d-Methadone Blocks Morphine Tolerance and N-Methyl-D-Aspartate-Induced Hyperalgesia[1]

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ABSTRACT

Previous in vitro and in vivo studies have determined that the *d* isomer of methadone has *N*-methyl-D-aspartate (NMDA) receptor antagonist activity. The present studies examined the ability of *d*-methadone to attenuate the development of morphine tolerance in mice and rats and to modify NMDA-induced hyperalgesia in rats. A decrease in the percentage of mice analgesic (tail-flick response) after 5 days of once-daily morphine (7 mg/kg s.c.) was completely blocked by coadministration of d-methadone given s.c. at 10 mg/kg. Morphine given s.c. to mice on an escalating three times per day dosing schedule resulted in a nearly 3-fold increase in the tail-flick ED_{50} dose of morphine which was prevented by s.c. coadministered *d*-methadone at 15 mg/kg. In rats, intrathecal (i.t.) morphine produced a 38-fold increase in the ED_{50}, which was completely prevented by the coadministration of i.t. *d*-methadone at 160 µg/rat. A decrease in thermal paw withdrawal latency induced by the i.t. administration of 1.64 µg/rat NMDA was completely blocked by pretreatment with 160 µg/rat *d*-methadone. Thus, systemically coadministered *d*-methadone prevents systemically induced morphine tolerance in mice, i.t. *d*-methadone attenuates tolerance produced by i.t. morphine in rats, and i.t. *d*-methadone, at the same dose which modulates morphine tolerance, blocks NMDA-induced hyperalgesia. These results support the conclusion that *d*-methadone affects the development of morphine tolerance and NMDA-induced hyperalgesia by virtue of its NMDA receptor antagonist activity.

Methadone, a synthetic analgesic with morphine-like properties, has been shown to be involved in several *N*-methyl-D-aspartate (NMDA) receptor-mediated activities. Choi and Viseskul (1988) found that methadone could dose-dependently decrease the neurotoxicity produced when NMDA is applied to cortical cells. Ebert et al. (1995) found that methadone could significantly displace the binding of (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*b*]cyclo-hepten-5,10-imine hydrogen maleate (MK-801), a potent NMDA receptor antagonist, at the noncompetitive NMDA receptor site. This inhibition was nearly equipotent to dextromethorphan, an in vivo NMDA receptor antagonist. In addition, in both a rat cortical wedge and a neonatal rat spinal cord electrophysiological preparation, methadone dose-dependently blocked NMDA induced depolarizations with no significant effects on either α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid or kainate receptor-mediated activities (Ebert et al., 1995).

Methadone has an asymmetric carbon atom resulting in two enantiomeric forms, the *d* and *l* isomers. The racemic mixture (*dl*-methadone) is the form commonly used clinically and in laboratory studies. Studies that compared the effects of the individual isomers after i.c.v. injection concluded that the *l* isomer possesses analgesic activity, whereas the *d* isomer is inactive or weak as an opioid (Scott et al., 1948; Ingoglia and Dole, 1970). Consistent with these observations, the affinity of *l*-methadone for the µ-opioid receptor is 10 times higher than that of *d*-methadone and twice that of the racemic mixture. Both isomers have a low affinity for δ- and κ-opioid receptors (Kristensen et al., 1995). Gorman et al. (1997) examined the NMDA receptor-binding ability of the two methadone isomers. Both the *d* and *l* isomers were found to bind, with similar affinities, to the noncompetitive but not to the competitive site of the NMDA receptor in rat forebrain and spinal cord synaptic membranes. Morphine, hydromorphone, and naltrexone did not show affinity for this site on the NMDA receptor, indicating that this type of binding is not a prototypical property of all opioids. Recently, Shimoyama et al. (1997) found that while intrathecal (i.t.) *d*-methadone is inactive in the tail-flick test, it is antinociceptive in the rat formalin test. This antinociception is not affected by the opioid antagonist, naloxone, and appears to be a result of the NMDA receptor antagonist activity of *d*-methadone.

