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The role of the NMDA receptor in alcohol relapse: a pharmacological mapping study using the alcohol deprivation effect

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Abstract

Modulators of glutamate receptors especially of the *N*-methyl-D-aspartate receptors (NMDARs) have recently been suggested as putative pharmacotherapeutic agents in the treatment of alcohol relapse. However, at present it is not clear, which binding and modulatory sites of the NMDAR are involved in relapse behavior. We, therefore, performed a pharmacological mapping study in long-term alcohol drinking rats using the alcohol deprivation effect (ADE) as a model for relapse behavior. In a comprehensive fashion, we studied dose–response curves, employing the following selective pharmacological agents: the NMDAR competitive antagonist CGP37849, the glycine binding site antagonist L-701.324, the NR2B subunit selective antagonist ifenprodil, which acts at the polyamine binding site, the NMDAR channel blocker neramexane, and ethanol, which acts as a functional antagonist at the NMDAR. Our data show that the animals' alcohol consumption inversely correlates with the dose of ethanol administered intraperitoneally. This indicates that under the present experimental conditions alcohol intake during an ADE is an entirely pharmacologically driven behavior that is not under the control of other factors such as taste or novelty of alcohol re-exposure. The effects of the administration of the aforementioned compounds were comparable to those of ethanol, suggesting a similar pharmacological impact on relapse behavior. Repeated administration of both competitive and uncompetitive NMDAR antagonist dose-dependently suppressed alcohol consumption during ADE. In addition, ifenprodil and L-701.324 dose-dependently reduced the expression of an ADE as well. In summary, the results suggest that an inhibition of NMDAR function in general, rather than a particular interference with a specific binding site of this receptor, is sufficient for the reduction of relapse behavior.
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Keywords: NMDA; CGP37849; Neramexane; L-701.324; Ifenprodil; Alcohol deprivation effect (ADE); Relapse

Glutamatergic systems are targets for the actions of alcohol via its antagonism of the *N*-methyl-D-aspartate receptor (NMDAR) and other mechanisms. Following chronic alcohol intake, adaptations within the glutamatergic systems appear to contribute to alcohol dependence. In particular, the up-regulation of different subunits of the NMDAR (Dodd et al., 2000; Henninger et al., 2003; Nagy, 2004a) may cause hyperexcitability of the central nervous system during withdrawal or conditioned withdrawal and further possibly represent one mechanism involved in the induction of relapse behavior (Tsai and Coyle, 1998; Spanagel and Bienkowski, 2002; Krystal et al., 2003).

In order to study alcohol relapse-like behavior in rats, a new model of long-term alcohol consumption with repeated deprivation phases has recently been developed

Abbreviations: NMDA, *N*-methyl-D-aspartate; ADE, alcohol deprivation effect.

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(Spanagel and Hölter, 1999). Alcohol deprivation is known to affect voluntary alcohol intake. Renewed access to alcohol solutions after a period of deprivation for several days/weeks leads to a pronounced, although temporary, increase in voluntary alcohol intake (Le Magnen, 1960; Sinclair and Senter, 1967). This robust phenomenon is called the alcohol deprivation effect (ADE) and is observed across several species including rats, mice and monkeys (Sinclair, 1971; Salimov and Salimova, 1993). Following repeated deprivation phases, ADE is characterized by an increased demand for the drug and is clearly dissociated from normal eating or drinking behavior (Spanagel et al., 1996; Hölter et al., 1998; Spanagel, 2003) and therefore resembles a typical lapse or relapse situation in alcoholic patients. In recent years, the ADE model has become a widely used paradigm in examining the efficacy of pharmacological agents in preventing relapse drinking (Hölter et al., 1996; Spanagel and Zieglgänsberger, 1997; Heyser et al., 1998; Hölter and Spanagel, 1999; Rodd-Henricks et al., 2000; Spanagel and Hölter, 2000; Serra et al., 2003). In particular, the functional NMDAR antagonist acamprosate (Rammes et al., 2001) has been found to be effective in reducing ADE in long-term alcohol drinking rats (Spanagel et al., 1996; Hölter et al., 1997; Heyser et al., 1998, 2003) and has also been demonstrated to reduce relapse rates in abstinent alcoholic patients (Mann et al., 2004), suggesting an important role of the NMDAR in mediating relapse behavior. However, at the moment it is not known, which binding or modulatory sites of the NMDAR are involved in the expression of an ADE.

The NMDAR is a heteromeric subunit complex, composed of NR1 and NR2 subunits (NR2A-D). Functional receptors in the adult mammalian CNS are formed via combinations of NR1 and NR2 subunits, expressing the glycine and glutamate binding sites, respectively. Glutamate acts as an agonist and glycine as a co-agonist at the NMDAR. The endogenous polyamines spermine and spermidine also influence the activity of the NMDAR, in particular at NR2B subunit-containing receptors (Johnson, 1996). Further, yet another important binding site lies within the NMDAR complex channel, where uncompetitive antagonists block the receptor in a use-dependent manner, i.e. the channel must first be opened by an agonist for the antagonist to bind (Parsons et al., 2002).

In the present study, we examined the role of the different binding and modulatory sites of the NMDAR in mediating alcohol relapse behavior using the ADE model in long-term alcohol drinking rats. In a comprehensive fashion, we studied dose–response curves with the following selective pharmacological agents: the competitive antagonist CGP37849, which acts on the glutamate binding site, the antagonist L-701.324, which acts at the glycine binding site, the NR2B subunit selective antagonist ifenprodil, acting at the polyamine binding site, the uncompetitive antagonist neramexane, which acts as a channel blocker, and finally ethanol, which acts as a functional antagonist at the NMDAR.

