Effects of N-Methyl-D-Aspartate Receptor Antagonists on Discriminative Stimulus Effects of Naloxone in Morphine-Dependent Rats Using the Y-Maze Drug Discrimination Paradigm\textsuperscript{1,2}

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ABSTRACT

The present study assessed the ability of various site-selective N-methyl-D-aspartate (NMDA) receptor antagonists to affect the discriminative stimulus properties of naloxone in morphine-dependent rats. Adult male Wistar rats were trained to discriminate 0.1 mg/kg of s.c. naloxone from saline using a Y-maze shock-avoidance procedure. Naloxone-appropriate responding was exhibited as a function of naloxone dose (0.01–1.0 mg/kg, ED\textsubscript{50} = 0.03 mg/kg) and was also observed when morphine treatment temporarily was discontinued (8–96 hr, peak at 24 hr). Discriminative stimulus effects of naloxone (0.1–3.0 mg/kg) were antagonized by morphine (10–100 mg/kg). Ligands of peripheral opioid receptors failed to either substitute for naloxone (methylnaloxone, 0.1–3.0 mg/kg) or attenuate naloxone's stimulus effects (loperamide, 1–30 mg/kg). In rats treated with the training dose of naloxone, administration of dizocilpine (0.03–0.3 mg/kg) and ε-CPPene (1–10 mg/kg) decreased levels of naloxone-appropriate responding, whereas memantine (1–30 mg/kg), ACEA-1021 (10 and 50 mg/kg) and eliprodil (3–30 mg/kg) seemed to have little or no effects. Meanwhile, all NMDA receptor antagonists produced a decrease in the occurrence of two or more of the following opioid withdrawal signs: weight loss, forelimb tremor, ptosis, diarrhea and “wet-dog”-like shaking. Additionally, dizocilpine (0.1 mg/kg), ε-CPPene (5.6 mg/kg) and ACEA-1021 (50 mg/kg) but not memantine (10 mg/kg) or eliprodil (30 mg/kg) significantly reduced the naloxone-appropriate escape area selection when administered during the period of suspended morphine treatment 24 hr after the last morphine injection. Thus, NMDA receptor antagonists appear to inhibit the discriminative stimulus effects of both naloxone-precipitated and spontaneous morphine withdrawal, and this ability depends on the type of antagonist applied.

Substantial experimental evidence indicates that the antagonists of NMDA subtype of EAA receptors can attenuate the development and expression of somatic, autonomic and some behavioral signs of opioid dependence (specifically of morphine dependence) (Herman et al., 1995; Popik and Skolnick, 1996). There also is evidence for the hyperactivity of EAAergic neurotransmission in animals withdrawn from opioids (Hong et al., 1993; Rasmussen, 1995). Recent reports suggest that the aversive place conditioning of opioid withdrawal state is retarded by treatment with NMDA receptor channel blockers (Higgins et al., 1992; Popik and Danyysz, 1997).

The present study sought to investigate the ability of NMDA receptor antagonists to modulate the discriminative stimulus effects of opioid antagonist naloxone in morphine-dependent rats trained to discriminate naloxone from saline using a Y-maze shock avoidance procedure. There were several reports on successful establishment of opioid antagonist discrimination in opioid agonist-dependent rats (Gellert and Holtzman, 1979; Holtzman, 1985, 1989), rhesus monkeys (France, 1994), and humans (Preston et al., 1987). Stimulus control of behavior by opioid antagonists in morphine-dependent subjects was characterized by long-term stability and reproducibility, orderly dose- and time-effect relationships, pharmacological specificity and central rather than peripheral origin (Gellert and Holtzman, 1979; Holtzman, 1985). As it was suggested by Holtzman (1985), “... discrimination paradigm may afford a specific animal model... for evaluating novel pharmacological approaches for treating opiate withdrawal...”.

ABBREVIATIONS: NMDA, N-methyl-D-aspartate; EAA, excitatory amino acid; PCP, phencyclidine; dizocilpine (MK-801), (\textsuperscript{-})-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate; ε-CPPene (SDZ EAA 494), 3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid; memantine, 3,5-dimethyladamantan-1-amine; ACEA-1021 (licostinel), 5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione.
Compared with the procedure described by Gellert and Holtzman (1979), the model used in the present study had several distinguishing features. First, rats were required to run in a Y-maze to escape/avoid electric shocks instead of completion of the two-lever press chains in the operant conditioning chambers. Second, subcutaneous injections of morphine were given rather than drinking solution containing morphine. And, finally, the opioid antagonist naloxone was selected as a training drug while naltrexone was used in other studies. Because of these differences, at the beginning of the study naloxone’s discriminative stimulus effects were characterized by (1) analyzing the interactions between naloxone and morphine, (2) evaluating the involvement of central vs. peripheral opioid receptors and (3) comparing the discriminative stimulus effects of naloxone and spontaneous withdrawal from morphine.

There is a large body of evidence suggesting that antagonists acting at pharmacologically distinct sites of NMDA receptor complex have remarkably different psychopharmacological profile (Balster and Willetts, 1996). For instance, drug discrimination studies clearly demonstrate that NMDA antagonists acting competitively at NMDA, glycine and polyamine sites fail to produce discriminative stimulus effects identical to PCP-like drugs (Balster et al., 1994, 1995; Mansbach and Balster, 1991). Therefore, for comparative purposes, the present study used antagonists acting at each of the following binding sites on the NMDA-receptor complex:

1. A transmitter-recognition site that binds competitive antagonists (δ-CPPene; Lowe et al., 1994),
2. A site located within the ion channel site that binds drugs such as PCP,
3. A strychnine-insensitive glycine coactivator site (ACEA-1021; Woodward et al., 1995) and
4. A polyamine binding site.

