N-Methyl-D-Aspartate Receptor Antagonists and the Development of Tolerance to the Discriminative Stimulus Effects of Morphine in Rats

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Accepted for publication March 9, 1999

This paper is available online at http://www.jpet.org

ABSTRACT

Several reports have indicated that N-methyl-D-aspartate (NMDA) receptor antagonists prevent the development of analgesic tolerance to opiates. Some effects of opiates, such as their discriminative stimulus effects, are known to be more resistant to tolerance induction. In this study, adult male Long-Evans rats were trained to discriminate 3.2 mg/kg of s.c. morphine from water (vehicle) using a standard, two-lever fixed ratio 10 schedule of food reinforcement. Subsequently, repeated morphine treatment (20 mg/kg; 14 days b.i.d.) was administered, which induced tolerance-like rightward shifts in the dose-effect curves for both morphine's discriminative stimulus and response rate-suppressing effects. Withdrawal-induced, response rate reductions indicative of behavioral dependence appeared as well. Separate groups were then treated repeatedly with a combination of morphine or its vehicle and one of the following competitive or noncompetitive NMDA antagonists: dizocilpine (0.1 mg/kg i.p.), 3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid (D-CPPene; 3 and 5.6 mg/kg i.p.), eliprodil (17.3 mg/kg i.p.), or R(+)3-amino-1-hydroxy-2-pyrrolidone ([+]-HA-966; 10 mg/kg i.p.). The development of tolerance to morphine's stimulus effects was attenuated by eliprodil and the higher dose of D-CPPene, but not by dizocilpine, the lower dose of D-CPPene, nor R(+)3-amino-1-hydroxy-2-pyrrolidone. All antagonists prevented the induction of tolerance to morphine's response rate effects. Dizocilpine and D-CPPene (5.6 mg/kg) appeared to prevent the induction of behavioral dependence as well. NMDA antagonists can prevent tolerance to the discriminative stimulus effects of morphine, and perhaps to its behavioral dependence effects, but their site of action on the NMDA receptor complex confers a different ability to do so.

Substantial evidence accumulated over the last 6 years suggests that N-methyl-D-aspartate (NMDA) receptors are implicated in the development of drug tolerance, sensitization, and dependence. Noncompetitive and competitive NMDA receptor antagonists have been shown to block the development of tolerance to morphine analgesia (Marek et al., 1991a; Trujillo and Akil, 1991a; Herman et al., 1995), diazepam's sedative effects (File and Fernandes, 1994), nicotine-induced locomotor depression (Shoaib et al., 1994), hypothermic and incoordinating effects of ethanol (Szabó et al., 1994), analgesic properties of Δ2-tetrahydrocannabinol (Thorat and Bhargava, 1994), and sensitization to locomotor stimulation produced by cocaine, morphine, amphetamines (Wolf and Jeziorski, 1993), and nicotine (Shoaib and Stolerman, 1992) as well as the development and/or expression of opiate (Trujillo and Akil, 1991a; Herman et al., 1995), ethanol (Liljequist, 1991), barbiturate (Rabbani et al., 1994), and benzodiazepine (Steppuhn and Turski, 1993) dependence.

At least for the opiates, this information has led to the consideration of NMDA antagonists as potential medications with possible clinical appeal for treatment of drug tolerance and dependence (Herman et al., 1995). However, there are toxicological problems associated with the administration of some of the NMDA antagonists, and some of them induce phencyclidine (PCP)-like effects (Herman et al., 1995; Balster and Willetts, 1996). In addition, there are some other concerns regarding the development of tolerance prevention medications. It is well known that tolerance easily develops to some effects of the opiates (e.g., depressant, analgesic, and respiratory effects) whereas other effects (mostly excitatory effects such as euphoria and miosis) seem to be more resistant (Reisine and Pasternak, 1996). Therefore, while developing medications for opiate analgesic tolerance, it would be important to know whether these treatments would affect alterations in the sensitivity to other effects of opiates.

ABBRviATIONS: NMDA, N-methyl-D-aspartate; PCP, phencyclidine; FR, fixed ratio; FFR, first completed fixed ratio; MLR, morphine-lever responding; D-CPPene, 3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid; (+)-HA-966, R(+)3-amino-1-hydroxy-2-pyrrolidone.
Although the attenuation of analgesic tolerance to opiates was repeatedly demonstrated in rats and mice after both systemic (Marek et al., 1991; Trujillo and Akil, 1991a; Elliott et al., 1994; Herman et al., 1995) and intrathecal (Gutstein and Trujillo, 1993) administration of NMDA antagonists, it is not clear whether NMDA antagonists would affect development of tolerance to the discriminative stimulus effects of opiates. The discriminative stimulus effects of opiates in laboratory animals are relevant to understanding opiate subjective effects in humans (Preston and Bigelow, 1991). Therefore, information about the ability of NMDA antagonists to alter changes in sensitivity to morphine discrimination will also be useful in assessing their possible role in pharmacotherapy of the addictions.

