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ORIGINAL INVESTIGATION

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Kappa-opioid receptors and relapse-like drinking in long-term ethanol-experienced rats

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Abstract *Rationale:* The role of the dynorphin/κ-opioid receptor system in ethanol reinforcement is unclear. *Objective:* Examination of the effects of the highly selective κ-opioid receptor agonist CI-977 (enadoline) and of the long-acting selective κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI) on relapse-like drinking measured by the alcohol deprivation effect (ADE) in long-term ethanol-experienced rats. *Methods:* Rats were either implanted with mini-osmotic pumps delivering 0 or 0.01 mg/kg per h CI-977 or received two injections (12 h apart) of nor-BNI (0 or 5 mg/kg i.p.) before representation of alcohol after 2 weeks of alcohol deprivation in a four-bottle home cage drinking paradigm. In a second experiment, long-term ethanol-experienced rats trained in an operant ethanol self-administration paradigm received either acute CI-977 treatment (0, 0.003–0.1 mg/kg i.p.) or two injections (12 h apart) of nor-BNI (0 or 5 mg/kg i.p.) before a 23-h session. *Results:* Chronic CI-977 potentiated ethanol intake and preference during the ADE. Acute CI-977 dose-dependently reduced total lever pressing activity demonstrating an unspecific sedative effect, except for the lowest dose (0.003 mg/kg), which selectively increased lever pressing for ethanol during basal drinking. Nor-BNI did not affect relapse-like drinking at all. *Conclusions:* Stimulation of κ-opioid receptors can increase ethanol intake, at least in long-term ethanol-experienced rats. Since κ-opioid receptor agonists have aversive motivational consequences, increased ethanol drinking might be an attempt to counteract the aversive effects of this treatment. On the other hand, the nor-BNI experiments indicate that endogenous κ-opioid receptor stimulation does not seem to be involved in relapse-like drinking after protracted abstinence.

Keywords Alcohol deprivation effect · Enadoline (CI-977) · κ-opioid receptor · Nor-binaltorphimine (nor-BNI) · Relapse · Voluntary ethanol self-administration

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Introduction

Numerous studies have shown that endogenous opioid systems are involved in the mediation of ethanol reinforcement (Gianoulakis 1990; Froehlich and Li 1994; Gianoulakis et al. 1996; Herz 1997). It is assumed that at least a part of the rewarding effect of ethanol is mediated by the activation of μ-, and possibly also δ-opioid receptors, which in turn reinforces ethanol intake. The role of the dynorphin/κ-opioid receptor system in ethanol reinforcement is less clear. In contrast to μ- and δ-opioid receptor stimulation, κ-opioid receptor stimulation has aversive motivational consequences in animals (Mucha and Herz 1985) and humans (Pfeiffer et al. 1986). These opposing motivational effects are probably mediated by the mesolimbic dopamine system which is assumed to mediate the reinforcing effects of drugs of abuse (Wise and Bozarth 1987; Spanagel and Weiss 1999). Thus, activation of μ-/δ-opioid receptors in the ventral tegmental area disinhibits dopamine neurons, resulting in an increase of dopamine release in the nucleus accumbens, which is rewarding. In contrast, activation of κ-opioid receptors in the nucleus accumbens results in a decrease of dopamine release in the nucleus accumbens, which is aversive (Spanagel et al. 1990, 1992; Bals-Kubik et al. 1993).

To further elucidate the role of the dynorphin/κ-opioid receptor system in ethanol reinforcement, the present study investigated the effects of the highly selective κ-opioid receptor agonist CI-977 (enadoline; Hunter

et al. 1990) and of the extremely long-acting selective κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI; Portoghese et al. 1987) on the alcohol deprivation effect (ADE) in long-term ethanol-experienced rats. The term ADE designates the phenomenon of a transient increase in ethanol consumption and preference after a period of imposed ethanol abstinence which has been observed in several species including humans (Sinclair and Senter 1967; Sinclair 1971; Burish et al. 1981). We described previously that the drug taking behaviour of long-term ethanol-experienced rats during the ADE is characterised by changes in intake patterns resulting in an immediate increase in consumption of highly concentrated ethanol solutions in a four-bottle free-choice home cage drinking paradigm (Spanagel et al. 1996; Hölter et al. 1998). In addition, an operant ethanol self-administration paradigm with weekly 23-h sessions was developed to match the characteristics of the drinking behaviour in the home cage paradigm. Thus, drinking behaviour is comparable in both, paradigms both under baseline drinking conditions and after alcohol deprivation (Spanagel et al. 1996; Hölter et al. 1997, 1998; Hölter and Spanagel 1999b; for reviews see Spanagel and Hölter 1999, 2000). Because the computer-controlled operant paradigm allows the detailed analysis of drinking behaviour in small time intervals, this paradigm is particularly suitable for detecting short-lasting drug effects. In comparison, the home cage paradigm is more suitable for studying long-lasting drug effects or the effects of chronic treatment, because it allows prolonged observations for consecutive days.

CI-977 has a very short duration of action (Hunter et al. 1990), therefore this drug was given chronically by osmotic mini-pumps in the present study in order to match the inevitable chronicity of nor-BNI effects after acute injection due to its extremely long-lasting duration of action (Endoh et al. 1992; Spanagel et al. 1994); effects of these drug treatments were studied in the home cage paradigm. Because effects of acute and chronic treatment may differ, the effects of acute CI-977 treatment were studied in the operant paradigm. Nor-BNI was also studied in the operant paradigm, because it did not yield any effect in the home cage paradigm. Therefore the operant paradigm, where even small or transient effects would be detected, was used to ensure this lack of effect.

Materials and methods

Subjects

Sixty-one male Wistar rats (Max Planck Institute of Biochemistry, Martinsried, Germany) weighing 220–250 g at the beginning of the experiment, were used in this study. All animals were housed individually in standard hanging rodent cages with food and water ad libitum. Artificial light was provided daily from 7:00 a.m. until 7:00 p.m. and room temperature and humidity were kept constant (temperature: 23±1°C; humidity: 60±5%). The experiments were approved by the Committee on Animal Care and Use of the relevant local governmental body and carried out following the German Law on the Protection of Animals.

