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Effects of NMDA receptor antagonists on acute μ-opioid analgesia in the rat

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Abstract

Mixed research findings have led to a debate regarding the effect of *N*-methyl-D-aspartate (NMDA) receptor antagonists on opiate analgesia. NMDA antagonists have been found in various studies to enhance, to inhibit, or to have no effect on opiate analgesia. The present research used a single protocol to explore the effects of six NMDA receptor antagonists on acute morphine (3.0 mg/kg sc) and fentanyl (0.05 mg/kg sc) analgesia in adult male Sprague–Dawley rats. NMDA receptor antagonists were selected based on their abilities to block various sites on the NMDA receptor complex, including the noncompetitive antagonists MK-801 (0.1 and 0.3 mg/kg ip), dextromethorphan (10.0 and 30.0 mg/kg ip), and memantine (3.0 and 10.0 mg/kg ip), a glycine site antagonist, (+)-HA-966 (10.0 and 30.0 mg/kg ip), a competitive antagonist, LY235959 (1.0 and 3.0 mg/kg ip), and a polyamine site antagonist, ifenprodil (1.0 and 3.0 mg/kg ip). Analgesia was assessed using the tail-flick test. A single dose of each opiate was used. The low doses of the antagonists, which are known to produce significant neural and behavioral actions at NMDA receptors, had no effect on morphine or fentanyl analgesia. At the higher doses, morphine analgesia was significantly enhanced by LY235959 (3.0 mg/kg), and fentanyl analgesia was significantly enhanced by LY235959 (3.0 mg/kg), dextromethorphan (30.0 mg/kg), and (+)-HA-966 (30.0 mg/kg). Enhancement of analgesia occurred without any apparent adverse side effects. None of the NMDA antagonists affected tail-flick responses on their own, except the higher dose of LY235959 (3.0 mg/kg), which produced a mild analgesic effect. Because no consistent effects were observed, the data suggest that NMDA receptors are not involved in acute μ-opioid analgesia. The mechanisms underlying the enhancement of opiate analgesia by selected NMDA antagonists, such as LY235959, dextromethorphan, and (+)-HA-966, remain to be determined.

Keywords: Opioid; Analgesia; Morphine; Fentanyl; Glutamate; NMDA

1. Introduction

There is increasing interest in the potential role of glutamate in the physiological and behavioral effects of opiates. This is particularly evident in research on opiate-induced behavioral and neural plasticity. Evidence suggests that *N*-methyl-D-aspartate (NMDA) receptors, a class of glutamatergic receptors, are involved in the neural plasticity underlying the development of opiate tolerance, sensitization, and physical dependence (Herman et al., 1995; Inturrisi, 1997; Mao, 1999; Trujillo, 2000, 2003; Wolf, 1998). In the majority of studies on this topic, NMDA receptor antagonists have been found to inhibit the development of these processes without altering the acute analgesic effects of opiates (see Trujillo, 2000 for review).

Despite the lack of effect reported in many studies, some research has shown modulation of acute morphine analgesia by NMDA receptor antagonists. In some of these studies, NMDA receptor antagonists have been found to inhibit the acute analgesic effects of morphine (Lipa and Kavaliers, 1990; Lutfy et al., 1993; Plesan et al., 1999), whereas in others these antagonists have been observed to potentiate acute morphine analgesia (Belozertseva et al., 2000; Bernardi et al., 1996; Bespalov et al., 1998; Bhargava, 1997; Carlezon et al., 2000; Celerier et al., 1999; Dambisya and Lee, 1994; Grass et al., 1996; Hoffmann and Wiesenfeld, 1996; Kozela et al., 2001; Larcher et al., 1998; Lutfy et al., 1999; Manning et al.,

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1996; Mao et al., 1996; Plesan et al., 1999). The latter findings are of particular clinical interest—if it can be demonstrated that NMDA receptor antagonists enhance acute opiate analgesia without enhancing side effects of opiates, then NMDA receptor antagonist/opiate combinations may be of use in the treatment of acute pain. In fact, several studies have already obtained intriguing clinical data suggesting that such combinations may be effective in humans (Bell, 1999; Buvanendran et al., 2002; Caruso, 2000; Chizh and Eide, 2002; Fu et al., 1997; Goldblum, 2000; Ilkjaer et al., 2000; Katz, 2000; Mercadante et al., 2000; Weinbroum et al., 2002).

Because of the conflicting findings, further research is needed to clarify the ability of NMDA receptor antagonists to modify acute opiate analgesia. The present studies explored the ability of six different NMDA receptor antagonists to affect the analgesic actions of two opiates, morphine and fentanyl. We took advantage of the rich pharmacology of the NMDA receptor in designing these experiments. The NMDA receptor is a large protein complex, consisting of an ion channel and a number of binding sites to which drugs and neurotransmitters can bind and influence the function of the receptor (Danysz and Parsons, 1998; Dingledine et al., 1999; Michaelis, 1998; Ozawa et al., 1998; Yamakura and Shimoji, 1999). Among the most prominent sites are the glutamate (or competitive) site, the glycine site, the channel blocking site (often referred to as the noncompetitive or uncompetitive site), and the polyamine site. The diversity of sites on the receptor allows for numerous pharmacological interventions to explore NMDA receptor function. As a result, studies aimed at understanding the behavioral and physiological roles of NMDA receptors have often used compounds acting at multiple sites on the receptor.