Given its in vitro and in vivo NMDA receptor antagonist activity, it is of interest to determine whether *d*-methadone can block morphine tolerance as has previously been shown

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; LY274614, MK-801, (+)-5 methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclo-hepten-5,10-imine hydrogen maleate; TPW, thermal paw withdrawal; i.t., intrathecal; PKC, protein kinase C.

for other NMDA receptor antagonists (Inturrisi, 1997). The purpose of this report is to determine whether systemic and/or i.t. *d*-methadone can block morphine tolerance. In addition, we demonstrate that *d*-methadone, at doses which modulate morphine tolerance, can also block NMDA-induced hyperalgesia.

Materials and Methods

Animals and Preparations

The i.t. studies were performed in adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 350 to 400 g and the systemic studies used adult male CD-1 mice ranging in weight from 30 to 35 g (Charles River Laboratories, Wilmington, MA). The rats were housed individually and the mice were housed five per cage. The animals were provided with ad libitum food and water. Temperature and light cycles were regulated. For the i.t. administration of drugs to the rat, a cannula was placed in the i.t. space at least 2 days before the experiments. Under halothane anesthesia, a PE-10 tube was inserted through a small hole made in the atlanto-occipital membrane and threaded 9 cm down the i.t. space to the lumbo-sacral level of the spinal cord (Shimoyama et al., 1996). A cannulated rat with any signs of paralysis was excluded from the study. At the end of the experiment, 10 μl of 1% methylene blue solution was introduced into the cannulas followed by 10 μl of saline to confirm the position of the cannulas and the spread of the dye in the i.t. space.

Drugs

The morphine sulfate was obtained from Mallinkrodt (St. Louis, MO) and was dissolved in 0.9% sterile saline. The *d*-methadone [(*S*)-(+)-methadone] was obtained from the Research Triangle Institute (Research Triangle Park, NC) through the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD). The free base was dissolved in 0.9% saline and the pH was adjusted to 6.0 with 1 N HCl. The *N*-methyl-D-aspartate was obtained from Research Biochemical International (Natrick, MA) and was dissolved in 0.9% saline. The pH of the final solution was adjusted to 7.0 with 1 N NaOH. For the i.t. studies, the morphine and *d*-methadone solutions were prepared alone and combined, as indicated below, to limit the total volume administered.

Tail-Flick Test

To assess the analgesic potency of morphine, the tail-flick test was used. The tail-flick apparatus (EMDIE, Richmond, VA) emits radiant heat to the tail at 2 cm from the tip in mice and 5 to 8 cm in rats. The time from the onset of heat to the withdrawal of the tail (tail-flick latency) was measured. The intensity of the radiant heat was adjusted so that the baseline latencies were between 2.5 and 3.5 s. To avoid causing tissue damage, the heat stimulus automatically turned off at 10 s (cut-off latency). A mean tail-flick latency was calculated from two repeated measurements. Baseline latency was obtained before drug administration. Subsequent response latencies were determined at the time of peak analgesia, which was 15 min after i.t. morphine and 30 min after s.c. morphine. The latency data were converted to a quantal form by determining the percentage of analgesic responders in each group (see below).

Dose-Response Studies

After measuring the baseline tail-flick latencies, increasing doses of morphine were administered until each animal responded to the analgesic [cumulative dose-response assessment (CDR)] (Elliott et al., 1994a,b). An analgesic responder was defined as one whose mean response tail-flick latency was at least twice the value of the mean baseline latency. The percentage of analgesic responders in the group for each cumulative dose was calculated, and a cumulative dose-response curve was constructed. The changes in the median effective dose (ED_{50}) of morphine determined from the curves were used to express the changes in the relative potency of morphine.

NMDA-Induced Hyperalgesia

The change in thermal paw withdrawal (TPW) latency (hyperalgesia) was used to assess the response to intrathecally administered NMDA (Hargreaves et al., 1988). In the TPW test, a rat is placed on an elevated glass surface covered by an inverted plastic cage. A radiant heat source is focused on the plantar surface of the hindpaw and the time until the rat withdraws its hindpaw is automatically determined. A cutoff time of 30 s is imposed to avoid tissue damage, and the intensity of the heat source is adjusted so that baseline latencies are between 10 and 15 s. The testing was done on each hindpaw and these results were averaged for each time point. The mean control TPW latency was 12.8 ± 1.4 (S.D.) s ($n = 14$). A preliminary dose-response study identified 1.64 μg/5 μl/rat (11.1 nM) as the dose of NMDA that produced a decrease in mean TPW latency of approximately 8.8 s ($n = 14$).