1. Materials and methods

1.1. Animals

Two-month-old male Wistar rats (from our own breeding colony at the CIMH, Mannheim, Germany) were used. All animals were housed individually in standard rat cages under a 12 h artificial light–dark cycle (lights on at 6:00 a.m.). Room temperature was kept constant (temperature: 22 ± 1 °C, humidity: $55 \pm 5\%$). Standard laboratory rat food and water were provided ad libitum throughout the experimental period. Body weights were measured weekly. 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.

1.2. Alcohol self-administration with repeated deprivation phases

After two weeks of habituation to the animal room, rats were given ad libitum access to tap water and to 5%, 10% and 20% ethanol solutions (v/v) as well (for more details see Spanagel and Hölter, 1999). Spillage and evaporation were minimized by the use of special bottle caps (TSE, Bad Homburg, Germany). With this procedure the ethanol concentration remains constant for at least one week (Hölter et al., 1998). The positions of bottles were changed weekly to avoid location preferences.

The alcohol drinking procedure with repeated deprivation phases lasted for a duration of 52 weeks. The total time period for concurrent access to alcohol solutions and water was 40 weeks. All animals underwent 6 two-week deprivation periods. The first deprivation period was introduced after eight weeks of continuous alcohol availability. After this deprivation period, rats were given free access to water and to ethanol solutions for five weeks. Then, a second two-week deprivation period was introduced. This five-week drinking and two-week deprivation cycle was performed repeatedly.

1.3. Pharmacological studies

The pharmacological studies were introduced at the end of the seventh alcohol deprivation.

In order to study the effects of different NMDAR antagonists, 115 rats were divided into 14 groups

($n = 7–12$, see the figures for exact number of animals used in each group) in a way that the mean baseline total alcohol intake would be approximately the same in each group (i.e. ~3 g/kg/day). Baseline drinking was monitored for at least three days. After the last day of baseline measurement, the alcohol bottles were removed from the cages leaving the animals with free access to food and water for 14 days. Thereafter, each animal was subjected to a total of five intraperitoneal (IP) injections (starting at 7 p.m. with 12 h intervals) of CGP 37849 (G1: vehicle, G2: 2 mg/kg and G3: 8 mg/kg), neramexane HCl (G4: 4 mg/kg, and G5: 6 mg/kg), L-701.324 (G6: vehicle, G7: 2 mg/kg and G8: 5 mg/kg), ifenprodil (G9: vehicle, G10: 5 mg/kg and G11: 10 mg/kg), and ethanol (G12: vehicle, G13: 0.8 g/kg and G14: 1.6 g/kg). Control groups (G1, G6, G9, G12) were run following the administration of the respective vehicle (see Section 1.4); Note that for CGP37849 and neramexane HCl treatment the same vehicle control group (G1) was used.

The drug injection schedule was based on: (i) the half-life of the drugs, (ii) our pilot studies, (iii) previous studies using the same treatment schedule (e.g., Hölter and Spanagel, 1999; Hölter et al., 2000a,b), (iv) practicability (drug treatment should be done by only one investigator and the given schedule is already a maximal workload), and (v) the stress load for the animals – more than two injections per day are too stressful for these kinds of experiments.

The alcohol bottles were reintroduced after the second injection (at ~9 a.m. on the 15th day of alcohol deprivation) and the occurrence of an ADE was determined. Total ethanol intake (g/kg of body weight/day) and water intake (ml/kg of body weight/day) was measured daily at ~9 a.m. for a subsequent week. Each rat's body weight was recorded 24 h before the first injection and 12 h after the last injection.

1.4. Drugs

Alcohol drinking solutions were prepared from 96% ethanol (Merck, Darmstadt, Germany) and then diluted with tap water. For injections, 96% ethanol was diluted to a 15% (v/v) solution with 0.9% saline.

The following drugs were used in the study: 1-amino-1,3,3,5,5-pentamethyl-cyclohexane HCl (neramexane; Merz Pharmaceuticals), (*E*)-(±)2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849; Tocris Cookson Inc., Bristol, UK), 7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(1*H*)-quinolinone (L-701.324; Tocris Cookson Inc., Bristol, UK), 2-(4-benzylpiperidino)-1-(4-hydroxyphenyl)-1-propananol hemitartrate (ifenprodil; Tocris Cookson Inc., Bristol, UK). Note that all doses of the drugs used in this study were calculated based on the salt of the drug. All substances were dissolved in 0.9% saline except for ifenprodil, which was dissolved in water (*aqua ad iniectabilia*, Braun, Melsungen AG, Germany), and L-701.324, which was dissolved in polyethylene glycol 400 (PEG 400, Sigma–Aldrich Co., St. Louis, MO, USA) and then diluted with *aqua ad iniectabilia* to the final PEG 400 concentration 20%, and slightly alkalinized with NaOH (final pH ~9). All substances were freshly prepared and injected as a volume of 3 ml/kg IP. Ethanol was injected in different volumes in order to obtain different doses, i.e. G13: 0.8 g/kg (6.78 ml/kg) and G14: 1.6 g/kg (13.56 ml/kg) IP.

1.5. Statistics

Data obtained during baseline drinking and the ADE were analyzed by using two-way analysis of variance (ANOVA) with repeated measures for each drug treatment separately (factors were: between subjects–group, and within subjects–time/day). Data analysis for the effects of deprivation on water intake and changes of animals' body weight was performed using one-way ANOVA for different drug treatments (factor–group). Whenever significant differences were found, post hoc Student–Newman–Keul's tests were performed. The chosen level of significance was $p < 0.05$.