Long-term ethanol self-administration (four-bottles paradigm)

After 1 week of habituation to the animal room, all rats were given continuous access to tap water and to 5%, 10% and 20% (v/v) ethanol solutions in their home cages. Spillage and evaporation were minimised by the use of bottle caps with ball bearings (Ehret, Emmendingen, Germany). With this procedure the ethanol concentration in a given solution stayed constant for at least 1 week (see Hölter et al. 1998). All drinking solutions were renewed weekly and at that time the positions of the four bottles were changed to avoid location preferences. After 8 weeks of continuous access, ethanol solutions were repeatedly withdrawn for 3 days (deprivation phase) every 4 weeks until the rats were used in the experiments as described below.

Experiment 1: home cage drinking paradigm

The effects of chronic CI-977 and the long-acting drug nor-BNI on the ADE were determined in 31 rats with 16–18 months of ethanol experience in the long-term four-bottles paradigm described above. Baseline measures were determined by daily weighing of the bottles, the food and the animals at 10:00 a.m. for 3 pre-abstinence days. Daily ethanol intake, food intake, weight changes, total fluid intake, total ethanol preference and preferences for the three ethanol solutions were calculated from these measurements. Total ethanol preference was calculated as the percentage share of the sum of consumption from the three ethanol solutions in total fluid consumption, and the preference for a particular ethanol concentration was calculated as the percentage share of consumption from this ethanol solution in total fluid consumption. After the last day of measurement the ethanol bottles were removed from the cages leaving the animals with food and tap water ad libitum. Ten days later, animals were either briefly anaesthetised with halothane and mini-osmotic pumps (Alzet, model number 2ML2, pumping rate 5 µl/h for 2 weeks) either filled with saline (n=8) or with CI-977 (0.01 mg/kg per h, n=8) were implanted subcutaneously, or animals were injected with either saline (n=8) or nor-BNI (5 mg/kg i.p., n=7) at 7:00 a.m. and a second time at 7:00 p.m. Four days after surgery or the first injection, respectively, the ethanol solutions were presented again to the animals at 10:00 a.m. and the daily weighing routine was reintroduced for 3 post-abstinence days to assess the ADE. Because these experiments were part of a bigger study concerning the effects of chronic opiate antagonist treatment with animals matched for age and ethanol experience, data of the saline control group implanted with mini-osmotic pumps has already been published (Hölter and Spanagel 1999a). Drinking behaviour of control groups after this kind of treatment (2 weeks of alcohol deprivation, implantation of mini-osmotic pumps) is well documented and essentially the same results have been obtained in previous studies (Hölter et al. 1996, 2000a).

Experiment 2: operant self-administration paradigm

Apparatus

Eight operant chambers (Med Associates, Georgia, Vt., USA) situated in sound-attenuating cubicles with background noise provided by a fan were used for the second experiment. Each chamber was equipped with a house light, two levers (one on each side of the chamber), two liquid dispensers adjacent to the levers and a food rack at the back wall. Pressing one lever resulted in the delivery of a drop of tap water and pressing the other in the delivery of a drop of 20% (v/v) ethanol. The volume per drop was 25–30 µl. The chambers were controlled and data automatically recorded by a personal computer.

Training and testing procedure

Effects of acute CI-977 treatment on the ADE were determined in an additional set of 16 rats with 10–12 months of ethanol experience. After 5 months of ethanol experience in the long-term, four-

bottles paradigm described above, the operant chamber sessions began. All sessions were started at 10:00 a.m. and lasted 23 h. During all sessions water and 20% ethanol were concurrently available on an FR1FR1 schedule and food was available ad libitum from the food rack. The house light in the chambers was turned off at 7:00 p.m. and on at 7:00 a.m. to keep the animals in their regular light/dark cycle. The animals were tested in the chambers once a week. They remained undisturbed in their home cages during the rest of the week.

All rats were liquid-deprived for 24 h before their first session in the operant chambers. The first session served as the shaping session, during which all rats learnt to lever press for liquid. Thereafter the animals were never liquid-deprived again. Sessions were continued until drinking behaviour and total lever pressing activity were stable in all animals before testing. All animals had equal numbers of training sessions before they were tested 15 min after the i.p. administration of either 0, 0.003, 0.01, 0.03 or 0.1 mg/kg CI-977 in a counterbalanced design. Because CI-977 strongly increased ethanol drinking during the ADE in experiment 1 contrary to our expectations, in experiment 2 also a baseline drinking group was included to test whether CI-977 affected only relapse-like drinking or also baseline drinking. The "basal" group (n=7) always had access to ethanol in their home cages (four bottles), thus representing baseline ethanol drinking conditions in the operant chamber sessions. The "ADE" group (n=7) had only access to ethanol in the operant chambers during their weekly session.

In an additional experiment, effects of nor-BNI on the ADE were determined in an additional set of 16 rats with 20 months of ethanol experience. Animals were divided into two groups. Both groups were "ADE" groups and drinking history (four-bottles free-choice) and training procedure were essentially the same as described for the "ADE" group above except for longer (13 months) ethanol experience in the four-bottles paradigm before the beginning of training. Before testing, rats were injected either with saline (n=8) or nor-BNI (5 mg/kg per injection i.p., n=8) at 7:00 a.m. and a second time at 7:00 p.m.. The test session was started at 10.00 a.m. on the following day.

Drugs

Ethanol solutions were made up from 96% pure ethanol diluted with tap water to the different concentrations. CI-977 (a generous gift from Parke-Davis, Cambridge, UK) and nor-BNI (synthesised by A.W. Lipkowski) were dissolved in 0.9% saline. The pH of the nor-BNI solution was adjusted to pH 7 using 1 N NaOH. Drug doses refer to the weight of the salt.