The present study sought to evaluate the ability of NMDA receptor antagonists to affect the development of tolerance to the discriminative stimulus effects of morphine in rats. Previous studies have shown that antagonists acting at various sites on the NMDA receptor complex affect the development and/or expression of opiate tolerance and dependence (Herman et al., 1995). Meanwhile, essential differences have been observed in the behavioral effects of various site-selective NMDA antagonists. For example, drug discrimination studies clearly demonstrate that NMDA antagonists acting competitively at NMDA, glycine, and polyamine sites fail to produce discriminative stimulus effects identical with PCP-like drugs (Mansbach and Balster, 1991; Balster et al., 1994, 1995). For the present study, we have selected the following four NMDA receptor antagonists that are known as site-selective and systemically active: 1) a competitive antagonist, 3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid (N-CPPen; Lowe et al., 1990), 2) a PCP-like noncompetitive antagonist, dizocilpine (MK-801, Wong et al., 1986), 3) a glycine site agonist/antagonist, R(+)3-amino-1-hydroxy-2-pyrrolidone ([+]-HA-966; Singh et al., 1990), and 4) a polyamine site NMDA antagonist, eliprodil, which may preferentially act at subtypes of NMDA receptors assembled from NR1 and NR2B subunits (Avenet et al., 1997).

Studies of the development of tolerance to the discriminative stimulus effects of morphine have shown the dosage regimen to be important, but since early in the 1970s tolerance has consistently been reported inducible in rats and pigeons (Hirschhorn and Rosecrans, 1974; Young et al., 1991, 1992). For this study, we initially followed a regimen of repeated morphine administration (10 mg/kg b.i.d. for 2 weeks) reported earlier by Young and colleagues (1991) to produce a 4.5-fold shift in morphine dose-response functions. However, in our studies this regimen appeared to be insufficient to induce tolerance to stimulus control by morphine. Therefore, for the rest of the study the amount of morphine per injection was doubled, resulting in a significant reversible tolerance to morphine’s discriminative stimulus effects.

Materials and Methods

Subjects. Twenty-six adult male Long-Evans rats (Harlan Sprague-Dawley, Inc., Dublin, VA) were used. Animals were housed individually in suspended wire cages with water available ad libitum. Food (Purina Rodent Chow) consumption was restricted to 10 to 12 g/day given after behavioral testing to maintain subjects’ body weights in the range of 360 to 424 g. All experiments were conducted during the light period of a 12-h light/dark cycle (lights on from 8:00 AM–8:00 PM). Ten subjects were drug-naive at the start of the experiments while sixteen other subjects had previously been tested with various NMDA and/or opiate antagonists (Bespalov et al., 1998). For these latter subjects, the last nonmorphine drug administration occurred at least 4 weeks before the start of this study.

Apparatus. Twelve standard two-lever operant conditioning chambers (BRS/LVE, Beltsville, MD) were connected to a microcomputer through an interface and controlled by MED-PC software (MED Associates, Inc., East Fairfield, VT). Each chamber was equipped with a white houselight centered above the levers, and a food dispenser that delivered 45-mg food pellets (Noyes Formula A, P.J. Noyes Company, Inc., Lancaster, NH). The chambers were housed within sound- and light-attenuating boxes.

Training Procedure. The naive rats were initially trained to press one of the levers for food pellet delivery according to a fixed ratio (FR) 1 schedule of reinforcement with this lever eventually becoming the vehicle-designated lever. All initial training and subsequent acquisition sessions were held daily, Monday to Friday. After rats had acquired the lever-press response (1–4 days), the FR value was gradually increased to 10. Then the active lever was switched to the opposite side and the FR value was reduced to FR1. As soon as rats showed evidence of responding on this lever, the FR value was rapidly increased to FR10.

