Concurrent Spinal Infusion of MK801 Blocks Spinal Tolerance and Dependence Induced by Chronic Intrathecal Morphine in the Rat

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Background: MK801, an N-methyl-D-aspartate receptor antagonist, has recently been reported to attenuate tolerance to, and withdrawal from morphine. This study analyzes tolerance and withdrawal in a chronic intrathecal infusion model of morphine and MK801.

Methods: Intrathecal catheters, attached to 7-day miniosmotic infusion pumps, were implanted in rats and infused with saline, 20 nmol/h morphine, MK801 (10 and 3 nmol/h) + morphine, and 10 nmol/h MK801. Analgesia was measured on the hot plate daily. On the day 7, groups received 3 mg/kg intraperitoneal naloxone and six signs of withdrawal were assessed: vocalization to air motion or light touch, abnormal posture, spontaneous vocalization, escape attempts, "wet dog shakes," and ejaculations. Similar groups were tested only on days 1 and 7. Intrathecal morphine dose-response curves were obtained on day 8. A separate morphine-tolerant group received 10 nmol MK801 on day 7. Rats from each group received 10 nmol intrathecal morphine 1 week later.

Results: Coinfusion of MK801 with morphine resulted in a dose-dependent preservation of effect, and attenuated three of six signs of withdrawal. Coinfusion of MK801 (10 and 3 nmol/h) prevented the reduction of potency observed with morphine alone. ED₅₀ values (maximum percent effect, nmol morphine) were: saline (16), morphine (496), MK801 (10 nmol/h) + morphine (4), and 10 nmol/h MK801 (0.3). Acute administration of MK801 was ineffective in restoring sensitivity to morphine. One week after cessation of infusion, there was no significant difference between groups.

Conclusions: Chronic spinal MK801 attenuates tolerance to, and withdrawal from spinal morphine in a dose-dependent fashion, supporting the hypothesis that N-methyl-D-aspartate receptor activity plays a role in the reorganization of spinal function produced by chronic opioid receptor activation. Chronic intrathecal MK801 appears to sensitize the spinal cord to intrathecal morphine. (Key words: Analgesics, narcotic; dependence; morphine; spinal; tolerance. Receptors: N-methyl-D-aspartate.)

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In previous work, we characterized the loss of antinociceptive activity of opioids as a consequence of continuous spinal infusion using chronically implanted spinal catheters coupled to subcutaneous osmotic pumps. In these studies, the initiation of a chronic infusion resulted in an initial concentration-dependent increase in escape latency, which declined daily, returning to baseline by 4–6 days. This loss of effect occurred in the absence of intervening testing and, in spite of a continued and measured presence of spinal drug. Importantly, consistent with the inactivation of opioid-sensitive sites, examination of the animals’ responses to a bolus “probe” dose of morphine after chronic exposure revealed a right shift in the probe drug dose–response curve, with the magnitude of the right shift being proportional to the infusion concentration of the toleragen. That this right shift is not a consequence of a general dysfunction of the animal is indicated by the absence of a similar change in sensitivity to probe drugs acting on a separate receptor (e.g., \(\alpha_2\) agonists).

The specific aims of the current study were: (1) to attempt to reproduce previous findings and assess the effects of NMDA receptor antagonism on the model of continuous spinal opioid exposure, as well as the effects of daily testing on this model; (2) to quantify the magnitude of the tolerance development induced by chronic opioid exposure by assessing the degree of shift in the bolus dose–response curves of each group after a week of infusion (“probe dose–response curve”); (3) to assess the duration of effect of MK801 on morphine tolerance after its discontinuation; and (4) to assess naloxone-precipitated withdrawal in MK801-treated morphine-tolerant rats in an intrathecal model.

Methods

Animals

Approval for this study was obtained from the University of California San Diego Animal Care Committee. Male Sprague-Dawley rats (weighing 350–400 g) were implanted and thereafter housed in individual standard cages at room temperature on 12 h/12 h light/dark cycle (lights on 7:00 AM). Testing was performed during the light cycle from 12:00 noon. Animals had free access to food and water. Each rat was implanted as described later with a subarachnoid catheter attached to a subcutaneous osmotic pump filled with saline or drug(s). Rats were assigned to one of the other group randomly. All rats received a 7-day infusion and after testing on the last day were killed by an overdose of barbiturate.

