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Effects of a novel uncompetitive NMDA receptor antagonist, MRZ 2/579 on ethanol self-administration and ethanol withdrawal seizures in the rat

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Abstract

It has been repeatedly reported that NMDA receptors may contribute to ethanol-induced discriminative stimulus effects and withdrawal syndrome. However, the role of NMDA receptors in the reinforcing properties of ethanol remains unclear. The aim of the present study was to evaluate effects of the novel low-affinity, uncompetitive NMDA receptor antagonist, 1-amino-1,3,3,5,5-pentamethyl-cyclohexane hydrochloride (MRZ 2/579), on ethanol self-administration and ethanol withdrawal-associated seizures in rats. Both an operant (lever pressing for ethanol) and non-operant two-bottle choice setups were employed to initiate ethanol self-administration. In another procedure, forced treatment with high doses (9–15 g/kg/day) was used to induce physical dependence on ethanol. MRZ 2/579 delivered chronically by osmotic minipumps (9.6 mg/day, s.c.) did not alter either operant or non-operant ethanol drinking behaviour in a maintenance phase of ethanol self-administration. In contrast, repeated daily injections of the drug (5 mg/kg, i.p.) led to a progressive decrease in operant responding for ethanol. MRZ 2/579 (0.5–7.5 mg/kg, i.p.) and another low-affinity NMDA receptor antagonist, memantine (1–10 mg/kg, i.p.) dose-dependently suppressed ethanol withdrawal seizures with efficacies comparable with that of a standard benzodiazepine derivative, diazepam. The results of the present study indicate that: (i) intermittent administration of MRZ 2/579 may lead to a gradual decrease of operant responding for ethanol; and (ii) the group of low-affinity uncompetitive NMDA receptor antagonists may be an interesting alternative to benzodiazepines in the treatment of alcohol withdrawal.

Keywords: Ethanol self-administration; Ethanol withdrawal; NMDA receptor antagonist; MRZ 2/579; (Rat)

1. Introduction

It has been shown repeatedly that acute exposure to clinically relevant concentrations of alcohol (ethanol) decreases the NMDA receptor-associated channel function in several in vitro preparations (for review, see Lovinger, 1997; Faingold et al., 1998). In contrast, chronic treatment with ethanol may increase the number of NMDA receptors in different brain structures (Gulya et al., 1991; Hoffman, 1995; Chandler et al., 1998). Moreover, it has been reported that withdrawal from chronic alcohol increases the extracellular glutamate concentration in the rat brain (Rossetti and Carboni, 1995).

Behavioural studies have revealed that the NMDA receptor complex may contribute to the discriminative stimulus effects, sedation, ataxia and myorelaxation induced by single doses of alcohol (Grant et al., 1991; Danysz et al., 1992; Bienkowski et al., 1996, 1998; Beleslin et al., 1997; Kostowski and Bienkowski, 1999). It has also been sug-

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gested that adaptive 'up-regulation' of the NMDA receptor system associated with chronic alcoholisation leads to neuronal hyperexcitability (Valverius et al., 1990; Morgan et al., 1992), neurodegeneration (Hoffman, 1995; Hoffman et al., 1995), and seizures in animals withdrawn from chronic ethanol (Grant et al., 1990; Riaz and Faingold, 1994). In line with this hypothesis, NMDA receptor antagonists potently attenuate various signs of ethanol withdrawal syndrome in rodents (Grant et al., 1990; Danysz et al., 1992; Kotlinska and Liljequist, 1996; Gatch et al., 1999).

