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# Prolongation of morphine analgesia by competitive NMDA receptor antagonist D-CPPene (SDZ EAA 494) in rats

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**Abstract**

A possible future clinical application of NMDA receptor antagonists is the control of the development of opiate analgesic tolerance. Therefore, the ability of NMDA receptor antagonists to modify the acute analgesic effects of opiates becomes increasingly important. The present study sought to evaluate the analgesic potency of combined administration of morphine (5–20 mg/kg) and a competitive NMDA receptor antagonist D-CPPene (SDZ EAA 494; 3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid; 0.3–5.6 mg/kg) in the tail-flick and tail-pinch tests with rats. It was found that D-CPPene significantly increased the duration of morphine analgesia, but there was hardly any evidence for potentiation of morphine analgesia shortly after morphine administration. This effect could only in part be attributed to the D-CPPene-induced disruption of the development of 'learned hyperresponsiveness' (i.e., acquisition of decreased latencies to escape from repeated exposures to noxious stimulation). In addition, the plasma concentration of morphine was not affected by concurrent treatment with D-CPPene. 

*Keywords:* NMDA receptor antagonist; D-CPPene ((3-(2-carboxypiperazin-4-yl)-1-propenyl-1- phosphonic acid); SDZ EAA 494; Morphine; Analgesia; Plasma concentration; Time course; (Rat)

## 1. Introduction

There is a considerable body of evidence suggesting that NMDA receptor antagonists prevent the development of tolerance to morphine-induced analgesia in rats and mice (Herman et al., 1995). Various NMDA receptor antagonists have been used in these studies including non-competitive antagonists (Marek et al., 1991a; Trujillo and Akil, 1991a,b; Ben-Eliyahu et al., 1992; Lutfy et al., 1993; Elliott et al., 1994; Bespalov et al., 1994; Bilsky et al., 1996), competitive antagonists (Kolesnikov et al., 1993; Elliott et al., 1994; Bilsky et al., 1996), and a partial agonist of the glycine site (Kolesnikov et al., 1994).

For tolerance studies, it is essential that NMDA receptor antagonists at the selected doses do not significantly interact with the acute analgesic effect of morphine (Marek et al., 1991a,b; Vaccarino et al., 1992; Kolesnikov et al., 1993, 1994; Elliott et al., 1994). However, for clinical considerations, it is highly important whether opiate analgesia is affected by concurrent treatment with NMDA receptor antagonists if these drugs are administered at the doses equal to or higher than those necessary for tolerance prevention. The existing literature is quite equivocal, and the reported results indicate that the analgesic activity of the combination of morphine and NMDA receptor antagonist depends on both the antagonist used for the study and the species of animals that served as experimental subjects. For a non-competitive NMDA receptor antagonist, dizocilpine, potentiation of morphine analgesia seemed to be more likely in rats (Ben-Eliyahu et al., 1992; Grass et al., 1996) while a reduction in morphine activity was more likely to be observed in mice (Lipa and Kavaliers, 1990; Lutfy et al., 1993; Saucier and Kavaliers, 1994). For competitive antagonists, there are reports demonstrating both an increase (mice: Saucier and Kavaliers, 1994; Bhargava, 1997; rats: Tiseo and Inturrisi, 1993; Grass et al., 1996) and no change (mice: Elliott et al., 1994; Bilsky et al., 1996) in the acute analgesic potency of morphine. There is relatively little information about other types of NMDA receptor antagonists (Kolesnikov et al., 1994; Bernardi et al., 1996).

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However, it should be noted that potentiation of morphine's acute effects may be a negative rather than a positive feature of adjunctive administration of NMDA receptor antagonists, since they were shown to potentiate lethality, catalepsy (Trujillo and Akil, 1991b) and hypothermia (Bhargava, 1997) produced by acute morphine injection.