Preparation of the Catheter with Infusion Pump and Implantation

The preparation of the catheter has been described previously. In brief, a 16-cm length of PE-10 tubing is connected by heat fusion with a hot air jet to a 2-cm length of PE-60 tubing. A 1-cm piece of Silastic tubing (Tucson, Norwalk, MA) previously soaked in chloroform to increase its internal diameter, is then passed over both ends of the PE-10 tubing so as to form a loop at a distance of 3 cm from the end of the PE10 tubing fused to the PE-60. The long end of the catheter is stretched to reduce diameter, soaked in alcohol (70%) overnight, and cut to a length of 9 cm from the silastic tubing. Alzet osmotic minipumps (model 2001 delivered 1 \(\mu\)l/h; Alza, Palo Alto, CA) were filled with drug(s) or saline and attached to the saline-flushed catheter. This pump is designed to deliver a constant infusion of 1 \(\mu\)l/h for 7 days after an initial activation period in the animal of 4 h. In vitro pilot studies at 37°C confirmed the accuracy of the pump with catheter attached. The catheter and pump were implanted between the hours of 9:00 AM and 12:00 PM, according to the procedure originally described for chronic catheterization of the rat spinal cord, with the additional modification of the subcutaneous osmotic pump. In brief, animals were anesthetized with halothane and placed in a stereotaxic head holder. A midline incision is made to expose the atlanto-occipital membrane. The membrane is pierced and the PE-10 end of the catheter is passed intrathecaally to a distance of 8.5 cm, that is, caudal to the level of the thoracolumbar junction. The pump is then attached to the PE-60 end of the catheter and implanted subcutaneously in a pouch to lie just behind one or the other shoulder. A 14-G needle is used to make a small hole in the forehead. The loop end of the catheter is passed through this hole. This PE-10 loop can then be cut and used to administer external doses of drug at the end of the 7-day infusion period. The wound is sutured, including a loose ligature at the base of the loop to prevent it from moving. Animals fully recovered 15–30 min after implantation. Those showing any signs of motor impairment were killed with an overdose of barbiturate.

Drugs and Injection

The following drugs were used for continuous spinal infusion: morphine sulfate (Merck, Sharp and Dohme, S. DUNBAR AND T. L. YAKSH

The study was divided into two phases: one during chronic morphine withdrawal designed to assess the effects of NMDA receptor antagonism on the subsequent right shift in probe dose–response curves of treatment groups. Phase I. In the first phase, rats were placed in the hot plate and then exposed at random on a daily basis to two 30 min sessions: 9:00 AM and 12:00 noon, corresponding to day 7; i.e., at the end of the 7th day of morphine administration. This phase of the experiment consisted of three groups, chosen, 3 nmol/h and 6 nmol/h saline, plus morphine. The higher dose was chosen to have the maximal withdrawal effect. The least number of rats was used in studies when administered saline. In our studies, we observed that 3 nmol/h resulted in moderate withdrawal symptoms. In cases of doses of 1 nmol/h, the effect on morphine withdrawal was significantly attenuated with 3 nmol/h morphine sulfate, 0.06 nmol/h of MK801, and saline. Two sets of this treatment group of rats were used to assess whether a smaller number of rats could be used to determine whether a drug restored morphine withdrawal. Bolus doses of 10 nmol/h bolus doses of morphine were ineffective and thus were not used to assess dependence. All three groups tested demonstrated a significant reduction in the withdrawal response following MK801 treatment.
SPINAL OPIOID TOLERANCE AND NMDA RECEPTOR

West Point, PA), and (+)MK801 hydrogen maleate (Research Biochemicals, Natick, MA). Drugs were dissolved in sterile normal saline. Drug doses, calculated as the free base, were expressed in nanomoles per hour (nm/h) for the infusion concentrations, or nanomoles per rat for the postinfusion dose-response studies. Morphine infusion concentration was 20 nm/μl/h in all animals receiving morphine because this dose was found in previous experiments to have yielded a near maximal increase in hot plate latency on day 7 after implantation.

Experimental Paradigms

The study was divided into two phases. Phase 1 was directed toward defining the change in response over time during chronic morphine infusion and the magnitude of the withdrawal response. Phase 2 was designed to assess the effects of daily handling on the nociceptive threshold at 7 days and to assess the subsequent right shift in probe dose-response curve as a function of treatment group.