Experimental Paradigms

The experimenter was blinded to the treatment groups in each experiment.

Study 1. The effects of systemically administered *d*-methadone on morphine tolerance was assessed using two different paradigms in mice. In the first paradigm (A) morphine tolerance was produced by a once-daily s.c. injection of 7 mg/kg morphine for 5 days. In addition to the saline + morphine treatment group, a second group received *d*-methadone at 10 mg/kg s.c. 30 min before morphine for 5 days (*d*-methadone + morphine). To complete the study, a group received *d*-methadone followed 30 min later by saline (*d*-methadone + saline) and another group received saline followed by saline (saline + saline). Each treatment group included 20 mice. The baseline tail-flick latency was determined for each group on days 1 and 5. The response to morphine was determined for the saline + morphine and the *d*-methadone + morphine groups on days 1 and 5.

In the second paradigm (B), tolerance to morphine and the ability of *d*-methadone to attenuate this response was also determined using a 3 times per day, escalating schedule of morphine dosage. On the morning of day 1, each animal received 10 mg/kg morphine s.c. at 10:00 AM or underwent a CDR using s.c. morphine to determine their ED_{50} value for morphine. Each analgesic responder was not subjected to further tail-flick assay but received the subsequent dose of morphine so that each animal received the same opioid dose, approximately 10 mg/kg on the morning of day 1. On that same day, 10 mg/kg morphine was administered two more times. On day 2, the animals received 20 mg/kg morphine s.c. three times per day; on day 3, the animals received 40 mg/kg morphine s.c. three times per day. *d*-Methadone at a dose of 15 mg/kg or saline was administered s.c. 30 min before each morphine injection on days 1 through 3. On day 1, the first dose of *d*-methadone or saline was administered before the CDR assessment. On day 4, all of the animals underwent the morphine CDR without first receiving *d*-methadone or saline. The development of morphine tolerance was determined by comparing the morphine ED_{50} values obtained on day 4 to those of day 1. There were 30 mice in each treatment group.

Study 2. This experiment was conducted to determine whether i.t. *d*-methadone could attenuate tolerance produced by i.t. morphine. A paradigm similar to study 1 (B) was used to produce i.t. morphine tolerance in rats. On the morning of day 1, each animal underwent a CDR with i.t. morphine to determine the morphine ED_{50} value. After this procedure, the animals received additional doses of i.t. morphine so that each animal received the same dose of morphine, approximately 10 μg on the morning of day 1. Ten micrograms of morphine or saline was administered twice more on day 1. Twenty and 40 μg of morphine or saline was administered intrathecally three times per day at 10:00, 14:00, and 19:00 on days 2 and 3, respectively. *d*-

Methadone at 160 μg or saline was administered intrathecally combined in solution with each morphine injection on days 1, 2, and 3. However, d-methadone was only administered after the CDR on the morning of day 1 with the subsequent dose of morphine. Thus, the treatment arms included saline + morphine, d-methadone + morphine, and d-methadone + saline. Each animal was tested for its analgesic response to morphine, and the morphine ED_{50} value was determined again on day 5. This was done on day 5 because the return of tail-flick latencies to baseline values required more than 24 h after the last 40-μg i.t. dose of morphine on day 3. The development of morphine tolerance was determined by comparing the morphine ED_{50} values obtained on day 5 to those obtained on day 1. The treatment groups averaged 18 rats each.

Study 3. This study was designed to determine whether d-methadone, at the same i.t. dose as was used in study 2 (160 μg/rat or 640 nM), can affect NMDA-induced hyperalgesia. There were four treatment groups for this study: saline + saline, saline + NMDA, d-methadone + saline, and d-methadone + NMDA. The procedure involved allowed the rat to remain on the test surface for 30 min before the measurement of a baseline withdrawal latency. The pretreatment, either saline or d-methadone, was then administered. The TPW latency was determined following the injection and 5 min later (time points labeled −10 min and −5 min, respectively, Fig. 3). NMDA, at 1.64 μg/rat or 11.1 nM, was then administered (labeled time 0, Fig. 3) and the TPW latency was determined every 5 min for the next 30 min. Fourteen rats were used in this complete crossover study and the treatment order was randomly assigned. At least 24 h was allowed between treatments. No difference was obtained in baseline TPW latencies as a function of treatment day.