2. Results

The pharmacological studies were performed at the end of the seventh alcohol deprivation phase. Following the re-introduction of alcohol solutions, all four vehicle treated groups (G1, G6, G9 and G12) showed a typical increase in alcohol consumption indicating the occurrence of an ADE. This increase was not different from that observed during the first six deprivation periods. The treatment with CGP 37849, neramexane and L-701.324 produced rather similar effects, i.e. the drug treatment caused a reduction of the ADE but it was still expressed in all three animal groups. In particular, a two-way ANOVA data analysis indicated a significant effect of group and day for CGP 37849 (G1: 0 mg/kg = vehicle, G2: 2 mg/kg and G3: 8 mg/kg) treated animals [factor group: $F(2,23) = 7.7$, $p < 0.01$; factor day: $F(3,69) = 58.9$, $p < 0.0001$] and significant group × day interaction effect [$F(6,69) = 5.8$, $p < 0.0001$] (Fig. 1). Similarly, there was a significant group and day effect for neramexane (G1: 0 mg/kg = vehicle, G4: 4 mg/kg, and G5: 6 mg/kg) treated animals [factor group: $F(2,25) = 10.7$, $p < 0.001$; factor day: $F(3,75) = 41.9$, $p < 0.0001$] and a significant group × day interaction effect on alcohol intake [$F(6,75) = 4.3$, $p < 0.001$] (Fig. 2). In the case of L-701.324 (G6: 0 mg/kg = vehicle, G7: 2 mg/kg and G8: 5 mg/kg) treated animals, there was a significant effect of group and day [factor group: $F(2,23) = 4.1$, $p < 0.05$; factor day: $F(3,69) = 27.8$, $p < 0.0001$] and a significant group × day interaction

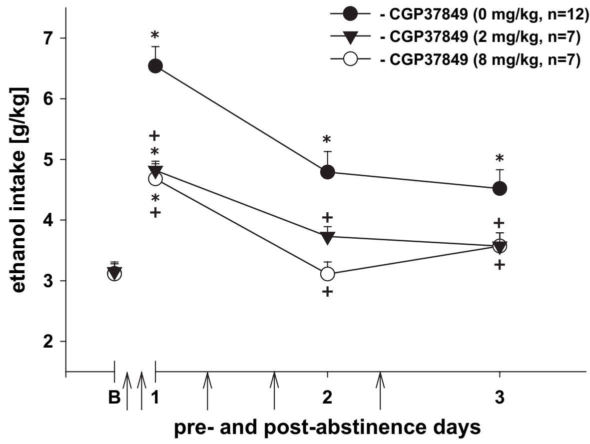

Fig. 1. Total ethanol intake (g/kg/day) before and after an alcohol deprivation period of two weeks. The average of three days measurements of ethanol intake is given as baseline drinking (B). Arrows indicate the administration of vehicle/0 mg/kg of CGP37849 ($n = 12$), 2 mg/kg of CGP37849 ($n = 7$), or 8 mg/kg of CGP37849 ($n = 7$). Data are presented as means ± S.E.M. * Indicates significant differences to baseline drinking; + indicates significant differences from vehicle control group, $p < 0.05$.

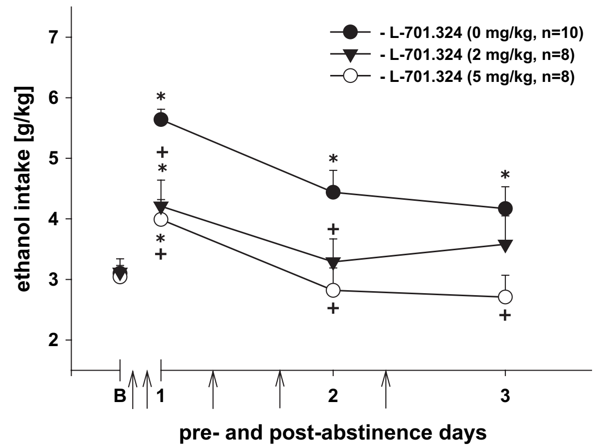

Fig. 3. Total ethanol intake (g/kg/day) before and after an alcohol deprivation period of two weeks. The average of three days measurements of ethanol intake is given as baseline drinking (B). Arrows indicate the administration of vehicle/0 mg/kg of L-701.324 ($n = 10$), 2 mg/kg of L-701.324 ($n = 8$), or 5 mg/kg of L-701.324 ($n = 8$). Data are presented as means ± S.E.M. * Indicates significant differences to baseline drinking; + indicates significant differences from vehicle control group, $p < 0.05$.

effect [$F(6,69) = 4.1$, $p < 0.01$] (Fig. 3). Interestingly, both lower and higher treatment doses of CGP 37849, neramexane and L-701.324 had a similar impact on alcohol intake.

The lower dose of ifenprodil (G10: 5 mg/kg) did not have any considerable effect on the expression of the ADE. The higher dose (G11: 10 mg/kg) reduced the ADE but to a lower extent as compared to the other compounds, i.e. alcohol intake remained greater than baseline level during the treatment procedure. Furthermore, the effect of treatment only caused a moderate reduction of alcohol intake on the first alcohol re-exposure day. Hence, a two-way ANOVA revealed a significant effect of group and day for ifenprodil (G9: 0 mg/kg = vehicle, G10: 5 mg/kg and G11: 10 mg/kg) treated animals during the ADE [factor group: $F(2,21) = 3.3$, $p < 0.05$; factor day: $F(3,63) = 109.7$, $p < 0.0001$], however, a group × day interaction effect was not significant (Fig. 4).