For the present studies, we used compounds that antagonize each of the major sites on the NMDA receptor complex: the competitive site (LY235959), the glycine site [(+)-HA-966], the polyamine site (ifenprodil), and three different compounds that act at the channel blocking site [MK-801, dextromethorphan, and memantine]. MK-801 is a high-affinity compound very useful as a pharmacological tool to explore NMDA receptor function but with little clinical potential, while dextromethorphan and memantine are both clinically available NMDA receptor antagonists. We used two doses of each antagonist—a relatively low dose, known to block NMDA receptors, but producing few motoric side effects and a dose $\sim$ 1/2 log higher.

In addition to six NMDA receptor antagonists, we selected two opiates for these studies, morphine and fentanyl. The vast majority of previous studies that have explored interactions between NMDA receptor antagonists and μ-opioid receptors have used morphine as the drug of choice and assumed that the findings would extend to other μ-opioids. However, there are suggestions that interactions with NMDA receptor antagonists may be restricted to morphine and may not extend to other drugs acting at μ-opioid receptors (Bilsky et al., 1996). On the other hand, other studies have suggested that such interactions may extend to μ-opioids other than morphine (Allen and Dykstra, 2000; Celerier et al., 2000).

The aim of these studies was to further explore the effects of NMDA receptor antagonists on acute opiate analgesia in an effort to determine (1) if different NMDA receptor antagonists acting in different manners with, and at different sites on, the NMDA receptor complex will affect opiate analgesia, (2) if NMDA receptor antagonists that do not produce significant motoric effects will affect opiate analgesia, and (3) if the analgesic effect of μ-opioids other than morphine will be affected by NMDA receptor antagonists. Although previous studies have explored acute interactions between selected NMDA receptor antagonists and selected opiates, to our knowledge, this is the first series of experiments to explore a broad selection of NMDA receptor antagonists and different opiates. Based on previous research, we expect that selected antagonists may enhance acute opiate analgesia but only at relatively high doses.

2. Methods

2.1. Subjects

Adult male Sprague–Dawley rats ($\sim$ 225–250 g at purchase; Harlan, San Diego, CA) were used in all studies. Animals were housed three per cage in standard plastic rat cages, with food and water available ad lib. A 12-h light/dark cycle was maintained. Following arrival, animals were allowed to acclimate to the vivarium for at least 1 week before testing. All experiments were conducted during the light cycle. The experimental protocol was approved by the California State University, San Marcos Institutional Animal Care and Use Committee (IACUC) and is in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Apparatus

An IITC Model 33 tail-flick apparatus was used to assess tail-flick latencies (D'Amour and Smith, 1941). Methods were similar to those used previously (Trujillo and Akil, 1991a, 1994). Briefly, rats were gently restrained by hand for tail-flick tests. Three tests in a row, 10–20 s apart, were performed for each animal, and the average was used in data analysis. The heat was focused 2.5–5 cm from the tip of the tail at a different location for each of the three tests. The heat intensity was set so that baseline latencies averaged 3–5 s. A cutoff latency of 8 s was used to prevent tissue damage. Animals were tested in groups, with all treatment groups represented on any individual test day.

2.3. Procedure

Six experiments were performed, each exploring the ability of a NMDA receptor antagonist to influence the analgesic effect of morphine and fentanyl. Two doses of each NMDA receptor antagonist and a single dose of each opiate were examined in each experiment. NMDA antagonists, their binding sites and doses included MK-801 (high-affinity channel blocker, 0.1 and 0.3 mg/kg), dextromethorphan (low-affinity channel blocker, 10.0 and 30.0 mg/kg), memantine (low-affinity channel blocker, 3.0 and 10.0 mg/kg), LY235959 (competitive site, 1.0 and 3.0 mg/kg), (+)-HA-966 (glycine site partial agonist, 10.0 and 30.0 mg/kg), and ifenprodil (polyamine site, 1.0 and 3.0 mg/kg). The lower dose was selected based on the ability of the compound to effectively block NMDA receptor function in vivo without producing significant motoric side effects; the higher dose was ~ 1/2 log higher. Doses were obtained from a variety of sources (Bilsky et al., 1996; Bubser et al., 1992; Carter, 1994; Cudennec et al., 1994; Dunn et al., 1992; Gotti et al., 1988; Lutfy et al., 1995, 1996; Mazzola-Pomietto et al., 1996; Popik and Danysz, 1997; Popik and Skolnick, 1996) and from previous experience in our laboratory (Trujillo et al., 2000, 2001a,b; Warmoth and Trujillo, 2002). Doses of morphine (3.0 mg/kg) and fentanyl (0.05 mg/kg) were selected based on their abilities to produce relatively equianalgesic effects across a similar time course, as determined by pilot studies (Watorski, 2002).

Each experiment consisted of six groups (pretreatment-treatment): vehicle-vehicle, NMDA antagonist-vehicle, vehicle-morphine, NMDA antagonist-morphine, vehicle-fentanyl, and NMDA antagonist-fentanyl. Animals ($n = 6$ per group) were assigned in a counterbalanced manner to each group. Two doses of each NMDA receptor antagonist were examined in each experiment across 2 consecutive weeks. The same experimental animals were used across weeks in each experiment. To prevent carryover effects between doses, the low dose of the antagonist preceded the high dose, at least 1 week separated the two doses, and animals were reassigned to treatment groups (in a counterbalanced manner) between doses.

A 3-day habituation phase preceded all studies to familiarize the animals with the tail-flick procedure and apparatus and to reduce variability during testing. On each of these days, animals were weighed and taken to the experimental room in their home cages. After 30 min in the experimental room, the experimenter went through the motions of the tail-flick process with each animal for three trials, without engaging the heat source. One day of habituation was also included between the first week and the second week.

