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

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ABSTRACT
Previous findings suggested that drugs modulating glutamatergic neurotransmission could be useful in the treatment of alcohol dependence. This study examined the effects of chronic and acute treatment with MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexane hydrochloride), a novel uncompetitive $N$-methyl-$d$-aspartate receptor antagonist, on voluntary ethanol intake in long-term ethanol-experienced rats. Rats were implanted with mini-osmotic pumps delivering either 9.6 mg/day MRZ 2/579 or vehicle, and the effects of treatment on the alcohol deprivation effect (ADE) were studied in a four-bottle home cage-drinking paradigm. The same rats were tested for a second ADE 3 weeks later in the absence of the drug. In a second experiment long-term ethanol-experienced rats trained in an operant free-choice ethanol self-administration paradigm with concurrent water received acute MRZ 2/579 treatment (0–4 mg/kg i.p.) before a 23-h session either during basal drinking or during the ADE. In an additional experiment, MRZ 2/579 (0.5–4 mg/kg i.p.) was tested for generalization to the ethanol cue in a drug discrimination procedure. Chronic MRZ 2/579 treatment selectively abolished the increased ethanol intake during the ADE. This effect depended on the presence of the drug. Acute MRZ 2/579 treatment (2 and 4 mg/kg) had a short-lasting reductive effect on lever pressing for ethanol, but not for water, both during the ADE and basal drinking. MRZ 2/579 dose dependently generalized to the ethanol cue in the drug discrimination experiment. It is concluded that MRZ 2/579 might exert its reducing effect on ethanol intake by substituting for some of the stimulus properties of ethanol.

There is increasing evidence that drug-induced changes in glutamatergic neurotransmission might play a role in the development of alcoholism (Tsai et al., 1995; Tsai and Coyle, 1998). Acutely, alcohol exerts antagonistic effects on $N$-methyl-$d$-aspartate (NMDA) receptor function, and chronic alcohol consumption leads to an increase in NMDA receptor-mediated neurotransmission, which is presumably not due to a simple increase in NMDA receptor density, but to differential up-regulation of different NMDA receptor subunits that could result in changes in the composition and functioning of NMDA receptor complexes (Tabakoff and Hoffman, 1996; Rudolph et al., 1997; Darstein et al., 1998; Faingold et al., 1998). Therefore, modulators of the glutamatergic/NMDA receptor system are now considered in the search for pharmacotherapeutic agents that may be useful in the treatment of alcohol dependence (Parsons et al., 1998).

The functional NMDA receptor antagonist acamprosate was effective in a series of preclinical studies (Spanagel et al., 1996; Hölter et al., 1997; Heyser et al., 1998) and clinical trials (Sass et al., 1996; Whitworth et al., 1996) in reducing alcohol consumption and relapse (for review, see Spanagel and Ziegglinsberger, 1997). Competitive NMDA receptor antagonists attenuated operant responding for ethanol without affecting baseline levels of water self-administration (Rassnick et al., 1992). However, the selectivity of such an effect was questioned by demonstrating that the competitive NMDA receptor antagonist CPPene decreased both ethanol and saccharin self-administration (Shelton and Balster, 1997). Uncompetitive NMDA receptor antagonists such as phencyclidine and memantine also reduced alcohol intake (Shelton and Balster, 1997; Piasecki et al., 1998) and prevented relapse (Hölter et al., 1996).

Encouraged by these findings, new compounds based on a cyclohexan structure with similar characteristics to memantine were developed, MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexane hydrochloride) being one of the most promising of these agents. MRZ 2/579 possesses rapid block-
ing/unblocking kinetics and a strong voltage dependence, neuroprotective properties in vitro and in vivo and antiparkinsonian-like activity, whereas it lacks psychotomimetic potential (Parsons et al., 1995, 1999; Danysh et al., 1997; Wenk et al., 1998). This profile suggests that MRZ 2/579 could prove to be useful in a wide range of central nervous system disorders that involve disturbances of glutamatergic neurotransmission because such biophysical properties may allow therapeutically relevant concentrations to block chronic, low-level pathological activation of NMDA receptors whereas leaving their synaptic activation intact (Parsons et al., 1998).