Data analysis

The effects of chronic CI-977 and nor-BNI on the occurrence of an ADE were analysed by three-way analysis of variance (ANOVA) with repeated measures (treatment×ADE×days). Since the ADE is defined as an increase in alcohol drinking after an abstinence period compared to pre-abstinence drinking levels, the factor ADE is a within-group comparison of 3 pre-abstinence days with 3 post-abstinence days. Pre-abstinence data represent baseline levels before drug treatment. Given that there were no significant group differences in baseline levels, treatment effects on post-abstinence days were assessed by two-way ANOVA with repeated measures (treatment×days). The effects of ethanol deprivation on preferences for different ethanol concentrations were assessed separately for each drug dose by two-way ANOVA with repeated measures (ADE×days). Drug effects on changes in body weight, food, water and fluid consumption were analysed by two-way ANOVA with repeated measures (treatment×days). Baseline values of these parameters were determined as the average of three pre-abstinence data points. Operant chamber data were analysed by two-way ANOVA with repeated measures (group×dose or dose×interval). The accepted level of significance was $P \leq 0.05$. Fisher's LSD (protected-t) test was applied for post hoc comparisons when appropriate.

Results

Experiment 1

Effects of CI-977

After 2 weeks of abstinence a significant ADE occurred, characterised by a transient increase in ethanol intake [factor ADE: $F(1,14)=267.9$, $P<0.0001$] and ethanol preference [factor ADE: $F(1,14)=74.67$, $P<0.0001$] during the 3 post-abstinence days in comparison with the 3 pre-abstinence days (Fig. 1). Chronic CI-977 treatment strongly increased ethanol intake [interaction treatment× ADE: $F(1,14)=88.78$, $P<0.0001$] and also increased ethanol preference [interaction treatment×ADE: $F(1,14)=4.56$, $P=0.05$] during the ADE.

Alcohol deprivation only significantly increased the preference for 20% ethanol compared to pre-abstinence levels [$F(1,14)=5.3$, $P<0.05$] in the saline group (Fig. 1 bottom right), and the preference for 10% ethanol [$F(1,14)=8.83$, $P<0.05$] and for 20% ethanol [$F(1,14)=11.74$, $P<.01$] in the CI-977 group (Fig. 1 top right). However, there were no significant drug effects on the preference for a particular ethanol solution during the ADE in a between-group comparison.

Chronic CI-977 treatment led to a significant reduction in body weight [$F(1,14)=127.53$, $P<0.0001$] and food intake [$F(1,14)=46.03$, $P<0.0001$] (Fig. 2 left). Total fluid consumption was increased by chronic CI-977 treatment [$F(1,14)=6.76$, $P<0.05$] (Fig. 2 top right). Water intake was strongly decreased in both groups during the ADE (Fig. 2 bottom right), but still chronic CI-977 significantly reduced water intake in comparison to saline treatment [$F(1,14)=6.86$, $P<0.05$].

Effects of nor-BNI

Also in this experiment a significant ADE occurred concerning ethanol intake [factor ADE: $F(1,13)=149.22$, $P<0.0001$] and ethanol preference [factor ADE: $F(1,13)=44.19$, $P<0.0001$] after 2 weeks of abstinence. However, post-abstinence levels of ethanol intake and ethanol preference were not affected by nor-BNI (Fig. 3 left). Alcohol deprivation only significantly increased the preference for 10% ethanol compared to pre-abstinence levels [$F(1,14)=6.1$, $P<0.05$] in the control group (Fig. 3 bottom right). In nor-BNI-treated rats, alcohol deprivation increased the preference for 20% ethanol compared to pre-abstinence levels [$F(1,12)=8.62$, $P<0.05$]. There were no significant effects of drug treatment on the preference for a particular ethanol solution during the ADE in a between-group comparison. Nor-BNI treatment neither affected body weight, food intake, total fluid intake or water intake during the ADE (Fig. 4).

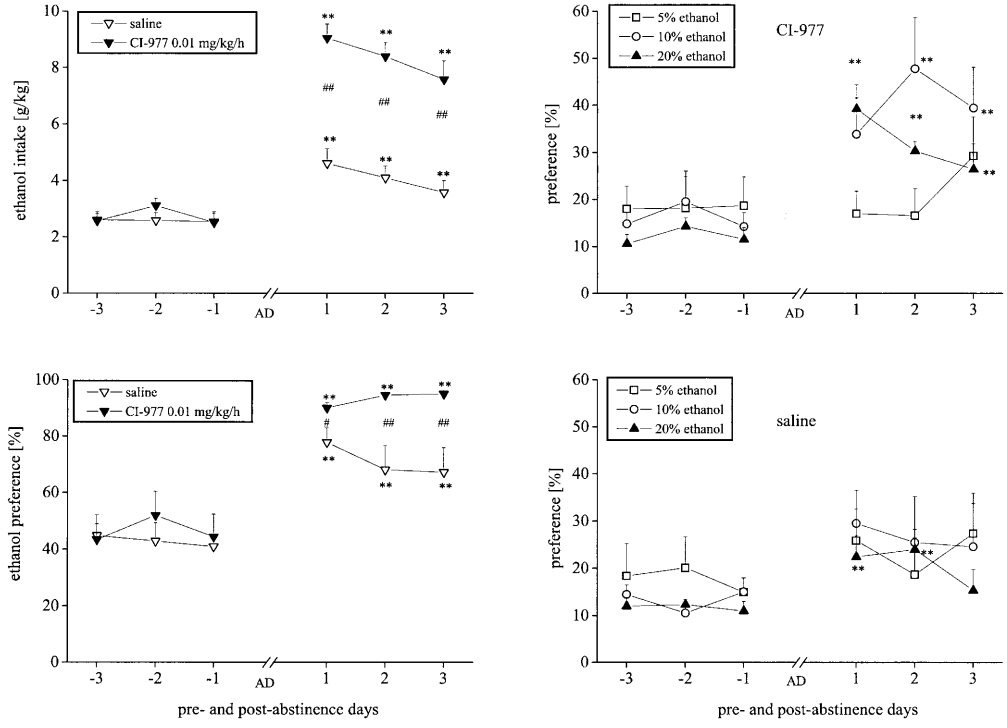

Fig. 1 Effects of chronic CI-977 treatment on ethanol intake (*top left*) and ethanol preference (*bottom left*) during the alcohol deprivation effect (ADE). Preferences for the different ethanol concentrations during the ADE after CI-977 treatment (*top right*) and in control rats (*bottom right*). Note that pre-abstinence data represent baseline levels of each group before drug treatment. *AD* Alcohol deprivation for 2 weeks. Data are presented as means + SEM of 24 h measurements; *n*=8. # $P<0.05$, ## $P<0.01$, significant difference vs saline; ** $P<0.01$, significant difference vs all 3 pre-abstinence days