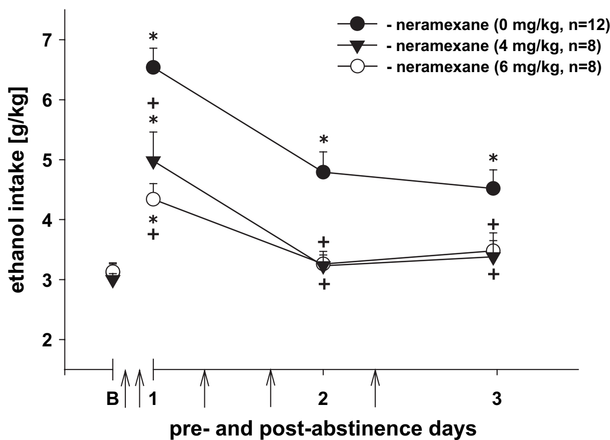

Fig. 2. Total ethanol intake (g/kg/day) before and after an alcohol deprivation period of two weeks. The average of three days measurements of ethanol intake is given as baseline drinking (B). Arrows indicate the administration of vehicle/0 mg/kg of neramexane ($n = 12$), 4 mg/kg of neramexane ($n = 8$), or 6 mg/kg of neramexane ($n = 8$). Data are presented as means ± S.E.M. * Indicates significant differences to baseline drinking; + indicates significant differences from vehicle control group, $p < 0.05$.

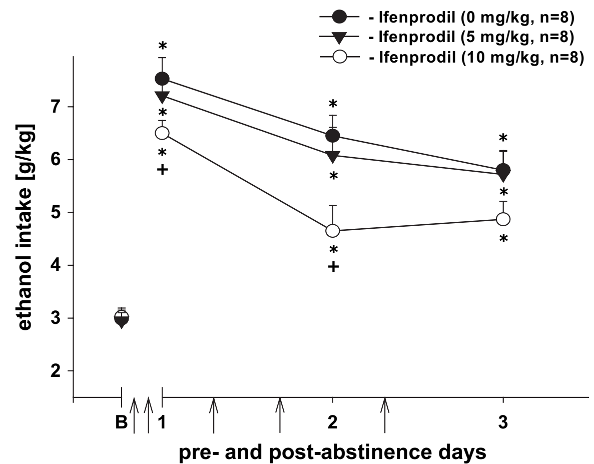

Fig. 4. Total ethanol intake (g/kg/day) before and after an alcohol deprivation period of two weeks. The average of three days measurements of ethanol intake is given as baseline drinking (B). Arrows indicate the administration of vehicle/0 mg/kg of ifenprodil ($n = 8$), 5 mg/kg of ifenprodil ($n = 8$), or 10 mg/kg of ifenprodil ($n = 8$). Data are presented as means ± S.E.M. * Indicates significant differences to baseline drinking; + indicates significant differences from vehicle control group, $p < 0.05$.

The effect of IP administration of alcohol was clearly dose-dependent. The lower dose (G13: 0.8 g/kg) caused a reduction of the ADE and it was completely abolished at the highest dose (G14: 1.6 g/kg). Thus, significant differences in alcohol intake between ethanol treated (G12: 0 g/kg = vehicle, G13: 0.8 g/kg and G14: 1.6 g/kg) rat groups during the ADE could be detected [factor group: $F(2,20) = 6.7$, $p < 0.01$]. Alcohol deprivation led to a significant increase in alcohol intake [factor day: $F(3,60) = 63.3$, $p < 0.0001$]. Moreover, a two-way ANOVA revealed a significant group × day interaction effect on alcohol intake during the treatment days [$F(6,60) = 8.3$, $p < 0.0001$] (Fig. 5).

Significant differences in water intake after reintroducing the alcohol were found in ethanol treated rats [factor group: $F(2,20) = 4.0$, $p < 0.05$] (Fig. 6). However, none of the other pharmacological treatments led to significant changes in water intake but a general trend towards enhanced water intake could be observed throughout the treatment groups following the administration of the highest dose of the respective compound.

Changes of animals' body weight following repeated drug treatment were found to be significantly different from the respective vehicle control groups (G1 and G6) in CGP37849 treated rats [factor group: $F(2,23) = 10.1$, $p < 0.01$] (G3: $-1.9 \pm 0.3\%$), in neramexane treated rats [factor group: $F(2,25) = 7.0$, $p < 0.01$] (G5: $-1.3 \pm 0.3\%$), and in L-701.324 treated rats [factor group: $F(2,23) = 18.5$, $p < 0.001$] (G8: $-1.4 \pm 0.3\%$). However, the lower treatment dose of any compound studied did not have any effect on body weight.

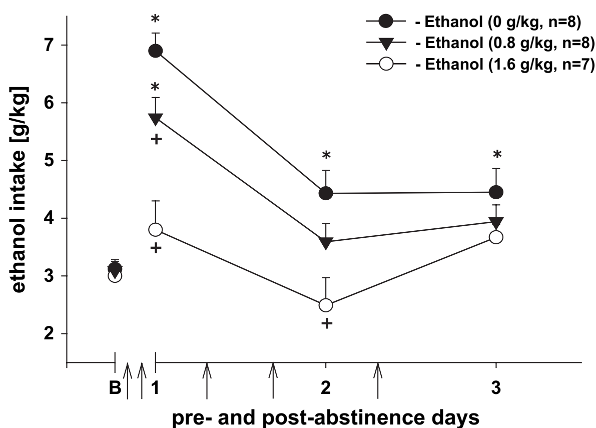

Fig. 5. Total ethanol intake (g/kg/day) before and after an alcohol deprivation period of two weeks. The average of three days measurements of ethanol intake is given as baseline drinking (B). Arrows indicate the administration of vehicle/0 g/kg of ethanol ($n = 8$), 0.8 g/kg of ethanol ($n = 8$), or 1.6 g/kg of ethanol ($n = 7$). Data are presented as means ± S.E.M. *Indicates significant differences to baseline drinking; + indicates significant differences from vehicle control group, $p < 0.05$.

