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Short communication

Effect of neramexane on ethanol dependence and reinforcement

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Abstract

It has been suggested that drugs modulating the glutamate/*N*-methyl-D-aspartate (NMDA) receptor system may be useful in the treatment of alcohol dependence. The effect of neramexane, a low-to-moderate affinity uncompetitive NMDA receptor antagonist, was examined on the development and expression of ethanol dependence (withdrawal-associated audiogenic seizures) and ethanol-induced conditioned place preference. Neramexane hydrochloride (3.5 mg/kg and higher) inhibited both the development and expression of ethanol dependence. Neramexane hydrochloride also inhibited the acquisition (1.75 mg/kg and higher) and expression (3.5 mg/kg and higher) of ethanol-induced place preference. Our data support therapeutic potential of neramexane as a treatment for alcohol abuse.

Keywords: NMDA receptor antagonist; Neramexane; Ethanol; Reinforcement; Dependence

1. Introduction

Currently available therapies for ethanol dependence (e.g., disulfiram, naltrexone) have shown limited efficacy (Johnson and Ait-Daoud, 2000), which obviates the need to explore novel treatments.

A growing body of evidence suggests that ethanol interferes with excitatory amino acid neurotransmission. While acute ethanol administration attenuates *N*-methyl-D-aspartate (NMDA) receptor function (Lovinger et al., 1989), chronic ethanol consumption may result in an increase in glutamatergic activity such as a up-regulation of different NMDA receptor subunits (Hoffman and Tabakoff, 1996) and increase in extracellular glutamate concentration following ethanol withdrawal (Rossetti and Carboni, 1995).

The recent data suggest that glutamate receptor antagonists may be also useful in treatment of ethanol dependence (Bisaga et al., 2000). Neramexane hydrochloride (MRZ 2/579, 1-amino-1,3,3,5,5-pentamethyl-cyclohexane hydrochloride), a novel uncompetitive NMDA receptor antagonist with low-to-moderate affinity (Danysz et al., 2002; Parsons et al., 1999), inhibits alcohol consumption in various animal models (Bienkowski et al., 1999; Bienkowski et al., 2001; Hölter et al., 2000). In drug discrimination experiments, neramexane hydrochloride substitutes for ethanol at doses between 2 and 4 mg/kg (Hölter et al., 2000). This observation suggests that neramexane could exert its effect through a substitution action since both ethanol and neramexane attenuate NMDA receptor function.

In the present study, we try to assess whether neramexane: (a) inhibits development and/or expression of alcohol dependence; (b) attenuates the reinforcing properties of ethanol in the acquisition and expression of ethanol-induced conditioned place preference; (c) induces physical dependence or exhibits reinforcing properties in the conditioned place preference paradigm.

Clinical trials have shown that acamprosate prevents relapse in alcohol-dependent patients (Johnson and Ait-Daoud, 2000). However, in animal models, alcohol-induced hypothermia, motor impairment and taste aversion were not altered by acamprosate. In other studies, it was found that acamprosate did not antagonize the discriminative stimulus properties of alcohol and did not substitute for alcohol in a discrimination task (see Spanagel and Zieglgänsberger,

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1997). In our experiments, acamprosate was used in the test evaluating the development and expression of alcohol dependence for comparison with the effect of neramexane.

2. Methods

2.1. Subjects

Male Wistar rats (HZL, Warsaw, Poland, 200–250 g) were housed at a standard laboratory conditions (22 °C, 12:12-h light–dark cycle). All procedures were in accordance with the Polish and European regulation and were approved by the Local Ethics Committee (No. 408/02).

2.2. Ethanol dependence

The currently used ethanol treatment regimen was based upon the modified procedures described by Adams et al. (1995). Ethanol was given orally at 3 g/kg ethanol (20% w/v; 95% ethyl alcohol) at 8 a.m. and then every 8 h for the first day, next day, the dose was increased to 3.5 g/kg. Then, a dose of 4 g/kg was given every 8 h for 5 days. Using this regimen, the animals (8–12 per group) received 10–12 g/kg ethanol per day. On day 8, 10–12 h after the last ethanol dose, rats were tested for ethanol withdrawal signs in a wide glass cylinder. The occurrence of audiogenic seizures (clonic–tonic episodes) was measured after sound stimuli (an electric buzzer, 92 dB, up to 90 s).

