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European Journal of Pharmacology 378 (1999) 1–8

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Low-affinity NMDA receptor channel blockers inhibit acquisition of intravenous morphine self-administration in naive mice

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Received 21 January 1999; received in revised form 8 June 1999; accepted 11 June 1999

Abstract

Experimental evidence suggests that NMDA receptor antagonists modulate behavioral effects of morphine in models assessing abuse potential of drugs. The present study sought to evaluate the ability of NMDA receptor channel blockers to affect the acquisition of morphine i.v. self-administration in drug- and experimentally naive mice. DBA/2 mice were allowed to self-administer morphine (0.125–4.0 mg/ml) or saline during the 30-min test. Each nose-poke of the active mouse resulted in a 1.6-μl infusion to both the active mouse and the passive (yoked control) mouse. In vehicle-treated mice, differences between operant activity of active and passive mice were most obvious when active mice were allowed to self-inject morphine at the concentration of 0.5 mg/ml (the optimum concentration). Pretreatment with MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexan hydrochloride; 1, 3.2 and 10 mg/kg) shifted the optimum concentration to 0.75 mg/ml. Memantine (1-amino-3,5-dimethyladamantane hydrochloride; 0.3, 1, 3.2 and 10 mg/kg) suppressed both the morphine intake and the difference in nose-poke activity of active vs. passive mice across all tested concentrations of morphine. Dizocilpine ((+)-5-methyl-10,11-dihydro-5*H*-dibenzocyclohepten-5,10-imine maleate; 0.1 mg/kg) was ineffective. Taken together with earlier reports, the present results suggest that low-affinity NMDA receptor channel blockers — in contrast to dizocilpine — attenuate the rewarding potential of morphine.

Keywords: Morphine; Self-administration; NMDA receptor antagonist; Dizocilpine; Memantine; MRZ 2/579

1. Introduction

Antagonists acting at the NMDA subtype of excitatory amino acid receptors are likely candidates for possible development as medication to treat drug dependence. Numerous reports have demonstrated that NMDA receptor antagonists effectively counteract the development and/or expression of tolerance and dependence on morphine (Herman et al., 1995) as well as on several other drugs (e.g., barbiturates; Rabbani et al., 1994; Oh et al., 1997).

Treatment with NMDA receptor antagonists modifies the acute effects of abused drugs such as morphine (Lutfy et al., 1993; Tzschentke and Schmidt, 1995; Popik and Danysz, 1997; Tzschentke and Schmidt, 1997) and cocaine (Cervo and Samanin, 1995; Kim et al., 1996; Barat and Abdel-Rahman, 1997; Matsumoto et al., 1997; Pulvirenti et al., 1997). However, the results seem to be highly dependent on both the abused drug and the NMDA receptor antagonist used. For instance, amphetamine-induced expression of c-*fos* in the nucleus accumbens (Dalia and Wallace, 1995) and amphetamine-induced place preference (Hoffman, 1994) are not blocked by the NMDA receptor channel blocker, dizocilpine, although earlier data suggested that NMDA receptors mediate the behavioral effects of amphetamine infused into the nucleus accumbens (Kelley and Throne, 1992).

Recently, NMDA receptor antagonists were shown to block morphine-induced conditioned place preference in rats (Tzschentke and Schmidt, 1995; Popik and Danysz, 1997; Tzschentke and Schmidt, 1997; Popik et al., 1998). The nonselective endogenous antagonist of excitatory amino acid receptors, kynurenic acid, has also been shown to modulate morphine's effects in conditioned place preference and electrical brain stimulation tests (Bespalov et al., 1994) and impaired the acquisition of intravenous mor-

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00431-8

phine self-administration in rats (Bespalov and Zvartau, 1996).

On the other hand, the abuse potential of phencyclidine (PCP)-like NMDA receptor antagonists was demonstrated using a variety of experimental models such as intravenous self-administration (Balster and Willetts, 1996), intracranial self-stimulation (Carlezon and Wise, 1996; Olds, 1996) and conditioned place preference (Papp and Moryl, 1994). In addition, PCP-like channel blockers (dizocilpine) and cocaine have synergistic effects on brain stimulation reward (Ranaldi et al., 1997) and dizocilpine reinstates previously extinguished cocaine-seeking behavior (De Vries et al., 1998).