Acquisition Procedure. All acquisition and subsequent test sessions were divided into two or three consecutive discrete trials, each consisting of a 30-min injection period followed by a 5-min period where lever pressing resulted in food delivery. At the start of each trial, rats were given a sham injection (i.e., standard injection procedure with no needle or fluid delivery) or injected s.c. with either 3.2 mg/kg of morphine or sterile water, returned to their home cages, and 30 min later placed into the operant chambers for 5 min. Training sessions varied in the sequence of the trials: type A, sham-vehicle–morphine; type B, sham-morphine; type C, vehicle–morphine. The rats experienced all three types of sessions in an alternating sequence predetermined for each 2-month block of training and testing. Half of the rats used in this study were trained to press the right lever for food reinforcement after receiving morphine and the left lever after vehicle or sham injection; the reverse pairing was used with the remaining half. Lever presses on the correct lever only were reinforced and incorrect responses reset the FR requirement on the correct lever.

Acquisition training proceeded until the following criteria were met: 1) during at least 8 of 10 consecutive training days the first completed FR (FFR) was on the correct lever for all trials; 2) the percentage of response emitted on the correct lever was more than 90% of the total lever presses during the above training days when the FFR was correct. After the criteria were met, the rats were given test days. Test days were held on Tuesdays and Fridays provided the FFR was correct. As soon as rats showed evidence of responding on this lever, the FR value was rapidly increased to FR10.

Each test period consisted of an injection, the return of the animals period of 2 days, beginning with an injection of vehicle. During these test days, lever-press rates were recorded and then averaged during the most recent training sessions of each type (A, B, and C) the following criteria were met: 1) the FR was correct for all trials; 2) the percentage of response emitted on the correct lever was more than 90% of the total lever presses; 3) overall response rate was greater than 0.4 lever presses/s. During test days, ten consecutive responses on either lever produced a pellet delivery.

Each rat was initially tested with either vehicle or the training dose of morphine (3.2 mg/kg) until four test sessions were completed that satisfied criteria 2 and 3 described immediately above. These tests were held to corroborate the successful acquisition of stimulus control of behavior, and the results were not used in the further analyses. Sixteen other subjects that had previously been tested with various NMDA antagonists were not tested with drugs for at least 4 weeks before the experiment and were also re-tested under both training conditions.

Morphine-Cumulative Dose-Effect Testing. Morphine dose-response testing was accomplished using a cumulative dosing procedure. Six cumulative doses of morphine were administered over a period of 2 days, beginning with an injection of vehicle. During these 2 days (Tuesday and Wednesday) there were six 35-min test periods. Each test period consisted of an injection, the return of the animals
to the home cage for 30 min, followed by placement in the operant chambers for a 5-min test. Subjects were then removed, given another injection, and 30 min later reintroduced to the test chamber, and so on. Cumulative doses of morphine for the six 5-min test trials were as follows: Day 1: 0 (vehicle), 1.0, and 3.2 mg/kg (actual injection doses: 0, 1.0, and 2.2 mg/kg); Day 2: 3.2, 5.6, and 9.0 (actual injection doses: 3.2, 2.4, and 3.4 mg/kg).

**Tolerance Procedure.** For each subject, morphine dose-effect determinations were obtained as described above 1 week before initiating chronic treatments (baseline), 24 h after the last chronic treatment (tolerance assessment), and 2 weeks after chronic treatment (recovery). Subjects were then given another chronic treatment regimen, with periodic morphine dose-effect determinations as described up to a maximum of four. Each subject was exposed to a maximum of four repeated treatment periods as well. During each of the chronic treatment periods, drug discrimination training was suspended. Periods of retraining of at least 2 weeks separated repeated treatment periods.

Initially, all subjects were exposed to chronic vehicle administration (water; 14 days b.i.d.) to assess the influence of the suspension of drug discrimination training on morphine dose-effect functions. Then, rats were subjected to the repeated treatments (14 days b.i.d.) with the following drugs and their combinations (see Table 1 for the treatment order): 1) 10 mg/kg of morphine (s.c.); 2) 20 mg/kg of morphine (s.c.); 3) dizocilpine (0.1 mg/kg i.p.) + s.c. water or morphine (20 mg/kg); 4) D-CPPene (3 or 5.6 mg/kg i.p.) + s.c. water or morphine (20 mg/kg); 5) eliprodil (17.3 mg/kg i.p.) + s.c. water or morphine (20 mg/kg); and 6) (±)-HA-966 (10 mg/kg i.p.) + s.c. morphine (20 mg/kg).

Drugs were administered in the morning (between 8:00 and 8:30 AM) and in the late afternoon (between 5:00 and 5:30 PM) with no interval between i.p. and s.c. injections.