Data Analysis

The quantal dose-response data were analyzed using the BLISS-21 computer program. This program maximized the log-likelihood function to fit a parallel set of Gaussian normal sigmoid curves to the dose-response data and provides ED_{50} values, 95% CL and relative potency estimates (Umans and Inturrisi, 1981). Single-dose comparisons were analyzed using Fisher's exact test. The data from study 3 were analyzed at each time point by a one-way ANOVA,

and the appropriate post hoc tests were conducted. Statistical significance was accepted at $P < .05$.

Results

Study 1: Effects of Systemic d-Methadone on Morphine Tolerance in Mice. Fig. 1 shows the percentage of mice analgesic on treatment days 1 and 5. In the saline + morphine group ,70% of the mice were analgesic on day 1 but by day 5 only 5% continued to respond to this dose of morphine. This difference is significant ($P < .05$) and indicates that on treatment day 5 tolerance has developed to the analgesic effects of morphine. Concurrent administration of d-methadone completely prevented the development of tolerance so that there was no significant difference in the response of the d-methadone + morphine group on treatment days 1 and 5. d-Methadone or saline given alone did not produce an analgesic effect in this paradigm (see also Elliott et al., 1994a). No significant difference was observed between the response of the saline + morphine group and the d-methadone + morphine group on day 1 (Fig. 1).

Table 1 shows that the escalating morphine dose paradigm resulted in a nearly 3-fold increase in the morphine ED_{50} value ($P < .05$) of the saline + morphine group on day 4 as compared to the day 1 saline group. In contrast, coadministration of d-methadone with morphine completely prevented the development of morphine tolerance as assessed by the ED_{50} value. Compared to the saline controls, d-methadone treatment alone did not alter the ED_{50} values for morphine as assessed on treatment days 1 and 4.

●   Day 1 Saline + Morphine

○   Day 5 Saline + Morphine

▼   Day 1 d-Methadone 160 ug/rat + Morphine

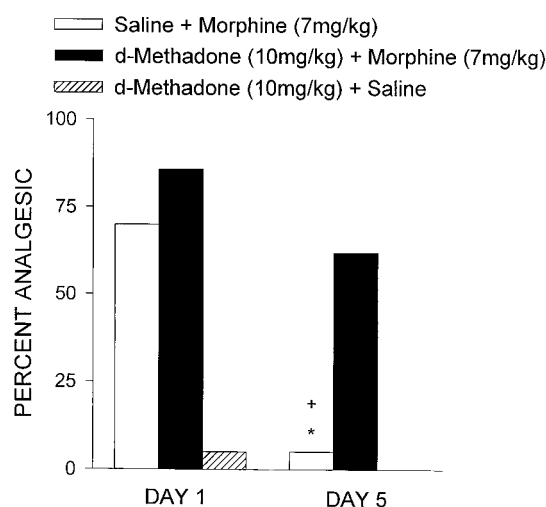▽   Day 5 d-Methadone 160 ug/rat + Morphine

Fig. 1. Attenuation of morphine tolerance by pretreatment with d-methadone. d-Methadone at a dose of 10 mg/kg or saline was administered s.c. 30 min before each morphine injection (7 mg/kg) on days 1 to 5. The percentage of analgesic responders in the d-methadone + morphine group was significantly different (*$P < .01$) from the saline + morphine group on day 5. The saline + morphine group response on day 5 was significantly different (*$P < .005$) from the saline + morphine group response on day 1. The saline + saline treatment group showed no analgesic response or change in tail-flick values on day 1 or 5 (data not shown).
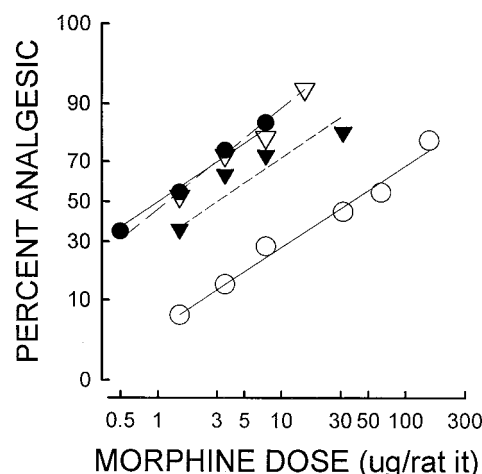