Dizocilpine and memantine were selected as representative high- and low-affinity channel blockers, respectively (Danysz et al., 1994), (2) a site located inside the ion channel site that binds drugs such as PCP, (3) a strychnine-insensitive glycine coactivator site (ACEA-1021; Woodward et al., 1995) and (4) a polyamine binding site. Dizocilpine and memantine were selected as representative high- and low-affinity channel blockers, respectively (Danysz et al., 1997; Wong et al., 1986). We also tested eliprodil, a polyamine-site NMDA receptor antagonist that may preferentially act at subtypes of NMDA receptors assembled from NMDA NR1 and NR2B subunits (Avenet et al., 1997).

Method

Subjects. Nine adult experimentally naive male Wistar rats (Rappolovo, St. Petersburg, Russia) were used. Animals were kept individually in plastic cages with water available ad libitum. Food (standard rodent chow from Volosovo, St. Petersburg, Russia) consumption was restricted to 14 to 18 g/day to maintain subjects' body weights under 400 g. All experiments were conducted during the light period of a 12:12 hr day/night cycle (8:00 a.m. to 8:00 p.m.).

Apparatus. The Y-maze had three equal-sized arms with a 120° angle between each two arms (fig. 1). The arms were 15.5 cm wide, 36 cm long and 16 cm high. The floor consisted of parallel stainless steel rods (d = 2 mm, spaced at 8 mm). The walls and ceiling were made of nontransparent light brown plastic. A guillotine door could be placed in front of either of escape areas and start area to prevent entry. Escape and start areas were 15.5 cm wide, 12 cm long and 16 cm high and had front transparent plastic doors to load/remove rats from the apparatus. All guillotine and front doors were operated manually. Illumination inside the apparatus was 36 (choice point) to 95 (escape and start areas) lux. General light intensity in the animal facility and experimental room was 300 to 320 lux. A model ES-51 constant current source provided 1.5-mA electric shocks (1-sec trains of positive pulses every 3 sec), which were applied to the floor of the maze.

Morphine dependence. Rats were injected s.c. with 15 mg/kg morphine twice a day at 11:00 a.m. and 5:00 p.m. It was shown earlier that the development of morphine physical dependence reaches a plateau after ~3 weeks of morphine intake (Gellert and Holtzman, 1979). After the initial 4 weeks of morphine administration, tests with opioid antagonist naloxone were conducted before commencement of the discrimination training to demonstrate the establishment of morphine dependence. Over the 2-week period, rats received treatments with various doses of naloxone 2 hr after the first daily morphine injection. Naloxone doses (vehicle, 0.01, 0.1, 1 and 10 mg/kg s.c.) were administered according to the Latin Square design with 3 to 4 days separating the tests. Total number of “wet dog”-like shakes and stretches and presence of forelimb tremor and ptosis were recorded for a 15-min period immediately after the naloxone administration. Additionally, body weight changes within 4 hr after the naloxone treatment were measured.

Discrimination training. Rats were initially trained to run from the start area to one of the escape areas to avoid or escape from electric shocks. Each training session consisted of three trials. At the start of each trial, the rat was placed into the start area with no electric current applied to the floor and all guillotine doors were closed, thus preventing the rat from going toward the choice point. The beginning of a trial was signaled 5 sec later by the removal of the guillotine door. Starting 0 to 60 sec later (the value was randomly selected for each trial), the maze floor was electrified everywhere except in the ones of the escape areas. As soon as the rat entered the appropriate escape area, sound generator (70 dB, frequency range 1 to 3 kHz) was turned on for 1 sec, and all three guillotine doors were closed. One minute later, a new trial was initiated by removing the rat from the escape area and placing it again in the start area. Rats were returned to the home cage after the completion of the third daily trial. All training and subsequent test sessions were held daily (Monday through Sunday) between 1:00 and 2:00 p.m. (i.e., 2–3 hr after the first daily morphine injection).

After rats had escaped from the electric shocks by running to the appropriate escape area on all three daily trials, the running direction was switched to another escape area that eventually would become the naloxone designated. As soon as an escape behavior had been established toward the second escape area, naloxone discrimination training was started.

All discrimination training sessions began 10 min after the s.c. injection of saline or 0.1 mg/kg naloxone. This dose of naloxone and the injection time of 10 min were selected on the basis of pilot
experiments. Daily saline (S) and naloxone (N) injections were given according to two, monthly alternating sequences: (1) SNSNSNSN, NSSNNSNN, NNNSNSN, NSSSNSSS and (2) NSNSNSS, SNNSNN, SSSSSNSS, NSSSSNNS, NSSSNN. Some of the rats (rats 1, 3, 5, 7 and 9) were trained to run toward the left escape area during each saline training session and toward the right escape area during the naloxone sessions. Other rats (rats 2, 4, 6 and 8) were trained under opposite conditions.