**Data Analysis.** Mean percentage of total presses of the drug lever and response rate (responses/s) were calculated separately for each dose of each drug. Data from rats emitting less than 0.03 responses/s were omitted from calculations of group percentage morphine-lever responses but were included in group response rate determinations. Response rate data from the tests conducted after vehicle injections were used to convert the drug test response rate to percentage of control levels. ED_{50} and ED_{30} doses with 95% CL were calculated using least squares regression of log dose based on individual rat data on percent morphine-lever responding (MLR) and response rate expressed as a percentage of control. To ensure minimal within- and between-subject variability, response rate calculations were based on vehicle tests trials conducted within each morphine cumulative dose test. ED_{50} values were compared using two-tailed Student's t test.

For each of the above described six treatment groups, a separate distribution-free two-way ANOVA was conducted on both MLR and response rate data with repeated measures on both factors. The two factors were morphine dose and previous postchronic treatment. For these analyses, the 3.2 mg/kg dose of morphine, which was tested twice in each dose-effect determination, was treated as two separate levels of the dose factor. Additional two-way ANOVAs were conducted to access differences between chronic regimens (e.g., repeated morphine alone versus repeated morphine in combination with an NMDA antagonist). Thus, statements in Results about whether an antagonist modified the effects of repeated morphine administration were made on the basis of comparisons between different groups of subjects. ANOVAs were performed using SAS-STAT software (version 6.10, SAS Institute Inc., Cary, NC). Individual comparisons were performed using a post hoc Duncan test (only when the ANOVA revealed significant effects). The null hypothesis was rejected at the p < .05 level.

**Drugs.** The drugs used were as follows: morphine sulfate (National Institute on Drug Abuse, Rockville, MD), D-CPPene (Novartis Pharma, Basel, Switzerland), dizocilpine maleate and (±)-HA-966 (Research Biochemicals International, Natick, MA), and eliprodil (Synthelabo Recherche, Bagneaux, France). Morphine and (±)-HA-966 were prepared in sterile water, dizocilpine in physiological saline, eliprodil in 0.1% Tween-80 in saline, and D-CPPene in equimolar NaOH in saline. Morphine and its vehicle were injected s.c. whereas the rest of the drugs and their vehicles were administered i.p.. All injections were delivered in a solution volume of 1 ml/kg. Doses are based on the forms of the drugs listed above.

**TABLE 1**

**Order of treatment periods**

Rats were subjected to the repeated treatments (14 days b.i.d.) with the following drugs and their combinations: a) water (WAT), b) 10 mg/kg of morphine (M10), c) 20 mg/kg of morphine (M20), d) dizocilpine + water (DIZ) or morphine (MD), e) D-CPPene (3 mg/kg) + water (C5) or morphine (MC5), f) D-CPPene (5.6 mg/kg) + water (C5) or morphine (MC5), g) eliprodil + water (ELI) or morphine (ME), and h) (±)-HA-966 + morphine (MHA). ID, individual rat number.

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Results

Morphine Dose-Response Determination. Morphine dose-dependently generalized from the training dose (Fig. 1, top) with an ED$_{50}$ of 1.3 mg/kg (CL: 1.1–1.6) and suppressed response rates with an ED$_{50}$ of 5.6 mg/kg (CL: 4.6–11.8). In addition, the effects of 3.2 mg/kg morphine given cumulatively (last injection of Day 1) and given as the first acute dose on Day 2 did not differ significantly.

Tolerance Studies. Suspending training by itself did not affect sensitivity to either the discriminative stimulus or response rate-decreasing effects of morphine because repeated treatment with vehicle did not alter the doses required for generalization and the production of rate suppression (Fig. 1, top). Chronic administration of 10 mg/kg b.i.d. of morphine during the tolerance induction period did not significantly affect stimulus control by morphine ($F_{(2,80)} = .42$, n.s.; Fig. 1). The ED$_{50}$ doses for pre- and postrepeated morphine administration were 1.3 and 1.6 mg/kg (CL: 1.0–2.1), respectively. However, some tolerance was obtained to the response rate effects of morphine (repeated morphine by morphine test dose interaction: $F_{(5,59)} = 4.7, p < .05$). The ED$_{50}$ for response rate suppression after this chronic regimen was 9.2 mg/kg (CL: 7.3–22.5) whereas for the chronically vehicle-treated subjects it was 4.1 mg/kg (CL: 3.0–5.3). Because our primary interest was in assessing drug effects of tolerance to the discriminative stimulus effects on morphine, all subsequent studies used the higher (20 mg/kg) dose of morphine during the chronic treatment regimen.