Fig. 2. Intrathecal d-methadone given in a dose of 160 μg (640 nM) prevents the rightward shift in the i.t. morphine dose-response curve on day 5. Tolerance was produced by administering morphine three times per day at 10 μg on day 1, at 20 μg on day 2, and at 40 μg on day 3. d-Methadone or saline was administered concurrently with each morphine dose. On day 1, cumulative morphine dose-response assessment preceded each treatment. On day 5, the saline + morphine curve shifted 38 times, whereas the curve for day 5 days d-methadone was not significantly different from the curve from day 1 before treatment (see Table 2 for ED_{50} values).

TABLE 1

d-Methadone prevents the development of tolerance to the analgesic effect of morphine

Tolerance was produced by the administration of morphine 3 times per day at 10 mg/kg on day 1, 20 mg/kg on day 2, and 40 mg/kg on day 3. *d*-Methadone at 15 mg/kg or saline was administered 30 min before each morphine dose. On day 4, the *d*-methadone or saline pretreatment was omitted and the ED_{50} values for morphine, with the 95% confidence interval, were determined via a cumulative dose-response assessment.

Treatment	Morphine		
	Day	ED_{50} (s.c.)	95% Confidence Interval
		mg/kg	
Saline	1	3.7	3.0–5.6
d-Methadone	1	4.0	3.0–5.1
Saline + saline	4	4.5	3.6–5.6
Saline + morphine	4	10.9[a]	8.9–13.3
d-Methadone + saline	4	3.4	2.7–4.2
d-Methadone + morphine	4	5.9[b]	4.8–7.2

[a] Significantly different ($P < .05$) from day 1 saline group.
[b] Significantly different ($P < .05$) from day 4 saline + morphine group.

Study 2: Effects of i.t. *d*-Methadone on i.t. Morphine Tolerance in Rats.

Figure 2 shows that the dosing paradigm which utilized an increasing i.t. morphine dose for 3 days resulted in a shift to the right of the morphine dose-response curve on day 5 (saline + morphine). The magnitude of this development of tolerance is given in Table 2 in terms of an increase in the ED_{50} values. The relative potency of morphine was decreased approximately 38-fold on day 5 in the saline + morphine group. In contrast, no significant shift was seen in the dose-response curve (Fig. 2) or in the morphine ED_{50} value (Table 2) when *d*-methadone was coadministered with each dose of morphine during the 3-day treatment period.

Study 3: Effects of *d*-Methadone on NMDA-Induced Hyperalgesia.

Figure 3 shows the time course of the paw withdrawal latency before and after the i.t. injection of NMDA. The injection of saline at −10 min did not alter subsequent measures over the 40 min. When the saline injection was followed by the administration of NMDA at time 0, a significant decrease in TPW latency was observed at 5, 10, and 15 min after NMDA. The peak of NMDA hyperalgesia occurred at 5 min after NMDA, the earliest time postdrug we were able to measure the TPW latency (Fig. 3). The NMDA-induced decrease in TPW was completely blocked by pretreatment (at −10 min) with *d*-methadone at 160 μg/rat.

TABLE 2

Intrathecal *d*-methadone prevents the development of tolerance to the analgesic effect of morphine

Tolerance was produced by the administration of escalating i.t. doses of morphine 3 times per day at 10 μg/rat on day 1, 20 μg/rat on day 2, and 40 μg/rat on day 3. The dose of *d*-methadone was 160 μg/rat. On day 5, the ED_{50} values for morphine, with their 95% confidence interval, were determined via cumulative dose-response assessment.

Treatment	Morphine		
	Day	ED_{50} (i.t.)	95% Confidence Interval
		μg/rat	
Saline + morphine	1	1.0	0.5–1.8
Saline + morphine	5	37.7[a]	25.9–56.7
d-Methadone + morphine	1	3.1	1.4–6.6
d-Methadone + morphine	5	1.4[a]	0.7–2.7