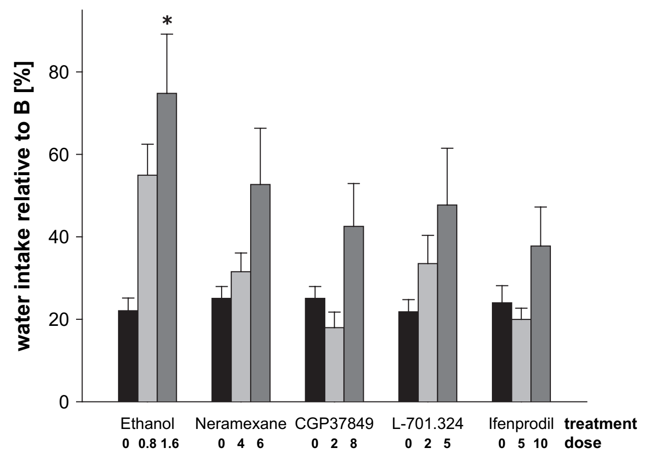

Fig. 6. Water intake during treatment days as the average of three days measurements. Water intake (ml/kg/day) after deprivation was calculated using drinking data before deprivation (B) as a reference. Data are presented as means ± S.E.M. *Indicates significant differences from control group, $p < 0.05$.

3. Discussion

In the present study, the repeated administration of the competitive NMDAR antagonist CGP37849, the NMDAR channel blocker neramexane, and the NR2B subunit selective antagonist ifenprodil, which acts at the polyamine binding site, as well as the glycine binding site antagonist L-701.324, produced a significant dose-dependent reduction of alcohol intake in long-term drinking rats during ADE. As well, IP administration of ethanol displayed a similar effect, as it completely abolished alcohol intake during ADE at the highest dose. The later finding is in accordance with a previous study, showing that the amount of orally consumed alcohol inversely correlates with the amount of alcohol infused intravenously in alcohol preferring rats (Waller et al., 1982). This indicates that under the present experimental conditions alcohol intake during an ADE is an entirely pharmacologically driven behavior that is not under the control of other factors such as taste or the novelty of alcohol re-exposure.

In recent years, the ADE paradigm in laboratory animals has become a standard model in measuring alcohol relapse behavior and in screening for new compounds, which have the ability to interfere with relapse mechanisms (Spanagel and Zieglgänsberger, 1997; Heyser et al., 1998; Rodd-Henricks et al., 2000; Spanagel and Hölter, 2000; Serra et al., 2003). Long-term alcohol drinking animals undergoing repeated alcohol deprivation phases exhibit an ADE, which is characterized by augmented alcohol intake and an uncontrolled demand for the drug that is clearly dissociated from normal eating and drinking behavior (Hölter et al., 1998; Spanagel and Hölter, 1999; Rodd-Henricks et al., 2001; Spanagel, 2003). Using this model, we studied the effects of repeated injections of ethanol

and different NMDAR antagonists on alcohol relapse behavior, finding that both ethanol as well as the other compounds may substitute for enhanced alcohol intake during the ADE. It should be noted, however, that at least one day following drug treatment enhanced alcohol intake remained in all groups indicating the involvement of other neurotransmitter systems in the expression of an ADE.

Ethanol inhibits the function of the NMDARs via a non-competitive mechanism (Ronald et al., 2001; Allgaier, 2002) and, thereby, produces specific discriminative stimulus properties. Accordingly, a number of NMDAR ligands were tested for substitution of the discriminative stimulus properties of ethanol. In general, it was found that uncompetitive NMDAR antagonists substituted for the discriminative stimulus effects of ethanol (Hundt et al., 1998). Thus, the NMDA channel blocker neramexane, in addition to a number of other antagonists acting at the NMDA channel, was found to have ethanol-like discriminative properties (Kotlinska and Liljequist, 1997; Hundt et al., 1998; Hölter et al., 2000a). The competitive NMDAR antagonist CGP37849 substituted only partially for ethanol (Bienkowski et al., 1996) and the glycine site antagonist L-701.324 showed a complete substitution for ethanol (Kotlinska and Liljequist, 1997; Bienkowski et al., 1998). In our study, ethanol, CGP37894, and L-701.324, as well as neramexane exhibited similar effects on alcohol intake during ADE. As mentioned above, all compounds may substitute for ethanol in a discrimination paradigm, suggesting that these drugs substitute for ethanol during ADE, thereby reducing in a dose-dependent manner relapse drinking behavior. Furthermore, our data suggest that the reduction of activity of the NMDARs in general, through different receptor binding and regulatory sites, plays a major role in modulating the effect of ethanol in the brain. Accordingly, it was demonstrated that the competitive antagonist CGP37849 reversed the anxiogenic effects of ethanol withdrawal (Gatch et al., 1999). L-701.324 produced a dose-dependent inhibition of alcohol withdrawal signs (Kotlinska and Liljequist, 1996; Kotlinska, 2001) and 5 mg/kg of L-701.324 co-administered repeatedly with alcohol, prevented the acquisition of the reinforcing properties of alcohol as measured in the conditioned place preference paradigm (Biala and Kotlinska, 1999). The channel blocker neramexane dose-dependently suppressed ethanol withdrawal seizures (Bienkowski et al., 2001), reduced ethanol intake and ADE when administered chronically via osmotic minipumps (Hölter et al., 2000a) and attenuated the responding for ethanol in an operant ethanol self-administration paradigm (Bienkowski et al., 1999). Furthermore, it prevented the acquisition of ethanol-induced conditioned place preference (Kotlinska, personal communication).