Sensitivity to the discriminative stimulus effects of morphine diminished when training was suspended and rats were treated with 20 mg/kg of morphine b.i.d. for 2 weeks ($F_{(2,116)} = 62.5, p < .01$; Fig. 1). These subjects showed a 4.1-fold increase in the ED$_{50}$ dose (5.4 mg/kg; CL: 4.3–23.9) for stimulus control compared with vehicle-treated controls ($p < .05$, Student’s $t$ test). The suspension of repeated morphine administration appeared to alter rates of responding because response rates were suppressed after injection of saline or low doses of morphine (1 mg/kg). Acute and cumulative doses of morphine equal to the training dose (3.2 mg/kg) restored response rates to control values. The dose of morphine (9 mg/kg) that produced more than 50% response rate suppression in vehicle-treated rats lacked effect on lever-pressing rates after 2 weeks of repeated morphine injection compared with vehicle-treated controls. Morphine’s response rate effects in tolerant rats did not show obvious dose dependence, suggesting development of tolerance (repeated morphine by morphine test dose interaction: $F_{(5,83)} = 14.9, p < .01$) and precluding calculation of a potency estimate for rate suppression. Sensitivity to both the discriminative stimulus and response rate effects of morphine recovered to initial levels when subjects were tested 2 weeks after the last repeated morphine administration (Fig. 1, bottom).

Dizocilpine. Dizocilpine-alone administration during the 2-week period of suspended discrimination training did not result in a statistically significant shift in the dose-effect function for either stimulus control by morphine ($F_{(2,56)} = .19$, n.s.) or for morphine-induced response rate suppression ($F_{(2,71)} = 3.17$, n.s.; Fig. 2, top). There was some evidence that the response rate-decreasing effects of 3.2 mg/kg morphine were enhanced after chronic dizocilpine treatment. This was evident during the second (noncumulative) determination of the effects of this dose (on Day 2 of postchronic dose-effect testing). Indeed, three of the four rats did not complete a FR during this test. These larger response rate effects with postchronic testing of 3.2 mg/kg morphine suggests that the small attenuation of morphine-lever selection may be attributable to a disruption of the discrimination performance.

Combined administration of dizocilpine and morphine for 2 weeks did not attenuate tolerance to morphine’s discriminative stimulus effects but did modify the change in sensitivity to morphine’s response-rate effects. Even with dizocilpine coadministration, the chronic morphine regimen resulted in a significant rightward shift of the morphine dose-effect function for MLR ($F_{(2,64)} = 31.4, p < .01$). On the other hand, when dizocilpine was given chronically, tolerance to the response rate-decreasing effects of morphine was not observed (dizocilpine by morphine test dose interaction: $F_{(5,131)} = 4.0, p < .01$).

Eliprodil. Repeated administration of eliprodil alone had little or no effect on subsequent testing of morphine’s discriminative stimulus and response rate-suppressing effects (Fig. 2). On the other hand, combined treatment with 20 mg/kg/injection morphine and eliprodil resulted in attenuation of morphine tolerance (Fig. 3). A significantly smaller
rightward shift occurred in the dose-effect function for morphine-lever selection in the eliprodil plus morphine group compared with that of the morphine-alone group ($F_{(1,61)} = 15.6, p < .01$). In fact, the postchronic effects of morphine did not differ significantly between the eliprodil plus morphine group and the vehicle-treated control group ($F_{(1,39)} = .61, \text{n.s.}$).

Response rates after the postchronic injection of vehicle were lower in both morphine-treated groups than in the vehicle-treated group (Fig. 3). These effects of discontinued morphine treatment were attenuated by low doses of morphine during the dose-effect determination. On the other hand, the response rate-decreasing effects of higher doses of morphine were reduced after chronic morphine-alone treatment. This tolerance was less in the morphine plus eliprodil group (repeated morphine by eliprodil treatment by test dose of morphine interaction: $F_{(5,131)} = 3.1, p < .05$).

**d-CPPene.** Repeated treatment with either dose (3 or 5.6 mg/kg/injection) of d-CPPene alone did not markedly affect dose-effect curves for stimulus control by morphine (Fig. 2). On the other hand, there is some evidence that these dosage regimens of d-CPPene had residual effects on rates of responding during the postchronic testing. After injection of saline and low doses of morphine, response rates were markedly lower in rats treated with d-CPPene (especially the lower dose) compared with vehicle-treated controls. Statistical analysis, however, indicated that neither treatment with 3 mg/kg ($F_{(1,47)} = 5.7, \text{n.s.}$) nor with 5.6 mg/kg d-CPPene ($F_{(1,47)} = 4.2, \text{n.s.}$) significantly altered overall rates compared with chronic vehicle treatment.