On test days, animals were weighed in the vivarium and then taken into the experimental room in their home cages. After 30 min of habituation to the experimental room, each animal was tested for baseline tail flick. Three tail-flick tests in a row, 10–20 s apart, were taken for each animal, and the average was used in data analysis. Baseline tests were followed by an injection of either saline or a NMDA receptor antagonist (intraperitoneal). Thirty minutes later, tail-flick latencies were again assessed followed by an injection of either saline or an opiate (subcutaneous). Then, every 30 min for 4.5 h, tail-flick latencies were assessed for each animal for a full time course of 5 h. At each testing interval, three tail-flick latencies were taken and the average of these was used in data analysis. Behavioral observations were made between testing intervals throughout the time course as time allowed. The experimenter was blind to the treatment group during testing in all studies.

2.4. Drugs

MK-801 hydrogen maleate, dextromethorphan hydrobromide, memantine hydrochloride, and ifenprodil tartrate were obtained from Sigma; LY235959 was obtained from Tocris. Morphine sulfate and fentanyl hydrochloride were generous gifts from the National Institute on Drug Abuse Drug Supply Program. All drugs were dissolved in 0.9% saline, with the exception of ifenprodil, which was dissolved in 1% Tween-80. Drugs were injected at a volume of 1 ml/kg. All dose calculations refer to the weight of the salts.

2.5. Data analysis

For statistical comparisons, mean tail-flick latencies were converted to percentage maximum possible effect (%MPE; see Dewey and Harris, 1975). Although %MPE was used for statistical analyses, the data are shown graphically as unconverted tail-flick latencies. Only data following the second injection (opioid or vehicle) were used in the statistical analyses to focus on the postopioid interval.

The effects of morphine and fentanyl were analyzed separately in each experiment, and each dose of the NMDA receptor antagonists was analyzed separately in each experiment to maximize the opportunity of finding an effect of the NMDA receptor antagonists on opiate analgesia. Thus, mixed-model [9×4 (within-subjects: Time intervals) $\times$ (between subjects: Treatment group)] repeated-measures ANOVAs were used to determine potential effects of the NMDA receptor antagonists on morphine and fentanyl analgesia (overall effect of treatment and interactions). Fisher's PLSD (post hoc) analyses were used to detect significant differences between treatment groups.

3. Results

In each experiment, morphine and fentanyl produced a short-lived increase in tail-flick latencies, peaking at 30–60

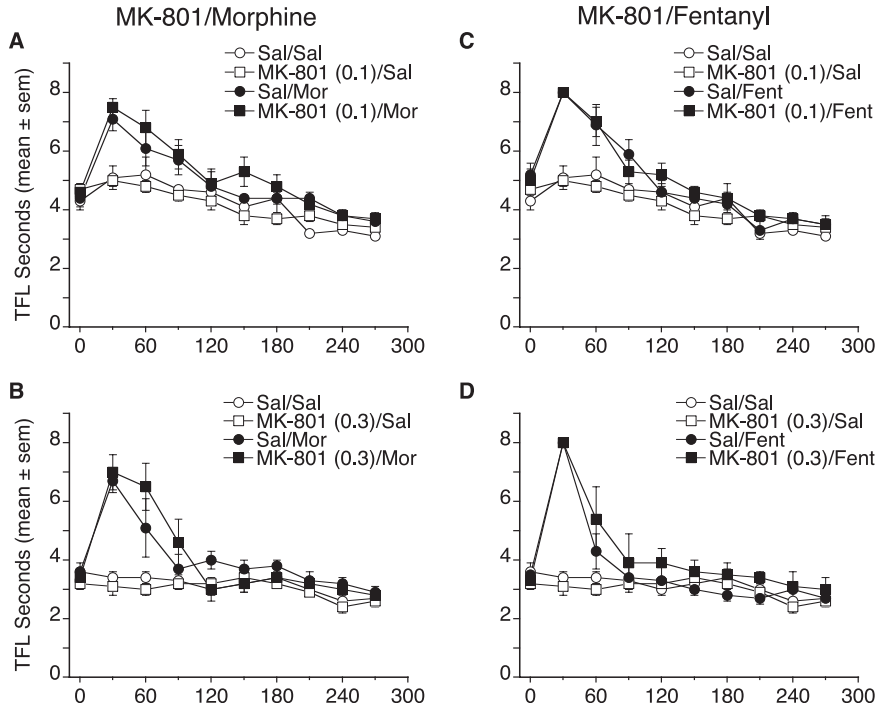

Fig. 1. MK-801 (0.1 or 0.3 mg/kg) had no significant effect on acute morphine (3.0 mg/kg; A and B) or fentanyl (0.05 mg/kg; C and D) analgesia as measured by the tail-flick test. Data are shown as means ± S.E.M. tail-flick latency.