Phase 1. In the first phase, animals were first tested on the hot plate and then implanted. Testing was carried out daily on the hot plate between the hours of 10:00 AM and 12:00 noon, the day of implantation, and 7 days after implantation. Two doses of MK801 were chosen, 3 nm/h and 10 nm/h as a coinfusion with morphine. The higher MK801 dose of 10 nm/h was found to have the maximal effect in attenuating tolerance with the least number of animals expressing side effects when administered in a pilot study. In preliminary studies, we observed that MK801 doses exceeding 25 nm/h resulted in weight loss and motor weakness, whereas doses of 1 nm/h or less were found to have little effect on morphine tolerance. Groups were thus examined with 3 nm/h MK801 + morphine sulfate, 10 nm/h MK801 + morphine sulfate, 10 nm/h MK801, morphine sulfate, and saline. A separate group of each treatment group of rats were tested only on days 0, 1, and 7 to assess if there was any effect on latencies from daily testing itself.

Finally, a group infused with morphine sulfate alone was given an intrathecal bolus dose of 10 nm MK801 on day 7 to determine whether acute intrathecal MK801 would restore morphine sensitivity in morphine-tolerant rats. Bolus doses higher than 10 nm produced side effects and thus were not studied.

To assess dependency in these animals, all rats in those groups tested daily on the hot plate were then entered into a withdrawal study as follows. At the end of the 7-day infusion period; i.e., on day 7 at 12:00 noon, rats from each group were given 0.3 mg/kg intraperitoneal naloxone and observed for a period of 1 h, in a circular transparent observation chamber, for the presence or absence of signs of withdrawal. The observer was blinded as to what group to which each rat belonged. Signs of withdrawal chosen for assessment had been previously determined to be prevalent in animals infused for 7 days with intrathecal morphine that received the same dose of naloxone. These signs were: (1) vocalization in response to air movement produced by blowing gently on the rat through a straw three times, or in response to light touch with a 3-cm piece of PE-10 tubing; (2) Spontaneous vocalization; (3) abnormal posture indicated by hind paw lift; 4. eczema; 5. had shaking, also often called 'wet dog shakes'; 6. escape attempts—one or more attempt to escape from the chamber. Animals were killed and dissected to assess the integrity of the catheter and the infusion system. Any animal found to have a blocked or misplaced catheter was eliminated from the study.

Phase 2. In phase 2, all groups were tested on the hot plate only on days 1, 2, and 7. Implanted rats were then entered into the following study. On day 7, after testing on the hot plate at approximately 12:00 noon, the external loop of catheter was cut, and the intrathecal part of the catheter flushed with 10 μl sterile normal saline. On day 8, 24 h after stopping the infusion, a single Probe dose of intrathecal morphine was administered. Based on preliminary studies, rats were given one of the following intrathecal probe doses of morphine; those infused with saline were given either 1 nm, 10 nm, or 100 nm/10 μl morphine; those infused with morphine alone were given 1 nm, 10 nm, 30 nm and 100 nm/10 μl morphine; those infused with MK801 10 nm/h + morphine were given either 0.01 nm, 0.1 nm, 1 nm, or 10 nm/μl morphine; those infused with MK801 alone were given either 0.01 nm, 0.1 nm, 1 nm, or 10 nm/μl morphine. Hot plate latencies were measured at 0, 30, 60, and 120 min. This assignment was continued until at least four or more rats in each group were obtained for each intrathecal probe dose, generating a dose-response curve for each group. Each rat was used only once.

Finally, rats from each of these groups were retested 1 week later, after cessation of infusion, to assess their response to an intrathecal probe dose of 10 nm of morphine on the hot plate. Hot plate latencies were measured at 0, 15, 30, and 60 min.
Antinociceptive Testing and Data Analysis

The effects of intrathecal infusions were assessed by the hot plate (HP) test. The HP apparatus was a water bath, the stainless steel surface of which was the test surface. This surface was maintained at a temperature of 52.5 ± 0.5°C by a proportional feedback controller. Licking of either hind paw, jumping with two feet off the hot plate, extreme agitation, or vocalization was taken as the end point. A cutoff time of 60 s was set to avoid tissue damage. Rats were assessed once for daily testing. For the second phase of the experiment after administration of the probe dose of morphine, testing was performed at 0, 30, 60, and 120 min.