Combined treatment with 3 mg/kg d-CPPene and 20 mg/kg morphine did not attenuate tolerance to morphine’s discriminative stimulus effects because the postchronic morphine dose-effect curve was nearly identical with the curve in the morphine-alone group, and both were shifted substantially to

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**Fig. 2.** Effects of repeated treatments with NMDA antagonists on morphine discrimination. Mean percentage ($\pm$S.E.M.) of MLR and mean ($\pm$S.E.M.) response rates after vehicle (V) and morphine (1–9 mg/kg, cumulative dosing) administration in rats trained to discriminate 3.2 mg/kg of morphine from water. Testing was conducted after 14 days of b.i.d. administration of water (○) or one of the following drugs (●): dizocilpine (0.1 mg/kg), eliprodil (17.3 mg/kg), or d-CPPene (3 and 5.6 mg/kg). For the sake of clarity, standard errors are not shown for all data points. n = 4 for each treatment group and for vehicle controls.

**Fig. 3.** Effects of NMDA antagonists on morphine tolerance. Mean percentage ($\pm$S.E.M.) of MLR and mean ($\pm$S.E.M.) response rates after morphine (1–9 mg/kg, cumulative dosing) administration in rats trained to discriminate 3.2 mg/kg of morphine from water (W). For all subjects, testing was conducted after each of the two subsequent 14-day (b.i.d.) treatment regimens: 1, administration of water (○) and 2, combined administration of morphine (20 mg/kg) and one of the following drugs (●): dizocilpine (0.1 mg/kg; n = 4), eliprodil (17.3 mg/kg; n = 4), d-CPPene (3 mg/kg; n = 3; 5.6 mg/kg; n = 4), or (+)-HA-966 (10 mg/kg; n = 4). For the purposes of convenient between-treatment comparisons, replicate data from Fig. 2 (repeated administration of 20 mg/kg of morphine; n = 7). For the sake of clarity, standard errors are not shown for all data points.
the right of that in the vehicle-treated group. One subject in the 3 mg/kg D-CPPene plus morphine group died during chronic treatment, leaving only three subjects in that group.

Unlike the results with 3 mg/kg D-CPPene, treatment with 5.6 mg/kg plus morphine did attenuate tolerance to morphine’s discriminative stimulus effects (Fig. 3). There was a significant difference \( F(1,40) = 16.3, p < .01 \) between the dose-effect curves in the D-CPPene plus morphine group compared with the morphine-alone group.

Interpretation of the response rate data after combined chronic treatment with D-CPPene and morphine (Fig. 3) is complicated by the residual effects of treatment with D-CPPene alone (Fig. 2). In the 3 mg/kg D-CPPene plus morphine group, the response rate-decreasing effects of morphine discontinuation were seen in both the morphine-alone and the morphine plus D-CPPene groups after postchronic vehicle testing. On the other hand, the response rate-decreasing effects of 5.6 and 9 mg/kg morphine were attenuated in the morphine-alone group but not in the morphine plus D-CPPene groups (repeated morphine by D-CPPene dose by test interaction \( F(10,167) = 3.6, p < .01 \)). At the higher test doses of morphine (5.6 and 9 mg/kg) not only was the tolerance observed in the morphine-alone group not evident in the morphine plus 5.6 mg/kg D-CPPene group, the effects of the doses of morphine on response rates were actually enhanced.

\((+)-HA-966\). \((+)-HA-966\) did not modify tolerance to morphine’s discriminative stimulus effects. Repeated administration of the combination of 10 mg/kg injection \((+)-HA-966\) and 20 mg/kg/injection morphine resulted in the morphine dose-effect curve shifting significantly to the right compared with the vehicle-treated group \( F(1,44) = 14.52, p < .05; \) Fig. 3). In fact, the dose-effect functions for stimulus control by morphine did not differ between groups that underwent repeated exposures to morphine alone or in combination with \((+)-HA-966\) \( F(1,59) = .26, \) n.s.). Tolerance to the response rate-decreasing effects of morphine was nonsignificantly diminished when morphine was given chronically in combination with \((+)-HA-966\) (repeated morphine by \((+)-HA-966\) treatment by test dose of morphine interaction; \( F(5,131) = 1.5, \) n.s.).

Discussion

The results of the present study demonstrate that when training is suspended after 14-day treatment with 20 mg/kg of morphine, a 4-fold shift to the right in the dose-effect curve occurs for the discriminative stimulus effects of morphine. After the repeated morphine treatment, response rate-decreasing effects of higher doses of morphine are also reduced, suggestive of tolerance development to this effect as well. The interpretation of the response rate data is somewhat confounded by the observation that disruption of responding can occur after discontinuation of chronic morphine treatment. This was evident in the extremely low response rates that occurred after vehicle administration when administered 24 h after the last chronic morphine dose in the morphine-alone group, and sometimes in the groups receiving chronic morphine plus NMDA receptor antagonists. Even though stereotypical signs of opiate withdrawal (e.g., jumping, “wet dog”-like shakes, ptosis, piloerection, diarrhea, etc.) were not observed in the present study, it should be noted that changes in operant performance can be a more sensitive behavioral measure of dependence (Ford and Balster, 1976).