All three binding sites at the NMDAR (competitive, uncompetitive, and glycine binding site) are therefore interesting for drug development in alcohol dependence. However, it should be mentioned that in recent studies on cue-induced reinstatement of ethanol-seeking behavior, competitive and uncompetitive NMDAR antagonists were not effective (Backström and Hyytiä, 2004; Bachteler et al., 2005), whereas L-701.324 dose-dependently reduced ethanol-seeking behavior (Backström and Hyytiä, 2004). Furthermore, since NMDARs are involved in almost all of the functions of the central nervous system, therapeutic intervention may potentially be associated with side effects (e.g., sedation and cognitive disturbances). Although the doses used in the present study do not usually produce sedation (Kotlinska and Liljequist, 1996; Przegalinski et al., 1996; Bienkowski et al., 1999), it should be noted that CGP37849, L-701.324 and neramexane caused a reduction (1–2%) in the animals' body weight at the highest dose tested. In contrast, treatment with the lower dose produced almost the same effect on alcohol intake without having any effect on the animals' body weight. Furthermore, water intake remained stable or even tended to increase with the higher treatment doses as a compensation due to the decreased ethanol consumption, indicating that no sedation following drug treatment occurred. However, even though it appears that these compounds had no sedative effects in the 24 h measurements, we cannot rule out that acute sedative effects occurred right after injections. In the future, a fully automated drinkometer system, adapted to the specific home cage drinking situation will help in determining the exact time-dependent intake patterns at the water bottle and at each of the alcohol bottles.

Polyamine site antagonists have earned attention because of their actions being restricted to NMDARs containing the NR2B subunit and due to better safety profiles (e.g., reduced liability to induce motor impairments) (Boyce et al., 1999; Chizh et al., 2001). It is also known that the NR1/NR2B subtype of the NMDA receptor is more sensitive to the inhibitory effect of ethanol and that chronic alcohol intake leads to an increase in the expression of this subtype (Dodd et al., 2000; Allgaier, 2002; Henniger et al., 2003; Nagy, 2004a). On the other hand, polyamines are not essential for the activity of the NMDAR. They can stimulate NMDARs by increasing the affinity for glycine. Conversely, they may also decrease the affinity for glutamate during chronic receptor activation (Parsons et al., 2002). However, polyamine site antagonists do not substitute for ethanol or show only partial substitution (Kotlinska and Liljequist, 1997; Hundt et al., 1998), even though they were shown to be potent inhibitors of ethanol withdrawal induced seizures and neurotoxicity (Malinowska et al., 1999; Nagy et al., 2004). In our study, the polyamine site antagonist

ifenprodil reduced the alcohol intake during ADE, although to a lower extent as compared to other compounds studied.

In summary, our present study demonstrates that a repeated administration of competitive and uncompetitive NMDAR antagonists dose-dependently suppresses alcohol consumption during an ADE, showing that an inhibition of the NMDAR in general, rather than particular subunits or binding sites, is essential for the reduction of relapse-like drinking behavior during an ADE. However, an NMDAR blockade via high affinity channel blockers or competitive NMDAR antagonists may also induce a variety of side effects. Therefore, low affinity channel blockers, glycine binding site and NR2B subunit selective antagonists, displaying a superior profile on side effects (Parsons, 2001; Chizh, 2002; Nagy, 2004b) may be more appropriate for the use as anti-relapse compounds.

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References

Allgaier, C., 2002. Ethanol sensitivity of NMDA receptors. Neurochemistry International 41, 377–382.

Bachteler, D., Economidou, D., Danysz, W., Ciccocioppo, R., Spanagel, R., 2005. The effects of acamprosate and neramexane on cue-induced reinstatement of ethanol-seeking behaviour in rat. Neuropsychopharmacology, in press.

Backstrom, P., Hyytia, P., 2004. Ionotropic glutamate receptor antagonists modulate cue-induced reinstatement of ethanol-seeking behavior. Alcoholism Clinical and Experimental Research 28, 558–565.

Biala, G., Kotlinska, J., 1999. Blockade of the acquisition of ethanol-induced conditioned place preference by *N*-methyl-D-aspartate receptor antagonists. Alcohol and Alcoholism 34, 175–182.

Bienkowski, P., Danysz, W., Kostowski, W., 1998. Study on the role of glycine, strychnine-insensitive receptors (glycineB sites) in the discriminative stimulus effects of ethanol in the rat. Alcohol 15, 87–91.

Bienkowski, P., Koros, E., Kostowski, W., Danysz, W., 1999. Effects of *N*-methyl-D-aspartate receptor antagonists on reinforced and nonreinforced responding for ethanol in rats. Alcohol 18, 131–137.