[a] Significantly different ($P < .05$) from day 1 saline + morphine group.
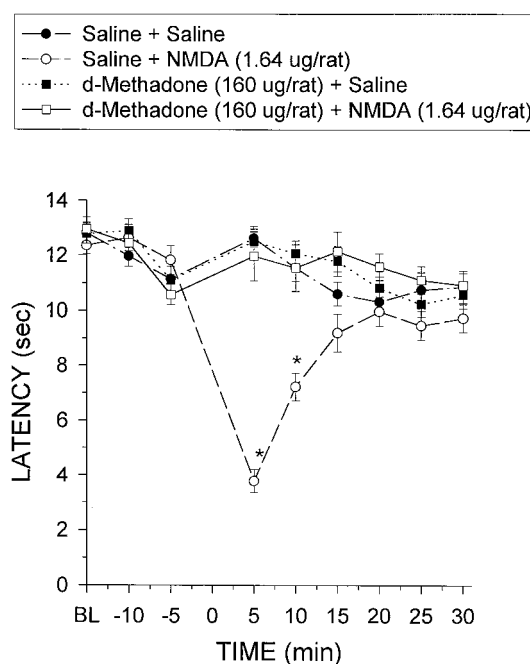[b] Significantly different ($P < .05$) from day 5 saline + morphine group.

Fig. 3. *d*-Methadone blocks the hyperalgesic effects of i.t. NMDA in the rat TPW test. *d*-Methadone at 160 μg/rat (640 nM) or saline was given 10 min before (time −10) the i.t. injection of 1.64 μg/rat NMDA (11.1 nM) at time 0. TPW latency was measured before drug administration (baseline) and every 5 min after administration for a total of 40 min. The saline + NMDA group showed a significant decrease in TPW latency at 5, 10, and 15 min after NMDA administration (*$P < .05$). Pretreatment with *d*-methadone completely prevented this effect. Saline + saline and *d*-methadone + saline treatments showed no change in TPW latency over the entire testing period.

The i.t. injection of *d*-methadone followed by saline did not alter the TPW latency (Fig. 3).

Discussion

It has been well established that NMDA receptor antagonists can block morphine tolerance (Inturrisi, 1997). NMDA receptor antagonists acting at the competitive [e.g., (±)-6-phosphonomethyl-decahydroisoquinolin-3-carboxylic acid (LY274614)], the noncompetitive (e.g., MK-801 and dextromethorphan), and glycine site (e.g., 1-aminocyclopropane carboxylic acid) can all attenuate the development of morphine tolerance (Inturrisi, 1997). Based on the work presented here, systemic *d*-methadone can also block morphine tolerance using well established paradigms (Elliott et al., 1994a,b). The doses of *d*-methadone (10 and 15 mg/kg) used in the once-daily and three times per day dosing paradigms, were selected after demonstrating that these doses of *d*-methadone do not significantly affect tail-flick latency (see also Fig. 1 and Table 1).

To define one site of action for *d*-methadone, it was administered intrathecally. Figure 2 and Table 2 show that *d*-methadone can block morphine tolerance when given intrathecally, as well as, systemically (Fig. 1 and Table 1). Other NMDA antagonists (e.g., ketamine and MK-801) have been shown to act intrathecally to block morphine tolerance (Shimoyama et al., 1996; Dunbar and Yaksh, 1996). The tolerance paradigm used in the Shimoyama et al. (1996) report is the same as the one used here in study 2.

To define a mechanism of action for *d*-methadone, an examination of its ability to block NMDA-induced hyperalgesia was conducted. Intrathecally administered NMDA results in thermal hyperalgesia, caudally directed scratching, biting, and licking, as well as vocalization at higher doses (Okano et al., 1993). We found that *d*-methadone, at the same dose (160 μg/rat) that could block morphine tolerance, could significantly attenuate NMDA-induced hyperalgesia. From this we can conclude that *d*-methadone is acting as an NMDA receptor antagonist both in its attenuation of hyperalgesia and its blockade of morphine tolerance both systemically and intrathecally. Furthermore, support for the NMDA antagonistic actions of *d*-methadone comes from a recent report that *d*-methadone can block phase 2 of the formalin test by a non-opioid (i.e., naloxone insensitive) mechanism (Shimoyama et al., 1997). Phase 2 of the formalin test is the result of central sensitization which has been shown to be an NMDA receptor-mediated phenomenon that can be blocked by an NMDA receptor antagonist (e.g., ketamine, dextromethorphan, or MK-801) (Dickenson, 1994; McCall et al., 1996). The same dose that was effective in the blockade of phase 2 of the formalin test by Shimoyama et al. (1997), 160 μg/rat i.t., was also shown to block i.t. morphine tolerance in study 2 of this work.