A long-term free-choice ethanol self-administration paradigm in Wistar rats is a promising animal model for the search for pharmacotherapeutic agents in relapse prevention. After several months of voluntary ethanol consumption, the drug-taking behavior following a deprivation phase is characterized by increased ethanol intake and preference and by changes in intake patterns, resulting in an immediate increase in consumption of highly concentrated ethanol solutions both under operant and nonoperant conditions (Spanagel et al., 1996; Hölter et al., 1997, 1998). The phenomenon of a transient increase in ethanol consumption and preference after a period of imposed ethanol abstinence has been termed the alcohol deprivation effect (ADE) and has been observed in several species, including humans (Sinclair and Senter, 1967; Sinclair, 1971; Burish et al., 1981). Because the ADE in a long-term ethanol self-administration model is characterized by an increased motivation to obtain ethanol, outlasts long abstinence phases, and is hardly modified by external stimuli such as taste adulteration or social factors, it can be regarded as an animal model of relapse and possibly of craving (Wolfgrämm and Heyne, 1991; Spanagel et al., 1996; Spanagel and Hölter, 1999). Moreover, it has been shown that the ADE can be suppressed by acamprosate (Spanagel et al., 1996; Hölter et al., 1997; Heyser et al., 1998) and naltrrexone (Kornet et al., 1991; Hölter and Spanagel, 1999), compounds that are also effective in reducing relapse in abstinent alcoholics (O'Malley et al., 1992; Volpicelli et al., 1992; Sass et al., 1996; Whitworth et al., 1996).

The aim of the present study was to investigate the effects of MRZ 2/579 on ethanol-drinking behavior in long-term ethanol-experienced rats. Thus, we tested the effects of chronic MRZ 2/579 on the ADE in a home cage-drinking paradigm, and the effects of acute MRZ 2/579 in an operant ethanol self-administration paradigm because this allows for detailed analysis of the time course of the acute drug effects without disturbing the animals. In an additional experiment, MRZ 2/579 was tested for generalization to the ethanol cue in a drug discrimination test because previous studies have shown that uncompetitive NMDA-receptor antagonists dose-dependently substitute for the ethanol cue (Colombo and Grant, 1992; Shelton and Balster, 1994; Hundt et al., 1998).

Materials and Methods

Subjects. Forty-two male Wistar rats (Max Planck Institute of Biochemistry, Martinsried, Germany), weighing 220 to 250 g at the beginning of the experiment, were used in this study. All animals were housed individually in standard hanging rodent cages with tap water ad libitum. Animals that were used for the long-term voluntary ethanol self-administration and for pharmacokinetic experiments received food ad libitum. The weight of animals used for the drug discrimination study was maintained at ~80% of the weight under free-feeding conditions by restricting their daily food consumption. Artificial light was provided daily from 7:00 AM until 7:00 PM, 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. After 1 week of habituation to the animal room, all rats were given continuous access to tap water, and 5, 10, and 20% (v/v) ethanol solutions in their home cages. Spillage and evaporation were minimized by the use of bottle caps with ball bearings (Ehret, Emmendingen, Germany). With this procedure the ethanol concentration in any of the solutions stayed constant for at least 1 week (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.

ADE Measurement in Nonoperant Self-Administration Paradigm. In the first experiment, we determined the effects of chronic MRZ 2/579 treatment on the alcohol deprivation effect in rats with 11 months of ethanol experience in the long-term paradigm described above.