The present study sought to evaluate the ability of low-affinity NMDA receptor channel blockers, MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexan hydrochloride) and memantine (1-amino-3,5-dimethyladamantane hydrochloride), to affect the acquisition of morphine i.v. self-administration in drug- and experimentally naive mice. Dizocilpine ((+)-5-methyl-10,11-dihydro-5*H*-dibenzocyclohepten-5,10-imine maleate; (+)-MK-801) was selected as a representative, selective PCP-like noncompetitive antagonist (Wong et al., 1986). MRZ 2/579 and memantine represent low-affinity NMDA receptor channel blockers that share discriminative stimulus effects with but are thought to be safer than high-affinity blockers (Rogawski, 1993; Parsons et al., 1995; Grant et al., 1996; Hudzik et al., 1996; Parsons et al., 1999). Dizocilpine (Tzschentke and Schmidt, 1995, 1997), memantine (Popik and Danysz, 1997) and MRZ 2/579 (Popik et al., 1998) were shown earlier to block acquisition of morphine conditioned place preference. DBA/2 mice repeatedly served as experimental subjects in this procedure and were shown to reliably initiate i.v. morphine self-administration (Semenova et al., 1995; Kuzmin et al., 1996, 1997).

2. Materials and methods

2.1. Subjects

Adult male drug- and experimentally naive DBA/2 mice (20–32 g; State Breeding Farm ''Rappolovo'', St. Petersburg, Russia) were used. The animals were housed in groups ($N = 5$) with food and water available ad libitum. All experiments were conducted during the light period of a 12/12-h day–night cycle (0900–2100 h). The experiments were approved by the Institutional Ethics Committee of Pavlov Medical University and were performed in accordance with the recommendations and policies of the US National Institutes of Health Guidelines for the Use of Animals.

2.2. Drugs

Morphine hydrochloride (''Endokrinnyj Zavod'', Moscow, Russia), dizocilpine maleate ((+)-MK-801; Research Biochemicals International, Natick, MA, USA), memantine (1-amino-3,5-dimethyladamantane) and MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexan hydrochloride; both from Merz, Frankfurt am Main, Germany) were dissolved in physiological saline. Dizocilpine, MRZ 2/579, memantine and their vehicles were administered intraperitoneally (i.p.) in a volume of 10 ml/kg.

2.3. Apparatus

Each of the three custom-made testing apparatuses consisted of four identical test cages ($8 \times 8 \times 8$ cm) for simultaneous testing of two pairs of mice (see below). The test cages were made from opaque plastic and were covered with an opaque lid during the test. Each cage had a frontal wall hole (diameter = 1 cm) for nose-poking and a vertical slot (width = 5 mm) in the back wall for immobilizing the mouse's tail. During the test, the mice were partially immobilized by fixing their tails with adhesive tape to a horizontal surface.

The nose-poke responses were recorded by means of infrared sensors interfaced to an operating computer which controlled the activation of the two-syringe infusion pumps. The volume and duration of infusions were constant at 1.6 μl and 1.0-s, respectively. During the injection, the nose-poke responses were recorded but the infusion pump was not activated.

2.4. Procedure

A preliminary test was conducted for each mouse to record the operant level of nose-poking. Mice were placed into the test cages for 10 min, their tails were immobilized but needles were not inserted. Based on these pre-tests, the mice were grouped in pairs so that both animals in a pair exhibited approximately equal levels of nose-poking.

Within 1 h after the pretest, pairs of mice were placed again into the experimental boxes and needles (OD = 0.4 mm) were inserted into the lateral tail veins of both animals of the pair. After 10 min of habituation to the test cages, intravenous deliveries of morphine or its vehicle were made contingent upon each nose-poke of the one animal per pair (''active'' mouse). Each nose-poke of the active mouse resulted in an infusion of 1.6 μl of the morphine solution or saline to both the active mouse and the passive (yoked control) mouse. Nose-pokes of the yoked control were counted but had no programmed consequences. Each test session lasted 30 min. The mice were returned to their home cage after the experiment. Each mouse was tested only once.