As mentioned above, the discriminative stimulus effects of ethanol may be related to a decrease of NMDA receptor function. In the drug discrimination procedure, different classes of NMDA receptor antagonists (e.g. competitive, uncompetitive or glycine$_B$ site antagonists) substitute fully for the ethanol cue (Grant et al., 1991; Hundt et al., 1998; Kostowski and Bienkowski, 1999; Hölter et al., 2000). Furthermore, a high-affinity uncompetitive NMDA receptor antagonist, ketamine partially mimics the subjective effects of ethanol in human alcoholics (Krystal et al., 1998). Thus, it seems that compounds decreasing NMDA receptor function could be considered as a form of substitution therapy of alcohol addiction. Unfortunately, little is known about the role of NMDA receptors in the positive reinforcing effects of alcohol and neural regulation of alcohol drinking behaviour. The few preclinical studies on these topics have yielded conflicting results. Danysz et al. (1992) have reported that a high-affinity uncompetitive NMDA receptor antagonist, dizocilpine (MK-801), does not affect two-bottle choice ethanol drinking in the rat. Other researchers (Shelton and Balster, 1997; Piasecki et al., 1998) have shown non-selective effects of NMDA receptor antagonists in operant oral ethanol self-administration studies. The doses needed to reduce ethanol self-administration suppressed other self-administration behaviours, i.e. sucrose (Shelton and Balster, 1997) or water self-administration (Piasecki et al., 1998).

Most NMDA receptor antagonists tested so far in humans have been shown to produce serious psychotropic side-effects (Luby et al., 1959; Kornhuber and Weller, 1997; Lees, 1997). In addition, high-affinity uncompetitive NMDA receptor antagonists [phencyclidine (PCP)-like compounds] have a clear-cut abuse potential (Luby et al., 1959; Corbett, 1989). In an apparent contrast, low-affinity uncompetitive antagonists ($K_d > 0.5$ μM), amantadine and memantine, have been used safely in humans in the treatment of Parkinson's disease and dementia, respectively (Kornhuber and Weller, 1997; Parsons et al., 1998; Danysz et al., 2000). In comparison with high-affinity compounds, memantine and amantadine show more rapid blocking kinetics and stronger voltage dependence that might explain their favourable clinical profile and low incidence of serious side effects (Parsons et al., 1998, 1999).

MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexane hydrochloride) is a novel low-affinity uncompetitive NMDA receptor antagonist with rapid blocking/unblocking kinetics and strong voltage dependence (Parsons et al., 1999; Danysz et al., 2000). The compound shows good systemic availability and penetration to the central nervous system (CNS) (Hesselink et al., 1999a) and its behavioural activity has been confirmed in animal models of opioid dependence (Popik et al., 1998; Semenova et al., 1999). Recently, we have demonstrated that in contrast to memantine which suppresses both ethanol and water self-administration, single doses of MRZ 2/579 may selectively attenuate lever pressing for ethanol (Piasecki et al., 1998; Bienkowski et al., 1999b). This latter finding might be a consequence of partially different NMDA receptor subtype selectivity of memantine and MRZ 2/579 (Parsons et al., 1999).

The aim of the present study was to further evaluate the potential usefulness of MRZ 2/579 in the treatment of alcohol addiction. First, we decided to evaluate the effects of acute MRZ 2/579 administration on the expression of ethanol withdrawal seizures. Second, effects of prolonged MRZ 2/579 administration (chronic infusion or repetitive injections) on ethanol drinking behaviour were tested.

2. Method

2.1. Animals

Male Wistar rats weighing 300–350 g at the beginning of each experiment were kept in a room with environmental conditions: temperature of 22 ± 1°C, ~60% humidity, and a 12-h light–dark cycle (light on at 6:00 a.m.). The animals were supplied by a breeder (HZL, Warsaw, Poland) at least 10 days before the start of the experiments. Food ('Labofeed H', WPiK, Kcynia, Poland) was always available ad libitum. Tap water was available ad libitum except as noted below.

Treatment of the rats in the present study was in full accordance with the ethical standards laid down in European and Polish regulations. All procedures were reviewed and approved by a local Ethics Committee.

2.2. Forced ethanol administration and assessment of ethanol withdrawal-associated seizures

The procedure developed by Majchrowicz (1975) with some minor modifications (for details, see Danysz et al., 1992; Kostowski et al., 1993) was used to induce physical dependence on ethanol. Ethanol (20% w/v; 96% Rectified Spirit; Polmos, Zielona Gora, Poland) was given for 5 days by gavage. The ethanol solution was administered three times a day at 7–8 a.m., 2–3 p.m. and 9–10 p.m. On day 1, a priming dose of 5 g/kg of ethanol was given. All subsequent doses were adjusted to behavioural signs of intoxication according to Majchrowicz (1975). Typically, the total dose of ethanol ranged from 9 to 15 g/kg/day.