Acquisition training proceeded until during 10 consecutive training days when the escape area selection corresponded to the preceding treatment condition on all trials. After the criterion was met, the rats were given test days. Test days were held provided that during at least two most recent saline and two most recent naloxone training sessions the escape area selection corresponded to the preceding treatment condition on all trials. At least three training sessions intervened between consecutive tests. On test days, single-trial sessions were held, and the floor was not electrified at any time, allowing rats to choose any of the escape areas. The test trial was terminated when rat chose one of the escape areas or after 180 sec, whichever came first. Each rat was repeatedly tested with either vehicle or the training dose of naloxone (0.1 mg/kg) until four test sessions were completed with rats selecting the escape area that corresponded to the preceding treatment.

Naloxone dose-response determination and morphine antagonism. Naloxone dose-response functions were initially obtained when naloxone (vehicle, 0.01, 0.017, 0.032, 0.056, 0.1, 0.32 and 1 mg/kg) was injected by itself and subsequently were reobtained when various doses of naloxone (0.1–3 mg/kg) were coadministered with morphine (10–100 mg/kg s.c.; preinjection time 30 min).

Tests with peripherally active opioids. Substitution tests were conducted with opioid receptors antagonist methylnaloxone (vehicle, 0.3, 1.0, 3.0 and 10 mg/kg s.c.; preinjection time 30 min) while opioid receptors agonist loperamide (1–30 mg/kg s.c., preinjection time 30 min) was administered in combination with the training dose of naloxone (0.1 mg/kg).

Tests with combinations of naloxone and NMDA receptor antagonists. During each test there were two injection groups: one s.c. with either naloxone (0.1 mg/kg) or saline (preinjection time 10 min) and one i.p. with the test drug or its vehicle. Control tests with the training dose of naloxone and with saline were conducted at intervals throughout the study and included i.p. vehicle (instead of NMDA receptor antagonist) as the second injection. Tests were conducted with the following drugs: dizocilpine (0.03–0.3 mg/kg; preinjection time 20 min), memantine (1–30 mg/kg; preinjection time 30 min), d-CPPene (1–10 mg/kg; preinjection time 60 min), ACEA-1021 (10 and 50 mg/kg; preinjection time 30 min) and eliprodil (3–30 mg/kg; preinjection time 30 min). The order of tests with dizocilpine, d-CPPene, memantine, and eliprodil and the dosing orders were determined according to the Latin Square design. Tests with ACEA-1021 were conducted at the end of the experiment. The dose and preinjection times for NMDA receptor antagonists were selected on the basis of pilot experiments and available literature.

With the selected doses of dizocilpine (0.1 mg/kg), d-CPPene (5.6 mg/kg), memantine (10 mg/kg), ACEA-1021 (50 mg/kg) and eliprodil (30 mg/kg), additional behavioral monitoring was conducted for 15 min immediately after the administration of the naloxone training dose while rats were in home cages. During these observation sessions, expression of various signs of opioid withdrawal was recorded. Preinjection times for various NMDA receptor antagonists are indicated above.

Spontaneous withdrawal. Repeated morphine treatment was discontinued by omitting daily morphine injections. Test sessions were conducted 10 min after an injection of saline at 2, 8, 24, 48, 72 and 96 hr after the last morphine administration over a single series of tests. To study the effects of NMDA receptor antagonists on the discriminative stimulus properties of spontaneous suspension of morphine treatment, rats were repeatedly withdrawn from morphine for 24 hr and tests were held provided the above-mentioned criterion was met on the preceding training sessions. Intervals between withdrawals were no shorter than 1 week. Selected doses of dizocilpine (0.1 mg/kg), d-CPPene (5.6 mg/kg), memantine (10 mg/kg), ACEA-1021 (50 mg/kg) and eliprodil (30 mg/kg) and their vehicles were administered in combination with s.c. saline injection 24 hr after the last morphine treatment. The order of tests with dizocilpine, d-CPPene, memantine and eliprodil was determined according to the Latin Square design. Tests with ACEA-1021 were conducted at the end of the experiment.

Data analysis. For quantal data, the percentage of rats per group with forelimb tremor, ptosis, salivation, diarrhea or rats selecting naloxone-appropriate escape area was determined. For gradual data, mean (±S.E.M.) total numbers of “wet dog-like” shakes, stretches and mean (±S.E.M.) body weight changes were calculated.

Data were analyzed using SAS-STAT software (version 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 test). Withdrawal signs and weight loss data were subjected to the distribution-free one- and two-factorial ANOVA with repeated measures using a combination of the RANK and General Linear Model (GLM) procedures (SAS Institute Inc., 1990). Briefly, data were ranked and the ranks were later subjected to ANOVA (GLM procedure for unbalanced design with unequal group sizes). Naloxone-ARM selection data were analyzed using probit analysis (PROBIT procedure) adjusted for repeated measures design. Wilcoxon’s test for gradual data and Fisher’s exact test for quantal data were used wherever between-group pairwise comparisons were needed. ED_{50} values were calculated using PROBIT (for quantal data) and REG (regression analysis; for gradual data) procedures.