At least two of the earlier studies pointed out that morphine-induced analgesia lasts longer in rats pretreated with NMDA receptor antagonists (Ben-Eliyahu et al., 1992; Grass et al., 1996). These studies did not evaluate the dose–effect relation for NMDA receptor antagonists with respect to the prolongation of morphine analgesia and did not question whether the effects of acute potentiation (e.g., 30–60 min after morphine injection) and prolongation (e.g., as observed several hours after morphine treatment) can be distinguished.

The aim of the present study was to explore further the ability of the competitive NMDA receptor antagonists to enhance morphine-induced analgesia. First, full dose–effect relations for both morphine and NMDA receptor antagonist were determined. Since there is enough data to suggest that competitive NMDA receptor antagonists increase the acute analgesic effects of morphine in both rats and mice, D-CPPene (3-(2-carboxypiperazin-4- yl)-1-propenyl-1-phosphonic acid) was selected as a selective competitive NMDA receptor antagonist (Aebischer et al., 1989; Lowe et al., 1994) as it is one of the most potent, systemically active compounds available of this type. There is currently no evidence that it affects amine re-uptake or receptors other than the NMDA-subtype of glutamate receptors.

Second, repeated tests of nociceptive thresholds (e.g., procedures such as 'hot-plate', 'tail-pinch') are likely to result in what may be called 'learned hyperalgesia' because the subjects acquire faster response latencies in order to escape from noxious stimulation. Since NMDA receptors antagonists are known to retard learning (Danysz et al., 1995), the hypothesis was tested that prolongation of morphine analgesia by the NMDA receptor antagonist is at least in part due to the development of 'learned hyperalgesia'. Third, although there is no published evidence that competitive NMDA receptor antagonists, such as D-CPPene, affect the plasma clearance of any drug, the time course of changes in morphine plasma concentrations was assessed in rats that received an injection of morphine in combination with either NMDA receptor antagonist or its vehicle.

## 2. Materials and methods

### 2.1. Animals

Adult male drug- and experimentally naive Wistar rats (220–250 g; State Breeding Farm 'Rappolovo', St. Petersburg, Russia) were used. Animals were housed in groups ($n = 3$) with food and water available ad libitum. All experiments were conducted during the light period of a 12/12-h day–night cycle (08:00–20:00 h). All testing was performed in accordance with the recommendations and policies of the International Association for the Study of Pain and the US National Institutes of Health Guidelines for the Use of Animals.

### 2.2. Drugs

Morphine hydrochloride was purchased from commercial sources and was dissolved in physiological saline. D-CPPene (3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid, SDZ EAA 494, Novartis Pharma, Basel, Switzerland) was dissolved in equimolar NaOH to form stock solutions ($pH = 7.1 \pm 0.2$). Further dilutions were made with 0.9% saline. D-CPPene and its vehicle (saline) were injected intraperitoneally while morphine and its vehicle (saline) were injected subcutaneously. All injections were given in a volume of 1 ml/kg.

### 2.3. Procedure

For the tail-pinch assay, a flattened clip (approximately 6 mm wide) was placed at the base of a rat's tail. The pressure produced by the clip on the tail was determined to be approximately 0.8 to 1 kg. Drug-naive rats responded to this pressure by immediately vocalizing and biting at the clip. The clip was never applied to the rat's tail for longer than 5 s. Nociceptive responding was scored on a scale of 0 to 5, where 0 = no response, 1 = head movement towards the clip, 2 = head and body movements towards the clip, 3 = vocalization, 4 = biting the clip, and 5 = generalized motor response and vocalization. The average ($\pm$S.E.M.) baseline score for drug-naive rats was $4.38 \pm 0.06$ ($n = 132$).