On the sixth day of the experiment, 15–16 h after the last ethanol administration, the intensity of audiogenic seizures was assessed. Each rat was tested in a circular Plexiglas chamber (height: 38 cm, diameter: 42 cm) in which an electric bell (100 dB) was secured. After the rat was put in the chamber, the bell was rung until the rat presented seizure response or 60 s elapsed whichever came first. Convulsions were scored according to Jobe et al. (1973).

Effects of diazepam (0.1–2.5 mg/kg, i.p.; 5 mg/ml solution, commercially available) and MRZ 2/579 (0.5–7.5 mg/kg, i.p.) on ethanol withdrawal seizures were investigated first. Control rats were injected with either original diazepam vehicle (obtained from Polfa, Warsaw, Poland; $n = 10$) or sterile distilled water ($n = 8$). Effects of memantine (1–10 mg/kg, i.p.) on withdrawal seizures were assessed in a separate experiment.

2.3. Effects of MRZ 2/579 on ethanol-induced sleep time

In this experiment, we aimed to investigate whether MRZ 2/579 (2.5–7.5 mg/kg) might potentiate the sedative/hypnotic effects of ethanol. Twenty one rats were randomly assigned to one of three experimental groups ($n = 7$ rats per group). The subjects were first given i.p. distilled water or MRZ 2/579. After 30 min, all rats received 3 g/kg ethanol (20% v/v, i.p.). After the alcohol injection, each animal was repeatedly placed on its back (once every 10 s) until it was unable to right itself within 30 s. Then, the rat was left undisturbed until it regained the righting reflex three times within a 30-s period. Duration of ethanol-induced sleep time was defined as the length of time from loss to recovery of the righting reflex (Christensen et al., 1996; Colombo et al., 1998).

2.4. Pharmacokinetics of MRZ 2/579

The pharmacokinetic properties of MRZ 2/579 during chronic infusion have been described in detail by Parsons et al. (1999) and Hesselink et al. (1999a). As smaller rats and relatively high doses of the drug were used in these studies we decided to evaluate plasma concentrations of MRZ 2/579 in our rats. For this purpose, two types of ALZET osmotic minipumps (Alza, Palo Alto, CA, USA) were used. The rats were anaesthetised with ketamine (75 mg/kg, i.p.; Gedeon Richter, Budapest, Hungary) and implanted s.c. with either 2ML2 (flow rate: 120 μl/24 h) or 2ML1 ALZET minipumps (flow rate: 240 μl/24 h; $n = 4$ rats per group). All pumps were filled with 2 ml of sterile MRZ 2/579 solution (40 mg/ml). On day 6 after the minipumps implantation, the animals were deeply anaesthetised with pentobarbital (50 mg/kg, i.p.; Biowet, Pulawy, Poland). Blood samples (2 ml) were taken by heart puncture into heparinised vials. Plasma was separated by centrifugation (4000 rpm) and immediately frozen at −40°C until analysis. MRZ 2/579 concentrations were assessed with a Hewlett-Packard gas chromatography system coupled with a mass selective detector (for details, see Hesselink et al., 1999a; Parsons et al., 1999).

2.5. Ethanol drinking in free-choice 24-h procedure: effects of chronic MRZ 2/579 administration by osmotic minipumps

The animals ($n = 36$) were housed singly and exposed to increasing concentrations of ethanol (2–7% v/v) and tap water in a two-bottle choice situation (for details, see Koros et al., 1998). Each concentration was offered for 2–4 days. For the next 21 days, the animals were presented with 8% ethanol and water. Drinking tubes were rotated daily to prevent position preference. All solutions were completely replaced twice a week. Only rats ($n = 14$; 39% of the whole experimental group) who consistently consumed more than 4 g/kg of alcohol per day were used in further experiments.

When 8% ethanol intake had stabilised, the ALZET 2ML1 minipumps were implanted. The minipumps were filled with sterile distilled water or the MRZ 2/579 solution (40 mg/ml; drug delivery rate: 9.6 mg/day; $n = 7$ rats per group). Ethanol intake (g/kg/day), ethanol preference (%) and total fluid intake (ml/kg/day) were monitored for the next 7 days. Because total fluid intake was clearly suppressed in all subjects on day 1 after the surgery, this day was not included in statistical analyses.