Drugs. The following drugs were used: morphine hydrochloride (Zdorov’e, Khar’kov, Ukraine), loperamide hydrochloride (Janssen Pharmaceutica, Beerse, Belgium), naloxone hydrochloride, methyl- naloxone hydrochloride, dizocilpine maleate (all from Research Biological Chemicals, Internal, Natick, MA), d-CPPene (SDZ EAA 494; gift from Novartis, Basel, Switzerland), eliprodil (gift from Synthelabo Recherche, Bagneux, France), memantine (gift from Merz, Frankfurt-am-Main, Germany) and ACEA-1021 (gift from CoCensys, Irvine, CA). Morphine, naloxone, methylnaloxone, dizocilpine and memantine were dissolved in physiological saline, loperamide and ACEA-1021 in 50% dimethylsulfoxide, d-CPPene in equimolar NaOH in saline and eliprodil in a vehicle of 5% ethanol and 5% Alkamuls EL-620 (castor oil ethoxylated; Rhone-Poulenc, Cranbury, NJ). Morphine, naloxone, methylnaloxone, loperamide and their vehicles were injected subcutaneously, whereas the rest of drugs and their vehicles were administered intraperitoneally. All injections delivered a solution in a volume of 1 ml/kg. Doses are based on the forms of the drugs listed above.

Results

Morphine dependence establishment. Administration of naloxone to rats that were repeatedly exposed to morphine for 4 to 6 weeks resulted in a dose-dependent weight loss, expression of forelimb tremor, ptosis and increased frequency of “wet dog-like” shakes and stretches (fig. 2). The degree of physical dependence of the rats appeared to be constant...
during the period of ~10 months when drug tests were conducted, as was reflected by the absence of consistent trends in naloxone-induced weight loss.

**Discrimination training.** All nine rats acquired the naloxone-saline discrimination within an average of 34 days (fig. 3). Rats chose the correct Y-maze arm on every occasion control tests with the training dose of naloxone and saline were conducted. Weight changes produced by naloxone remained stable during the discrimination training period: $-11.2 \pm 1.3$ g on day 1, $-11.2 \pm 1.0$ g on day 22, and $-10.2 \pm 1.7$ g on day 44. The mean naloxone-induced weight loss was $-2.7\%$ to $4.0\%$ (each $1\%$ represents $3$ g).

**Naloxone dose-response determination and morphine antagonism.** Naloxone dose-dependently generalized from the training dose (fig. 4, left), with ED$_{50}$ of $0.029$ mg/kg (CL, $0.021–0.041$ mg/kg). The percentage of rats selecting naloxone-appropriate escape area was higher with the increase in naloxone dosage ($\chi^2 = 11.07, P = .0009$). Similarly, the magnitude of naloxone-induced weight loss change was dependent on the naloxone dose [$F(7,49) = 16.18$, $P = .0001$], with ED$_{50}$ for this effect of $0.07$ mg/kg (CL, $0.024–0.200$ mg/kg).

The naloxone dose-effect function was also evaluated when naloxone ($0.1–3.0$ mg/kg) was coadministered with $100$ mg/kg of morphine (fig. 4). These tests yielded the significant rightward shift in naloxone dose-effect curve for both naloxone-appropriate escape area selection ($\chi^2 = 16.21, P = .0001$) and weight loss [$F(1,7) = 12.36, P = .0098$]. In morphine-pre-treated rats, ED$_{50}$ values for naloxone increased $1.5$ log units (naloxone-appropriate escape area selection: $0.86$ mg/kg, CL, $0.43–1.62$ mg/kg; weight loss: $2.14$ mg/kg, CL, $0.67–6.76$ mg/kg).

**Tests with peripherally active opioids.** Loperamide produced a dose-dependent suppression of naloxone-induced body weight loss [fig. 5, right, $F(4,28) = 18.96$, $P = .0001$] but failed to modify escape area selection (fig. 5, left). In contrast to the effects of loperamide, morphine dose-dependently blocked the effects of naloxone’s training dose [fig. 5; naloxone-appropriate escape area selection: $\chi^2 = 8.92, P = .0028$; weight loss: $F(3,21) = 11.57, P = .0001$].

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**Fig. 2.** Effects of naloxone in morphine-dependent rats. Various doses of naloxone or its vehicle (V, saline) were administered s.c. 2 hr after the last morphine injection, and rats' behavior was monitored for 15 min immediately thereafter. A, Change in body weight in grams was determined over an interval of 4 hr following naloxone administration. B, Percent of rats per group with ptosis (filled bars) and forelimb tremor (hatched bars). C, Mean ($\pm$S.E.M.) number of “wet-dog”-like shakes (filled bars) and stretches (hatched bars) per 15-min observation period. $n = 9$. * $P < .05$ (Wilcoxon’s test for A and C; Fisher’s exact test for B), compared with vehicle-treated controls.

**Fig. 3.** Acquisition of naloxone discrimination behavior in morphine-dependent rats. To escape or avoid from electric footshocks, rats were required to run toward one escape area of the Y-maze when drugged (0.1 mg/kg naloxone s.c.) and to run toward another escape area when not drugged (saline injection s.c.). Data are represented as percentage of rats selecting the naloxone-designated escape area after naloxone (triangles) and saline (circles) administration. $n = 9$.

**Fig. 4.** Percentage of the naloxone-appropriate escape area selection (left) and mean ($\pm$S.E.M.) body weight change (right) after the s.c. administration of various doses of naloxone alone (●) and in combination with morphine (100 mg/kg s.c.; ▲ represent the results of saline test sessions. Each point is based on observations made in 8 rats. The S.E.M. is not shown where it is less than the radius of the marker.)
Results of stimulus generalization tests with methylnaloxone are shown in figure 5. Methylnaloxone produced dose-dependent body weight loss \( F(4,28) = 10.29; P < .0001 \) but did not substitute for the training drug (i.e., naloxone). At the highest tested dose of methylnaloxone of 10 mg/kg, only 2 of 8 rats selected naloxone-appropriate escape area.