For the tail-flick assay, a rat's tail (about 3 cm from the base) was exposed to a focused heat source (300 W white bulb). By withdrawing or removing the tail from the path of the stimulus and thereby exposing a photocell located in the apparatus ('Farmakolog', St. Petersburg, Russia) immediately below the tail, the rat could terminate the noxious stimulation and the reaction time was then recorded. An animal that failed to respond before 10 s (cut-off time) was removed from the apparatus and assigned a latency of 10 s. The average ($\pm$S.E.M.) baseline tail-flick latency for drug-naive rats was $3.69 \pm 0.10$ s ($n = 60$). Tests were conducted by an investigator blinded to the drug treatment conditions.

The intensity of the tail-pinch response and the tail-flick latencies were determined 60 min prior to and at various times up to 240 min after the injection of morphine (5, 10 or 20 mg/kg) or saline. There is substantial evidence that the doses used in the present study are adequate to produce brain concentrations sufficient and selective for NMDA

receptor blockade (Van Amsterdam and Lemaire, 1997). D-CPPene (0.33, 1.0, 3.2 or 5.6 mg/kg) or its vehicle was administered 30 min prior to morphine injection. All doses of morphine were tested using the tail-pinch procedure, while only a single dose was assessed in the tail-flick tests. The single dose of 10 mg/kg was selected based upon the results obtained in the tail-pinch test.

In separate groups of rats, the intensity of the tail-pinch response was determined once 30 min before the treatment with D-CPPene (1.0 mg/kg) or its vehicle and once 180 min after the injection of morphine (5, 10 or 20 mg/kg) or saline. D-CPPene and its vehicle were administered 30 min prior to morphine injection. The dose of D-CPPene of 1.0 mg/kg was selected because no signs of behavioral toxicity, but significant prolongation of morphine analgesia were observed at this dose level.

### 2.4. Measurement of morphine plasma concentration

Blood sampling occurred 30, 60, 120, 180 and 240 min after morphine administration. Rats were pretreated with either D-CPPene (5.6 mg/kg, the highest dose used in this study) or its vehicle (saline) 30 min prior to morphine injection. Blood (200 $\mu$l) was collected into heparin-coated 1-ml plastic vials by cutting the tail tip. Blood was centrifuged at 8000 rpm for 3 min and the plasma was collected. High-performance liquid chromatography (HPLC) analysis was conducted according to the method described earlier (Tebbett, 1987). The HPLC apparatus was Gold System (Beckman, USA) with model 126 double pump and model 168 U.V. detector.

### 2.5. Statistics

Both tail-pinch scores and tail-flick latencies were converted to percentages of the maximal possible effect. For the tail-pinch test, the percent analgesia was calculated per rat according to the formula: $(S_{BL} - S_{EXP}) \times 100/S_{BL}$, where $S_{BL}$ = baseline score, $S_{EXP}$ = test score. For the tail-flick test, the percent analgesia was calculated per rat according to the formula: $(T_{EXP} - T_{BL}) \times 100/(10 - T_{BL})$, where $T_{EXP}$ = test tail-flick latency (s), $T_{BL}$ = baseline latency (s). Data were analyzed using SPSS software (ver. 6.1). Data were subjected to a one- and two-factorial analysis of variance (ANOVA; morphine and D-CPPene doses as independent variables) with repeated measures on the time factor. Duncan's multiple range test was used for post-hoc between-group pairwise comparisons. For analgesia studies, all experimental groups consisted of 6 rats. For kinetics experiments, each group consisted of 5 rats.

## 3. Results

Fig. 1 shows the time-course of the tail-pinch score in rats pretreated with a combination of various doses of morphine and D-CPPene. Overall, global ANOVA revealed that both morphine and D-CPPene doses, as well as time were significant determinants of the intensity of the tail-pinch response ($F(3,90) = 77.72$, $P < 0.001$, $F(4,90) = 12.52$, $P < 0.001$, $F(7,630) = 8.04$, $P < 0.001$, respectively). The inhibitory effects of morphine on the tail-pinch response reached a maximum at 60 min after the morphine injection and disappeared in a time- and dose-dependent manner ($F(21,630) = 5.29$, $P = 0.001$). No significant analgesia was observed 90 min after the administration of the dose of 5 mg/kg, 180 min after the administration of the dose of 10 mg/kg, or 240 min after the administration of the dose of 20 mg/kg of morphine (Duncan's test, compared to saline-treated controls, $P < 0.05$).