Assuming that the low response rates after discontinued morphine treatment was a manifestation of a withdrawal effect, coadministering dizocilpine or 5.6 mg/kg D-CPPene with morphine prevented the induction of dependence because response rates were preserved, relative to the morphine-alone group, when compared one day after the last injection of morphine. Interestingly, both dizocilpine and D-CPPene but not glycine (ACEA-1021) or polyamine (eliprodil) site antagonists were shown to impair stimulus control by naloxone in morphine-dependent rats (Medvedev et al., 1998).

Our results indicate that the noncompetitive NMDA receptor antagonist, dizocilpine, failed to block the induction of tolerance to the stimulus properties of morphine, which is in apparent contrast to other reports of dizocilpine preventing the development of tolerance to the analgesic effects of morphine. Although in the present study NMDA receptor antagonists were administered simultaneously with morphine, in most earlier studies NMDA receptor antagonist was administered before morphine with interinjection interval of 20 to 30 min (e.g., Marek et al., 1991a; Trujillo and Akil, 1991a). However, these differences are unlikely to explain the failure of dizocilpine to attenuate development of tolerance to discriminative stimulus effects of morphine. First, there was a clear evidence that dizocilpine attenuated response rate effects of repeated morphine exposures. Second, development of tolerance to morphine analgesia is blocked even when NMDA receptor antagonist is administered 2 h after each of the repeated morphine injections (Marek et al., 1991b; Belozertseva, Danysz and A.Y.B., unpublished).

The dose of dizocilpine selected for the present study was previously shown to be effective in preventing the induction of analgesic tolerance in various species of experimental subjects including rats. For example, Trujillo and Akil (1991a) showed a significant inhibition of tolerance development when morphine (10 mg/kg b.i.d. for 9 days) was administered to rats in combination with dizocilpine (0.1 mg/kg). To our knowledge, previous studies reporting the effects of dizocilpine on the development of tolerance to the analgesic effects of morphine (discrete dosing procedures) have used a morphine regimen reduced in either dosage or duration than the regimen used in the present study (20 mg/kg b.i.d. for 2 weeks). For instance, Marek and colleagues (1991a) treated rats with morphine at the dose of 15 mg/kg once a day for 4 days whereas Elliott and coauthors (1994) administered morphine to mice at the dose of 5 mg/kg once daily for 5 days. One possibility is that the dose of dizocilpine used in the present experiment was insufficient for producing reliable antitolerance effects. We have observed, however, that the development of tolerance to opiate analgesia is prevented by concurrent administration of dizocilpine when rats do receive morphine at a dose of 20 mg/kg twice a day for 8 days (Bespalov, 1994). It should be noted that increasing the dose of dizocilpine in the present study was contraindicated due to potentiation of the lethal and cataleptic effects of morphine by dizocilpine. In an earlier study, Trujillo and Akil (1991b) have shown that dizocilpine at a dose of 0.3 mg/kg reduced the \( ED_{50} \) for morphine-induced catalepsy from approximately 30 mg/kg to less than 10 mg/kg, and reduced the \( LD_{50} \) for morphine from approximately 100 mg/kg to approxi-
mately 10 mg/kg. Lower doses of dizocilpine did not affect morphine catalepsy or lethality. Similar to dizocilpine, (+)-HA-966, a partial agonist at the glycine site on the NMDA receptor complex, failed to attenuate tolerance to the discriminative stimulus effects of morphine. Glycine site antagonists such as ACEA-1328, and partial agonists such as ACPC, have been previously shown to prevent the development of tolerance to the analgesic effects of morphine (Kolesnikov et al., 1994; Lutfy et al., 1995). There are several considerations regarding why (+)-HA-966 failed to inhibit the development of tolerance to the discriminative stimulus properties of morphine. First, although intraventricular administration of (+)-HA-966 is capable of preventing the development of some chronic effects of psychoactive drugs (e.g., sensitization to the locomotor-stimulating properties of cocaine), (+)-HA-966 does have intrinsic activity at the glycine site of the NMDA receptor. For instance, systemic injection of (+)-HA-966 heightens the occurrence of some opiate withdrawal signs, whereas the expression of opiate withdrawal is known to be suppressed by NMDA receptor antagonists including the glycine site antagonists and partial agonists such as felbamate, d-cycloserine, and HA-966 (Kosten et al., 1995). Second, the dose of (+)-HA-966 selected for the present study may be thought insufficient to produce tolerance prevention. However, in pilot studies (data not shown) we observed that use of higher doses resulted in marked behavioral depression. The coadministration of morphine at 20 mg/kg and (+)-HA-966 at 17.3 and 30 mg/kg appeared to exert extremely toxic effects on the rats’ behavior as they remained motionless for up to 3 h after the drug injections and did not overtly respond to exteroceptive stimulation (e.g., noise). As was mentioned above, at least for some of the NMDA receptor antagonists (e.g., dizocilpine), dramatic potentiation of morphine’s depressant effects was observed, thus limiting the NMDA antagonist dose range that could be used during the present study.