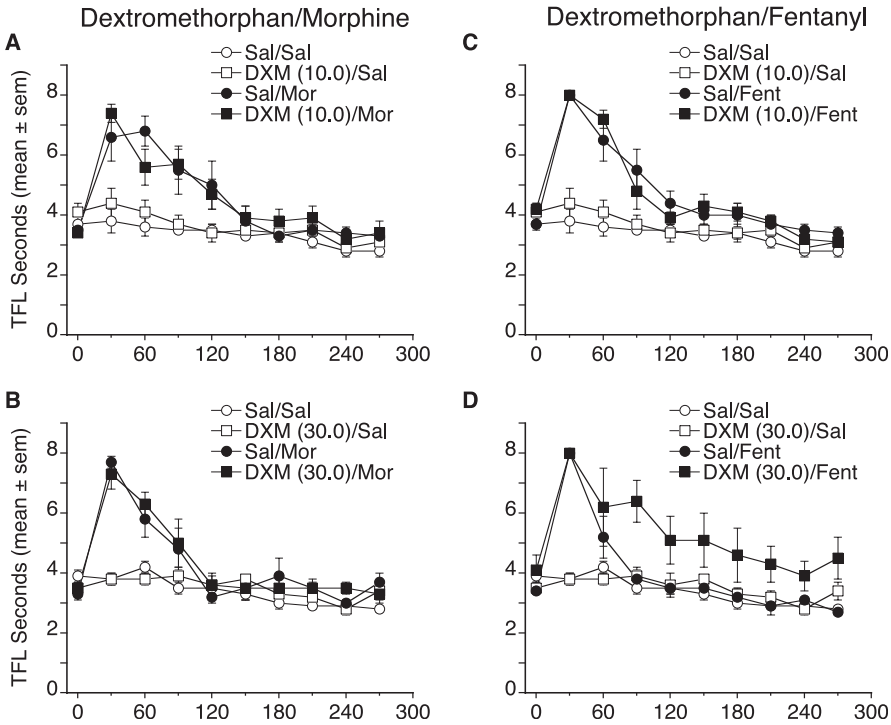

Fig. 2. Dextromethorphan, at the lower dose (10 mg/kg), had no significant effect on acute morphine (3.0 mg/kg; A) or fentanyl (0.05 mg/kg; C) analgesia as measured by the tail-flick test. The higher dose of dextromethorphan had no effect on morphine analgesia (B) but significantly enhanced fentanyl analgesia (D). Data are shown as means ± S.E.M. tail-flick latency.

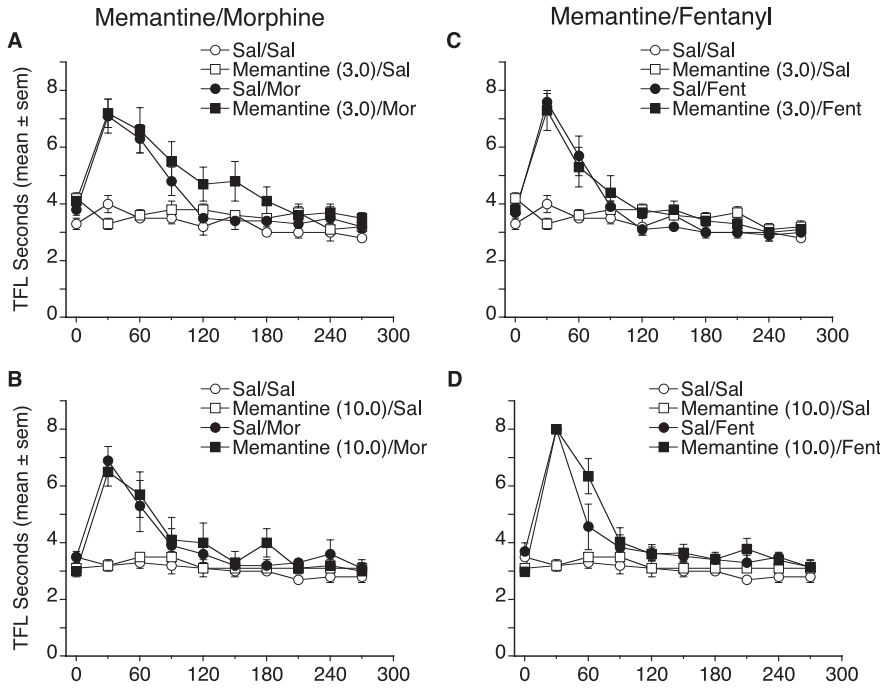

Fig. 3. Memantine (3.0 or 10.0 mg/kg) had no significant effect on acute morphine (3.0 mg/kg; A and B) or fentanyl (0.05 mg/kg; C and D) analgesia as measured by the tail-flick test. Data are shown as means ± S.E.M. tail-flick latency.

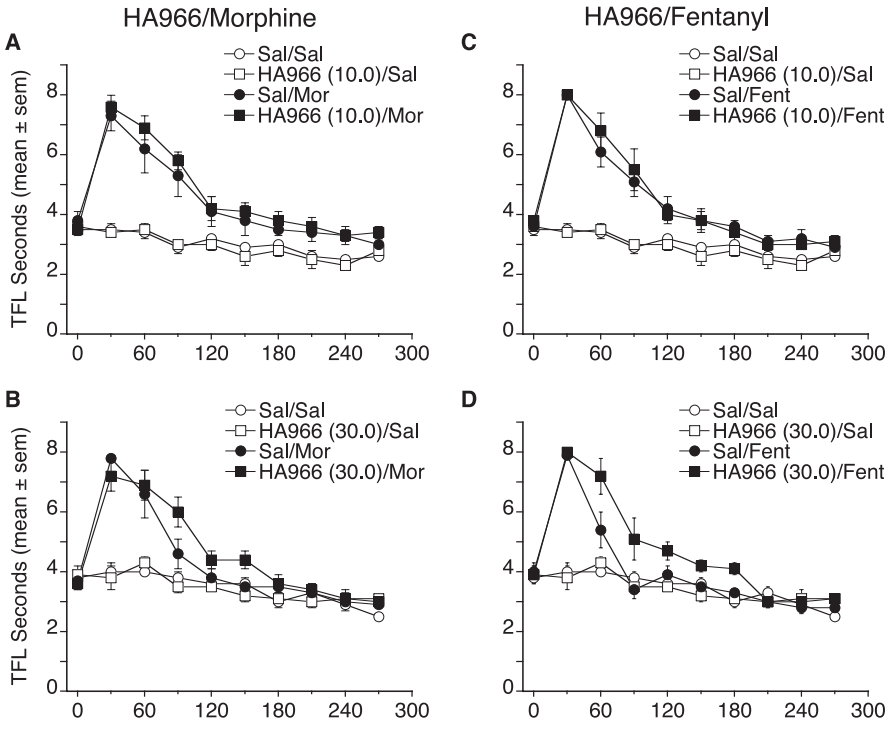

Fig. 4. (+)-HA-966, at the lower dose (10 mg/kg), had no significant effect on acute morphine (3.0 mg/kg; A) or fentanyl (0.05 mg/kg; C) analgesia as measured by the tail-flick test. The higher dose of (+)-HA-966 had no effect on morphine analgesia (B) but significantly enhanced fentanyl analgesia (D). Data are shown as means ± S.E.M. tail-flick latency.