D-CPPene significantly affected the analgesic effect of morphine ($F(10,90) = 1.51$, $P = 0.149$). There was also an overall significant morphine dose by D-CPPene dose by time interaction ($F(70,630) = 1.46$, $P = 0.011$). There was no evidence for potentiation of morphine-induced analgesia when data for the 30-min post-morphine time point were analyzed ($F(4,107) = 1.85$, $P = 0.127$). However, the analgesic effect of morphine (10 and 20 mg/kg) lasted longer in rats pretreated with D-CPPene. This prolongation was most obvious in rats treated with 10 mg/kg of morphine, where D-CPPene (1.0, 3.2 or 5.6 mg/kg)-pretreated subjects appeared significantly less responsive to noxious stimulation at 150 min, 180 min and 210 min after morphine (compared to vehicle-pretreated controls; $F(4,175) = 24.22$, $P < 0.001$).

Although D-CPPene by itself was found to have an effect on tail-pinch behavior in rats treated with saline instead of morphine ($F(4,175) = 3.89$, $P = 0.014$), this effect was time-dependent ($F(7,175) = 10.85$, $P < 0.001$) and disappeared by the time when a significant prolongation of morphine-induced analgesia was noted. These results (D-CPPene-induced hypoalgesia) could be attributed to the behavioral impairment seen with the dose of 5.6 mg/kg, but not with lower doses of D-CPPene. In support of this view, statistical analysis revealed a significant interaction between D-CPPene dose and time factors ($F(28,175) = 1.29$, $P = 0.0166$).

Similarly to the tail-pinch test results, the tail-flick test results demonstrated that D-CPPene significantly prolonged the analgesic action of morphine (Fig. 2; main effect of D-CPPene dose: $F(4,175) = 5.25$, $P = 0.003$; D-CPPene by time interaction: $F(28,175) = 1.87$, $P = 0.008$). In contrast to the tail-pinch data, the tail-flick data did not reveal any effects of D-CPPene itself (main effect of D-CPPene dose: $F(4,175) = 1.29$, $P = 0.302$; main effect of time: $F(7,175) = 1.53$, $P = 0.161$; D-CPPene dose by time interaction: $F(28,175) = 0.42$, $P = 0.996$).

For the next experiment, we selected the dose of D-CPPene of 1.0 mg/kg, which did not cause any behavioral impairment, and chose the time point of 180 min, when the prolongation of morphine (10 mg/kg)-induced analgesia by D-CPPene was most evident (Fig. 1). As shown in