2.6. Ethanol drinking in operant oral ethanol self-administration procedure

2.6.1. Apparatus

Ethanol-reinforced behaviour was studied in standard operant chambers (Coulbourn Instruments, Allentown, PA, USA). The chambers consisted of modular test cages enclosed within sound-attenuating cubicles with fans for ventilation and background white noise (for details, see Bienkowski and Kostowski, 1998). A white house light was centered near the top of the front of the cage. The start of training or test sessions was signalled by turning the house light on. The cage was also equipped with two response levers, separated by a liquid delivery system (a liquid dipper, E14-05, Coulbourn). Only one lever ('active' lever) activated the liquid dipper. Presses on the other lever ('inactive' lever) were recorded but not reinforced. The liquid delivery system presented 8% v/v ethanol in a 0.1-ml portion for 5 s. The availability of reinforcer was signalled by a brief audible click and a small white light (4 W) located inside the liquid dipper hole. Programming of training and test sessions as well as data recording made use of the L2T2 Software package (Coulbourn) running on an IBM-compatible PC.

2.6.2. Acquisition of operant oral ethanol self-administration

The rats ($n = 32$) were trained to respond for 8% v/v ethanol according to the sucrose-fading procedure (Samson, 1986) with some minor modifications (for details, see Bienkowski et al., 1999a,b). The animals were deprived of water for 22 h/day during the first 4 days of training and shaped to lever press for 10% w/v sucrose solution on a FR1 schedule of reinforcement. As soon as lever pressing was established, water started to be freely available in the home cages. Food was always available ad libitum. All training sessions were 30 min long and one session was given each day. Starting on day 5, the animals received 2.5% ethanol–10% sucrose. Then over the next 12–16 sessions ethanol concentrations were gradually increased from 2% to 8%, and sucrose concentrations were decreased from 10% to 0%.

2.6.3. Effects of chronic MRZ 2/579 infusion

The rats were allowed to stabilise their 8% ethanol consumption for at least 30 days. Only rats ($n = 13$; 41% of the whole experimental group) consistently responding more than 30 times on the 'active' lever/30 min were implanted with the ALZET 2ML1 minipumps. The minipumps were filled with 2 ml of sterile distilled water or the MRZ 2/579 solution ($n = 6$–7 rats per group).

Preliminary experiments showed that manipulations associated with s.c. minipump implantation may non-specifically disrupt operant behaviour in our rats for at least 48 h. For this reason, in the final experiment, the ethanol self-administration sessions were restarted on day 4 after minipump implantation.

2.6.4. Effects of repeated MRZ 2/579 injections

Another group of rats was trained to lever press for ethanol as described above. When the ethanol intake was stabilised, MRZ 2/579 (5 mg/kg, i.p.) or its vehicle was injected 30 min before the start of four consecutive self-administration sessions ($n = 8$ rats per group).

2.7. Drugs

Ethanol solutions were prepared daily from a 96% stock solution (Polmos). MRZ 2/579 hydrochloride and memantine hydrochloride (Merz, Frankfurt am Main, Germany) were dissolved in sterile distilled water (Polpharma, Starogard Gdanski, Poland). Diazepam (2-ml ampoules, 5 mg/ml) and its original vehicle (Polfa) was diluted to final concentrations with 1% Tween. All solutions were prepared immediately prior to use. The drugs were injected in a volume of 1 ml/kg and the doses refer to the salt forms. The parameters of chronic MRZ 2/579 infusion in the self-administration experiments were described above.

2.8. Statistics

A Kruskal–Wallis analysis of variance (ANOVA) and Mann–Whitney *U*-test were used to evaluate the intensity of ethanol withdrawal seizures and duration of ethanol-induced sleep.

A two-way ANOVA (Treatment × Days) with repeated measures was employed to analyse data from the self-administration experiments. Newman–Keuls test was used for post-hoc comparisons. Student's *t*-test was used when averaged data from two group were compared. The 'Statistica' software package for Windows was used to analyse all data. A probability level (P) less than 0.05 was considered significant.