Bienkowski, P., Krzascik, P., Koros, E., Kostowski, W., Scinska, A., Danysz, W., 2001. Effects of a novel uncompetitive NMDA receptor antagonist, MRZ 2/579 on ethanol self-administration and ethanol withdrawal seizures in the rat. European Journal of Pharmacology 413, 81–89.

Bienkowski, P., Stefanski, R., Kostowski, W., 1996. Competitive NMDA receptor antagonist, CGP 40116, substitutes for the discriminative stimulus effects of ethanol. European Journal of Pharmacology 314, 277–280.

Boyce, S., Wyatt, A., Webb, J.K., O'Donnell, R., Mason, G., Rigby, M., Sirinathsinghji, D., Hill, R.G., Rupniak, N.M., 1999. Selective NMDA NR2B antagonists induce antinociception without motor dysfunction: correlation with restricted localisation of NR2B subunit in dorsal horn. Neuropharmacology 38, 611–623.

Chizh, B.A., 2002. Novel approaches to targeting glutamate receptors for the treatment of chronic pain: review article. Amino Acids 23, 169–176.

Chizh, B.A., Headley, P.M., Tzschentke, T.M., 2001. NMDA receptor antagonists as analgesics: focus on the NR2B subtype. Trends in Pharmacological Sciences 22, 636–642.

Dodd, P.R., Beckmann, A.M., Davidson, M.S., Wilce, P.A., 2000. Glutamate-mediated transmission, alcohol, and alcoholism. Neurochemistry International 37, 509–533.

Gatch, M.B., Wallis, C.J., Lal, H., 1999. Effects of NMDA antagonists on ethanol-withdrawal induced "anxiety" in the elevated plus maze. Alcohol 19, 207–211.

Henniger, M.S., Wotjak, C.T., Holter, S.M., 2003. Long-term voluntary ethanol drinking increases expression of NMDA receptor 2B subunits in rat frontal cortex. European Journal of Pharmacology 470, 33–36.

Heyser, C.J., Moc, K., Koob, G.F., 2003. Effects of naltrexone alone and in combination with acamprosate on the alcohol deprivation effect in rats. Neuropsychopharmacology 28, 1463–1471.

Heyser, C.J., Schulteis, G., Durbin, P., Koob, G.F., 1998. Chronic acamprosate eliminates the alcohol deprivation effect while having limited effects on baseline responding for ethanol in rats. Neuropsychopharmacology 18, 125–133.

Hölter, S.M., Danysz, W., Spanagel, R., 1996. Evidence for alcohol anti-craving properties of memantine. European Journal of Pharmacology 314, R1–R2.

Hölter, S.M., Danysz, W., Spanagel, R., 2000a. Novel uncompetitive *N*-methyl-D-aspartate (NMDA)-receptor antagonist MRZ 2/579 suppresses ethanol intake in long-term ethanol-experienced rats and generalizes to ethanol cue in drug discrimination procedure. Journal of Pharmacology and Experimental Therapeutics 292, 545–552.

Hölter, S.M., Engelmann, M., Kirschke, C., Liebsch, G., Landgraf, R., Spanagel, R., 1998. Long-term ethanol self-administration with repeated ethanol deprivation episodes changes ethanol drinking pattern and increases anxiety-related behaviour during ethanol deprivation in rats. Behavioural Pharmacology 9, 41–48.

Hölter, S.M., Henniger, M.S., Lipkowski, A.W., Spanagel, R., 2000b. Kappa-opioid receptors and relapse-like drinking in long-term ethanol-experienced rats. Psychopharmacology (Berl) 153, 93–102.

Hölter, S.M., Landgraf, R., Zieglgansberger, W., Spanagel, R., 1997. Time course of acamprosate action on operant ethanol self-administration after ethanol deprivation. Alcoholism: Clinical and Experimental Research 21, 862–868.

Hölter, S.M., Spanagel, R., 1999. Effects of opiate antagonist treatment on the alcohol deprivation effect in long-term ethanol-experienced rats. Psychopharmacology (Berl) 145, 360–369.

Hundt, W., Danysz, W., Holter, S.M., Spanagel, R., 1998. Ethanol and *N*-methyl-D-aspartate receptor complex interactions: a detailed drug discrimination study in the rat. Psychopharmacology 135, 44–51.

Johnson, T.D., 1996. Modulation of channel function by polyamines. Trends in Pharmacological Sciences 17, 22–27.

Kotlinska, J., 2001. NMDA antagonists inhibit the development of ethanol dependence in rats. Polish Journal of Pharmacology 53, 47–50.

Kotlinska, J., Liljequist, S., 1996. Oral administration of glycine and polyamine receptor antagonists blocks ethanol withdrawal seizures. Psychopharmacology 127, 238–244.

Kotlinska, J., Liljequist, S., 1997. The NMDA/glycine receptor antagonist, L-701.324, produces discriminative stimuli similar to those of ethanol. European Journal of Pharmacology 332, 1–8.

Krystal, J.H., Petrakis, I.L., Mason, G., Trevisan, L., D'Souza, D.C., 2003. N-methyl-D-aspartate glutamate receptors and alcoholism: reward, dependence, treatment, and vulnerability. Pharmacology & Therapeutics 99, 79–94.

Le Magnen, J., 1960. Etude de quelques facteurs associés à des modifications de la consommation spontanée d'alcool éthylique par le rat. Journal of Physiology, Paris 52, 873–884.

Malinowska, B., Napiorkowska-Pawlak, D., Pawlak, R., Buczko, W., Gothert, M., 1999. Ifenprodil influences changes in mouse behaviour related to acute and chronic ethanol administration. European Journal of Pharmacology 377, 13–19.