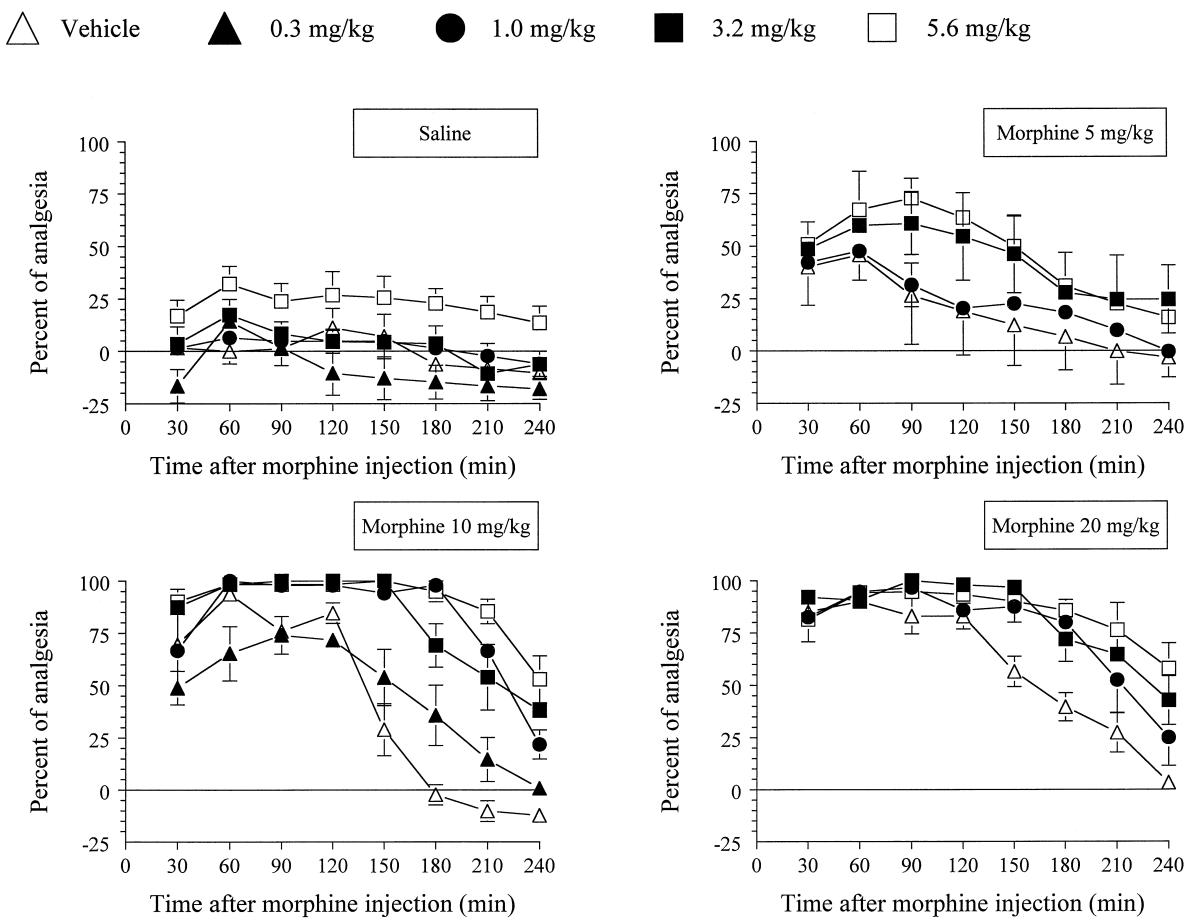


Fig. 1. Time-course of the tail-pinch test response of rats treated with a combination of D-CPPene and morphine. Morphine (5, 10 or 20 mg/kg) or saline was administered s.c. 30 min after the injection of 0.3, 1.0, 3.2 or 5.6 mg/kg of D-CPPene or its vehicle. Data are expressed as mean (±S.E.M.) percent analgesia. For the sake of clarity, error bars are indicated not for all data points. $n = 6$.