The following concentrations of morphine were used to characterize the dose-effect relationship for morphine: 0.125, 0.25, 0.5, 0.75, 1.0, 2.0 and 4.0 mg/ml. Dizocilpine (vehicle, 0.1 mg/kg), MRZ 2/579 (vehicle, 1, 3.2 and 10 mg/kg) or memantine (vehicle, 0.3, 1, 3.2 and 10 mg/kg)

Table 1
Concentration-effect relations for morphine i.v. self-administration
Data are represented as medians with interquartile ranges for group numbers of nose-pokes during test (active and passive mice), Δ-criterion, R-criterion, as well as percent of mice per group that displayed a decrease in threshold below the criterion (percent of mice per group that displayed an increase in threshold above the criterion, $N+/N-$), and cumulative dose of morphine self-administered per session (see text for additional details).

Morphine concentration (mg/ml)	N	Active mice (test)	Passive mice (test)	Δ-criterion	R-criterion	$N+/N-$	Cumulative dose (mg/kg)
0	12	19 (5)	19 (7)	−1.5 (8.4)	−0.05 (0.13)	8 (8)	–
0.125	8	43 (11)	37 (14)	8.5 (27)	0.05 (0.22)	25 (0)	0.28 (0.09)
0.25	12	29 (28)	23 (10)	2.5 (22.8)	0.03 (0.31)	17 (0)	0.33 (0.18)
0.5	12	62 (26)	25 (6)	32.5 (24.8)[a]	0.32 (0.18)[a]	75 (0)[b]	1.39 (0.48)[a]
0.75	12	37 (53)	27 (12)	9.5 (41.5)	0.13 (0.45)	25 (0)	0.68 (0.84)[a]
1.0	12	34 (18)	36 (16)	−2.0 (27.5)	−0.03 (0.37)	25 (8)	1.30 (0.80)[a]

[a,b] $P < 0.05$ (Wilcoxon's and Fisher's tests, respectively), compared to control saline group (0.125 mg/ml for cumulative dose data).

was administered 15 min prior to the 30-min test session. Each treatment group consisted of 8 mice ($n = 7–10$ for dizocilpine-treated groups).

2.5. Data analysis

The data analysis was based on the assumption that the number of nose pokes of the active mouse exceeds the corresponding value in the yoked control animal when the delivery line is loaded with the reinforcing drug solution. The ratio (R) criterion was calculated for each pair of the experimental animals according to the formula: $R = \log(A_T/P_T) - \log(A_{BL}/P_{BL})$, where A_T — the total number of nose-poke responses of the active mouse, P_T — the total number of nose-poke responses of the passive mouse during the 30-min test, A_{BL} — the total number of nose-poke responses of the active mouse, P_{BL} — the total number of nose-poke responses of the passive mouse during the 10-min pretest (baseline).

Differences in absolute values of total numbers of nose-pokes between active and passive mice were calculated for each pair (Δ-criterion). In addition, the cumulative doses of self-injected morphine were recorded.

Quantal measures were also used to analyze the data. The $N+$ criterion is the percentage of pairs in the group with the value of the R-criterion higher than the 95th percentile of the R-criterion in the control group allowed to self-administer saline (0.24, $n = 12$). The $N-$ criterion is the percentage of pairs in the group with the value of the R-criterion lower than the 5th percentile of the R-criterion in the control group allowed to self-administer saline (-0.48, $n = 12$).

The data were analyzed using SAS-STAT software (ver. 6.11, SAS Institute, Cary, NC). Analysis of the descriptive statistics produced by the SAS-STAT UNIVARIATE procedure demonstrated that some of the data were not distributed normally (Wilks–Shapiro's test). Following the rank transformation, R-criterion, Δ-criterion and cumulative dose data were subjected to the distribution-free one- and two-factorial analysis of variance (ANOVA). The General Linear Models (GLM) procedure was selected because of the unbalanced design with unequal group sizes. Quantal data ($N+$ and $N-$ criteria) were analyzed using probit analysis adjusted for repeated measures design. Wilcoxon's test for gradual data and