3. Results

3.1. Ethanol withdrawal-associated audiogenic seizures

The two control groups used in the first experiment did not differ in terms of the intensity of audiogenic seizures ($P > 0.3$; Mann–Whitney *U*-test) and thus were pooled before further analyses.

Diazepam dose-dependently suppressed ethanol withdrawal-associated audiogenic seizures ($\chi^2 = 14.23$, $df = 3$, $P < 0.01$; Table 1). Similar dose-dependent and almost complete inhibition of audiogenic seizures was observed after MRZ 2/579 administration ($\chi^2 = 10.65$, $df = 3$, $P < 0.02$).

Table 1
Effects of diazepam, MRZ 2/579 and memantine on intensity of ethanol withdrawal-associated audiogenic seizures

Dose (mg/kg)	*N*	Seizure intensity
Diazepam (ED_{50} = 3.7 μM/kg)		
Vehicle	18	2.2±0.4
0.1	8	2.2±0.5
1	9	0.3±0.2*
2.5	9	0.1±0.1**
MRZ 2/579 (ED_{50} = 13.9 μM/kg)		
Vehicle	18	2.2±0.4
0.5	7	1.85±0.6
2.5	9	0.45±0.3*
7.5	9	0.1±0.1**
Memantine (ED_{50} = 13.0 μM/kg)		
Vehicle	10	3.9±0.6
1	9	2.0±0.5
5	9	1.0±0.4*
10	9	0±0**

Values represent mean (±S.E.M.) seizure intensity scores.

* $P < 0.05$ vs. respective vehicle-treated control groups; Mann–Whitney *U* test.

** $P < 0.01$ vs. respective vehicle-treated control groups; Mann–Whitney *U* test.

Table 2
Effects of MRZ 2/579 on 24-h ethanol intake, ethanol preference and total fluid intake for 6 consecutive days of s.c. drug delivery (days 2–7 after minipump implantation)

Day of measurement					
1	2	3	4	5	6
Ethanol intake (g / kg / 24 h)					
Infusion of water vehicle					
4.44 ± 0.78	4.37 ± 0.58	4.18 ± 0.57	4.34 ± 0.78	4.10 ± 0.72	4.54 ± 0.67
Infusion of MRZ 2/579					
4.78 ± 0.75	4.60 ± 0.45	3.99 ± 0.58	4.22 ± 0.64	4.15 ± 0.75	4.10 ± 0.59
Ethanol preference (%)					
Infusion of water vehicle					
78.0 ± 10.3	78.3 ± 9.5	79.0 ± 9.5	77.0 ± 11.6	77.1 ± 12.1	76.9 ± 9.4
Infusion of MRZ 2/579					
86.3 ± 7.9	92.8 ± 4.5	80.4 ± 11.1	82.8 ± 9.5	78.4 ± 12.7	82.7 ± 8.4
Total fluid intake (ml / kg / 24 h)					
Infusion of water vehicle					
99.7 ± 5.3	100.7 ± 4.3	97.6 ± 6.5	99.4 ± 5.1	95.0 ± 5.1	103.8 ± 2.8
Infusion of MRZ 2/579					
94.1 ± 7.3	88.4 ± 4.5	90.0 ± 5.9	89.2 ± 3.5	92.9 ± 4.1	84.3 ± 3.8

Results are shown as the mean (±S.E.M.) ethanol intake, ethanol preference and total fluid intake; $n = 7$ rats.

Memantine dose-dependently attenuated ethanol withdrawal seizures ($\chi^2 = 17.94$, $df = 3$, $P < 0.01$; Table 1). The highest dose of memantine (10 mg/kg) completely eliminated audiogenic seizures in the animals withdrawn from the chronic ethanol treatment.

3.2. Effects of MRZ 2 / 579 on ethanol-induced sleep time

The doses of MRZ 2/579 (2.5 and 7.5 mg/kg) which suppressed ethanol withdrawal-associated seizures did not alter the duration of ethanol-induced sleep ($\chi^2 = 0.38$, $df = 2$, $P = 0.83$). The mean (±S.E.M.) duration of sleep was: 79 ± 20, 94 ± 19, and 68 ± 18 min, for the subjects injected with water, 2.5 and 7.5 mg/kg of MRZ 2/579, respectively.