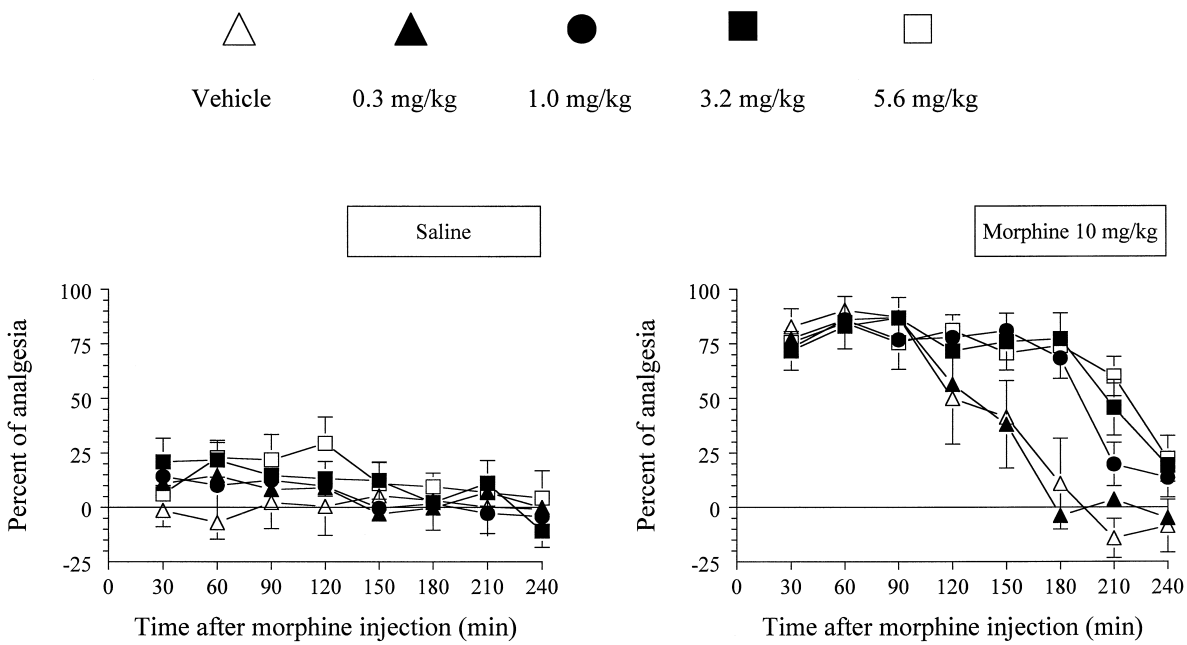


Fig. 2. Time-course of the tail-flick test response of rats treated with a combination of D-CPPene and morphine. Morphine (10 mg/kg) or saline was administered s.c. 30 min after the injection of 0.3, 1.0, 3.2 or 5.6 mg/kg of D-CPPene or its vehicle. Data are expressed as mean (±S.E.M.) percent analgesia. For the sake of clarity, error bars are indicated not for all data points. $n = 6$.

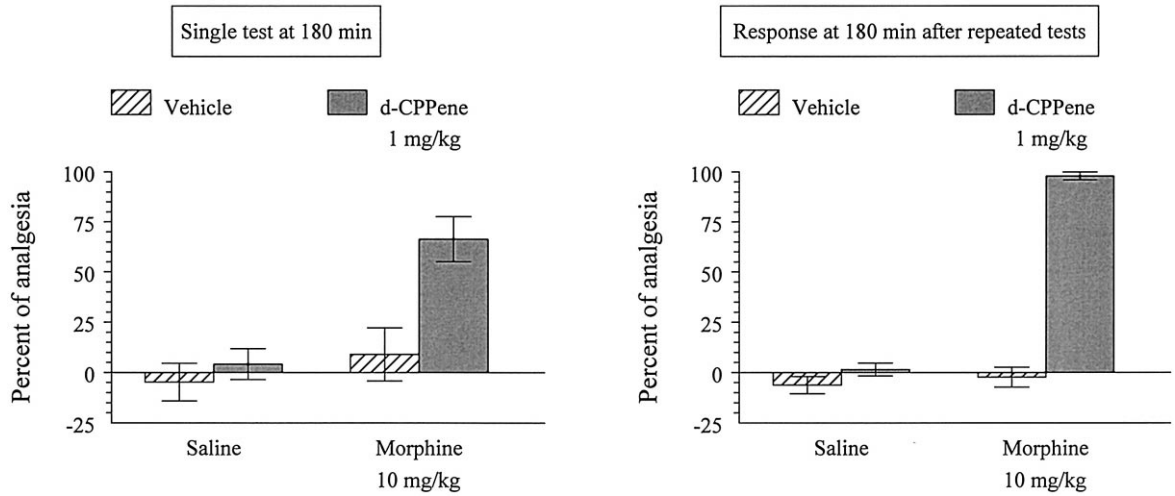


Fig. 3. Tail-pinch response of rats pretreated with morphine 180 min prior to the test. Left panel: no tests up to 180 min after morphine administration. Right panel: repeated tail-pinch tests performed up to 180 min after morphine administration (data extracted from Fig. 1). Morphine (10 mg/kg) or saline was administered s.c. 30 min after the injection of 1.0 mg/kg of D-CPPene or its vehicle. Data are expressed as mean (±S.E.M.) percent analgesia. $n = 6$.