Baseline measures were determined by daily weighing of the bottles, the food, and the animals at 10:00 AM for 4 preabstinence 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. Thirteen days later, the animals were briefly anesthetized with halothane and mini-osmotic pumps (model 2 ML1, pumping rate 10 μl/h for 1 week; Alzet, Palo Alto, CA) were implanted s.c. Half of the mini-osmotic pumps had been filled with MRZ 2/579 (40 mg/ml, resulting in a dose of 9.6 mg/day; n = 8) and the other half with vehicle (n = 8). One day after surgery, the ethanol solutions were presented again to the animals at 10:00 AM and the daily weighing routine was reintroduced to assess the ADE. This was done for six postabstinence days until the mini-osmotic pumps were empty. Then ethanol bottles were removed again for 2 weeks and after these 2 weeks, when ethanol bottles were given back to the animals, the ADE was assessed a second time by the daily weighing routine for four postabstinence days, this time in the absence of MRZ 2/579.

Operant Chambers. Eight operant chambers (Med Associates Inc., East Fairfield, VT) 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. The pressing of one lever resulted in the delivery of a drop of tap water and pressing of the other lever resulted in the delivery of a drop of 20% (v/v) ethanol (FR1FR1). The volume per drop was 25 to 30 μl. The chambers were controlled and data automatically recorded by a personal computer.

Training and Testing Procedure in Operant Self-Administration Paradigm. In this experiment, we determined the effects of acute MRZ 2/579 treatment on the alcohol deprivation effect in rats with at least 16 months of ethanol experience. After 5 months of ethanol experience in the long-term, four-bottle paradigm described above, the operant chamber sessions began. All sessions were started at 10:00 AM 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 PM and on at 7:00 AM to keep the room dark.
the animals in their regular light/dark cycle. The animals were tested in the chambers once a 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 learned to lever press for liquid. After that, the animals were never liquid deprived again. The “basal” group (n = 6) had always access to ethanol in their home cage, thus representing baseline ethanol-drinking conditions in the operant chamber sessions. The “ADE” group (n = 8) was always tested in the operant chambers after 1 week of ethanol abstinence. Sessions were continued until drinking behavior and total lever-pressing activity were stable within both groups before testing. Both groups had equal numbers of training sessions before the animals were tested 30 min after the i.p. injection of 0, 1, 2, and 4 mg/kg MRZ 2/579 in a counterbalanced design.

Drug Discrimination. Standard operant chambers (Coulburn Instruments, Lehigh Valley, PA) were used for drug discrimination training. Each chamber was equipped with two levers, one on either side and equidistant from a food cup. The chambers were contained in ventilated, sound-attenuated cubicles equipped with a house light. The experiments were controlled by a computer (Med Associates Inc.) with a modified version of the software package described by Spencer and Emmett-Oglesby (1985).

For discrimination training, the responding of the animals (n = 8) was first shaped by reinforcement of lever pressing with food delivery according to an increasing fixed ratio (FR). Once animals had reached a fixed ratio of 10 responses for each food pellet (FR 10) (45-mg pellets; Bioserve, Frenchtown, NJ), drug- and vehicle-training sessions began. Training sessions began 10 min after an injection of either ethanol (1 g/kg i.p., injection volume 10 ml/kg) or the appropriate volume of saline and ended after 15 min. Responses on the correct lever were reinforced and recorded and those on the incorrect lever recorded only. The left lever was designated as the drug lever for half of the animals and the right one for the remaining half. During each training session, the first 10 presses on either lever, designated the “selected lever,” were used to ascertain acquisition of stimulus control. Rats received a randomized sequence of training sessions (one session per day, 5 days a week) with a maximum of three consecutive drug- or vehicle-training sessions. The criterion for stimulus control was set at eight consecutive correct lever selections of the last 10 sessions, with at least 90% drug- or vehicle-appropriate responses during these sessions.