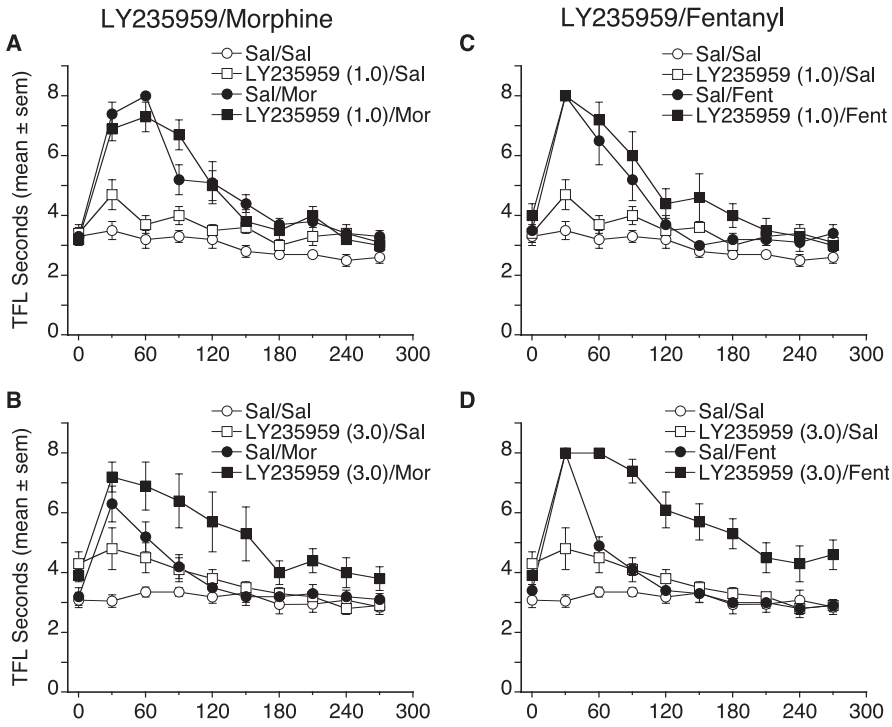

Fig. 5. LY235959, at the lower dose (1.0 mg/kg), had no significant effect on acute morphine (3.0 mg/kg; A) or fentanyl (0.05 mg/kg; C) analgesia as measured by the tail-flick test. The higher dose of LY235959 produced a mild analgesic on its own and significantly enhanced both morphine (B) and fentanyl analgesia (D). Data are shown as means ± S.E.M. tail-flick latency.

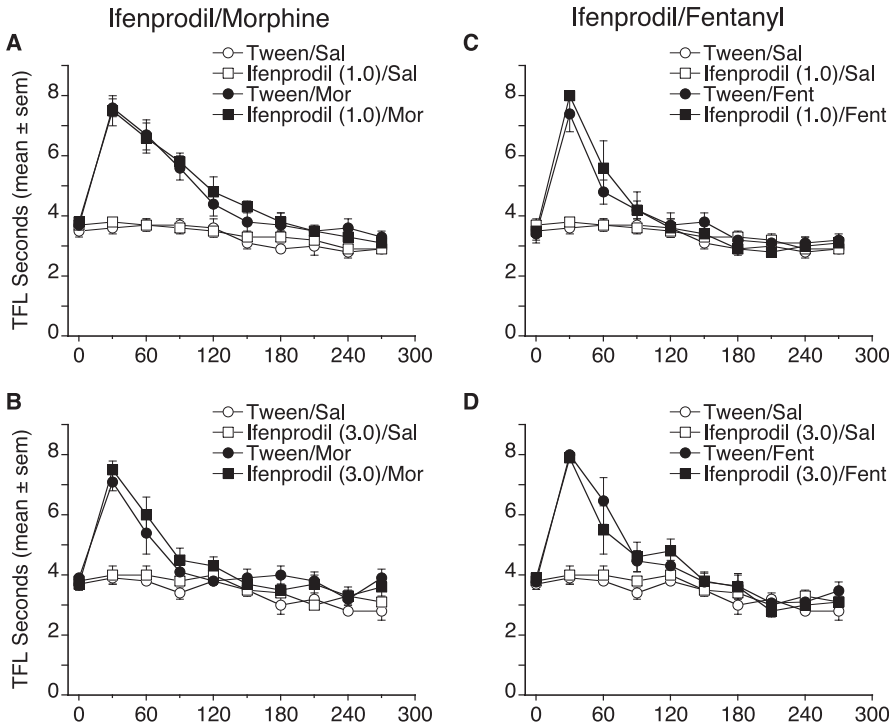

Fig. 6. Ifenprodil (1.0 or 3.0 mg/kg) had no significant effect on acute morphine (3.0 mg/kg; A and B) or fentanyl (0.05 mg/kg; C and D) analgesia as measured by the tail-flick test. Data are shown as means ± S.E.M. tail-flick latency.