Tests with combinations of naloxone and NMDA receptor antagonists. None of the rats pretreated with NMDA receptor antagonists alone (s.c. saline injection instead of naloxone) selected naloxone-appropriate escape area selection when tested alone (figs. 6, data above the A point). There was observed only one instance of the rat selecting naloxone-designated escape area after the administration of 10 mg/kg of D-CPPene. These tests also revealed that dizocilpine (0.1 mg/kg), memantine (30 mg/kg) and D-CPPene (10 mg/kg) per se caused body weight loss \( P < .05 \), compared with vehicle-treated controls), whereas ACEA-1021 (50 mg/kg) and eliprodil (30 mg/kg) did not (table 1).

Among two tested NMDA receptor channel blockers (fig. 6), dizocilpine but not memantine significantly decreased the naloxone-appropriate escape area selection when combined with the administration of the training dose of naloxone \( (x^2 = 7.39, P = .0066, x^2 = 3.48, P = .0692, \text{respectively}) \). The dose of dizocilpine of 0.3 mg/kg produced severe behavioral toxicity and only one rat was tested at this dose. Administration of the highest dose of memantine also resulted in marked alteration of rats’ behavior and motor function, with one rat not completing the test trial. Dizocilpine dose-dependently facilitated the naloxone-induced weight loss, whereas memantine at low doses had an opposite effect [table 2; dizocilpine: \( F(3,29) = 14.96, P = .0001 \); memantine: \( F(5,45) = 4.78, P = .0022 \)]. Memantine at the dose of 3 mg/kg was found to reduce significantly the capability of naloxone to induce weight loss \( P < .05 \), compared with controls treated with vehicle instead of memantine). Both dizocilpine and memantine had the suppressive effects on the expression of various signs of opioid withdrawal (table 3).

Similarly to dizocilpine, D-CPPene dose-dependently attenuated naloxone’s discriminative stimulus effects (fig. 6; \( x^2 = 5.89, P = .0387 \)) but did not prevent naloxone-induced weight loss. However, D-CPPene dose was still found to be a significant determinant of body weight changes in naloxone-pre-treated rats [table 2; \( F(4,36) = 4.84, P = .0005 \)]. D-CPPene significantly suppressed diarrhea as well as some other symptoms of opioid withdrawal (ptosis, diarrhea, shaking; table 3).

Effects of either ACEA-1021 or eliprodil on naloxone-appropriate responding did not reach the level of statistical significance (fig. 6; \( x^2 = 2.73, P = .0986; x^2 = 1.84, P = .1746, \text{respectively} \)). Three of seven rats treated with a combination of 50 mg/kg of ACEA-1021 and training dose of naloxone selected naloxone-designated escape area. Similarly, 6 of 8 rats treated with a combination of 30 mg/kg of eliprodil and training dose of naloxone selected naloxone-designated escape area. Meanwhile, both ACEA-1021 and eliprodil completely prevented the naloxone-induced weight loss [table 2; \( F(2,20) = 7.58, P = .0074; F(3,30) = 12.86, P = .0001, \text{respectively} \)]. In addition, ACEA-1021 seemed to reduce the

| Table 1 |
|-----------------|----------------|-----------------|-----------------|
| **NMDA antagonist dose (mg/kg)** | **Vehicle** | **0.1** | **0.5** |
| **Dose (mg/kg)** | **Weight loss** | **Weight loss** | **Weight loss** |
| | **0.2 ± 0.4** | **−1.8 ± 0.4** | **−2.1 ± 0.5** |
| **n** | **8** | **8** | **8** |
| **n** | **8** | **8** | **8** |
| **n** | **8** | **8** | **8** |
| **n** | **8** | **8** | **8** |
| **n** | **7** | **7** | **7** |

\* P < .05 (Wilcoxon’s test), compared with Vehicle tests.
occurrence of diarrhea and “wet dog-like”-shaking in naloxone-treated morphine-dependent rats (table 3). Eliprodil exerted significant suppressant effects on the expression of forelimb tremor, ptosis, diarrhea, and shaking precipitated by naloxone injection in morphine-dependent rats.

| Spontaneous withdrawal. | Figure 7 shows that when morphine treatment was temporarily discontinued, rats were selecting naloxone-appropriate Y-maze arm. Group levels of naloxone-appropriate responding increased gradually, reaching a peak at 24 hr and returning toward saline levels at 96 hr ($\chi^2 = 11.7$, $P = .0006$). Changes in body weight that were monitored at each time point closely paralleled the changes in naloxone-appropriate responding [$F(4,28) = 16.11$, $P = .001$]. Twenty-four hours after the last morphine injection, the body weight loss averaged 7.9% (~25 g).

To study the effects of NMDA receptor antagonists on the discriminative stimulus properties of spontaneous withdrawal, selected doses of NMDA receptor antagonists or their vehicles were administered in combination with s.c. saline injection 24 hr after the last morphine treatment (fig. 8). Dizocilpine (0.1 mg/kg), D-CPPene (5.6 mg/kg) and ACEA-1021 (50 mg/kg) but not memantine (10 mg/kg) or eliprodil (30 mg/kg) significantly reduced the percentage of rats selecting naloxone-appropriate escape area when suspended from morphine treatment ($P < .05$, Fisher’s exact test).

