically, more than 75% of the ethanol solution was consumed within the first 10 min of the session.

Acute injections of naltrexone (1 or 3 mg/kg) did not alter operant responding for ethanol [$F(2,19) = 0.64$, $P = 0.53$; Fig. 2A].

Repeated administration of the drug before three consecutive self-administration sessions led to progressive decrease in the ethanol consumption. The ANOVA showed a significant effect of Days [$F(8,112) = 2.42$, $P < 0.05$], and a significant Days × Treatment interaction [$F(8,112) = 3.28$, $P < 0.01$]. Post-hoc analysis revealed that the effects of naltrexone reached significance on the third day of treatment (Day 6; Fig. 3).

Naltrexone decreased responding to ethanol in the extinction procedure [$F(2,20) = 5.13$, $P < 0.05$]. Post-hoc analysis revealed that single administration of 3 mg/kg naltrexone significantly suppressed lever-pressing in extinction (Fig. 2B).

Non-contingent presentations of the ethanol-associated stimulus complex potently reinstated ethanol-seeking after extinction ($P < 0.01$ vs. the sham presentations; *t*-test). The ANOVA revealed that naltrexone attenuated reinstatement of ethanol-seeking [$F(2,18) = 6.19$, $P < 0.05$]. Post-hoc analysis indicated that the higher dose of the opioid antagonist (3 mg/kg) significantly reduced resumption of responding (Fig. 4).

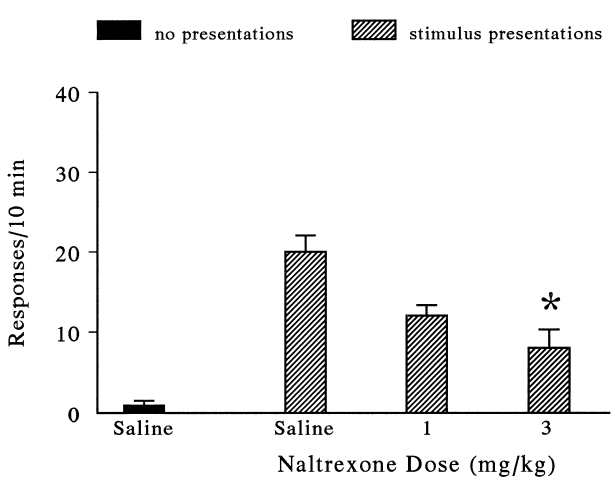

Fig. 4. Reinstatement of ethanol-seeking induced by 15 non-contingent presentations of the liquid dipper containing 8% ethanol as a function of naltrexone dose (hatched bars). For comparison, the effects of sham stimuli (no stimulus deliveries) are shown (black bar). Stimulus presentations started after 20-min extinction. Results are expressed as the mean (with SEM) number of responses on the previously 'active' lever in the last 10 min of the 30-min reinstatement session; $n = 6–8$ rats; $^*P < 0.05$ vs. subjects pre-treated with saline and presented with ethanol-associated stimuli.

The mean number of 'inactive' lever presses in all the above experiments was negligible and did not exceed 1.5 presses/30 min (data not shown).

4. Discussion

The results of the control experiments revealed that 10 mg/kg naltrexone suppressed water self-administration. Accordingly, the subjects responding for ethanol were not administered with the highest dose of the drug. The lower doses of the opioid antagonist (1–3 mg/kg) did not affect either water self-administration or responding for water in extinction.

The ethanol consumption and the pattern of ethanol drinking in the present experiments were comparable with those of previous studies (Files et al., 1997; Piasecki et al., 1998). The acute injections of naltrexone did not alter ethanol self-administration. However, the subchronic treatment with the drug progressively suppressed ethanol-taking behaviour. Some authors have found suppression of ethanol consumption after single doses of opioid antagonists in rats drinking ethanol in non-operant procedures (Herz, 1997; Hill and Kiefer, 1997). In contrast, Hyytiä and Sinclair (1993) have reported that only prolonged treatment with naloxone led to decreases in ethanol intake in rats responding for ethanol in the operant procedure. Our results seem to corroborate these latter findings. It has been suggested (e.g., Sinclair, 1998) that administration of naltrexone attenuates some primary reinforcing effects of ethanol, thus leading to extinction of alcohol drinking. However, other authors using non-operant procedures did not find any extinction of ethanol-drinking behaviour after prolonged naltrexone administration (Davidson and Amit, 1997; Phillips et al., 1997).

Progressive decrease in ethanol drinking found in the present study might result from accumulation of naltrexone. In rats, naltrexone has a mean elimination half-life of 4.6 h (Yoburn et al., 1986) but the possibility exists that the drug occupies central opioid receptors for a longer period of time. Notably, Lee et al. (1988) have reported that the half-time blockade of brain opioid receptors by naltrexone ranged from 72 to 108 h in humans.