Fig. 3 (left panel), combined administration of D-CPPene (1.0 mg/kg) and morphine (10 mg/kg) yielded a significant suppression of the tail-pinch response 180 min after the morphine injection (main effect of D-CPPene treatment: $F(1,23) = 6.04$, $P = 0.023$; main effect of morphine treatment: $F(1,23) = 14.93$, $P = 0.001$; D-CPPene by morphine interaction: $F(1,23) = 4.08$, $P = 0.057$). These results were obtained in rats that did not undergo any tests during the 180-min period after morphine administration.

The right panel of Fig. 3 depicts the tail-pinch test results obtained in rats that received exactly the same drug treatments but which were repeatedly exposed (four times) to tail-pinch tests during the period between morphine administration and the tail-pinch test held at 180-min after morphine administration (data were extracted from Fig. 1). A global ANOVA was performed on the data presented in both panels of Fig. 3. It was found that the analgesic activity of the D-CPPene plus morphine combination was less in rats that had only a single exposure to the test situation than it was in rats that underwent repeated tail-pinch tests (D-CPPene treatment factor by repeated testing factor: $F(1,47) = 6.20$, $P = 0.017$; D-CPPene by morphine by repeated testing interaction: $F(1,47) = 4.90$, $P = 0.033$).

Pretreatment with 5.6 mg/kg of D-CPPene did not affect plasma concentrations of morphine when compared to those of vehicle-treated controls (Fig. 4; $F(1,49) = 0.01$, $P = 0.933$). For both D-CPPene- and vehicle-treated rats, the morphine concentration was significantly dependent upon the time after morphine injection ($F(4,49) = 26.42$, $P < 0.001$).

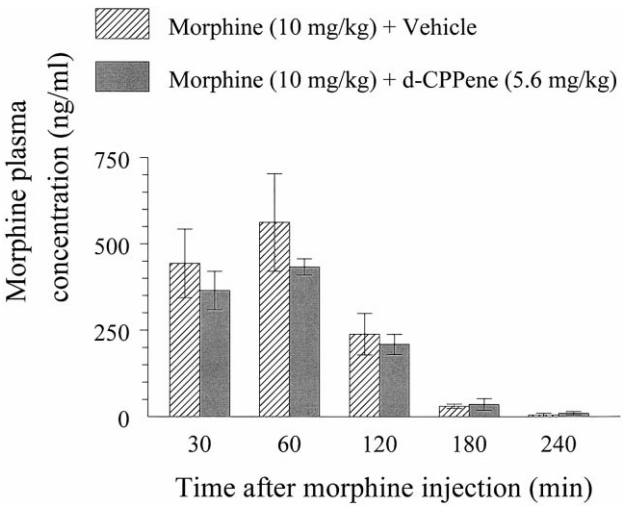


Fig. 4. Time course of the morphine plasma concentration (ng/ml) in rats treated with a combination of D-CPPene and morphine. Morphine (10 mg/kg) was administered s.c. 30 min after the injection of 5.6 mg/kg of D-CPPene or its vehicle. Data are expressed as mean (±S.E.M.) concentration per group. $n = 5$.