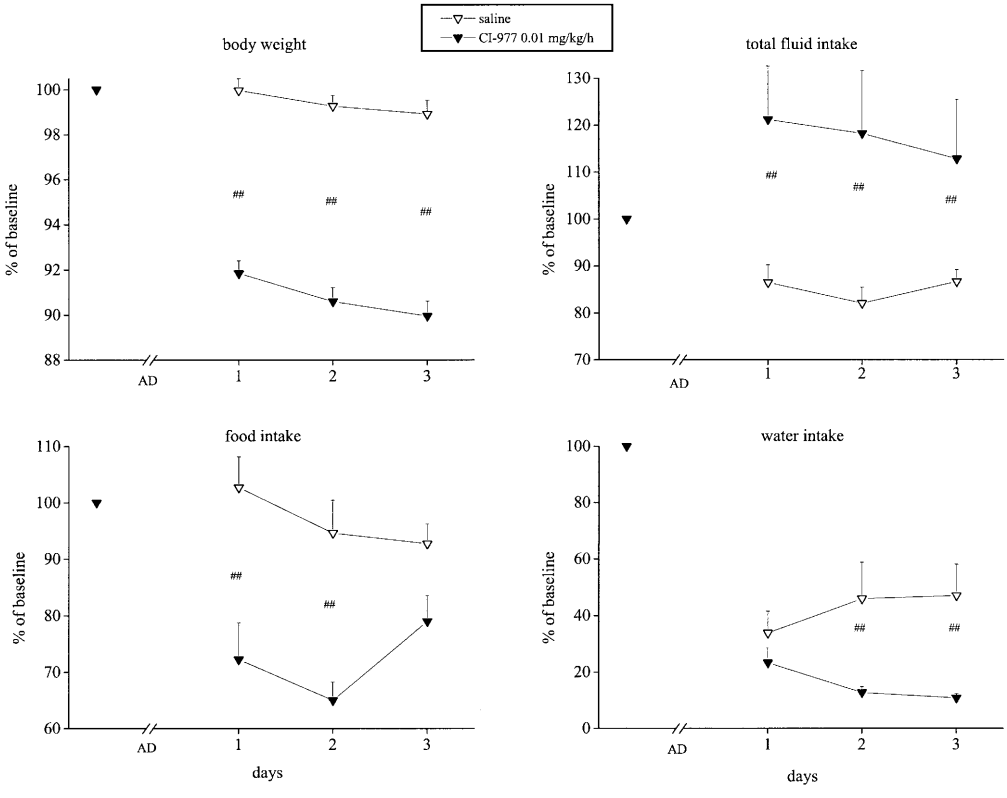

Fig. 2 Effects of chronic CI-977 treatment on body weight (*top left*), food intake (*bottom left*), total fluid intake (*top right*) and water intake (*bottom right*). *AD* Alcohol deprivation for 2 weeks. Data are presented as means + SEM of 24 h measurements; *n*=8. ## $P<0.01$, significant difference vs saline

To ensure that possible nor-BNI effects had not worn off at the time of ethanol re-presentation despite its reported long-lasting action, the same experiment was replicated with nor-BNI injections not 4, but 1 day before re-presentation of ethanol solutions. This second experiment yielded the same results as the first one (data not shown).

Experiment 2

Effects of CI-977

Because previous experiments with this paradigm have shown that drug effects on operant behaviour are most

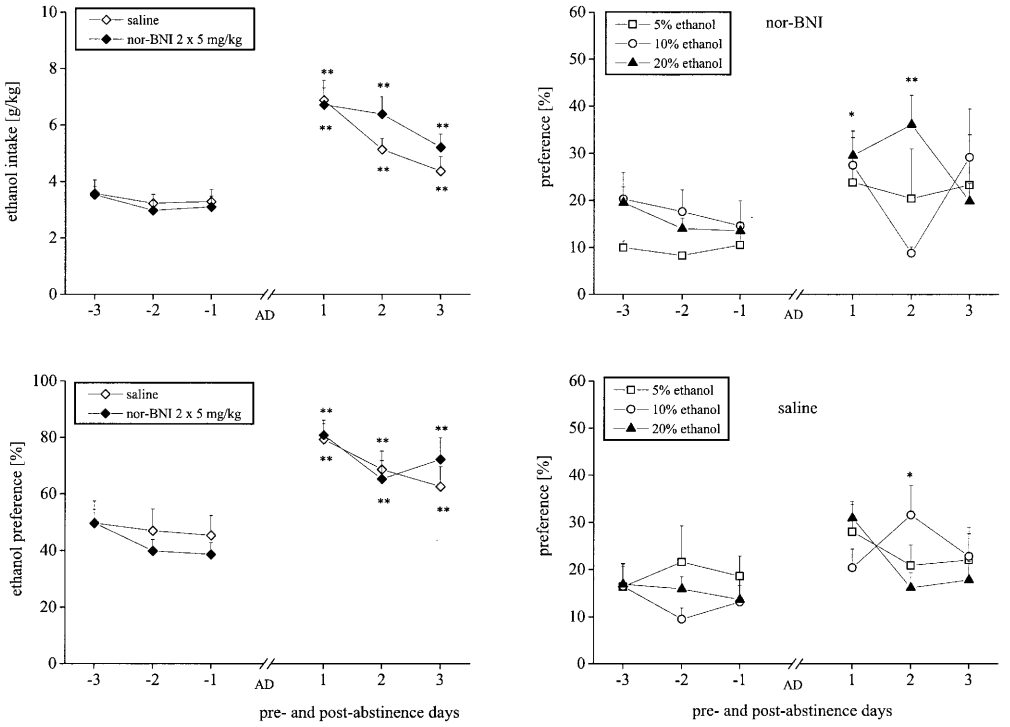

Fig. 3 Effects of nor-binaltorphimine (nor-BNI) on ethanol intake (*top left*) and ethanol preference (*bottom left*) during the ADE. Preferences for the different ethanol concentrations during the ADE after nor-BNI treatment (*top right*) and in control rats (*bottom right*). Note that pre-abstinence data represent baseline levels of each group before drug treatment. *AD* Alcohol deprivation for 2 weeks. Data are presented as means + SEM of 24 h measurements; n=7–8. * P<0.05, ** P<0.01, significant difference vs all 3 pre-abstinence days