Discrimination tests were conducted twice weekly, with either ethanol or vehicle training on the intervening days. The day before testing, all rats were trained with saline. Testing commenced after the rats had been placed in the chambers, which was 10 min after either ethanol or saline administration. Test sessions were terminated either after the completion of one FR (10 presses) or 5 min had elapsed. Responses were not reinforced during these sessions. Two measures of discrimination were obtained. A quantal measure, which was derived from the percentage of animals tested that selected the ethanol lever, and a graded measure, which was calculated from the number of responses on the drug lever and the total number of responses on both levers until the first FR was completed (first 10 presses on either lever designated it as selected lever). Thus, 19 possible responses could be elicited, and a percentage score was determined for each treatment. In the figure, the graded measure is used. The time required to complete the first ratio (lever response latency) served as an additional measure of responding, indicating a possible impairment of responding by the substance injected. Animals that did not reach a minimum of 10 responses on either the saline or the ethanol lever within 5 min were excluded from data analysis. Following acquisition of drug discrimination, generalization tests were conducted with four doses of ethanol (0.25–1.5 g/kg) to obtain a dose-response relationship for ethanol discrimination. Thereafter, a generalization test was conducted with MRZ 2/579 instead of ethanol animals were injected i.p. with different doses of MRZ 2/579 (0.5, 1, 2, or 4 mg/kg) in the same injection volume as ethanol-training sessions and placed into the operant chambers 30 min after injection. Doses were injected in a randomized order in all tests.

Determination of MRZ 2/579 Concentrations. To determine brain and serum levels of MRZ 2/579 after chronic infusion, four age-matched ethanol-naive control rats with 11 months of individual housing were briefly anesthetized with halothane and mini-osmotic pumps (model 2 ML1, pumping rate 10 μl/h for 1 week; Alzet) filled with MRZ 2/579 (40 mg/ml, resulting in a dose of 9.6 mg/day) were implanted s.c. Six days after surgery, the animals were sacrificed by decapitation after halothane anesthesia, a blood sample was taken, and the brain was removed.

Brain tissue was homogenized in a 4-ml vial with a disposable spoutula. Then 2 ml of 2.5 M H2SO4 was pipetted to 0.2 g of brain sample, mixed on a Vortex mixer, and heated for 60 min at 90°C in a heating block. Then 250 μl of homogeneous brain, 250 μl of internal standard (ISTD) solution (Aramantadine-HCl, –1 mg/l H3O), 500 μl water, and 1 ml of n-hexane were pipetted into a 4-ml vial and extracted for 30 min on a roller mixer. After centrifugation for 10 min at 4000 rpm, the organic phase was rejected. After that, 1 ml n-hexane and 0.5 ml 10 M NaOH were pipetted to the remaining solution. This mixture was extracted on a roller mixer for 30 min. Subsequently, the vial with the mixture was centrifuged for 10 min at 4000 rpm, the organic phase transferred into a gas chromatography vial, and 50 μl of N-methyl-bis-trifluoroacetamide was added. The sample was mixed on a Vortex mixer and heated for 30 min at 70°C in a heating block.

Serum samples (50 μl) were added with 250 μl of 2 N HCl and 250 μl of ISTD solution (Aramantadine-HCl, –1 mg/l H3O), pipetted into a 4-ml vial with a screw cap, and heated for 30 min at 70°C in a heating block. After cooling to room temperature, 1 ml of n-hexane and 0.25 ml of 10 M NaOH were added and the mixture was extracted for 30 min on a roller mixer. Subsequently, the test tube with the mixture was centrifuged for 10 min at 4000 rpm, the organic phase was transferred into a vial, and 15 μl of N-methyl-bis-trifluoroacetamide was added. After that, the sample was mixed on a Vortex mixer, and the organic phase was reduced to 70°C to 150 μl in a heating block.