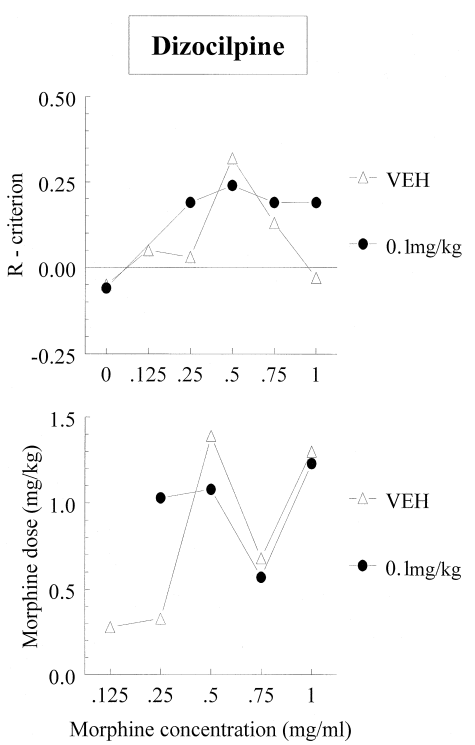

Fig. 1. Effects of dizocilpine on acquisition of morphine i.v. self-administration in naive mice. Dizocilpine (DIZ, 0.1 mg/kg) or vehicle (saline) was administered i.p. 15 min prior to the test. Data are represented as median R-criterion (*upper panel*) and median cumulative dose of morphine self-injected during the 30-min test session (*lower panel*). $N = 7–12$ for each data point.

Table 2
Effects of dizocilpine on morphine i.v. self-administration
Data are represented as medians with interquartile ranges for group numbers of nose-pokes during test (active and passive mice), Δ-criterion, *R*-criterion, percent of mice per group that displayed a decrease in threshold below the criterion (percent of mice per group that displayed an increase in threshold above the criterion, $N+/N-$), and cumulative dose of morphine self-administered per session (see text for additional details).

Morphine concentration (mg/ml)	Dizocilpine dose (mg/ml)	*N*	Active mice (test)	Passive mice (test)	Δ-criterion	*R*-criterion	$N+/N-$	Cumulative dose (mg/kg)
0	0.1	9	33 (19)	29 (13)	−6.0 (30)	−0.05 (0.41)	33 (11)	–
0.25	0.1	7	48 (40)	29 (17)	19.0 (21)[a]	0.19 (0.19)	29 (0)	1.03 (0.77)[b]
0.5	0.1	9	45 (31)	29 (11)	22.0 (34)[a]	0.24 (0.33)	56 (0)	1.08 (0.73)
0.75	0.1	10	50 (34)	27 (13)	14.5 (23.5)	0.19 (0.17)	30 (0)	0.57 (0.48)
1.0	0.1	8	39 (23)	35 (20)	11.0 (13.8)	0.19 (0.27)	50 (0)	1.23 (0.88)

[a,b]$P < 0.05$ (Wilcoxon's test), compared to corresponding control groups treated with saline instead of morphine and dizocilpine, respectively.

Fisher's exact test for quantal data were used wherever between-group pairwise comparisons were needed.

3. Results

There were no overall differences between treatment groups with regard to their performance during the 10-min pretest. The nose-poke activity of active and passive mice did not differ for any treatment group and on average was 26 responses per 10-min period (data not shown).

Nose-poke activity during the test depended significantly upon the concentration of morphine in the delivery line (Table 1; Fig. 1; one-way ANOVA: *R*-criterion — $F(5,67) = 2.4$, $P < 0.05$; Δ-criterion — $F(5,67) = 2.5$, $P < 0.05$; cumulative dose — $F(4,55) = 14.7$, $P < 0.01$). *R*-criterion and Δ-criterion were significantly higher in mice allowed to self-inject morphine at the concentration of 0.5 mg/ml (compared to saline self-administration controls) (Table 1; Fig. 1, *upper panel*). The cumulative dose of self-administered morphine was significantly higher at the concentrations of 0.75, 0.5 and 1.0 mg/ml than at concentrations of 0.125 and 0.25 mg/ml (Fig. 1, *lower panel*). Thus, the optimum concentration (concentration yielding highest *R*-criterion value) was 0.5 mg/ml.

The effects of dizocilpine were studied following administration of a single dose of 0.1 mg/kg (Table 2; Fig. 1).This dose seemed sufficient to produce behavioral effects, and motor impairment and ataxia were overtly present in mice pretreated with dizocilpine. However, none of the analyzed measures of self-administration behavior were

Table 3
Effects of MRZ 2/579 on morphine i.v. self-administration
Data are represented as medians with interquartile ranges for group numbers of nose-pokes during test (active and passive mice), Δ-criterion, *R*-criterion, percent of mice per group that displayed a decrease in threshold below the criterion (percent of mice per group that displayed an increase in threshold above the criterion, $N+/N-$), and cumulative dose of morphine self-administered per session (see text for additional details).