Table 1
ANOVA results for all experiments

NMDA antagonist	Dose	Opiate	Treatment	Time	Interaction	Post hoc
MK-801	0.1	Morphine	2.98 (P=.057)	40.26 (P<.05)	2.62 (P<.05)	n.s.
		Fentanyl	1.82 (n.s.)	60.16 (P<.05)	5.72 (P<.05)	n.s.
	0.3	Morphine	1.97 (n.s.)	30.18 (P<.05)	8.07 (P<.05)	n.s.
		Fentanyl	3.88 (P<.05)	45.59 (P<.05)	11.91 (P<.05)	n.s.
Dextromethorphan	10.0	Morphine	6.74 (P<.05)	32.37 (P<.05)	4.07 (P<.05)	n.s.
		Fentanyl	8.20 (P<.05)	60.24 (P<.05)	8.52 (P<.05)	n.s.
	30.0	Morphine	8.40 (P<.05)	44.65 (P<.05)	7.34 (P<.05)	n.s.
		Fentanyl	12.75 (P<.05)	14.69 (P<.05)	2.76 (P<.05)	**P<.05**
Memantine	3.0	Morphine	4.15 (P<.05)	33.73 (P<.05)	7.70 (P<.05)	n.s.
		Fentanyl	8.41 (P<.05)	32.57 (P<.05)	7.47 (P<.05)	n.s.
	10.0	Morphine	9.22 (P<.05)	22.04 (P<.05)	5.18 (P<.05)	n.s.
		Fentanyl	9.18 (P<.05)	11.96 (P<.05)	2.71 (P<.05)	n.s.
LY235959	1.0	Morphine	16.92 (P<.05)	47.49 (P<.05)	7.86 (P<.05)	n.s.
		Fentanyl	12.51 (P<.05)	48.56 (P<.05)	7.30 (P<.05)	n.s.
	3.0	Morphine	9.44 (P<.05)	24.66 (P<.05)	3.85 (P<.05)	**P<.05**
		Fentanyl	19.55 (P<.05)	43.92 (P<.05)	8.76 (P<.05)	**P<.05**
(+)-HA-966	10.0	Morphine	19.58 (P<.05)	47.21 (P<.05)	7.11 (P<.05)	n.s.
		Fentanyl	14.10 (P<.05)	75.39 (P<.05)	12.15 (P<.05)	n.s.
	30.0	Morphine	12.70 (P<.05)	62.05 (P<.05)	8.26 (P<.05)	n.s.
		Fentanyl	12.20 (P<.05)	66.61 (P<.05)	9.87 (P<.05)	**P<.05**
Ifenprodil	1.0	Morphine	11.82 (P<.05)	51.90 (P<.05)	8.03 (P<.05)	n.s.
		Fentanyl	9.70 (P<.05)	39.83 (P<.05)	6.66 (P<.05)	n.s.
	3.0	Morphine	8.85 (P<.05)	33.12 (P<.05)	4.66 (P<.05)	n.s.
		Fentanyl	5.07 (P<.05)	58.27 (P<.05)	8.99 (P<.05)	n.s.

Post hoc results represent the comparison between the experimental group (NMDA receptor antagonist + opiate) and its respective opiate control group (vehicle + opiate); significant differences between these groups are shown in bold.

min postinjection and returning to baseline by ~ 2 h postinjection (Figs. 1–6). None of the NMDA receptor antagonists affected tail-flick latencies in the absence of the opiates, with the exception of the competitive antagonist LY235959, which produced a mild increase in latencies at the higher dose (3.0 mg/kg; Fig. 5B and D).

The ability of morphine or fentanyl to increase tail-flick latencies was not affected by the low dose of any of the antagonists (Figs. 1–6 and Tables 1 and 2). LY235959 at the higher dose (3.0 mg/kg) enhanced the ability of both morphine and fentanyl to increase tail-flick latencies; however, as noted above, this dose also produced an increase in tail-flick latencies on its own. Dextromethorphan and (+)-HA-966, at the higher doses of each, enhanced the ability of fentanyl, but not morphine, to increase tail-flick latencies. Statistical analyses are summarized in Table 1, and overall findings are summarized in Table 2.

4. Discussion

To summarize the present results, there was a lack of consistency in the ability of NMDA receptor antagonists to modify acute opiate analgesia. First and foremost, there was a complete lack of effect of the lower doses of the NMDA receptor antagonists on both morphine and fentanyl analgesia. These doses were selected based on their ability to effectively block NMDA receptor function in vivo while

Table 2
Summary: the effects of NMDA receptor antagonists on acute μ-opioid analgesia

	MK-801 (0.1 and 0.3 mg/kg)	Dextromethorphan (10.0 and 30.0 mg/kg)	Memantine (3.0 and 10.0 mg/kg)	(+)-HA-966 (10.0 and 30.0 mg/kg)	LY235959 (1.0 and 3.0 mg/kg)	Ifenprodil (1.0 and 3.0 mg/kg)
Morphine[a]						
Low	0	0	0	0	0	0
High	0	0	0	0	↑	0
Fentanyl[b]						
Low	0	0	0	0	0	0
High	0	↑	0	↑	↑	0

0 = no effect; arrow up = potentiation of opiate analgesia.

[a] Acute morphine (3.0 mg/kg) analgesia was enhanced by a high dose of LY235959 (3.0 mg/kg).

[b] Acute fentanyl (0.05 mg/kg) analgesia was enhanced by high doses of dextromethorphan (30.0 mg/kg), (+)-HA-966 (30.0 mg/kg), and LY235959 (3.0 mg/kg).

producing minimal motoric side effects. The findings suggest that NMDA receptors are not involved in acute opiate analgesia. At the higher doses of the antagonists, the analgesic effects of both morphine and fentanyl were enhanced by the competitive antagonist LY235959 (3.0 mg/kg); however, at this dose, the compound produced mild analgesic effects on its own. Thus, the enhancement may represent a simple additive effect of two analgesics rather than a synergistic interaction. Fentanyl but not morphine analgesia was enhanced by the higher doses of (+)-HA-966 (30 mg/kg) and dextromethorphan (30 mg/kg), demonstrating the lack of consistency between the two opiates. The lack of effect at the lower doses of the antagonists, and the lack of consistency at the higher doses, argues against a role for NMDA receptors in acute opiate analgesia. If these receptors were involved, we would expect a more consistent effect of NMDA receptor antagonists on opiate analgesia.