Hot plate data are expressed either as mean latencies for each group, or as maximum percent effect (MPE). Maximum percent effect is calculated as follows:

\[
MPE = \frac{\text{post-drug latency} - \text{baseline} \times \text{cutoff time} - \text{baseline}}{\text{cutoff time}} \times 100
\]

where postdrug latency is the response measured at the particular time after initiation of infusion or after intrathecal dose of probe drug. Baseline is the preinfusion or preprobe latency, and the cutoff time is 60 s.

**Statistics**

Analysis of the dose-response curves and statistics were obtained with computer software programs (Abacus Concepts, StatView, Abacus Concepts, Berkeley, CA). Where applicable, data from hot plate testing, i.e., absolute latencies or calculated %MPE, were analyzed using one- or two-way analysis of variance (ANOVA) to detect differences between groups. When differences were found, these findings were subjected to Scheffe's F test (significant at 95%). Unless stated otherwise, single points of comparison were made using a standard paired or unpaired t-test. Using linear regression, calculation of the ED₅₀ (95% confidence intervals) test for relative potency was carried out where applicable. The tolerance ratio (the ratio of ED₅₀ in drug-infused animals to ED₅₀ of saline infused animals), and 95% confidence intervals were calculated. Differences yielding critical values corresponding to P = 0.05 were considered statistically significant.

**Results**

**Daily Hot Plate Response Latencies in Chronically Infused Rats**

**Saline-infused Rats.** There was no significant difference between latencies on days 1 and 7 in rats that were tested daily, i.e., on days 0 through 7 (phase 1 rats: 15 ± 1 vs. 15 ± 1 s, n = 5, P > 0.1; fig. 1A), or in those that were tested only on days 0, 1, and 7 (phase 2 rats) (15 ± 1 vs. 17 ± 1 s, n = 11, P > 0.1; fig. 1B).

Neither was there a significant difference between these two saline-infused groups (ANOVA, P > 0.1), demonstrating no significant effect after day 1, from implantation, infusion of the vehicle saline, or from daily testing. All saline-infused rats showed a small, but statistically significant, decrease in latency on day 1 compared with baseline (12 ± 1 vs. 15 ± 1 s, P < 0.05; fig. 1C).

**Morphine-infused Rats.** There was no difference between saline-infused rats and those tested on days 0, 1, and 7 (ANOVA, P > 0.1; fig. 1C). Neither was there a significant difference in saline-infused rats between testing days 1 and 7 (11 ± 1 and 12 ± 1 s, P > 0.1; fig. 1C).

**MK801-infused Rats.** There was no difference between saline-infused rats and those tested on days 1 and 7 (ANOVA, P > 0.1; fig. 1C). Neither was there a significant difference in saline-infused rats between testing days 1 and 7 (11 ± 1 and 12 ± 1 s, P > 0.1; fig. 1C).

**MK801 + Morphine-infused Rats.** There was a significant difference between saline-infused rats and those tested on days 1 and 7 (ANOVA, P < 0.05; fig. 1C). Neither was there a significant difference in saline-infused rats between testing days 1 and 7 (11 ± 1 and 12 ± 1 s, P > 0.1; fig. 1C).
testing. All saline-infused rats \( n = 16 \) were used, however, showed a small, but statistically significant rise in latencies on day 1 compared to baseline values on the day 0; \( 12 \pm 1 \text{ vs. } 15 \pm 1 \text{ s, } P < 0.05 \).

**Morphine-infused Rats.** There was no significant difference between morphine-infused rats tested daily \( n = 15 \), and those tested only on days 0, 1, and 7 \( n = 15 \); ANOVA \( P > 0.1 \), fig. 1). All morphine-infused rats, i.e., those included in phases 1 and 2 of the study, showed a maximal increase in HP latencies on day 1 over baseline on day 0 \( (45 \pm 3 \text{ vs. } 13 \pm 1 \text{ s}) \), returning to baseline values on day 7 \( (7 \pm 16 \text{ s, } 0 \pm 13 \text{ s}) \). There was no significant difference between latencies of all saline-infused rats \( n = 16 \) and all morphine-infused rats \( n = 30 \) on day 7 \( 16 \pm 1 \text{ vs. } 16 \pm 1 \text{ s, ANOVA } P > 0.1 \).