Neramexane hydrochloride (MRZ 2/579, Merz Pharmaceuticals, Frankfurt/Main, Germany, in all cases doses refer to hydrochloride salt of neramexane) was dissolved in physiological saline and in case of experiment focusing on the development of dependence given i.p., three times a day, 30 min before ethanol administration, at the doses of 0.9, 1.75, 3.5 and 7.0 mg/kg. Acamprosate (calcium acetylhomotaurinate) was prepared from tablets of Campral (Merck, Darmstadt, Germany), dissolved in water and given i.p. twice a day (morning and night) during the development of ethanol dependence, 30 min before ethanol at doses of 50, 100, 200 and 400 mg/kg. For the expression study, neramexane or acamprosate was given 30 min before the test (sound application).

2.3. The conditioned place preference (CPP) apparatus

Rectangular wooden boxes (60×35×30 cm) divided in two large compartments (25×35 cm) were separated from a small central gray area (10×10 cm) by removable guillotine doors. The walls of the one large compartment were black while the other was white.

The ethanol-induced CPP procedure was described earlier (Biala and Kotlinska, 1999). This procedure was preceded by single daily injections of 10% (w/v) ethanol (0.5 g/kg, i.p.) or saline each day for 15 days. Then, during the habituation phase (pre-conditioning) the time spent in the two large compartments was recorded for 15 min. The initially non-preferred compartment was later paired with ethanol.

During the conditioning phase (8 days), the rats were pretreated with neramexane (1.75, 3.5 or 7.0 mg/kg, i.p.), 30 min before ethanol injection. Five minutes later, the animals were confined to the non-preferred compartment for 30 min. On alternate days the rats were exposed for 30 min to the preferred compartment after the injection of distilled water. During the last phase (test), the guillotine door was removed and the time spent in each of large compartments was recorded for 15 min without any injection.

To investigate the effect of neramexane on the expression of ethanol-induced CPP, rats that had developed ethanol-induced CPP were pretreated with neramexane 30 min before placement in the CPP apparatus on the test day.

2.4. Statistical analysis

The frequency of audiogenic seizures was analyzed using the chi-square test with Yates' correction. CPP results were assessed by one-way analysis of variance (ANOVA) which in case of significant (P=0.05 level) main effect was followed by the Tukey–Kramer multiple comparisons test (P<0.05 was considered significant).

3. Results

3.1. Effect of neramexane and acamprosate on ethanol dependence

Acute administration of neramexane, 30 min before the test, attenuated alcohol withdrawal-induced audiogenic seizures at 3.5 and 7 mg/kg (Fig. 1 upper panel). Chronic administration of neramexane, at the doses of 3.5 and 7 mg/kg, administered before each injection of ethanol, also attenuated the development of ethanol dependence (Fig. 1 lower panel). Acute injection of acamprosate (400 mg/kg), but not at the lower doses tested, also inhibited withdrawal-associated audiogenic seizures (Fig. 1 upper panel). Similar to neramexane, acamprosate given 30 min before the oral administration of ethanol over a 7-day period attenuated the development of ethanol dependence, but only at the highest dose tested (400 mg/kg) (Fig. 1 lower panel). Administration of neramexane alone (7 mg/kg, i.p., three times a day) or acamprosate alone (200 mg/kg, i.p., twice a day), each for 7 days, did not induce physical dependence in rats and did not change the rat's behaviour. That is, in each drug group only one rat (1/10) showed a clonic–tonic seizure episode, an outcome comparable to the control (water) group. However, chronic injection of acamprosate and neramexane, 30 min before ethanol, induced middle sedation and in some rats motor incoordination was observed.