After transfer of a sample in a vial insert, the measurement was carried out with gas chromatography/mass spectrometry detection (Hewlett-Packard HP 5890 II with mass spectrometry detection 5971A/5972). Measurement conditions were as follows: column, HP1 (Hewlett-Packard) Cl-methylsilicone, 25 m × 0.2 mm i.d.; carrier gas, helium 12 psi; injection mode, splitless 3 μl; injection temperature, 250°C; detector temperature, 280°C; detected ions, TFA MRZ 2/579 137 (±0.2 amu) and TFA Aramantadine 247 (±0.2 amu).

Drugs. Ethanol-drinking solutions were made up from 96% pure ethanol diluted with tap water to the different concentrations. For injections, 96% pure ethanol was diluted with 0.9% saline to a 12% (v/v) solution. MRZ 2/579 (Merz) was dissolved in water ad inexabilia (Braun, Meisenburg, Germany). Drug doses refer to the weight of the salt.

Data Analysis. The effects of chronic MRZ 2/579 treatment on the occurrence of an ADE were analyzed by three-way ANOVA with repeated measures (treatment × ADE × days). Given that there were no significant group differences in baseline levels, treatment effects on postabstinence days were assessed by two-way ANOVA with repeated measures (treatment × days). Drug effects on food and fluid consumption were analyzed by two-way ANOVA with repeated measures (treatment × days). Concerning operant chamber data, dose-response effects of drug treatment on ethanol intake, ethanol preference, and the total number of lever presses were assessed individually for each group by one-way ANOVA with repeated measures. Dose-response effects on accumulated first-hour data were analyzed by two-way ANOVA with randomized blocks (dose × time interval). Lever latency data of drug discrimination testing was analyzed by a one-way ANOVA. The chosen level of significance was P < .05. Fisher’s least-significant difference (Protected-t) test was applied for post hoc comparisons when appropriate.
**Results**

**Effects of Chronic MRZ 2/579 on ADE.** After 2 weeks of abstinence, a significant ADE occurred in the vehicle group (Fig. 1), characterized by a transient increase in ethanol intake (factor ADE: $F_{1,42} = 12.59, P < .01$) and a transient increase in ethanol preference (factor ADE: $F_{1,42} = 7.49, P < .05$). Chronic MRZ 2/579 treatment completely suppressed the occurrence of an ADE in terms of the increased ethanol intake (interaction treatment $\times$ ADE: $F_{1,42} = 12.48, P < .01$), but did not affect ethanol preference (interaction treatment $\times$ ADE: $F_{1,42} = 0.05$, NS). After a second alcohol deprivation phase of 2 weeks, again an ADE occurred in the vehicle group, but the suppressant effect on ethanol intake in the MRZ 2/579 group was no longer present in the absence of the drug (Fig. 1). Water and food intake were not affected by chronic MRZ 2/579 treatment (Fig. 2). After 6 days of chronic infusion, serum levels of MRZ 2/579 were $0.52 \pm 0.046 \mu M$ and its concentration in brain tissue was $87.8 \pm 7.79 \mu mol/kg$ tissue (mean $\pm$ S.E., $n = 4$).

**Effects of Acute MRZ 2/579 on ADE.** Previous experiments in this paradigm have shown that drug effects are most pronounced at the beginning of the session (Hölter et al., 1997). Furthermore, at lower doses drug effects can wear off before the end of the session. Thus, data were analyzed for both the first hour and the cumulative total for the 23-h test session. During vehicle treatment, animals of the basal and ADE groups differed in ethanol intake and preference in the operant 23-h session, which constitutes the alcohol deprivation effect in this operant paradigm, but not in total lever-pressing activity and, therefore, not in total fluid intake (Fig. 3). During the first hour (Fig. 3, left), MRZ 2/579 reduced ethanol intake during the ADE (factor dose: $F_{3,31} = 6.16, P < .01$) as well as during basal drinking (factor dose: $F_{3,23} = 3.47, P < .05$). Ethanol preference was only affected in the basal-drinking group (factor dose: $F_{3,23} = 4.84, P < .05$) and total lever-pressing activity was only significantly reduced during the ADE (factor dose: $F_{3,31} = 6.2, P < .01$). Concerning the whole 23-h session (Fig. 3, right), the drug had no lasting effect on ethanol intake or preference in either group. Only total lever-pressing activity remained reduced in the ADE group until the end of the session (factor dose: $F_{3,31} = 3.88, P < .05$). This reduction in total activity was composed of reductions in lever pressing for both ethanol and water, which were not significant on their own. Detailed analysis of the time course of lever-pressing activity for ethanol did not reveal any rebound in ethanol intake during the course of the session in either group.