3.3. Pharmacokinetics of MRZ 2 / 579

The mean (±S.E.M.) plasma concentrations of MRZ 2/579 were 0.48 ± 0.03 and 1.2 ± 0.09 μM after the implantation of the 2ML2 and 2ML1 minipumps, respectively. The implantation of the 2ML1 minipumps resulted in a mean MRZ 2/579 concentration comparable with that observed 30 min after acute i.p. administration of 5 mg/kg MRZ 2/579 (~1.5 μM; Hesselink et al., 1999a). The 2ML1 minipumps were selected for further experiments.

3.4. Ethanol drinking in free-choice 24-h procedure: effects of chronic MRZ 2 / 579 administration by osmotic minipumps

The ANOVA revealed that MRZ 2/579 delivered by the 2ML1 minipumps did not alter 24-h ethanol drinking and preference (all Fs < 1.63, Ps > 0.16; Table 2). Total fluid intake tended to be decreased by MRZ 2/579. However, this trend did not reach significance [an effect of Treatment: $F(1,12) = 3.79$, $P = 0.075$]. MRZ 2/579 significantly reduced total fluid intake averaged across the whole period of drug infusion (the MRZ 2/579 group: 90.5 ± 3.4 ml/kg/24 h; the control group: 99 ± 3.4 ml/kg/24 h; $P < 0.02$; Student's t-test).

3.5. Ethanol drinking in operant oral ethanol self-administration procedure

The mean baseline number of ethanol deliveries ranged from 50 to 80 dipper deliveries/30 min with individual ethanol intakes ranging between 0.6–0.8 g/kg/30 min. Typically, more than 80% of lever presses occurred within the first 15 min of the self-administration session. This pattern of operant behaviour was similar to that previously found (Bienkowski et al., 1999a,b).

Table 3
Effects of MRZ 2/579 on operant oral ethanol self-administration for 4 consecutive days of s.c. drug delivery (days 4–7 after minipump implantation)

Day of measurement			
1	2	3	4
Infusion of water vehicle			
49.5 ± 7.9	70.1 ± 13.6	61.6 ± 8.6	62.8 ± 10.7
Infusion of MRZ 2 / 579			
50.6 ± 10.9	51.0 ± 11.2	47.3 ± 6.6	57.0 ± 11.2

Results are shown as the mean (±S.E.M.) number of responses/30 min; $n = 6$–7 rats.

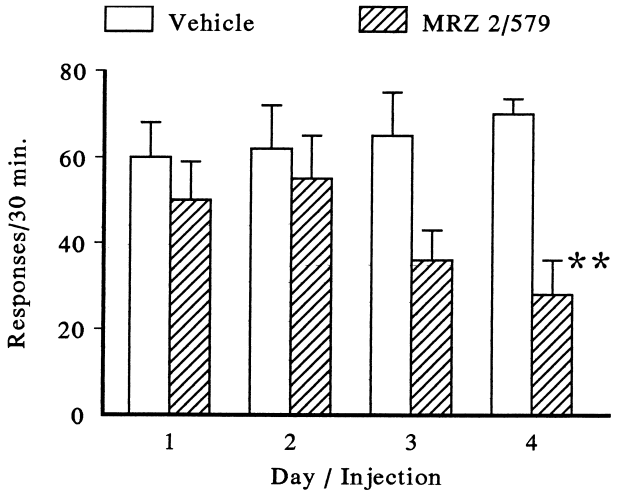

Fig. 1. Effects of repeated MRZ 2/579 injections on operant oral ethanol self-administration. MRZ 2/579 (5 mg/kg, i.p.) or its vehicle was administered 30 min before four consecutive self-administration sessions. Bars represent the mean (±S.E.M.) number of lever presses/30 min. $^{**}P < 0.01$ vs. the vehicle-treated control group; $n = 8$ rats.

The two-way ANOVA revealed that chronic s.c. infusion of MRZ 2/579 did not alter operant responding for ethanol (Fs < 1, Ps > 0.4; Table 3). In contrast, repeated injections of MRZ 2/579 led to progressive reduction of ethanol-reinforced behaviour [a significant effect of Treatment: $F(1,14) = 5.29$, $P < 0.05$; Fig. 1]. A non-significant decrease in ethanol self-administration was observed after the first drug administration. This effect became significant after subsequent drug injections [a Treatment × Days interaction: $F(3,42) = 3.10$, $P < 0.05$]. The post hoc analysis confirmed that ethanol self-administration was significantly reduced after the 4th injection of 5 mg/kg MRZ 2/579 (Fig. 1).