Morphine concentration (mg/ml)	MRZ 2/579 dose (mg/ml)	*N*	Active mice (test)	Passive mice (test)	Δ-criterion	*R*-criterion	$N+/N-$	Cumulative dose (mg/kg)
0	1	8	30 (19)	33 (18)	−8.0 (6)	−0.16 (0.07)	13 (0)	–
0	3.2	8	30 (22)	34 (12)	0.0 (23)	−0.01 (0.34)	13 (0)	–
0	10	8	24 (18)	34 (8)	−8.5 (19.3)	−0.17 (0.30)	13 (13)	–
0.25	1	8	29 (19)	28 (11)	8.5 (16.8)	0.10 (0.29)	25 (0)	0.36 (0.25)
0.25	3.2	8	29 (10)	27 (18)	3.5 (14.8)	0.05 (0.24)	13 (0)	0.31 (0.15)
0.25	10	8	23 (12)	25 (23)	−8.0 (21.3)	−0.15 (0.39)	13 (0)	0.28 (0.16)
0.5	1	8	46 (24)	26 (21)	21.0 (18.8)[a]	0.24 (0.33)[a]	50 (0)	0.87 (0.45)
0.5	3.2	8	26 (13)	23 (11)	−3.0 (23.8)[b]	−0.05 (0.43)[b]	25 (13)	0.42 (0.23)[b]
0.5	10	8	20 (16)	24 (17)	−5.0 (24)[b]	−0.14 (0.53)[b]	13 (25)[c]	0.36 (0.35)[b]
0.75	1	8	21 (9)	22 (15)	−4.5 (6.5)	−0.12 (0.14)	0 (0)	0.60 (0.28)
0.75	3.2	8	21 (8)	18 (16)	10.0 (24.5)	0.22 (0.57)	50 (13)	0.66 (0.35)
0.75	10	8	46 (27)	54 (51)	12.5 (68)	0.11 (0.61)	38 (25)	1.37 (0.79)
1.0	3.2	12	21 (15)	22 (20)	4.0 (18.8)	0.05 (0.40)	25 (17)	0.66 (0.49)
1.0	10	8	27 (13)	38 (29)	−23.0 (39.5)	−0.29 (0.55)	25 (13)	0.96 (0.39)

[a,b,c]$P < 0.05$ (Wilcoxon's/Fisher's test), compared to corresponding control groups treated with saline instead of morphine and MRZ 2/579, respectively.

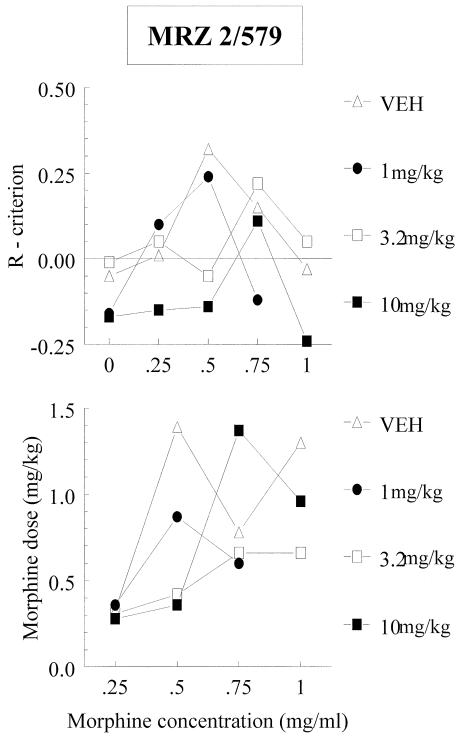

Fig. 2. Effects of MRZ 2/579 on acquisition of morphine i.v. self-administration in naive mice. MRZ 2/579 (1–10 mg/kg) was administered i.p. 15 min prior to the test. Data are represented as median *R*-criterion (*upper panel*) and median cumulative dose of morphine self-injected during the 30-min test session (*lower panel*). $N = 8$ for each data point.

found to be altered by dizocilpine administration (two-way ANOVA: *R*-criterion — $F(1,110) = 1.5$, $P = 0.22$; Δ-criterion — $F(1,110) = 1.0$, $P = 0.31$; cumulative dose — $F(1,89) = 0.2$, $P = 0.66$). Interestingly, the cumulative dose of self-administered morphine seemed to be increased when dizocilpine-pretreated mice were allowed to self-inject lower concentrations of morphine (0.25 mg/ml; morphine concentration by dizocilpine dose interaction: $F(3,89) = 5.0$, $P < 0.01$).