Mann, K., Lehert, P., Morgan, M.Y., 2004. The efficacy of acamprosate in the maintenance of abstinence in alcohol-dependent individuals: results of a meta-analysis. Alcoholism Clinical and Experimental Research 28, 51–63.

Nagy, J., 2004a. The NR2B subtype of NMDA receptor: a potential target for the treatment of alcohol dependence. Current Drug Targets. CNS and Neurological Disorders 3, 169–179.

Nagy, J., 2004b. Renaissance of NMDA receptor antagonists: do they have a role in the pharmacotherapy for alcoholism? The Investigational Drugs 7, 339–350.

Nagy, J., Horvath, C., Farkas, S., Kolok, S., Szombathelyi, Z., 2004. NR2B subunit selective NMDA antagonists inhibit neurotoxic effect of alcohol-withdrawal in primary cultures of rat cortical neurones. Neurochemistry International 44, 17–23.

Parsons, C.G., 2001. NMDA receptors as targets for drug action in neuropathic pain. European Journal of Pharmacology 429, 71–78.

Parsons, C.G., Danysz, W., Lodge, D., 2002. Introduction to glutamate receptors, their function and pharmacology. In: Lodge, D., Danysz, W., Parsons, C.G. (Eds.), Ionotropic Glutamate Receptors as Therapeutic Targets. FP Graham Publishing Co., Johnson City, TN, pp. 1–27.

Przegalinski, E., Tatarczynska, E., Deren-Wesolek, A., Chojnacka-Wojcik, E., 1996. Anticonflict effects of a competitive NMDA receptor antagonist and a partial agonist at strychnine-insensitive glycine receptors. Pharmacology Biochemistry and Behavior 54, 73–77.

Rammes, G., Mahal, B., Putzke, J., Parsons, C., Spielmanns, P., Pestel, E., Spanagel, R., Zieglgansberger, W., Schadrack, J., 2001. The anti-craving compound acamprosate acts as a weak NMDA-receptor antagonist, but modulates NMDA-receptor subunit expression similar to memantine and MK-801. Neuropharmacology 40, 749–760.

Rodd-Henricks, Z.A., McKinzie, D.L., Edmundson, V.E., Dagon, C.L., Murphy, J.M., McBride, W.J., Lumeng, L., Li, T.K., 2000. Effects of 5-HT(3) receptor antagonists on daily alcohol intake under acquisition, maintenance, and relapse conditions in alcohol-preferring (P) rats. Alcohol 21, 73–85.

Rodd-Henricks, Z.A., Bell, R.L., Kuc, K.A., Murphy, J.M., McBride, W.J., Lumeng, L., Li, T.K., 2001. Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of alcohol-preferring rats. Alcoholism Clinical and Experimental Research 25, 1140–1150.

Ronald, K.M., Mirshahi, T., Woodward, J.J., 2001. Ethanol inhibition of N-methyl-D-aspartate receptors is reduced by site-directed mutagenesis of a transmembrane domain phenylalanine residue. Journal of Biological Chemistry 276, 44729–44735.

Salimov, R.M., Salimova, N.B., 1993. The alcohol deprivation effect in hybrid mice. Drug and Alcohol Dependence 32, 187–191.

Serra, S., Vacca, G., Tumatis, S., Carrucciu, A., Morazzoni, P., Bombardelli, E., Colombo, G., Gessa, G.L., Carai, M.A., 2003. Anti-relapse properties of IDN 5082, a standardized extract of *Salvia miltiorrhiza*, in alcohol-preferring rats. Journal of Ethnopharmacology 88, 249–252.

Sinclair, J.D., 1971. The alcohol-deprivation effect in monkeys. Psychonomic Science 25, 21–22.

Sinclair, J.D., Senter, R.J., 1967. Increased preference for ethanol in rats following alcohol deprivation. Psychonomic Science 8, 11–12.

Spanagel, R., 2003. Alcohol addiction research: from animal models to clinics. Best Practice and Research Clinical GA 17, 507–518.

Spanagel, R., Bienkowski, P., 2002. Alcohol dependence and addiction, in ionotropic glutamate receptors as therapeutic targets. In: Lodge, D., Danysz, W., Parsons, C.G. (Eds.), Ionotropic Glutamate Receptors as Therapeutic Targets. FP Graham Publishing Co., Johnson City, TN, pp. 375–400.

Spanagel, R., Hölter, S.M., 1999. Long-term alcohol self-administration with repeated alcohol deprivation phases: an animal model of alcoholism? Alcohol and Alcoholism 34, 231–243.

Spanagel, R., Hölter, S.M., 2000. Pharmacological validation of a new animal model of alcoholism. Journal of Neural Transmission 107, 669–680.

Spanagel, R., Hölter, S., Allingham, K., Landgraf, R., Zieglgänsberger, W., 1996. Acamprosate and alcohol: I. Effects on alcohol intake following alcohol deprivation in the rat. European Journal of Pharmacology 305, 39–44.

Spanagel, R., Zieglgansberger, W., 1997. Anti-craving compounds for ethanol: new pharmacological tools to study addictive processes. Trends in Pharmacological Sciences 18, 54–59.

Tsai, G., Coyle, J.T., 1998. The role of glutamatergic neurotransmission in the pathophysiology of alcoholism. Annual Review of Medicine 49, 173–184.

Waller, M.B., McBridge, W.J., Lumeng, L., Li, T.K., 1982. Effects of intravenous ethanol and of 4-methylpyrazole on alcohol drinking in alcohol-preferring rats. Pharmacology Biochemistry and Behavior 17, 763–768.