Development of tolerance to the discriminative stimulus effects of morphine was inhibited when each repeated 20 mg/kg morphine injection was accompanied by either d-CP-Pene (5.6 mg/kg) or eliprodil (17.3 mg/kg). Moreover, response rate data indicated that both d-CP-Pene and eliprodil prevented alterations in dose-effect curves for morphine-induced rate suppression.

The present experiments seem to be the first to demonstrate the ability of a competitive NMDA receptor antagonist and a polyamine site antagonist to affect the development of drug tolerance in a procedure in which a noncompetitive NMDA receptor antagonist was ineffective. Previously, several competitive NMDA receptor antagonists were found to prevent the development of tolerance to morphine analgesia but, to our knowledge, d-CP-Pene was not among the drugs studied (Herman et al., 1995). However, other evidence suggests that d-CP-Pene affects the development of tolerance and/or sensitization to other psychoactive drugs such as nicotine (Shoaib et al., 1994), cocaine (Haracz et al., 1995), and methamphetamine (Ohmori et al., 1994). Additionally, we failed to find reports on the ability of polyamine site antagonists (i.e., eliprodil) to inhibit the development of drug tolerance/sensitization.

Overall, the present study provides evidence suggesting that the development of tolerance to the discriminative stimulus properties of morphine may not parallel alterations in other effects of morphine. Repeated administration of 10 mg/kg morphine did not result in any significant alterations in the dose-effect functions for morphine stimulus control. Concurrently, however, the ED50 for the response rate-suppressing effects of morphine was increased 2-fold, suggesting the development of tolerance to the rate effects of morphine. Analgesic potency of morphine is also known to decrease after a similar regimen of subchronic morphine administration (Fernandes et al., 1982). Further evidence comes from the observation that dizocilpine, d-CP-Pene (3 mg/kg), and (+)-HA-966 were able to block the development of tolerance to the response rate, but not to the discriminative stimulus effects of morphine. Similarly, Bhargava and Matwysyn (1993) reported that dizocilpine inhibited tolerance to the analgesic but not to the hyperthermic effects of morphine in rats. There are many indications that tolerance develops differentially to various effects of opiates. For example, Fernandes et al. (1982) showed that analgesic, motor, and temperature effects of opiates differed with regard to the threshold doses inducing tolerance to their effects, the degree of tolerance, etc. Moreover, tolerance disappeared at different rates for thermal, locomotor depressant, and hormonal effects in rats (Rauhala et al., 1995). Miczek (1991) found that brief social defeat induced tolerance to morphine analgesia but not to its discriminative stimulus effects in rats. In addition, 4 months of drug discrimination training did not affect dose-response functions for morphine or fentanyl as training drugs, but did result in marked tolerance to opiate analgesia (Colpaert et al., 1976).

The present results suggest that ligands acting at different sites on the NMDA receptor complex as antagonists or partial agonists have differential ability to prevent the induction of tolerance to the discriminative stimulus effects of morphine and to its response rate effects, and perhaps to morphine’s ability to induce behavioral dependence as well. These results, when considered with other studies that have reported NMDA-antagonist prevention of tolerance to the analgesic effects of morphine, also suggest that it is likely that different neurochemical mechanisms mediate tolerance to the analgesic and discriminative stimulus effects of morphine. This latter speculation, if true, will be an important consideration if NMDA antagonists are used as medication with morphine in the treatment of chronic pain.

Acknowledgments
We thank Novartis Pharma and Synthelabo Recherche for their generous contribution of drugs used in this study, (+)-HA-966 was provided by Research Biochemicals International as part of the Chemical Synthesis Program of the National Institute of Mental Health (Contract N01 MH30003).

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