Pretreatment with MRZ 2/579 dose dependently suppressed morphine self-administration (Table 3; Fig. 2). This effect was most evident when the *R*-criterion and the Δ-criterion, i.e., criteria based on the difference in performance of active vs. passive mouse, were subjected to analysis (two-way ANOVA: $F(3,183) = 3.6$, $P < 0.05$; $F(3,183) = 3.9$, $P < 0.01$, respectively). Analysis of the cumulative dose of self-administered morphine suggested that the optimum concentration was shifted to 0.75 mg/ml (Table 3; Fig. 2, *lower panel*; two-way ANOVA: $F(3,147) = 6.5$, $P < 0.01$). Indeed, statistical analysis supported this conclusion by showing a significant interaction between MRZ 2/579 dose and morphine concentration ($F(8,147) = 4.2$, $P < 0.01$). In mice pretreated with MRZ 2/579 (10 mg/kg), the cumulative dose of self-administered morphine was not different from that for the controls treated with vehicle and was significantly higher at the concentrations of 0.75 and 1.0 mg/ml compared to 0.25 mg/ml.

Table 4
Effects of memantine on morphine i.v. self-administration
Data are represented as medians with interquartile ranges for group numbers of nose-pokes during test (active and passive mice), Δ-criterion, *R*-criterion, percent of mice per group that displayed a decrease in threshold below the criterion (percent of mice per group that displayed an increase in threshold above the criterion, $N+/N-$), and cumulative dose of morphine self-administered per session (see text for additional details).

Morphine concentration (mg/ml)	Memantine dose (mg/ml)	*N*	Active mice (test)	Passive mice (test)	Δ-criterion	*R*-criterion	$N+/N-$	Cumulative dose (mg/kg)
0	0.3	8	19 (3)	21 (8)	−4.5 (4.3)	−0.10 (0.10)	0 (0)	–
0	1	8	21 (14)	24 (14)	−5.0 (20)	−0.10 (0.38)	25 (0)	–
0	3.2	8	16 (6)	21 (8)	−5.0 (8.8)	−0.16 (0.21)	13 (0)	–
0	10	8	23 (10)	22 (13)	0.0 (6)	−0.02 (0.14)	0 (0)	–
0.25	0.3	8	27 (8)	21 (6)	8.5 (11.5)	0.16 (0.21)	13 (0)	0.27 (0.07)
0.25	1	8	27 (6)	20 (8)	6.5 (11)	0.11 (0.24)	25 (0)	0.23 (0.06)
0.25	3.2	8	25 (14)	34 (14)	−8.5 (17.8)	−0.13 (0.29)	0 (0)	0.20 (0.17)
0.25	10	8	19 (18)	31 (20)	−7.5 (14.5)	−0.16 (0.25)	13 (0)	0.17 (0.23)
0.5	0.3	8	50 (23)	27 (28)	23 (37.3)[a]	0.28 (0.42)[a]	63 (0)[c]	0.98 (0.46)
0.5	1	8	25 (12)	19 (5)	8.5 (11.3)	0.09 (0.21)	25 (0)	0.41 (0.24)[c]
0.5	3.2	8	24 (4)	20 (12)	1.5 (9.5)[c]	0.02 (0.21)[c]	25 (0)	0.44 (0.10)[c]
0.5	10	8	18 (18)	27 (24)	−7.5 (16)[c]	−0.15 (0.27)[c]	13 (0)[c]	0.31 (0.28)[c]
0.75	1	8	20 (17)	17 (9)	6.0 (13.3)	0.16 (0.27)	38 (0)	0.54 (0.41)
0.75	3.2	8	15 (10)	17 (6)	−2.0 (7.5)	−0.09 (0.14)	13 (0)	0.39 (0.24)
0.75	10	8	15 (14)	17 (16)	1.5 (17.5)	−0.00 (0.41)	0 (25)	0.44 (0.30)
1	0.3	8	22 (6)	22 (11)	5 (15)	0.07 (0.27)	13 (0)	0.80 (0.29)
1	1	8	18 (8)	16 (5)	−3 (13.8)	−0.10 (0.36)	13 (0)	0.66 (0.42)
1	3.2	8	17 (2)	25 (5)	−8.5 (11.5)	−0.21 (0.19)	0 (0)	0.61 (0.18)[c]
1	10	8	7 (7)	14 (9)	−3.5 (4)	−0.22 (0.14)	0 (13)	0.29 (0.28)[c]
2	1	8	14 (16)	14 (10)	−5.5 (5.5)	−0.19 (0.19)	0 (13)	1.06 (1.34)
4	1	8	12 (8)	15 (6)	−1.5 (8.8)	−0.17 (0.30)	0 (13)	1.98 (1.25)