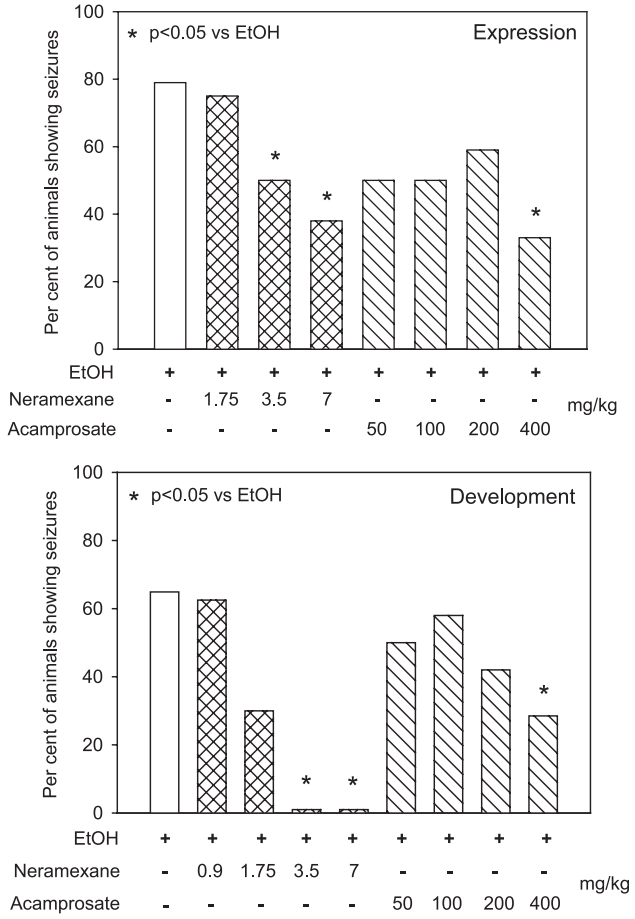

Fig. 1. Effect of neramexane and acamprosate on the expression (upper panel) and development (lower panel) of alcohol dependence as measured by alcohol withdrawal-associated audiogenic seizures (N=10–22) *P<0.05 vs. EtOH alone (chi-square test with Yates' correction).

3.2. Effects of neramexane on of CPP induced by ethanol

Data was expressed as a score, i.e. post-conditioning minus pre-conditioning time (in s) spent in the drug-associated (white) compartment. One-way ANOVA indicated significant differences between groups [F(5,47)=4.32, P<0.01]. Post hoc analysis showed that ethanol-treated rats during conditioning induced a significant preference for the alcohol-associated compartment during the test (P<0.01). Acute injection of neramexane at the doses of 3.5 or 7.0 mg/kg inhibited the expression of ethanol-induced CPP (Fig. 2, upper panel). Injection of neramexane alone (7 mg/kg) did not produce place conditioning (Fig. 2, upper panel).

In experiments designed to evaluate the effect of drug treatment on the development of conditioned place preference, one-way ANOVA showed a significant difference between drug treatment groups [F(5,43)=6.34, P<0.001]. Post hoc analysis revealed that ethanol treatment during conditioning days induced significant place preference (P<0.01) during the test (Fig. 2, lower panel). Chronic injection of neramexane at the doses of 1.75 (P<0.05), 3.5 (P<0.01) or 7.0 (P<0.001) mg/kg, 30 min before ethanol during the conditioning phase, significantly inhibited the acquisition of ethanol-induced CPP (Fig. 2, lower panel). Eight days treatment with neramexane alone (7 mg/kg, i.p.) did not produce CPP (Fig. 2, lower panel).

4. Discussion

Neramexane attenuated the development and expression of ethanol dependence measured as audiogenic withdrawal seizures in rats. Neramexane also suppressed the rewarding effect of ethanol measured in CPP paradigm (development and expression) itself exhibiting any apparent having rewarding or aversive effect. Acamprosate showed similar effects on the development and expression of ethanol dependence but a significant effect was obtained only at a relatively high dose (400 mg/kg).