**Discussion**

To our knowledge, there were no reports on naloxone discrimination in morphine-dependent rats based on escape behavior in the Y- (or T-)maze. Thus, the initial experiments

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**TABLE 2**

Effects of NMDA receptor antagonists on the weight loss produced by naloxone injection in morphine-dependent rats

<table>
<thead>
<tr>
<th>Mean of Weight loss % body weight change (±S.E.M.) after the administration of naloxone (0.1 mg/kg s.c.) with i.p. NMDA receptor antagonists.</th>
<th>Dose (mg/kg)</th>
<th>Vehicle</th>
<th>0.03</th>
<th>0.056</th>
<th>0.1</th>
<th>0.3</th>
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</thead>
<tbody>
<tr>
<td>Dizocilpine</td>
<td>Weight loss</td>
<td>$-3.0 \pm 0.1$</td>
<td>$-2.8 \pm 0.9$</td>
<td>$-5.9 \pm 0.9$</td>
<td>$-7.3 \pm 1.0$</td>
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<td></td>
<td>$n$</td>
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<td>7</td>
<td>7</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>D-CPPene</td>
<td>Weight loss</td>
<td>$-2.7 \pm 0.2$</td>
<td>$-1.5 \pm 0.5$</td>
<td>$-1.3 \pm 0.6$</td>
<td>$-2.4 \pm 0.8$</td>
<td>$-4.8 \pm 1.0$</td>
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<td>1</td>
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<tr>
<td>Memantine</td>
<td>Weight loss</td>
<td>$-2.7 \pm 0.5$</td>
<td>$-2.5 \pm 0.4$</td>
<td>$-0.8 \pm 0.5$</td>
<td>$-1.4 \pm 0.3$</td>
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<tr>
<td>Eliprodil</td>
<td>Weight loss</td>
<td>$-3.0 \pm 0.2$</td>
<td>$-3.2 \pm 0.9$</td>
<td>$1.3 \pm 0.5$</td>
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<tr>
<td></td>
<td>$n$</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>ACEA-1021</td>
<td>Weight loss</td>
<td>$-2.8 \pm 0.2$</td>
<td>$-2.5 \pm 0.6$</td>
<td>$-0.8 \pm 0.4$</td>
<td>$-0.8 \pm 0.4$</td>
<td>$-0.8 \pm 0.4$</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

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**TABLE 3**

Effects of NMDA receptor antagonists on the expression of tremor, ptosis, diarrhea, salivation and shaking precipitated by naloxone injection in morphine-dependent rats

The occurrence of forelimb tremor, ptosis, diarrhea, salivation and shaking (mean ± S.E.M.) was monitored for 15 min starting immediately after the administration of 0.1 mg/kg naloxone (NLX; 2 hr after the last morphine injection). Rats received combinations of intraperitoneal (i.p.) and subcutaneous (s.c.) injections. DIZ, dizocilpine (0.1 mg/kg), MEM, memantine (10 mg/kg), CPP, D-CPPene (5.6 mg/kg), ACEA, ACEA-1021 (50 mg/kg), ELI, eliprodil (30 mg/kg).

<table>
<thead>
<tr>
<th>Mean of Tremor, Ptosis, Diarrhea, Salivation and Shaking %</th>
<th>i.p.</th>
<th>Vehicle</th>
<th>n</th>
<th>Tremor</th>
<th>Ptosis</th>
<th>Diarrhea</th>
<th>Salivation</th>
<th>Shaking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle for DIZ</td>
<td>Saline</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Vehicle for DIZ</td>
<td>Nlx 0.1 mg/kg</td>
<td>7</td>
<td>71.4 $^a$</td>
<td>85.7 $^a$</td>
<td>85.7 $^a$</td>
<td>28.6</td>
<td>10.7 ± 2.2 $^a$</td>
<td></td>
</tr>
<tr>
<td>DIZ 0.1 mg/kg</td>
<td>Nlx 0.1 mg/kg</td>
<td>7</td>
<td>71.4</td>
<td>0 $^b$</td>
<td>0 $^b$</td>
<td>71.4</td>
<td>2.4 ± 1.3 $^b$</td>
<td></td>
</tr>
<tr>
<td>Vehicle for CPP</td>
<td>Saline</td>
<td>8</td>
<td>0</td>
<td>12.5</td>
<td>0</td>
<td>0</td>
<td>1.4 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Vehicle for CPP</td>
<td>Nlx 0.1 mg/kg</td>
<td>8</td>
<td>62.5 $^a$</td>
<td>100 $^a$</td>
<td>75 $^b$</td>
<td>25</td>
<td>13.0 ± 3.1 $^a$</td>
<td></td>
</tr>
<tr>
<td>CPP 10 mg/kg</td>
<td>Nlx 0.1 mg/kg</td>
<td>8</td>
<td>25</td>
<td>12.5 $^b$</td>
<td>12.5 $^b$</td>
<td>50</td>
<td>3.6 ± 1.3 $^b$</td>
<td></td>
</tr>
<tr>
<td>Vehicle for MEM</td>
<td>Saline</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.0 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Vehicle for MEM</td>
<td>Nlx 0.1 mg/kg</td>
<td>7</td>
<td>71.4 $^a$</td>
<td>85.7 $^a$</td>
<td>85.7 $^a$</td>
<td>14.3</td>
<td>13.9 ± 4.1 $^a$</td>
<td></td>
</tr>
<tr>
<td>MEM 10 mg/kg</td>
<td>Nlx 0.1 mg/kg</td>
<td>7</td>
<td>57.1</td>
<td>42.9</td>
<td>14.3 $^b$</td>
<td>14.3</td>
<td>6.4 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Vehicle for ELI</td>
<td>Saline</td>
<td>8</td>
<td>0</td>
<td>12.5</td>
<td>0</td>
<td>0</td>
<td>0.4 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Vehicle for ELI</td>
<td>Nlx 0.1 mg/kg</td>
<td>8</td>
<td>87.5 $^a$</td>
<td>87.5 $^a$</td>
<td>75 $^b$</td>
<td>25</td>
<td>15.9 ± 5.4 $^a$</td>
<td></td>
</tr>
<tr>
<td>ELI 30 mg/kg</td>
<td>Nlx 0.1 mg/kg</td>
<td>8</td>
<td>25 $^b$</td>
<td>62.5</td>
<td>0 $^b$</td>
<td>0</td>
<td>4.9 ± 2.4 $^b$</td>
<td></td>
</tr>
<tr>
<td>Vehicle for ACEA</td>
<td>Saline</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Vehicle for ACEA</td>
<td>Nlx 0.1 mg/kg</td>
<td>7</td>
<td>85.7 $^a$</td>
<td>85.7 $^a$</td>
<td>85.7 $^a$</td>
<td>28.6</td>
<td>12.1 ± 3.1 $^a$</td>
<td></td>
</tr>
<tr>
<td>ACEA 50 mg/kg</td>
<td>Nlx 0.1 mg/kg</td>
<td>7</td>
<td>28.6</td>
<td>28.6</td>
<td>14.3 $^b$</td>
<td>14.3</td>
<td>3.7 ± 2.2 $^b$</td>
<td></td>
</tr>
</tbody>
</table>