[a,c] $P < 0.05$ (Wilcoxon's/Fisher's test), compared to corresponding control groups treated with saline instead of morphine and MRZ 2/579, respectively.

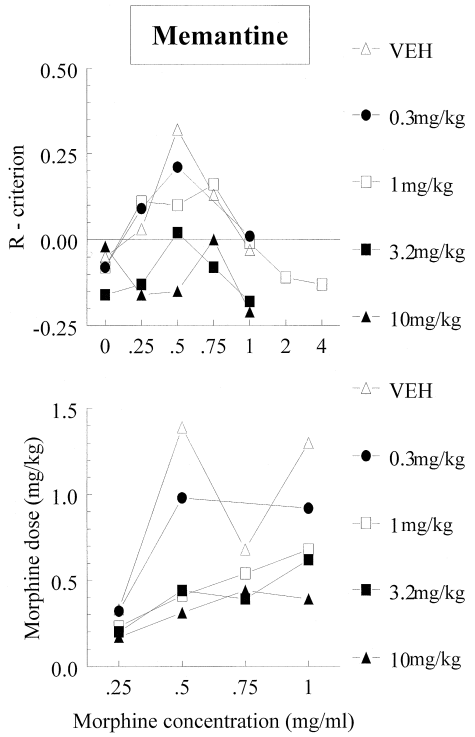

Fig. 3. Effects of memantine on acquisition of morphine i.v. self-administration in naive mice. Memantine (1–10 mg/kg) was administered i.p. 15 min prior to the test. Data are represented as median *R*-criterion (*upper panel*) and median cumulative dose of morphine self-injected during the 30-min test session (*lower panel*). $N = 8$ for each data point.

Results obtained in tests with memantine are shown in Table 4 and Fig. 3. There were no differences between active and passive mice across all morphine concentrations when mice were pretreated with memantine (two-way ANOVA: *R*-criterion — $F(4,211) = 6.2$, $P < 0.01$; Δ-criterion — $F(4,211) = 6.1$, $P < 0.01$). The cumulative dose of self-administered morphine was also significantly reduced in mice treated with memantine (two-way ANOVA: $F(4,167) = 15.4$, $P < 0.01$).

4. Discussion

Pretreatment with both MRZ 2/579 and memantine attenuated the acquisition of morphine i.v. self-administration in drug- and experimentally naive mice. There were no differences between active and passive mice across all morphine concentrations and the cumulative dose of self-administered morphine was significantly reduced when mice were pretreated with memantine. Meanwhile, in mice pretreated with MRZ 2/579 (10 mg/kg), the cumulative dose of self-administered morphine was not lower than that in control animals treated with vehicle and clearly depended on the morphine concentration, since it was significantly higher at the concentrations of 0.75 and 1.0 mg/ml compared to 0.25 mg/ml. The optimal concentration (yielding highest *R*-criterion) in vehicle-tested mice was 0.5 mg/ml and pretreatment with MRZ 2/579 shifted the optimum concentration to 0.75 mg/ml.