On day 8, 24 h after stopping the infusion, latencies of morphine-infused rats, tested only on days 0, 1, and 7, were significantly lower than those on day 7 \( (7 \pm 16 \text{ s, } 0 \pm 16 \text{ s}) \), ANOVA \( P < 0.05 \), Scheffe) and significantly less than those of the group of similarly tested rats receiving saline, on day 8 \( 7 \pm 16 \text{ s, } 0 < 0.05 \). (Scheffe, fig. 1B), demonstrating an increased thermal sensitivity in the morphine-infused rats only after discontinuation of the morphine infusion.

**Rats infused with 10 nm/h MK801.** Although 10 nm/h MK801-infused rats tested daily showed a small but significant increase in latencies on days 0, 1, 4, and 6, as compared to saline-infused rats tested daily (ANOVA \( P < 0.05 \), Scheffe), there was no significant difference in calculated % MPE between these two groups (ANOVA \( P > 0.1 \), fig. 1A). Also, there was no significant difference between the same group of MK801-infused rats on day 0, as compared to those on day 7 \( 15 \pm 1 \text{ s, } P < 0.01 \), or compared to those of saline-infused rats on day 7 \( 15 \pm 1 \text{ s, } P < 0.1 \). In addition, there was no significant difference between rats infused with MK801 alone and tested daily \( n = 6 \), as compared to a separate group of rats infused with MK801 alone, but only tested on days 0, 1, and 7 \( n = 17 \); ANOVA \( P > 0.1 \), fig. 1B).

**MK801 + Morphine-coinfused Rats.** Rats coinfused with 10 nm/h MK801 + 20 nm/h morphine that were tested daily showed a comparable increase in latencies on day 1 \( (37 \pm 5 \text{ s, } n = 11 \) that was not significantly different from morphine-infused rats that were also tested daily \( 10 \pm 4 \text{ s, } n = 15 \), \( P > 0.1 \), fig. 2). Latencies remained elevated in 10 nm/h MK801 + morphine-coinfused rats tested daily from days 1 through 7, and there was no significant difference between days 1 and 7 \( 37 \pm 5 \text{ vs. } 37 \pm 4 \text{ s, } P > 0.1 \), \( \text{n = 11} \). On day 8, 24 h after the infusion was stopped, latencies in rats tested only on days 0, 1, and 7, declined to near baseline values with only a small, but significant increase as compared to baseline on day 0 \( 18 \pm 1 \text{ vs. } 18 \pm 1 \text{ s, } P < 0.05 \). However, these latencies were not significantly different from saline-infused rats tested similarly \( (16 \pm 2 \text{ vs. } 11 \text{, } P > 0.1) \), but they were significantly higher than morphine-infused rats tested similarly \( (11 \pm 1 \text{ s, } n = 15 \), fig. 1B) .

Thus, coinfusion of MK801 at 10 nm/h with morphine resulted in maintenance of elevated latencies during infusion, which, on cessation of infusion, returned toward baseline but did not go below baseline values, as seen in morphine-infused rats.

Latencies in rats tested daily and infused with 3 nm/h MK801 and 20 nm/h morphine \( n = 9 \), were significantly increased over saline-infused rats, except on days 3 and 7 (ANOVA \( P < 0.05 \), Scheffe; fig. 2). These latencies also were significantly increased over those of morphine-infused rats from days 4 through 7 (ANOVA \( P < 0.05 \), Scheffe). Latencies observed in this group were numerically less but not significantly different than those of rats coinfused with the larger dose of 10 nm/h MK801 + morphine, except on day 1 (ANOVA \( P > 0.05 \)).

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Morphine-infused Rats Given Intrathecal MK801 Acutely on Day 7. Rats infused with morphine alone that were given a 10-nm bolus of intrathecal MK801 on day 7 (n = 4), failed to showed a significant increase in latencies when measured at 0, 30, 60, and 120 min; (17 ± 0.4 vs. 20 ± 1 vs. 19.5 ± 1, P > 0.1), thus demonstrating a lack of acute effect of MK801 in restoring sensitivity to morphine (fig. 3).

Withdrawal Studies. All groups tested daily on the hot plate were entered into a withdrawal study receiving 0.3 mg/kg naloxone intraperitoneally on day 8 (fig. 4). None of the rats infused with saline (n = 5) showed any of the six withdrawal signs examined. Two rats in the group that received MK801 alone (n = 6) showed one sign of withdrawal each. In contrast, morphine-infused rats (n = 7), all exhibited three or more of the six signs of withdrawal assessed. MK801 and morphine-infused rats (n = 11) showed a significant reduction in three of the six signs of withdrawal; i.e., vocalization to air motion or light touch, spontaneous vocalization, and paw lift when compared to morphine-infused rats (P < 0.05). MK801 (3 nm/h) and morphine-infused rats (n = 8) showed only a significant reduction in one sign of withdrawal, i.e., ejaculation when compared to morphine-infused rats.