$a, b P < .05$ (Wilcoxon’s test for shaking; Fisher’s exact test for tremor, ptosis, diarrhea and salivation) compared with Vehicle ± Saline and Vehicle ± NLX tests, respectively.
The vehicle-treated controls. Dizocilpine and D-CPPene can be interpreted as a blockade of the withdrawal state (dizocilpine: Higgins et al., 1992; memantine: (10 mg/kg); CPP, D-CPPene (5.6 mg/kg); ACEA, ACEA-1021 (50 mg/kg); ELI, eliprodil (30 mg/kg). Each point is based on observations made in 7 or 8 rats. * P < .05 (Fisher’s exact test), compared with the vehicle-treated controls.

Fig. 8. Effects of NMDA receptor antagonists on percentage of the naloxone-appropriate escape area selection after the temporary discontinuation of repeated morphine treatment in morphine-dependent rats trained to discriminate 0.1 mg/kg naloxone from saline. NMDA receptor antagonists (filled bars) or their vehicles (hatched bars) were administered 24 hr after the last morphine injection. DIZ, dizocilpine (0.1 mg/kg); MEM, memantine (10 mg/kg); CPP, D-CPPene (5.6 mg/kg); ACEA, ACEA-1021 (50 mg/kg); ELI, eliprodil (30 mg/kg). Each point is based on observations made in 7 or 8 rats. * P < .05 (Fisher’s exact test), compared with the vehicle-treated controls.

Several findings emerge from the present experiments. First, discriminative stimulus effects of naloxone in morphine-dependent rats were antagonized by NMDA receptor channel blocker dizocilpine and competitive antagonist D-CPPene. The naloxone-appropriate escape area selection in rats withdrawn from morphine for 24 hr was significantly affected by dizocilpine, D-CPPene and ACEA-1021.

These results are in agreement with earlier reports on the ability of NMDA receptor antagonists to block various signs of morphine withdrawal (Herman et al., 1995). Taken together with the reports on inhibitory effects of NMDA receptor antagonists on aversive place conditioning of opioid withdrawal state (dizocilpine: Higgins et al., 1992; memantine: Popik and Danyasz, 1997), the aforementioned effects of dizocilpine and D-CPPene can be interpreted as a blockade of the subjective effects of morphine withdrawal.

NMDA receptor antagonists are well known for their memory-impairing properties (Danyasz et al., 1995). Thus, one may suggest that the effects of dizocilpine and D-CPPene described in the present report can rather be due to memory deficits. However, none of the tested NMDA receptor antagonists affected saline-appropriate escape area selection suggesting that disturbances in spatial navigation can hardly account for the selective reduction in naloxone-appropriate escape area selection after combined administration of naloxone and dizocilpine (or D-CPPene). Moreover, we did not observe a significant reduction in stimulus control of escape behavior by naloxone following treatment with memantine, although amnestic effects are seen with high doses of this drug (above 10 mg/kg) (Miształ and Danysz, 1995). It can also be argued that NMDA receptor antagonists are less likely to disrupt the storage or recall of associations that are well established (Caramanos and Shapiro, 1994; Danysz et al., 1995).