In the operant procedure, the mean number of 'inactive' lever presses did not exceed 0.7 responses/session and was not altered by MRZ 2/579 (data not shown).

4. Discussion

It has been repeatedly demonstrated that compounds decreasing NMDA receptor function may inhibit ethanol withdrawal seizures in rodents (Grant et al., 1990; Morrisett et al., 1990; Liljequist, 1991; Riaz and Faingold, 1994; Kotlinska and Liljequist, 1996). More recently, Erden et al. (1999) have reported that a low-affinity uncompetitive NMDA antagonist, dextromethorphan, attenuated the ethanol withdrawal syndrome in the rat. Doses of dextromethorphan which inhibited withdrawal-associated seizures did not alter locomotor behaviour in ethanol-naive subjects.

In the present study, two low-affinity uncompetitive NMDA receptor antagonists, MRZ 2/579 and memantine, potently suppressed ethanol withdrawal-associated seizures. The anti-seizure efficacies of the NMDA antagonists were comparable to that of the $GABA_A$/benzodiazepine receptor positive modulator, diazepam. Notably, the doses of MRZ 2/579, memantine and diazepam which suppressed withdrawal seizures in the present study did not alter locomotor activity and self-administration behaviour in various behavioural procedures used in our laboratory (Stefanski et al., 1992; Bienkowski et al., 1997, 1999b; Bienkowski and Kostowski, 1998; Piasecki et al., 1998). In the present study, MRZ 2/579 did not influence the duration of ethanol-induced sleep. In line with the above, we have reported that MRZ 2/579 does not alter spontaneous locomotor activity when given in combination with ethanol (Bienkowski et al., 1999b). Thus, it seems that the drug may eliminate ethanol withdrawal seizures in the range of doses which do not enhance central depressant effects of alcohol. In contrast, it has been shown that the high-affinity uncompetitive NMDA receptor antagonist, dizocilpine reduces ethanol withdrawal seizures at the doses (≥ 0.3 mg/kg) which exert various non-specific behavioural effects in the rat (Morrisett et al., 1990; Danysz et al., 1992; Bienkowski et al., 1996). Even lower doses of dizocilpine (≤ 0.1 mg/kg) may enhance behavioural effects of ethanol including its locomotor stimulant and sedative/hypnotic properties (Danysz et al., 1992; Kuribara, 1994; Bienkowski et al., 1997; Toropainen et al., 1997). These and other differences between high-and low-affinity uncompetitive NMDA receptor antagonists observed in preclinical studies (e.g. Hyytiä et al., 1999) are consistent with a favourable clinical profile of low-affinity compounds (Rogawski, 1993; Lees, 1997; Kornhuber and Weller, 1997; Parsons et al., 1998).

It has been found repeatedly that acute administration of various NMDA receptor antagonists did not alter alcohol drinking in a behaviourally selective manner (Shelton and Balster, 1997; Piasecki et al., 1998; Bienkowski et al., 1999b). In the present study, neither operant nor non-operant ethanol drinking behaviour was altered by chronic delivery of MRZ 2/579 by the ALZET minipumps. Considering the receptor profile and the pharmacokinetic properties of MRZ 2/579 described recently by Hesselink et al. (1999a) and Parsons et al. (1999), one may suspect that the plasma concentrations achieved in the present study were most likely sufficient for selective NMDA receptor blockade. Moreover, it seems that the doses delivered by the minipumps were sufficient to produce measurable behavioural effects as chronic infusion of the drug reduced the total fluid intake in the non-operant procedure.