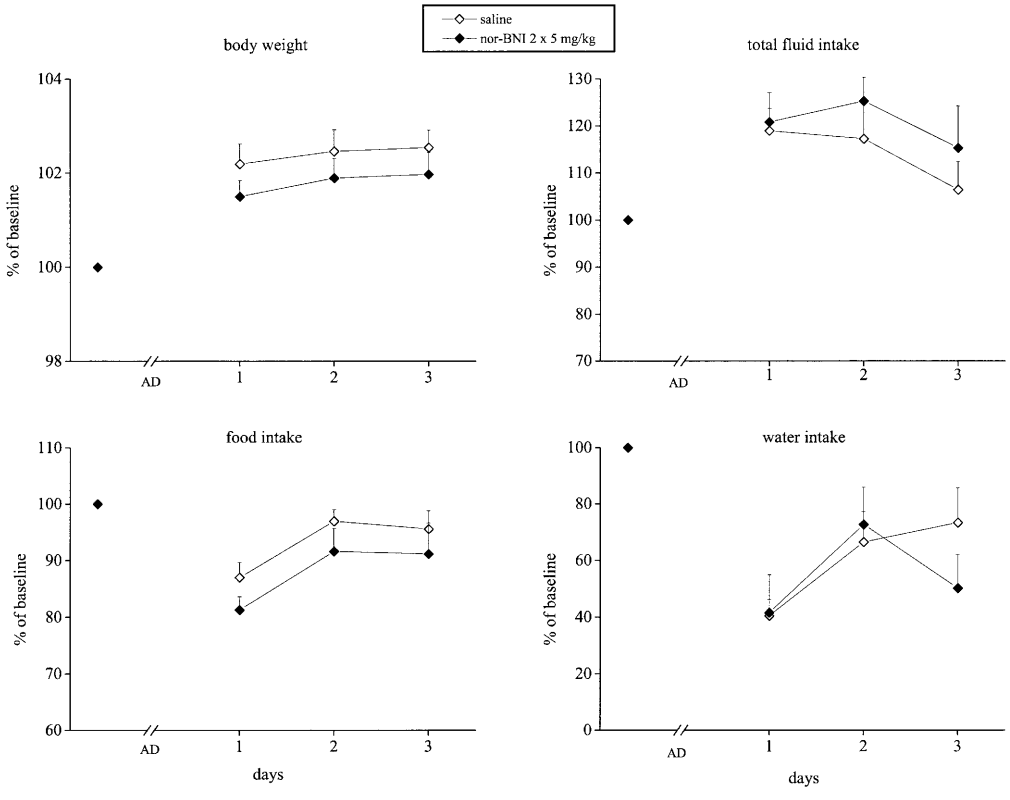

Fig. 4 Effects of nor-BNI on body weight (*top left*), food intake (*bottom left*), total fluid intake (*top right*) and water intake (*bottom right*). *AD* Alcohol deprivation for 2 weeks. Data are presented as means + SEM of 24 h measurements; n=7–8

pronounced at the beginning of the operant session and, depending on the dose used, can wear off before the end of the session resulting in no significant change within the 23-h session (Hölter et al. 1997), data were analysed both for the first hour of the operant session and for the total 23-h session.

During saline treatment, animals of the "basal" and "ADE" groups differed in ethanol intake and preference in the operant 23-h session, which constitutes the ADE in this operant paradigm, but not in total lever pressing activity and therefore not in total fluid intake (Fig. 5 right). During the first hour (Fig. 5 left), CI-977 reduced

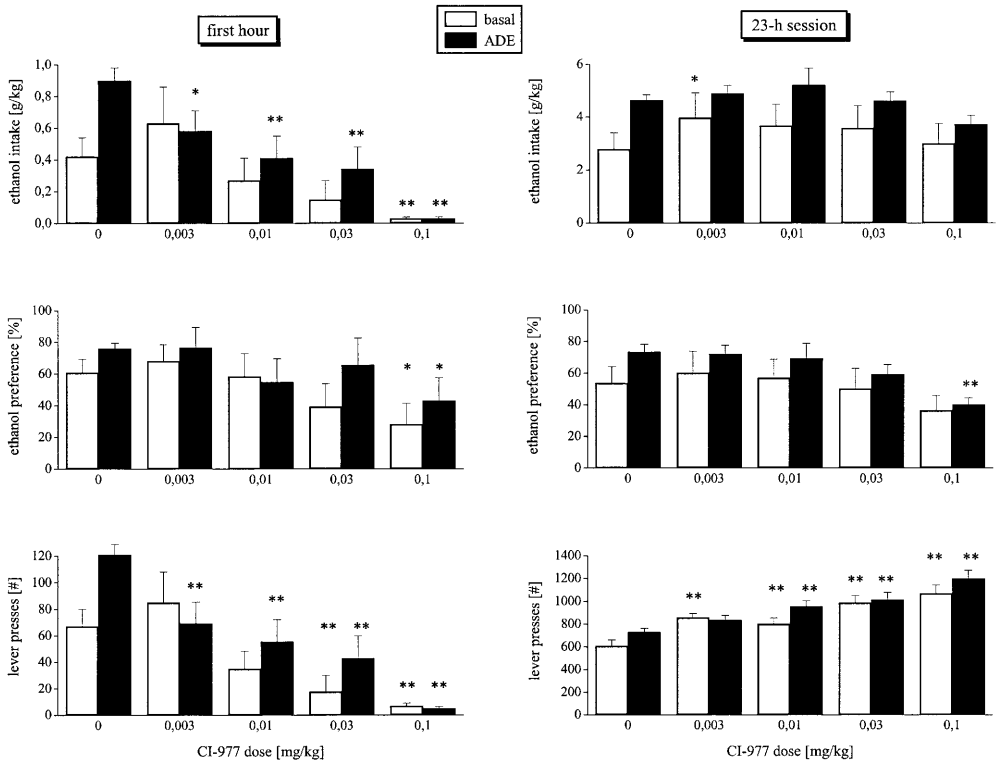

Fig. 5 Effects of acute CI-977 treatment on basal drinking and the ADE in an operant 23-h session. *Left* Effects during the first hour, *right* effects during the whole 23-h session. *Top panels* Effects on ethanol intake, *middle* effects on ethanol preference, *bottom* effects on total lever pressing activity. Data are presented as means + SEM; n=7. * P<0.05, ** P<0.01, significant difference vs saline