This study examined the broadest range of NMDA receptor antagonist/μ-opioid drug combinations to date in analgesia, including six different antagonists and two opiates. To systematically explore NMDA receptor function, we selected compounds known to effectively block each of the key sites on the NMDA receptor complex (the competitive site, the glycine site, the channel blocking site, and the polyamine site). Moreover, to examine clinical potential, we included two clinically available compounds, dextromethorphan and memantine. The findings are strengthened considerably by the fact that comparisons across compounds were made by the same experimenter, in a single laboratory, using a consistent experimental protocol, and the same species and strain of rat throughout. Previous findings on interactions between NMDA receptor antagonists and opiates have typically used a single antagonist (or a limited number of antagonists) and a single opiate (almost exclusively morphine) and have assumed that the findings would extend to other NMDA receptor antagonist/opiate combinations.

The results of the present studies do not rule out the possibility that NMDA receptors may be involved in opiate analgesia under certain circumstances. The tail-flick test is a good assay for spinally mediated acute opiate analgesia (Berge and Hole, 1981; Dewey and Harris, 1975; Gutstein and Trujillo, 1993); however, this test may not adequately assess other forms of analgesia, such as those mediated at higher brain sites. Additionally, we used modest doses of the opiates and relatively high heat settings for these studies. Thus, it may be that NMDA receptor antagonists affect different doses of opiates and different intensities of pain than those tested in the present experiments. Furthermore, the maximum analgesic effect in the present studies was close to the cutoff values; the present approach may therefore have been insensitive to a short-lived enhancement of the peak analgesic effect (on the other hand, the results demonstrate that the approach was sensitive to an enhancement of analgesia as expressed in a prolongation of the analgesic effect). It might be argued that we aimed our doses of the antagonists too low and that higher doses are required to enhance opiate analgesia. However, the doses were selected based on their ability to selectively block NMDA receptor function; higher doses of these compounds are less selective and in many cases produce significant side effects (see below). There are number of other factors that may have influenced the results, ranging from strain of rat to handling of the animals. Previous research has suggested that rat strain is an important factor in the ability of NMDA receptor antagonists to potentiate opiate analgesia, with Sprague–Dawley, Wistar–Kyoto, and Spontaneously Hypertensive sensitive to enhancement and Dark-Agouti insensitive to enhancement (Plesan et al., 1999). In a more recent study, Kozela et al. (2001) found that the tail-flick test was a sensitive assay, whereas the paw-withdrawal test was insensitive to NMDA receptor antagonist facilitation of morphine analgesia. The present study therefore used a strain (Sprague–Dawley) and a behavioral assay (tail flick) that in previous work has been shown to be sensitive to the potentiation produced by such combinations.

Given that some NMDA receptor antagonists produce potent motoric effects raises the possibility that the enhancement of analgesia may be related to motor impairment. However, this is unlikely, at least in the present experiments. First, the tail-flick test is a spinal reflex and is resistant to motor impairment, remaining intact even in animals with a severed spinal cord (Berge and Hole, 1981; Dewey and Harris, 1975; Gutstein and Trujillo, 1993). Second, and more importantly, the enhancement of analgesia did not correlate with known motoric actions of the compounds. In our hands, neither dextromethorphan, (+)-HA-966, nor ifenprodil produce motoric effects at the doses used; memantine produces mild stimulation, and MK-801 produces potent stimulation at the higher doses (but not the lower doses); and LY235959 produces mild sedation at the higher dose (but not the lower dose) (Trujillo et al., 2000, 2001a,b; Warmoth and Trujillo, 2002; Trujillo et al., unpublished results). Although systematic motor ratings were not made in the present experiments, behavior of the animals was carefully observed and there was no evidence of motor impairment for any individual drug or drug combination. On a related note, no enhanced catalepsy or lethality was seen with the drug combinations in the current studies, as has been reported previously (Trujillo and Akil, 1991b; Tzschentke and Schmidt, 1996). However, as discussed previously, such enhancement is typically seen only with higher dose combinations (e.g., 0.3 mg/kg of MK-801 with 10 mg/kg of morphine or more) (Trujillo and Akil, 1991b). Another factor to consider concerning the inconsistent effects of the different compounds is pharmacokinetics, as differences in time course among the different antagonists might have contributed to the differing results. However, previous work in our laboratory (Trujillo et al., 2000, 2001a,b; Warmoth and Trujillo, 2002) and others (Bilsky et al., 1996; Bubser et al., 1992; Carter, 1994; Cudennec et al., 1994; Dunn et al., 1992; Gotti et al., 1988;

Lutfy et al., 1995, 1996; Mazzola-Pomietto et al., 1996; Popik and Danysz, 1997; Popik and Skolnick, 1996) suggests that these compounds are effective within the time frame examined in the present studies. Thus, neither differences in motoric effects nor differences in time course appear to be responsible for the inconsistent effects of the antagonists.