A plethora of data indicate that NMDA receptor antagonists suppress various signs of ethanol withdrawal in animals (Dodd et al., 2000) and only few data that they also prevent the development of ethanol dependence (Kotlinska, 2001). Neramexane is a low-to-moderate affinity, uncompetitive NMDA receptor antagonist with rapid blocking/unblocking kinetics and strong voltage depend-

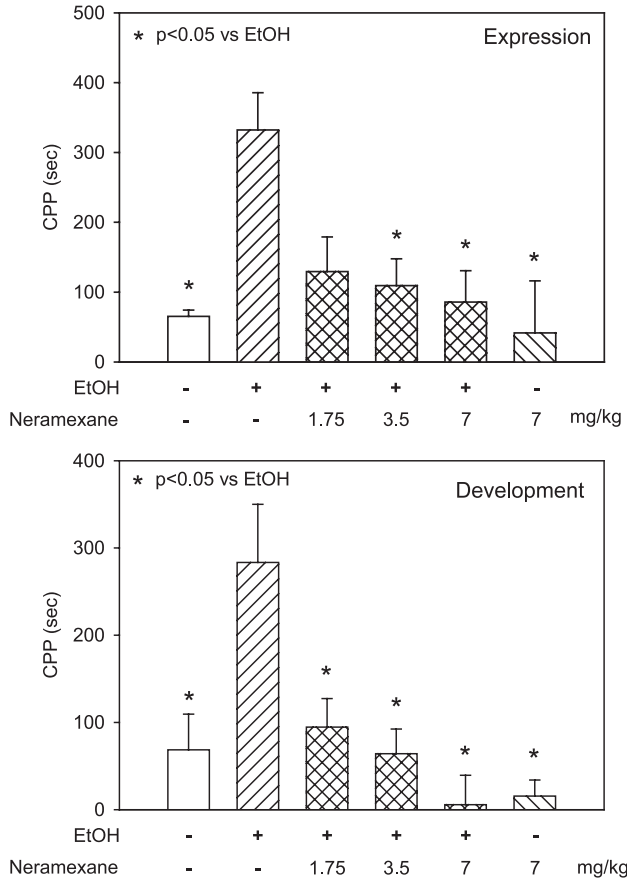

Fig. 2. Effect of neramexane on the expression (upper panel) and development (lower panel) of alcohol CPP. Results are expressed as mean±S.E.M. (N=7–12). *P<0.05 vs. EtOH alone (Tukey–Kramer test).

ence (Danysz et al., 2002; Parsons et al., 1999). It has been reported that neramexane selectively inhibits consumption of ethanol in various paradigms (Bienkowski et al., 1999; Hölter et al., 2000). However, Hölter et al. (2000) also showed that neramexane dose-dependently generalized to an ethanol cue in a drug discrimination paradigm which may suggest that neramexane reduces ethanol intake by substituting for some of the stimulus properties of ethanol.

However, other studies have demonstrated that neramexane does not appear to exhibit abuse potential since it is not self-administered nor does it produce CPP in mice (see (Danysz et al., 2002); present study). Furthermore, our study shows that chronic administration of neramexane does not induce physical dependence, even though both ethanol and neramexane have been shown to attenuate NMDA receptor channel activity.

Similarly to neramexane, acamprosate alone does not induce physical dependence in our study. However, other experiments performed on rodents indicate that acamprosate attenuates ethanol consumption without affecting water intake (Boismare et al., 1984) and inhibits the rewarding effect of ethanol in the CPP paradigm (McGeehan and Olive, 2003). It has been shown that acamprosate is a functional antagonist of the NMDA receptor having complex mechanism of action (Rammes et al., 2001). These data demonstrate, for the first time, that acamprosate attenuates the development and expression of alcohol dependence in rats as measured by a decrease in withdrawal-associated audiogenic seizures.

Our results showed that neramexane, like acamprosate, inhibits the development and expression of alcohol dependence, but at lower doses compared with acamprosate. Inhibition of the development of ethanol dependence by neramexane suggests that its mechanism of action cannot be solely explained by a substitution-like effect. In support of this, neramexane alone, unlike ethanol, did not exhibit physical dependence or reinforcing properties (lack of place preference) following chronic administration. Neramexane was also shown to inhibit the acquisition and expression of ethanol-induced place preference, suggesting that neramexane may attenuate the reinforcing properties of alcohol. Together, these data suggest that neramexane may be a promising therapy for the treatment of alcohol dependence.

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