Interestingly, as in the study by Hill and Kiefer (1997), ethanol self-administration quickly returned to control levels with discontinuation of the opioid antagonist. As a matter of fact, some individual rats consumed unusually high amounts of ethanol the day after the last naltrexone injection (Day 7; Fig. 3). In this respect, the present data support the results of Gardell et al. (1996) who observed rebound effect after discontinuance of chronic naltrexone in rats drinking ethanol in a limited access procedure.

Opioid receptor antagonists may possess some intrinsic aversive properties as shown by the conditioned place aversion paradigm (Herz, 1997). Moreover, naloxone has been found to enhance aversive actions of ethanol in C57BL/6J mice (Broadbent et al., 1996). Thus, one could hypothesise that repeated pairings of naltrexone injections with ethanol consumption would result in development of conditioned aversion to ethanol taste. The development of conditioned taste aversion might explain progressive decrease in ethanol intake after chronic treatment with opioid antagonists (Hyytiä and Sinclair, 1993; present study). However, this explanation seems to be rather unlikely as in the present and other studies (Gardell et al., 1996; Hill and Kiefer, 1997), ethanol consumption quickly reached baseline levels after termination of naltrexone administration. In contrast, conditioned aversion to gustatory cues is a persistent phenomenon lasting weeks or even months (Hunt and Amit, 1987; Bienkowski et al., 1997a).

The acute administration of 3 mg/kg naltrexone decreased lever- pressing for ethanol in the extinction procedure. As responding in extinction is cued and maintained by ethanol-associated context stimuli (Bouton and Swartzentruber, 1991), the above finding suggests that naltrexone attenuates some learned aspects of ethanol reinforcement. Notably, the same single dose of the opioid antagonist did not alter ethanol self-administration. However, responding in the self-administration session might be more directly controlled by discrete ethanol-associated cues (e.g., taste and smell). Even if naltrexone eliminated some of the primary and secondary reinforcing effects of ethanol, these cues were still sufficiently strong to maintain responding during a single session.

In agreement with our previous results (Bienkowski et al., in press), the non-contingent, random presentations of the ethanol-associated stimulus complex (i.e., the dipper filled with 8% ethanol) reinstated lever-pressing previously reinforced with ethanol. Interestingly, deliveries of the empty dipper did not produce any reinstatement (Bienkowski et al., in press). Accordingly, we hypothesised that it was mainly taste and/or smell of 8% ethanol which primed operant responding after extinction. However, although the consumption of ethanol during the reinstatement sessions was very low (< 0.15 g/kg), the possibility existed that some central effects of ethanol were sufficiently strong to prime ethanol-seeking.

In accord with the results from the extinction procedure, the resumption of ethanol-seeking was significantly and dose-dependently decreased by naltrexone. The effects of naltrexone in the extinction and the reinstatement procedure may be considered relatively selective as 3 mg/kg naltrexone did not alter any parameters of water-reinforced behaviour in the control animals. Besides, as discussed above, the acute administration of 3 mg/kg naltrexone did not influence ethanol self-administration.

The results from both the extinction and the reinstatement procedure seem to agree with the data published recently by Cunningham et al. (1998). In this latter study, naloxone selectively accelerated extinction of ethanol's conditioned rewarding effects in the conditioned place preference paradigm. Notably, naltrexone has been also suggested to reduce conditioned reinforcing effects of ethanol in social drinkers (Davidson et al., 1996).

A number of studies have shown that acute ethanol administration increases release of endogenous opioids in different regions of the mammalian brain (Van Ree et al., 1994; Gianoulakis, 1996; Herz, 1997). Interestingly, consumption of placebo instead of expected alcohol drink has led to naloxone-reversible analgesia in experienced alcohol drinkers (Cutter and O'Farrell, 1987). This latter result seems to indicate that opioid peptides may be released not only in response to alcohol but also to alcohol-associated stimuli. Therefore, it is possible, that in the present study, ethanol-paired conditioned cues maintained lever-pressing in the extinction and/or the reinstatement procedure by an enhancement of opioid transmission. If this is true, one could suggest that naltrexone suppressed the conditioned reinforcing effects of ethanol by altering consequences of this learned opioid activation (Spanagel and Zieglgänsberger, 1997). Certainly, further studies are needed to evaluate this hypothesis.

Taken together, the results from the present study seem to indicate that acute administration of naltrexone, even at the doses which do not affect ethanol self-administration, reduces some conditioned aspects of ethanol reinforcement. These effects of naltrexone might be expressed as decreased motivation to drink and reduced probability of relapse in human alcoholics (Volpicelli et al., 1992; Davidson et al., 1996; Spanagel and Zieglgänsberger, 1997).

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