Thus, memantine and MRZ 2/579 caused downward and rightward shifts in morphine concentration-effect relationships, respectively. To our knowledge differences in the binding profile of MRZ 2/579 and memantine are unknown. The differences in the kinetics of their interactions with the binding site within the NMDA receptor channel can hardly account for the effects observed. Both MRZ 2/579 and memantine are highly voltage-dependent low-affinity channel blockers with IC_{50} values of 1.4 and 2.3 μM, respectively (Parsons et al., 1995; Blanpied et al., 1997; Parsons et al., 1999). It is important that MRZ 2/579 and memantine inhibited the morphine-conditioned place preference with nearly equal efficacy but MRZ 2/579 was more potent than memantine (Popik et al., 1998). In the present study, MRZ 2/579 and memantine were studied over a fairly wide range of doses. Both compounds significantly interfered with morphine-taking behavior, starting at relatively low doses (3.2 and 1 mg/kg, respectively). Brain microdialysis studies in rats with in vivo recovery indicate that MRZ 2/579 reaches 0.8 μM concentration in the brain after i.p. administration of 5 mg/kg which should be sufficient to block NMDA receptors (Hesselink et al., 1999) and does not produce any behavioral ''side-effects''. Also based on serum levels of memantine, the dose effective in the present study against acquisition of morphine self-administration was clearly within the therapeutic range (see Danysz et al., 1997 for discussion).

The mechanisms by which NMDA receptor antagonists affected the acquisition of morphine self-administration are not clear. For instance, NMDA receptor antagonists were shown to have minimal interactions with the discriminative stimulus effects of morphine (Popik and Danysz, 1997; Bespalov et al., 1998). To date there is no evidence for binding of either memantine or MRZ 2/579 to opioid receptors. The role of the drug-induced learning deficits is also questionable since dizocilpine, which has stronger learning impairing effects (Misztal and Danysz, 1995), had no effect but failed to attenuate the acquisition of morphine self-administration in the present study. Although effects of dizocilpine were studied following administration of a single dose of 0.1 mg/kg, this dose did seem sufficient to produce behavioral effects. For instance, the cumulative dose of self-administered morphine appeared to increase when dizocilpine-pretreated mice were allowed to self-inject lower concentrations of morphine. This increase of the dose consumed, without parallel changes in *R*-criterion (active vs. passive mice performance), can be interpreted as a result of general motor stimulation produced by dizocilpine.

There have been numerous reports of different behavioral profiles for high- vs. low-affinity NMDA channel blockers (e.g., Parsons et al., 1995, 1999). Fast unblocking

kinetics of the low-affinity channel blockers (such as those used in this study) was suggested to be essential for their therapeutic effectiveness. However, there are not enough data to speculate any further on the contribution of channel trapping to the observed differences between dizocilpine and memantine/MRZ 2/579.

Nucleus accumbens septi was suggested as a possible substrate for reinforcement learning (Kelley et al., 1997). Administration of the competitive NMDA receptor antagonist, 2-amino-5-phosphonopentanoic acid, into the nucleus accumbens impaired response-reinforcement learning for the acquisition of a simple lever-pressing task to obtain food. Data from this laboratory also support this view. For instance, there is evidence of impaired acquisition of intracranial self-stimulation in rats with bilateral kainic acid-induced lesions of nucleus accumbens septi (Bespalov and Zvartau, 1995). However, controversy still remains since NMDA receptors in the nucleus accumbens were shown to modulate intravenous cocaine but not heroin self-administration (Pulvirenti et al., 1992).

On the other hand, it is known that restrained animals may show a higher propensity to initiate drug self-administration (Shaham, 1993). Thus, taking into account an anxiolytic potential of NMDA receptor antagonists (Wiley, 1997), this would suggest alternative explanations for the present results. However, if anxiolytic effects were essential for reducing morphine self-administration behavior, dizocilpine (and any other drug with anxiolytic-like activity) would have to exert effects similar to those of memantine and/or MRZ 2/579. Moreover, one should note that the low-affinity channel blockers (e.g., memantine) may be devoid of anxiolytic activity (Karcz-Kubicha et al., 1997).

Overall, taken together with earlier reports, the present results suggest that low-affinity NMDA receptor channel blockers may attenuate the rewarding potential of morphine. Our data also support the idea that different forms of reinforcement learning are involved in i.v. self-administration and place conditioning procedures since NMDA receptor channel blockers differentially influenced the effects of morphine and displayed distinct potency ranks in these models.

The present data suggest that, for memantine, that has been used clinically for many years in dementia and spasticity, the treatment of opioid abuse could be another valuable therapeutic use. In fact, preliminary studies in humans performed at Columbia University suggest that this might be the case (Bisaga et al., 1998). In case of MRZ 2/579, a drug that is currently under development, the anti-abuse potential should be investigated further.

Acknowledgements

Supported in part by Fogarty International Research Collaboration Award #1R03TW00714-01.

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