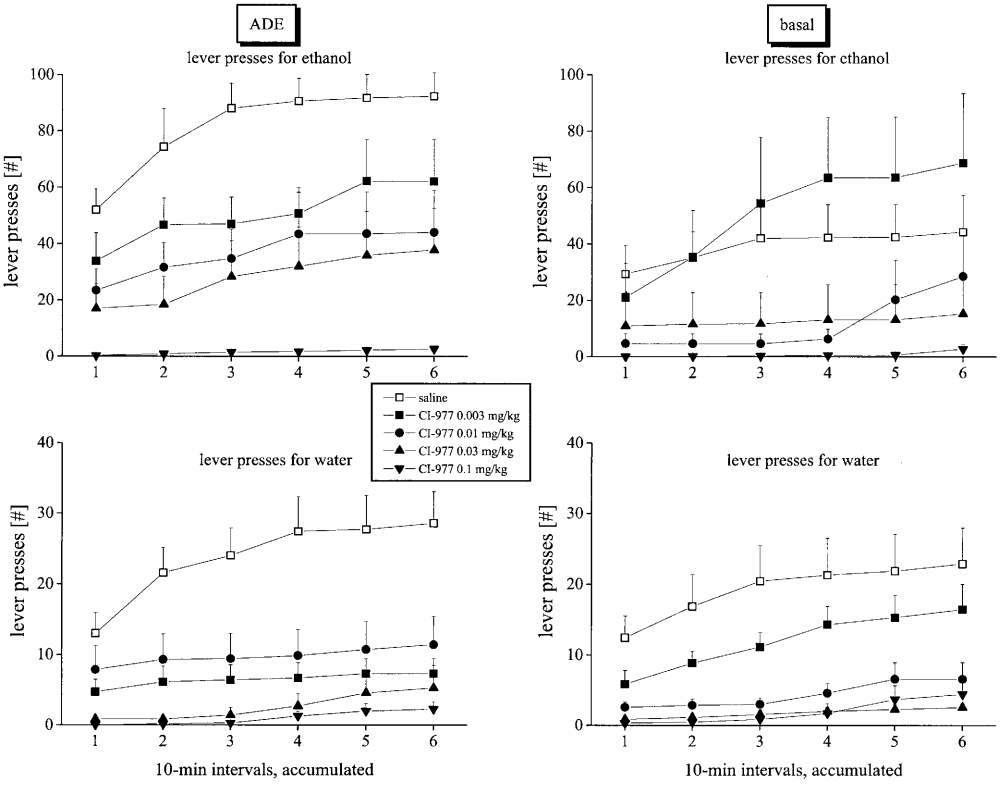

Fig. 6 Effects of acute CI-977 treatment on basal drinking (*right*) and the ADE (*left*) during the first hour of the operant 23-h session. *Top* Effects on lever pressing for ethanol, *bottom* effects on lever pressing for water. Data are presented as means + SEM of accumulated lever pressing activity; n=7. Asterisks are omitted for clarity

ethanol intake and total lever pressing activity in a dose-dependent fashion during the ADE, whereas during basal drinking only the two highest doses led to a significant reduction [factor dose: $F(4,48)=11.32$, $P<0.0001$ for ethanol intake, and factor dose: $F(4,48)=17.66$, $P<0.0001$ for total lever pressing activity]. At the lowest dose (0.003 mg/kg) there was a tendency towards an increase of ethanol intake and total lever pressing activity in the basal group. Ethanol preference was only reduced by the highest dose in both groups [factor dose: $F(4,48)=3.21$,

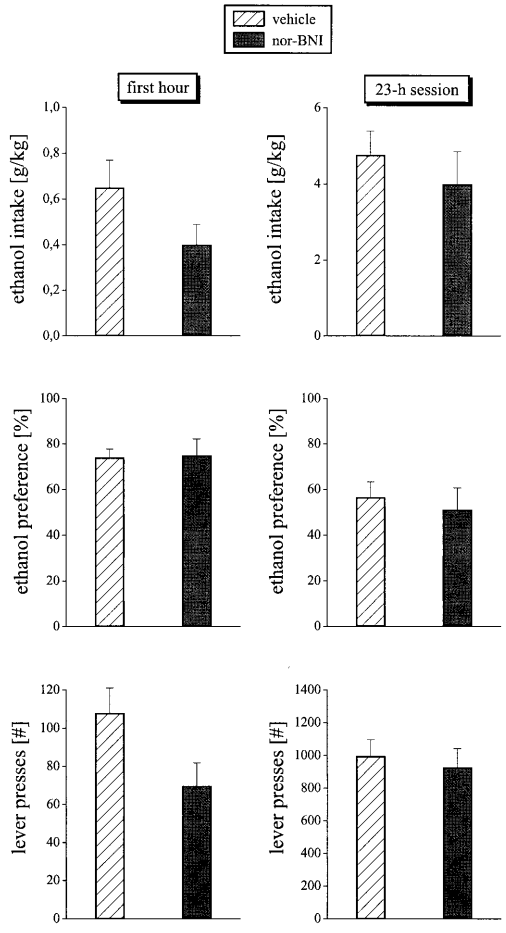

Fig. 7 Effects of nor-BNI on the ADE in an operant 23-h session. *Left* Effects during the first hour, *right* effects during the whole 23-h session. *Top panels* Effects on ethanol intake, *middle* effects on ethanol preference, *bottom* effects on total lever pressing activity. Data are presented as means + SEM; *n*=8