At the cellular level, there are several lines of evidence linking opioid receptor events and NMDA receptor-mediated activities to the induction of morphine tolerance. The slow time course (2–4 days) necessary for the reversal of morphine tolerance by an NMDA receptor antagonist is suggestive of a mechanism of morphine tolerance involving intracellular biochemical events mediated by NMDA receptor activation leading to plastic changes in the central nervous system (Inturrisi, 1997). Support for this mechanism comes from Chen and Huang (1991) who show that within a single neuron, expressing both the μ and NMDA receptors, the magnitude of the NMDA receptor-mediated inward membrane current is enhanced by the selective μ-opioid receptor agonist D-Ala^2-MePhe^4-Gly-ol^5-enkephalin. Furthermore, they demonstrate that μ receptor activation results in the translocation and activation of protein kinase C (PKC). The translocated PKC can then phosphorylate the NMDA receptor, leading to the removal of the Mg^{2+} blockade and allowing the NMDA receptor to be activated (Chen and Huang, 1992). Opening of the NMDA receptor channel causes an increase in the influx of Ca^{2+}, thereby increasing the intracellular Ca^{2+} concentration. Increases in intracellular Ca^{2+} have been shown to occur in the brain synaptosomes of morphine-tolerant mice (Welch and Olson, 1991). The PKC may also be involved in additional downstream events including the modulation of G protein-coupled K$^+$ channels and the uncoupling of G proteins at the μ receptor (Mao et al., 1995). Support for this comes from studies that demonstrated during morphine tolerance the spinal cord levels of membrane-bound PKC increase and GM1 ganglioside, an inhibitor of PKC translocation and activation, and H-7, a PKC inhibitor, can both attenuate morphine tolerance (Mao et al., 1994, 1995; Mayer et al., 1995).

Recently, Cai et al. (1997) found that incubation of cells with NMDA leads to an attenuation of the acute responsiveness of the δ-opioid receptor as evidenced by the inability of a δ-opioid receptor agonist, [D-Pen^2,D-Pen^5]enkephalin, to inhibit cyclic AMP production in the system. This effect of NMDA could be blocked by ketamine, a specific NMDA receptor antagonist. Furthermore, PKC inhibitors could also prevent the NMDA effect implicating the involvement of PKC in this phenomenon. No information is available as yet for cell lines expressing the μ-opioid receptor; however, if the NMDA receptor contributes to μ-opioid receptor responsiveness in a similar manner, this could explain the effectiveness of in vivo NMDA receptor antagonists in the prevention of the development of morphine tolerance.

The studies presented in this article demonstrate more directly that *d*-methadone has NMDA receptor antagonist properties. When NMDA is introduced directly into the spinal space, it results thermal hyperalgesia (Okano et al., 1993). This in vivo effect of NMDA involves the NMDA/nitric oxide cascade as evidenced from the ability of NMDA receptor antagonists and nitric oxide synthase inhibitors to block NMDA-induced hyperalgesia (Aanonsen and Wilcox, 1986; Kitto et al. 1992; Malmberg and Yaksh, 1993). Activation of NMDA receptors also causes the release of Substance P (Liu et al., 1997) and i.t. administration of Substance P itself can cause hyperalgesia. However, the hyperalgesia produced by i.t. NMDA is mediated by the activation of the NMDA receptor and not the Substance P receptor NK-1, since coadministration of a Substance P antagonist inhibits the hyperalgesic response induced by i.t. Substance P but not that induced by the i.t. NMDA (Okano et al., 1993). The primary role of the Substance P released in response to NMDA is to exacerbate and prolong the hyperalgesic response generated when NMDA interacts with postsynaptic receptors (Liu et al., 1997).

In conclusion, the results presented here support the conclusion that *d*-methadone affects the development of morphine tolerance and NMDA-induced hyperalgesia by virtue of its NMDA receptor antagonist activity. Our results suggest that *d*-methadone could be used alone or in combination with morphine to treat the hyperalgesic component of neuropathic pain, as well as improve the analgesic efficacy of chronically administered morphine by attenuating the development of morphine tolerance (Inturrisi, 1997).

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