As discussed above, previous research has shown mixed results on interactions between NMDA receptor antagonists and opiates in analgesia. On the one hand, studies have shown that competitive antagonists (LY235959, CGS19755, NPC12626, and D-CPPene), glycine site antagonists (ACEA-1021, ACEA-1328, and MRZ 2/576), channel-blocking antagonists (MK-801, ketamine, dextromethorphan, dextrorphan, memantine, and MRZ 2/579), and polyamine site antagonists (ifenprodil) have the ability to enhance the analgesic effect of morphine (Belozertseva et al., 2000; Bernardi et al., 1996; Bespalov et al., 1998; Bhargava, 1997; Celerier et al., 1999; Dambisya and Lee, 1994; Grass et al., 1996; Hoffmann and Wiesenfeld, 1996; Kozela et al., 2001; Larcher et al., 1998; Lutfy et al., 1999; Manning et al., 1996; Mao et al., 1996; Plesan et al., 1999). However, other studies have shown that these compounds, as well as other NMDA receptor antagonists, do not affect morphine analgesia at doses that effectively block NMDA receptors (Allen and Dykstra, 1999; Bilsky et al., 1996; Dunbar and Yaksh, 1996; Elliott et al., 1994a,b; Fairbanks and Wilcox, 1997; Gonzalez et al., 1997; Kolesnikov et al., 1993; Marek et al., 1991; Tiseo and Inturrisi, 1993; Trujillo and Akil, 1991a, 1994). Still, others (a minority of studies) have shown that NMDA receptor antagonists decrease morphine analgesia (Lipa and Kavaliers, 1990; Lutfy et al., 1993; Plesan et al., 1999). Given this mix of findings, there is apparently an as yet unidentified experimental factor (or factors) responsible for the contrasting observations.

To determine the potential factors that might contribute to positive versus negative findings in similar experiments, we performed a broad exploration of the literature on interactions between NMDA receptor antagonists and opiates in analgesia (Watorski, 2002). Among the many experiments that have been performed thus far, no specific factor stood out as critical to the presence or absence of interactions, including the NMDA receptor antagonist used, the NMDA receptor site at which the antagonist acts, the opiate examined, the mode of injection, the time separating the two injections, the analgesic test used, the intensity of the pain stimulus, or the species, strain, or source of animal (Watorski, 2002). It appears that there may be particular receptor sites (glycine, polyamine, or competitive), specific NMDA receptor antagonists (e.g., dextromethorphan), analgesia tests (tail flick), or species (rats or squirrel monkeys) that hold more promise than others. However, identifiable differences in research protocol and species are not enough to explain the differences among the many studies. Further research is therefore necessary to isolate the factor or factors responsible. Attention may need to be given to factors that are not normally included in the methods section of a paper, such as animal handling before testing.

In the current studies, only one of the NMDA receptor antagonists tested produced an analgesic response by itself. The high dose (3.0 mg/kg) but not the low dose (1.0 mg/kg) of the competitive antagonist LY235959 produced a mild increase in tail-flick latencies. These results are consistent with the results of Bhargava and Thorat who found that LY235959 produced analgesia at 2.0 mg/kg (Bhargava and Thorat, 1997). On the other hand, others have found no analgesia from systemic administration of LY235959 in this dose range (Allen and Dykstra, 1999; Bhargava, 1997; Bilsky et al., 1996). The reason for these differences is presently unclear. Given that other competitive NMDA receptor antagonists have not been found produce analgesia, it appears that this effect may be specific to LY235959 rather than due to site-specific blockade of the NMDA receptor. However, future studies should carefully assess the analgesic response to other competitive NMDA receptor antagonists to determine if these compounds have the ability to produce analgesia under conditions similar to those in the present experiments.

An additional notable observation from the present experiments is that enhancement of analgesia occurred more often when combining NMDA antagonists with fentanyl than with morphine. The literature offers some interesting contrasts between fentanyl and morphine that might help explain the differences between these opiates. Fentanyl is much more potent than morphine (Reisine and Pasternak, 1996; also present results) and more selective and more efficacious than morphine at μ-opioid receptors (Muijsers and Wagstaff, 2001; Reisine and Pasternak, 1996; Zuurmond et al., 2002). Additionally, recent research demonstrates that administration of morphine and fentanyl are accompanied by different downstream biochemical changes (Bot et al., 1998; Zaki et al., 2000), which could potentially produce different interactions with NMDA receptors. Differences between the effect of NMDA antagonists on morphine and fentanyl analgesia might also be explained by the degree to which the opiates produce hyperalgesia as an opponent process to their analgesic actions (Bespalov et al., 2001; Bespalov and Trujillo, 2002); as suggested by Celerier et al. (1999, 2000), fentanyl may induce NMDA receptor-mediated hyperalgesia to a greater extent than morphine. If this is the case, by inhibiting this NMDA receptor-mediated opponent process, enhancement of fentanyl analgesia by NMDA receptor antagonists would be expected to be more pronounced than morphine analgesia.

In conclusion, pharmacological adjuncts that enhance the analgesic effects of opiates would be of benefit for the treatment of pain, and the potential use of NMDA antagonists for this indication is alluring (Caruso, 2000; Chizh and Eide, 2002; Goldblum, 2000; Katz, 2000). This type of cotreatment could allow for a decrease in opiate dose, resulting in a reduction of side effects of acute opiate use

as well as problematic chronic consequences, including tolerance, sensitization, and physical dependence (Ahmedzai, 1997; Bespalov and Trujillo, 2002; Trujillo, 2003). Thus, opiate/adjunct combinations, if found to be safe and effective, have the potential to dramatically improve the lives of people in pain. However, the results of these studies suggest that enhancement of opiate analgesia by NMDA antagonists is idiosyncratic and therefore likely unrelated to NMDA receptor blockade. It therefore may be other properties of selected antagonists that allow them to potentiate analgesia to selected opiates. Future work should be aimed at identifying the factor or factors responsible for this potentially useful effect.

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