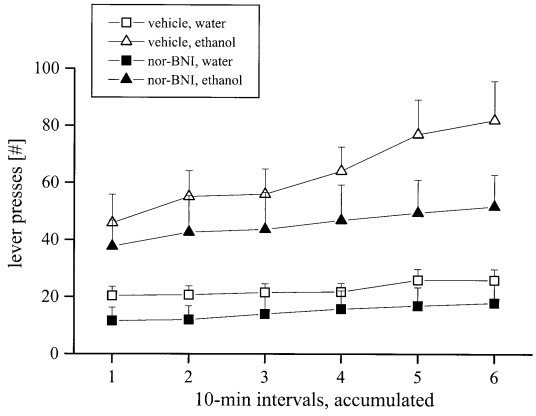

Fig. 8 Effects of acute nor-BNI on lever pressing for ethanol and water during the ADE during the first hour of the operant 23-h session. Data are presented as means + SEM of accumulated lever pressing activity; *n*=8

P<0.05]. Concerning the whole 23-h session (Fig. 5 right), only the lowest dose (0.003 mg/kg) significantly increased ethanol intake in the basal group [factor dose: $F(4,48)=3.14$, $P<0.05$]. Ethanol preference was only significantly reduced at the highest CI-977 dose [factor dose: $F(4,48)=7.06$, $P<0.001$], and total lever pressing activity was increased in a dose-dependent manner by CI-977 [factor dose: $F(4,48)=24.31$, $P<0.0001$]. Analyses of the time course of lever pressing activity for ethanol and water during the whole session revealed that the increase in 23-h total lever pressing activity was due to a dose-dependent rise in lever pressing for water in both groups starting about 6 h after drug injection while lever pressing for ethanol remained unchanged (data not shown). This delayed, water-specific effect was also responsible for the subsequent reduction of ethanol preference at more or less unchanged ethanol intakes (see Fig. 5 right).

Analyses of lever pressing activity for ethanol (Fig. 6 top) and water (Fig. 6 bottom) during the first six 10-min intervals of the first hour of the session revealed that CI-977 depressed lever pressing for ethanol [factor dose: $F(4,30)=9.13$, $P<0.0001$] and water [factor dose: $F(4,30)=11.18$, $P<0.0001$] in a dose-dependent manner during the ADE (Fig. 6 left). During basal drinking, lever pressing for ethanol was significantly increased after the first 30 min of the session at the lowest dose of CI-977 (0.003 mg/kg), whereas all other doses significantly decreased lever pressing for ethanol right from the beginning of the session [factor dose: $F(4,30)=3.55$, $P<0.05$] (Fig. 6 top right). Lever pressing for water during basal drinking was dose-dependently reduced by CI-977 [factor dose: $F(4,30)=10.44$, $P<0.0001$] (Fig. 6 bottom right).

Effects of nor-BNI

Although there was a tendency towards a decrease of ethanol intake and lever pressing activity by nor-BNI during the first hour of the operant session (Fig. 7 left), these effects were not significant. Nor-BNI did not affect 23-h values of ethanol intake, ethanol preference or total lever pressing activity (Fig. 7 right). Analyses of the time course of lever pressing activity for ethanol and water during the first hour (Fig. 8) and during the whole session (data not shown) did not reveal any significant drug effect.

Discussion

The results of this study show that: (1) chronic treatment with the selective κ-opioid receptor agonist CI-977 strongly increased the ADE in long-term ethanol-experienced rats, (2) acute CI-977 treatment reduced lever pressing for both ethanol and water except for the lowest dose, which increased ethanol intake during basal drinking and (3) the long-acting selective κ-opioid receptor antagonist nor-BNI had no effect on the ADE in long-term ethanol-experienced rats.

Like other κ-opioid receptor agonists, CI-977 does induce diuresis (Hunter et al. 1990). The diuresis induced by κ-opioid receptor agonists is characterised by a selective increase of water elimination without any associated increase in electrolyte elimination, which is due to an inhibition of vasopressin release and a direct effect on renal function (Dykstra et al. 1987; Leander et al. 1987; Hamon and Jouquey 1990). It can not be excluded that the diuretic action contributed to the increased ethanol intake. However, it is unlikely that the increase of the ADE by chronic CI-977 may solely be explained by the diuretic action of this drug, because then water intake should have increased and ethanol preference subsequently decreased, but ethanol preference was increased and water intake decreased by CI-977, rather suggesting an increase in the demand for ethanol.

Chronic CI-977 also reduced food intake. We have frequently observed that food intake is adjusted to the availability of ethanol. Thus, ethanol-drinking rats consume less food than control rats, but increase their food intake to control levels during ethanol deprivation, and strongly decrease it again during the ADE (Hölter et al. 2000b). This can not only be explained by the maintenance of a certain level of caloric intake, because ethanol-drinking rats have a higher caloric intake than controls, particularly during the ADE. We conclude that food intake is adjusted to ethanol intake due to a maximum of caloric need. Therefore the reduction of food intake by chronic CI-977 is most likely a result of the strong increase in ethanol intake and not due to a direct effect of the drug on food intake. This conclusion is supported by findings indicating that stimulation of κ-opioid receptors rather increases than decreases food intake (Cooper et al. 1985; Morley and Levine 1985; Gulati et al. 1992; Lee and Clifton 1992).

In contrast to chronic treatment, acute CI-977 reduced both ethanol and water intake at the beginning of the operant sessions, which is most likely due to the dose-dependent sedative effect of κ-opioid receptor agonists (Hunter et al. 1990; Davis et al. 1992; Wollemann et al. 1993). Even at the highest dose the sedative effect disappeared after 6 h when a dose-dependent rise in lever pressing for water set in which is most likely the consequence of the diuretic action of this drug. Thus, any possible specific effect on ethanol reinforcement is likely to be masked by the early unspecific sedative effect and the delayed consequences of the diuretic effect of acute CI-977 injections. Only the lowest, the least sedative and diuretic CI-977 dose (0.003 mg/kg) had an ethanol-specific effect, which was an increase of ethanol intake. It can not be excluded that even lower doses may also have yielded ethanol-specific increases.