Analysis of lever-pressing activity for ethanol (Fig. 4, top) and water (Fig. 4, bottom) during the first six 10-min intervals of the first hour of the session revealed that MRZ 2/579 dose dependently (Fig. 4, top left) depressed lever pressing for ethanol during the ADE (factor dose: $F_{3,15} = 9.43, P < .001$). During basal drinking, MRZ 2/579 also reduced lever pressing for ethanol (factor dose: $F_{3,15} = 6.58, P < .01$), but a dose-dependent relationship was less obvious (Fig. 4, top).
right). The highest dose almost completely suppressed lever pressing for ethanol in this group. Lever pressing for water was not significantly reduced by MRZ 2/579 during the ADE (factor dose: $F_{3,15} = 0.56$, NS) or in the basal-drinking group (factor dose: $F_{3,15} = 1.36$, NS). The dispersion in lever presses for water in the ADE group is due to one outlier, whereas the dispersion in the basal group is rather genuine.

Effects of MRZ 2/579 on Drug Discrimination. As shown in Fig. 5, MRZ 2/579 dose dependently generalized to the ethanol cue in rats trained to discriminate ethanol from saline. Drug treatment also affected lever response latency ($F_{4,34} = 3.99$, $P < .05$), but only at the highest dose tested (4 mg/kg) (Fig. 5, bottom). At this dose, only four of eight animals completed the test session.

Discussion

The results of this study show that: 1) chronic MRZ 2/579 treatment abolished the increased ethanol intake during the ADE in long-term ethanol-experienced rats without affecting water and food intake; 2) this effect required the presence of the drug and did not affect a second ADE in the absence of the drug; 3) acute MRZ 2/579 treatment had a short-lasting, reductive effect on ethanol intake both during basal drinking and during the ADE without affecting water intake; and 4) in the dose range used for acute treatment, MRZ 2/579 generalized to the ethanol cue in a drug discrimination test.

The acute effects of MRZ 2/579 were only significant at the beginning of the session, probably due to the short half-life of this drug. Plasma and brain extracellular fluid $t_{1/2}$ values for a dose of 5 mg/kg i.p. are 1.63 and 1.95 h, respectively (Hesselink et al., 1999). The dose range used in the present study was even lower (1–4 mg/kg). With longer-acting drugs, reductions in lever pressing for ethanol also can be seen at later hours of the session (Hölter et al., 1997; Hölter and Spanagel, 1999).

At the doses used in this study, both acute and chronic administration of MRZ 2/579 specifically reduced ethanol intake without affecting water or food intake. Similar doses of acute MRZ 2/579 (2.5–7.5 mg/kg) also inhibited the reinforcing effects of morphine (Popik et al., 1998) and starting at 1 mg/kg, NMDA receptors were blocked sufficiently to provide neuroprotection from NMDA toxicity in the nucleus basalis magnocellularis (Wenk et al., 1998). Higher doses of acute MRZ 2/579 were not used because they were likely to