The possibility that dizocilpine and D-CPPene exerted their effects by interfering with the discrimination task by a state-dependent mechanism should also be considered (Koek et al., 1993). The intermediate levels of drug-appropriate responding has been seen in various drug discrimination studies where NMDA antagonists have been tested for substitution, regardless of the training drug; this list includes morphine (Koek and Woods, 1989), cocaine (A. Y. Bespalov and P. M. Beardsley, unpublished), NMDA (Willetts and Balster, 1989a), pentylentetrazol (Emmett-Oglesby and Herz, 1987), pentobarbital (Willetts and Balster, 1989b) and Δ-9-tetrahydrocannabinol (Browne and Weissman, 1981). Therefore, it appears that this effect is rather characteristic for NMDA receptor antagonists and may have contributed to the effects observed in this study even though we did not observe any appreciable levels of naloxone-appropriate responding after the administration of either dizocilpine or D-CPPene per se. It should also be noted that at least for dizocilpine there was no evidence for intermediate levels of naloxone-appropriate responding when rats were treated with combinations of naloxone and various doses of dizocilpine. Dizocilpine dose-dependently suppressed the selection of naloxone-appropriate escape area, with only one rat of eight exhibiting naloxone-like response after the injection of 0.1 mg/kg dizocilpine followed by the training dose of naloxone.

Dizocilpine and D-CPPene appeared to be the only antagonists tested that exerted consistent effects on naloxone-induced stimulus control of escape area selection. Memantine administration resulted in intermediate levels of responding, whereas ACEA-1021 and eliprodil seemed to have little or no effect. Numerous studies have shown differences in the behavioral effects of NMDA receptor antagonists acting at different sites of the NMDA receptor complex (Balster and Willetts, 1996). For instance, for both ACEA-1021 (Balster et al., 1995) and eliprodil (Balster et al., 1994), there is experimental evidence suggesting that these drugs do not produce even partial substitution for PCP-like drugs. Meanwhile, full or partial substitution is usually seen with dizocilpine (Willetts and Balster, 1988a) and competitive NMDA receptor antagonist (Willetts and Balster, 1988b) in rats trained to discriminate PCP from saline. Interestingly, memantine was found to substitute for dizocilpine cue in rats trained to discriminate dizocilpine from saline (Zajaczkowski et al., 1996). Our data with memantine seem to be rather in contrast with a recent report by Popik and Danyasz (1997) that demonstrated the blockade of morphine withdrawal-conditioned place aversion by memantine. Procedural differences and morphine treatment regimen in particular may be in part responsible for such discrepancy.

It should be noted that all the earlier studies on the effects of NMDA receptor antagonists in morphine-dependent rats were based on much shorter exposure to morphine (within 2 weeks) compared with the present study (over 5 months of daily morphine administration before the tests with
NMDA receptor antagonists). The significance of this difference is supported by the naloxone-induced body weight loss data.

Neither channel blockers nor competitive antagonists were reported to induce body weight loss in drug-naïve rats. Moreover, dizocilpine was found to either prevent morphine withdrawal-associated weight loss (rats: Rasmussen et al., 1991) or to have no effect (mice: Thorat et al., 1994). The competitive NMDA receptor antagonist (LY 274614) was also found to prevent naloxone-induced weight loss in morphine-dependent rats (Rasmussen et al., 1991), whereas no data are available for memantine. Meanwhile, in our experiments both dizocilpine and N-CPePene failed to reduce naloxone-induced weight loss and dizocilpine even facilitated the effects on weight loss. Moreover, treatment with high doses of dizocilpine (0.1 mg/kg), N-CPePene (10 mg/kg) or memantine (30 mg/kg) without accompanying injections of naloxone led to significant body weight loss in morphine-dependent rats. There are no obvious explanations for these data. For example, alimentary tract effects can be suggested because gastric motility is reported to increase after systemic administration of NMDA receptor antagonists, including dizocilpine (Shinozaki et al., 1990). However, our results indicated that all NMDA receptor antagonists tested had suppressive effects on withdrawal-associated diarrhea.

Increased motor activity levels and possibly a corresponding increase in general metabolic activity may be proposed as an alternative explanation for the weight loss data. Noncompetitive NMDA receptor antagonists, as well as to the lesser degree certain competitive antagonists, are capable of significantly elevating locomotor activity (Svensson et al., 1991), and such stimulatory influences were observed during the present study after the administration of the highest doses of dizocilpine and N-CPePene. Interestingly, low doses of memantine (3 mg/kg) significantly attenuated naloxone-induced weight loss, whereas higher doses were rather ineffective. These results with memantine are in agreement with existing information on memantine resembling high-affinity weight loss data. It is noteworthy that dizocilpine, N-CPePene and, to a lesser degree, memantine attenuated discriminative stimulus effects of naloxone and caused weight loss per se. Meanwhile, eliprodil and ACEA-1021 both failed to modify naloxone's discriminative stimulus effects and prevented naloxone-induced weight loss. It is known that NMDA receptor channel blockers as well as competitive antagonists produce behavioral effects similar to those of PCP, albeit these effects are evident to the different extent (see discussion above). Specific interoceptive stimulation after the administration of these drugs may overshadow the discriminative stimulus effects of naloxone and thus result in apparent reduction in stimulus control over behavior. On the other hand, PCP-like properties are associated with psychomotor activation and, as suggested above, possibly account for weight loss.

In summary, the present results suggest that the subjective effects of morphine withdrawal can be affected by the treatment with NMDA receptor antagonists. Combined with the evidence for the involvement of EAergic neurotransmission in the mechanisms underlying morphine’s rewarding potential (Bespalov et al., 1994; Popik and Danysz, 1997; Tschantke and Schmidt, 1995) and establishment of morphine dependence (Herman et al., 1995), the present data indicate that NMDA receptor antagonists may become an effective approach in the treatment of opioid dependence.

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References


Rasmussen K, Fuller RW, Stockton ME, Perry RW, Swinford RM and Ornstein PL (1993) NMDA receptor antagonists suppress behaviors but not norepinephrine...


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