Probe Dose-response curves
After severing the pump catheter connection on day 7 and flushing the catheter with 10 μl saline, 24 h was allowed to elapse and the rat was then given one of several doses of intrathecal morphine to generate the probe dose response curve.

Saline and MK801-infused Rats and Probe Dose Response. Twenty-four hours after terminating saline infusion, bolus doses of intrathecal morphine revealed a monotonic dose response curve with an ED_{50} of %MPE of 11 nm. In rats receiving MK801, the intrathecal morphine %MPE probe dose-response curve showed a 36-fold parallel left shift, as compared to saline-infused rats. The ED_{50} as measured by percent area under the curve (%AUC) also was decreased by 26-fold, showing an increased duration of effect, as well as peak effect (fig. 5 and table 1).

Morphine-infused Rats and Probe Dose Response. The intrathecal morphine %MPE probe dose-response curve in rats treated previously with 20 nm/h intrathecal morphine infusions displayed a significant right shift, such that the ED_{50} was increased by a factor of 14 as compared to the dose-response curve of saline-infused rats (fig. 5A and table 1). Probe doses greater than 150 nm of morphine began to produce a hyperalgesic response (e.g., spontaneous vocalization) as previously described. Thus, it was not possible to test even half log dose increase of intrathecal morphine probe over that of the highest dose given, i.e. 100 nm. The ED_{50} as measured by %AUC also was increased by a factor of 200 as compared to saline-infused rats (fig. 5B and table 1). This indicates that in morphine-tolerant animals, the peak effect as well as the duration of effect of the probe dose of spinal morphine is considerably reduced.

Morphine + MK801-infused Rats and Probe Dose Response. MK801 + morphine sulfate-infused rats showed a significant left shift in their dose-response curve, as measured by %MPE, such that the ED_{50} was significantly decreased by a factor of 60 as compared to that of morphine-infused rats (fig. 5A). ED_{50} as measured by %AUC was also significantly decreased by a factor of 264 as compared to morphine-infused rats (fig. 5B). ED_{50} as measured by %MPE or %AUC showed a small but not significant decrease compared to saline-infused rats (table 1). This indicates that in rats infused with morphine and MK801, sensitivity to peak effect and duration of morphine was not significantly different from that of saline-infused rats.

Response to Single Intrathecal Probe Dose of Morphine 7 Days after Cessation of Infusion. Four
or more rats in each group infused with saline, morphine, MK801 + morphine, and MK801 alone, and tested on days 0, 1, 7, and 8, were given a 10-nm intrathecal probe dose of morphine 7 days after discontinuation of their infusion, i.e., on day 15. There was no significant difference in HP latencies as measured by %MPE or %AUC between saline-infused rats (n = 4) and MK801 + morphine-infused rats (n = 4), MK801-infused rats (n = 5), or morphine-infused rats (n = 8) (P > 0.1, ANOVA, fig. 6). Thus, there was no residual evidence of hypersensitivity to morphine 7 days after termination of MK801 infusion, or of tolerance in morphine-infused rats when compared to saline control rats.

Discussion

Previous work has shown that the administration of MK801 can attenuate tolerance to, and withdrawal

Fig. 4. Time course of the antinociceptive effect expressed as percent maximum effect, of a single bolus of intrathecal morphine 1 week after discontinuation of a chronic infusion for 7 days of intrathecal morphine, 10 nm/h MK801, 10 nm/h MK801 with morphine, and saline. Each line presents the mean ± SE of five or more rats.