Fig. 3. Effects of acute MRZ 2/579 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, effects on ethanol intake; middle, effects on ethanol preference; and bottom, effects on total lever-pressing activity. Data are presented as means ± S.E. ($n = 6–8$). *$P < .05$, **$P < .01$, significant difference versus vehicle.
induce unspecific reductions of lever-pressing activity due to an impairment of motor coordination (Danysz et al., 1997). However, chronic s.c. infusion of MRZ 2/579 at a dose leading to steady-state brain levels that are sufficient to inhibit NMDA receptors (Danysz et al., 1997) was well tolerated without visible side effects, which is in line with previous findings with memantine, another uncompetitive NMDA receptor antagonist with similar biophysical properties (Parsons et al., 1995; Hölter et al., 1996). Although MRZ 2/579 is strongly accumulated in brain tissue, free brain (extracellular fluid) levels are only 2-fold lower than free serum levels as found by microdialysis with in vivo recovery (Hesselink et al., 1999). Thus, considering the serum levels attained in this study after chronic infusion, free brain levels can be estimated to have reached 0.26 μM. Higher concentrations can be expected after acute injections (e.g., at 4 mg/kg) because injection of 5 mg/kg i.p. resulted in a free brain concentration of 0.70 μM (Hesselink et al., 1999). MRZ 2/579 selectively blocked NMDA receptor-mediated responses with an IC₅₀ value of 1.11 μM in cultured hippocampal neurons (Hesselink et al., 1999) and with an IC₅₀ value of 0.42 μM in Xenopus oocytes (Parsons et al., 1999). Hence, the doses used in the present study were most likely sufficient for selective NMDA receptor blockade. Therefore, we conclude that the observed behavioral effect of selective reduction of ethanol intake is due to selective NMDA receptor blockade.

Because NMDA receptors are involved in the mediation of learning and memory processes, it could have been possible that the suppression of the ADE by chronic MRZ 2/579 had long-lasting consequences leading also to a reduction of subsequent ADEs in the absence of the drug, but this was not the case. Thus, the suppression of one ADE by chronic MRZ 2/579 did not lead to extinction of the ADE.

The finding that MRZ 2/579 generalized to the ethanol cue in the drug discrimination test suggests that the reductive effects of this drug on ethanol intake might be due, at least in part, to a substitution of the stimulus properties of alcohol. This is not surprising, bearing in mind that alcohol also antagonizes NMDA receptor function, and it fits in with the lack of any effects in the absence of the drug (see above). These data are in line with previous drug discrimination studies that showed that a variety of uncompetitive NMDA receptor antagonists generalized to the ethanol cue (Colombo and Grant, 1992; Shelton and Balster, 1994; Hundt et al., 1997).

**Fig. 4.** Effects of acute MRZ 2/579 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 ± S.E. (n = 6–8). *P < .05, **P < .01, significant difference versus vehicle.
Fig. 5. Results of discrimination testing with different doses of MRZ 2/579 in rats discriminating ethanol from saline. The mean percentage of responses on the ethanol lever was used as a measure of ethanol-appropriate responding (top). Drug effects on response latency (bottom). Data are presented as means ± S.E. (n = 4–8). *P < .05, significant difference versus vehicle.

Moreover, it has recently been demonstrated that ketamine produced dose-related ethanol-like subjective effects in detoxified alcoholics (Krystal et al., 1998), supporting the clinical importance of NMDA receptor antagonism among the mechanisms underlying the subjective effects of ethanol in humans.

In summary, in the treatment regimen used in this study, MRZ 2/579 specifically reduced ethanol intake without visible side effects independent of the strength of the motivation to consume ethanol. We conclude that this novel uncompetitive NMDA-receptor antagonist might exert its ethanol-consumption-reducing effect by substituting for some of the subjective effects of ethanol. This does not necessarily reduce the potential therapeutic usefulness of this compound in the treatment of alcohol dependence because one of the main goals of treatment is the reduction of alcohol consumption to reduce the pathological consequences of chronic alcohol abuse. In contrast, the lack of obvious side effects of chronic treatment with MRZ 2/579 within the therapeutic dose range encourages hope that this drug might be therapeutically applicable. Further studies are necessary to clarify this issue.

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References


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