Dose and treatment regimen seem to determine the direction of the effects of κ-opioid receptor agonists on ethanol drinking. In this study, either continuous stimulation of κ-opioid receptors or acute stimulation by a very low dose of CI-977 with minimal side effects induced an increase in ethanol drinking, whereas acute injections with higher, more sedating doses temporarily decreased ethanol drinking. The latter finding is in line with a study showing that acute maximal stimulation of κ-opioid receptors by a sedative dose of the agonist U50,488H induced a small decrease in ethanol intake in voluntarily ethanol drinking rats on the 1st treatment day, but not on later treatment days (Nestby et al. 1999).

Assuming that activation of κ-opioid receptors inhibits mesolimbic dopamine neurons (Spanagel et al. 1992; Herz 1997), our results are consistent with the notion that a decrease of dopamine release in the nucleus accumbens by continuous stimulation of κ-opioid receptors can lead to an increased demand for ethanol, at least in long-term ethanol-experienced rats. Hence, κ-opioid receptor stimulation might be able to increase the reinforcer value of ethanol. This effect might depend on ethanol experience. Studies with ethanol-naive alcohol-preferring and non-preferring rats and mice indicate that greater activity in the dynorphin/κ-opioid receptor system is rather associated with low alcohol intake and preference (Jamensky and Gianoulakis 1997; Winkler and Spanagel 1998; Soini et al. 1999). Thus, the dynorphin/κ-opioid receptor system seems to play a role in controlling the initial alcohol intake in naive animals. Therefore it is possible that an increase in voluntary ethanol drinking after κ-opioid receptor stimulation may only occur in animals which had the opportunity to learn about ethanol's pharmacological effects during prolonged voluntary ethanol drinking.

Kappa-opioid receptor agonists reliably produce aversive effects in rodents in place-conditioning and taste-conditioning procedures (Mucha and Herz 1985; Bals-Kubik et al. 1993; Funada et al. 1993). Moreover, place aversion can not be induced in mice lacking a functional κ-opioid receptor, indicating that this receptor is critical for the mediation of the aversive effects of κ-opioid receptor agonists (Simonin et al. 1998). Increased ultrasonic distress vocalisation in rat pups constitutes further evidence for aversive effects of κ-opioid receptor agonist treatment (Barr et al. 1994). In the elevated plus-maze, the most widely used animal model of anxiety-related behaviour, κ-opioid receptor agonists increase measures of anxiety (Motta et al. 1995). In humans, aversive effects of κ-opioid receptor agonists are often summarised as "dysphoria" (Kumor et al. 1986; Pfeiffer et al. 1986; Rimoy et al. 1994). This includes non-specific bodily complaints such as weakness, sweating, vertigo and dizziness as well as an increase in anxiety. Racing thoughts, feelings of body distortion and severe discomfort were also reported (Pfeiffer et al. 1986). Although the evidence is still controversial, it is generally assumed that unpleasant affect, including dysphoria and anxiety, can drive ethanol consumption in experienced drinkers, possibly in an attempt to utilise the stress dampening and/or tension reducing effects of ethanol (Conger 1956; Stockwell et al. 1982; Stewart et al. 1997; Sinha et al. 1998). Thus, assuming that κ-opioid receptor stimulation induces aversion in our long-term ethanol-experienced rats, these animals might be attempting to counteract this effect by increased voluntary ethanol intake.

The long-acting selective κ-opioid receptor antagonist nor-BNI did not affect the ADE at all in the home cage drinking paradigm and there was only a non-significant trend towards a reduction during the first hour of testing in the operant paradigm. It is highly unlikely that κ-selective drug effects had already worn off at the time of ethanol re-presentation, since these effects of nor-BNI can last for up to 30 days (Horan et al. 1992; Spanagel et al. 1994). The last nor-BNI injection was given 15 h before the test to ensure κ-selectivity of effects because nor-BNI does also exert μ-antagonistic actions during the first hours after injection which decrease with time, whereas the κ-antagonistic action is of slow onset, increases gradually and lasts longer (Endoh et al. 1992). The effectiveness of κ-selective antagonistic action peaks at 1–3 days after a single i.c.v. injection (Horan et al. 1992). After a single systemic injection, the κ-selective antagonistic effect of nor-BNI was shown to last for at least 4 days at 5 mg/kg and for at least 8 days at 20 mg/kg in mice (Endoh et al. 1992). Even longer durations of action after lower nor-BNI doses were observed in rhesus monkeys (Butelman et al. 1993). In conclusion, our results are in line with previous findings in monkeys where nor-BNI reduced ethanol-reinforced responding only on the day of injection, which was interpreted as a μ-receptor mediated, κ-unselective effect, but not on subsequent days (Williams and Woods 1998). Thus, endogenous κ-opioid receptor stimulation does not seem to be involved in baseline operant responding for ethanol. The lack of effect of nor-BNI treatment on the ADE after 1 (operant paradigm) or 2 weeks (home cage paradigm) of abstinence extends the above-mentioned findings by suggesting that endogenous κ-opioid receptor stimulation is also not involved in relapse-like drinking after protracted abstinence. It remains to be determined whether it might play a role earlier in withdrawal.

In conclusion, the κ-opioid receptor system can play a role in ethanol reinforcement in cases of increased receptor activation as it was experimentally induced in this study by chronic treatment with a selective κ-opioid receptor agonist. The subsequent increase in ethanol intake might be an attempt to counteract the aversive motivational consequences of this treatment. It is possible that the occurrence of an increase in ethanol intake after κ-opioid receptor stimulation does depend on long-term ethanol experience and/or prolonged receptor stimulation. It is not clear whether prolonged receptor stimulation occurs under physiological conditions. Previous findings indicate that an increased dynorphin activity might occur during the early phases of alcohol withdrawal, but disappear later on (Przewlocka et al. 1997). Hence, an increased endogenous κ-opioid receptor activation might enhance the probability of relapse during the early phases of alcohol withdrawal, and κ-opioid receptor antagonists might be useful at this time. However, since endogenous κ-opioid receptor stimulation does not seem to be involved in relapse-like drinking after protracted abstinence, it is unlikely that κ-opioid receptor antagonists may be useful to prevent relapse in the long run.

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