## 4. Discussion

The present results demonstrate that the competitive NMDA receptor antagonist D-CPPene significantly prolonged the analgesic effect of morphine. This effect was dependent on the dose of D-CPPene and was observed with doses of D-CPPene that were lower than those that produced behavioral impairment. The prolongation of morphine analgesia by D-CPPene was observed in both the tail-flick and tail-pinch tests. Our results suggest that prolongation of morphine analgesia is unlikely to result from alterations in the pharmacokinetic parameters of morphine since D-CPPene did not affect the time course of changes in morphine concentrations in plasma. In addition, earlier studies reported that within the dose range used in the present study D-CPPene: (a) blocks seizures induced by intracerebroventricular administration of NMDA (Bespalov, 1994; $ED_{50} = 0.94$ mg/kg), as well as handling-in-

duced, strychnine-potentiated convulsions (McAllister, 1993; $ED_{50} = 0.72$ mg/kg); (b) completely substitutes for another competitive NMDA receptor antagonist, NPC 12626, in rats trained to discriminate NPC 12626 from saline (Bobelis and Balster, 1993; $ED_{50} = 0.8$ mg/kg); and (c) exerts several effects that are characteristic for NMDA receptor antagonists, such as retardation of the development of tolerance or sensitization (sensitization to nicotine-induced dopamine release: Shoaib et al., 1994; tolerance to morphine analgesia: Belozertseva and Bespalov, in preparation), inhibition of somatic and subjective components of morphine withdrawal (Medvedev et al., submitted).

Our findings confirm that morphine-induced analgesia lasts longer in rats pretreated with NMDA receptor antagonists (Ben-Eliyahu et al., 1992; Grass et al., 1996). However, we found no evidence for an interaction between D-CPPene and morphine shortly after morphine administration (30–60 min). These data are in apparent contrast with other reports suggesting that there is potentiation of morphine analgesia by competitive NMDA receptor antagonists (mice: Saucier and Kavaliers, 1994; Bhargava, 1997; rats: Tiseo and Inturrisi, 1993; Grass et al., 1996).

Prolongation of morphine-induced analgesia by NMDA receptor antagonist can be viewed as a sign of retardation of the development of acute tolerance (Ben-Eliyahu et al., 1992). This hypothesis is based upon experimental evidence, indicating that after single morphine injection recovery from analgesia occurred much faster than did the decrease in morphine brain concentration (Kissin et al., 1991).

It is worth noting that it is not quite clear how NMDA receptor antagonists affect the development of acute tolerance to morphine analgesia and whether there is any relevance to the chronic tolerance effects. Of importance is the fact that pretreatment with dizocilpine completely blocked the development of chronic opiate tolerance but had only partial effects on the development of acute tolerance (Lutfy et al., 1993). It is interesting to note that antagonists of cholecystokinin, 5-HT receptors, GABA receptor agonists and $Ca^{2+}$ channel blockers also have the ability to both enhance morphine analgesia (Contreras and Tamayo, 1985; Contreras et al., 1988; Santillan et al., 1994; Xu et al., 1996; Verbitskaja and Kudryashova, 1997) and prevent the development of opiate tolerance (Contreras and Tamayo, 1985; Contreras et al., 1988; Xu et al., 1992; Zarrindast et al., 1995). Thus, drugs known to retard the development of opiate tolerance (including NMDA receptor antagonists) may share another common feature, such as potentiation of morphine analgesia.

## 5. Conclusion

The present study demonstrated that D-CPPene significantly increased the duration of morphine analgesia, but there was hardly any evidence of a potentiation of morphine analgesia shortly after morphine administration. The prolongation of morphine analgesia could only in part be attributed to the D-CPPene-induced disruption of the development of 'learned hyperresponsiveness' (i.e., acquisition of decreased response latencies to escape from repeated exposures to noxious stimulation). In addition, the morphine plasma concentration was not affected by concurrent treatment with D-CPPene. Taken together with the effects on chronic tolerance, the data on the prolongation of morphine analgesia favor the development of NMDA receptor antagonists as adjuncts for chronic opiate pain management.

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