In contrast to the effects of chronic drug infusion, repeated injections of 5 mg/kg MRZ 2/579 resulted in a progressive decrease of operant responding for ethanol. The plasma levels of MRZ 2/579 after a single administration of 5 mg/kg reported previously by Hesselink et al. (1999a) for Sprague–Dawley rats were similar to the level reached in the present study after chronic infusion. As the half-life of MRZ 2/579 does not exceed 4 h (Parsons et

al., 1999) it is rather unlikely that repeated injections of the drug led to its accumulation in the rat body. It has been shown that acute administration of 5 mg/kg MRZ 2/579 does not alter water self-administration and spontaneous locomotor activity in the rat (Bienkowski et al., 1999b). Accordingly, it is likely that the results of the present study reflect selective interactions of the drug with mechanism(s) regulating operant ethanol self-administration. However, further experiments are needed to examine whether repeated injections of 5 mg/kg MRZ 2/579 may also decrease the reinforcing effects of other reinforcers such as food or water.

As mentioned above, the mode of drug administration (continuous infusion vs. repeated injections) may, at least partially, determine the effects of MRZ 2/579 on ethanol-reinforced behaviour. Although the reason for this is not clear at the moment, at least two basic interpretations of our data are possible. First, continuous drug infusion leading to steady state plasma levels could mask any potential anti-alcohol effects of MRZ 2/579 by favouring the quick development of tolerance to these effects. Recently, Hölter and Spanagel (1999) have demonstrated that chronic infusion of an opioid receptor antagonist, naloxone, increases, while both acute and intermittent treatment with another opioid antagonist, naltrexone, decreases the so-called 'alcohol deprivation effect' in the rat (defined as a short-term enhancement of ethanol intake after forced abstinence). Second, it is possible that repeated injections of the NMDA receptor antagonist produced 'sensitisation' to its anti-alcohol properties or any other behavioural effect(s) which might alter ethanol self-administration. Notably, Hesselink et al. (1999b) have reported that intermittent i.p. administration but not chronic infusion of memantine produced sensitisation to its locomotor stimulant effects.

Alternatively, MRZ 2/579 might block primary reinforcing properties of ethanol, thus leading to progressive extinction of ethanol-reinforced behaviour. A similar mechanism is thought to explain the extinction of ethanol-reinforced behaviour associated with repeated naltrexone administration (Sinclair, 1998; Bienkowski et al., 1999a). This possibility could be supported by a recent report that NMDA receptor antagonists block the acquisition of ethanol-induced conditioned place preference (Biala and Kotlinska, 1999). However, one should bear in mind that MRZ 2/579 has been shown to substitute for the ethanol cue in the drug discrimination procedure (Hölter et al., 2000). Hölter et al. have also found that chronic MRZ 2/579 infusion by osmotic minipumps eliminated the alcohol deprivation effect in rats with a long-term history (11 months) of ethanol self-administration. The authors concluded that MRZ 2/579 reduces the alcohol deprivation effect by mimicking the cueing properties of ethanol. Direct comparison between the results of this latter study and our findings is difficult due to obvious procedural differences. Hölter et al. (1996) used rats which, according to previously described criteria, presented signs of 'psychological' dependence on alcohol. In addition, the mean plasma concentration of MRZ 2/579 reported by Hölter et al. (2000) (0.52 μM) was considerably lower than that achieved in the present study (1.2 μM).

Considering the similarities between the discriminative stimulus effects of ethanol and of MRZ 2/579 (Hölter et al., 2000), one must mention that the low-affinity uncompetitive NMDA receptor antagonists, memantine and amantadine, are free of abuse potential in humans (Kornhuber and Weller, 1997; Parsons et al., 1998). In line with the above, it has been reported that MRZ 2/579 is not self-administered (Semenova et al., 1999) and does not produce conditioned place preference in mice (Popik et al., 1998). Although the above findings argue against any risk of MRZ 2/579 abuse in humans, it seems clear that such a possibility should be carefully monitored in future clinical trials.

Concluding, the results of the present study, together with the findings of other authors (e.g. Erden et al., 1999), would indicate that low-affinity, strongly voltage-dependent uncompetitive NMDA receptor antagonists might be a safe and effective treatment for alcohol withdrawal seizures in humans. Clinical studies with dextromethorphan, memantine or MRZ 2/579 should help to verify the above hypothesis. In addition, our results suggest that MRZ 2/579 might reduce alcohol consumption in human alcoholics. However, one can predict that MRZ 2/579 will reduce alcohol intake after intermittent but not chronic administration.

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