Fig. 5. Withdrawal signs after chronic infusion for 7 days of intrathecal morphine, 10 nm/h MK801, 10 nm/h MK801 + morphine, 3 nm/h MK801 + morphine, or saline. Each column presents the percent of each group of five or more rats ± SE, showing one of the following six signs of withdrawal: (1) vocalization in response to air motion or light touch, (2) spontaneous vocalization, (3) abnormal posture, (4) ejaculation, (5) "wet dog head shakes," (6) escape attempts.
Table 1. ED₉₀ and 95% Confidence Interval for Probe Intrathecal Morphine Response Curves (% MPE) Assessed after 7-Day Infusions of Saline, Morphine, MK-801 10 nmol/h + Morphine, and MK-801 Alone-infused Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>ED₉₀ % MPE (95% CI)</th>
<th>Tolerance Ratio (ED₉₀ drug/saline ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (nmol)</td>
<td>11 (6–20)</td>
<td>1.0</td>
</tr>
<tr>
<td>Morphine (nmol)</td>
<td>159 (42–594)</td>
<td>15 ± 12</td>
</tr>
<tr>
<td>MK-801 + morphine (nmol)</td>
<td>4 (1–15)</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>MK-801 (nmol)</td>
<td>0.3 (0.1–0.5)</td>
<td>0.27 ± 0.01</td>
</tr>
</tbody>
</table>

from, chronic systemic and spinal morphine administration. These previous studies, while insightful, typically employ bolus drug delivery or pellet systems that produce widely changing drug concentrations over a brief period of time. To define the properties of the reported effects of NMDA antagonism on opioid tolerance and dependence, we initiated studies employing the well-characterized model of continuous spinal delivery in the rat. Continued exposure of the spinal cord to a fixed concentration of morphine will result in (1) a progressive loss of spinal opioid receptor-mediated antinociception, with a reliable right shift in the dose-response curve for bolus doses of spinal morphine, and (2) signs of withdrawal after precipitation with naloxone, including vocalization to touch, spontaneous vocalization, abnormal posture, ejaculation, head shaking, or escape attempts. Intrathecal MK-801, a use-dependent blocker of the NMDA ionophore, has no effect on the hot plate response, and will not acutely increase the response latency when given in a rat that has been rendered tolerant by chronic exposure to spinal morphine (current study). However, as demonstrated in the current study, when delivered concurrently with morphine, but not as a bolus after loss of opioid activity, it preserves, in a dose-dependent fashion, the antinociception produced by morphine. These results suggest that during continued opioid exposure there is a change in spinal function leading to a reduction in opioid responsiveness and this reorganization is mediated in an essential fashion by the activation of the NMDA glutamate receptor.

Spinal Opioid Tolerance

In the current model, loss of effect, and a right shift in the dose-response curve to intrathecal morphine, was observed in morphine-infused animals that were tested daily or tested only at the beginning and end of the infusion. Thus, it appears that the significant effects are not attributable to behavioral conditioning, where pairing of drug administration and environmental cues may play a role in the apparent progressive loss of drug effect. Changes in spinal morphine kinetics and metabolism also appear unlikely to account for the progressive reduction. Similarly, the ability to induce antinociception when agonists for other receptors are delivered in a morphine-tolerant rat appears to exclude a "nonspecific" change in nociceptive processing. Pharmacologically, chronic opioid exposure results in a right shift of the dose-response curve and frequently a reduction in the maximum achievable effect. Importantly, the magnitude of the right shift is inversely proportional to the intrinsic efficacy of the agonist as determined in experiments with spinally delivered μ-opioid and δ-opioid agonists. Such changes are consistent with a reduction in the number of receptors, or in coupling of the receptors to the intracellular second messengers that mediate the respective actions of these agonists. Binding studies often have shown a modest reduction in binding with chronic opioid exposures, but these results have not been consistent.

Mechanisms of N-methyl-D-aspartate Antagonist-Morphine Interaction

The current studies were carried out only with MK-801. However, results with systemic delivery also have been observed with a structurally distinct competitive NMDA antagonist, LY-274614. This joint action supports the synergistic role for the role of the NMDA ionophore with which these two agents are known to interact with high affinity. Hot plate latencies in morphine + MK-801-infused rats returned to baseline after discontinuation of infusion. This argues against any permanent neurologic effect that might have accounted for the observed persistence of antinociception. Any synergistic action between MK-801 and morphine would appear unlikely because: (1) MK-801 given alone had no observable effect, (2) MK-801 when administered with morphine as a coinfusion had no effect on latencies on the second day of infusion, (3) MK-801 when delivered as a bolus failed to increase hot plate latencies in the morphine-tolerant rat (4) Investigations by other previous studies have shown no simple additive effect of MK-801 and morphine in intrathecal studies, or in systemic studies, or in spinalized rats. In the formalin test in which MK-801 is active, bolus spinal injections of MK-801 and morphine do not appear to interact to any observable degree.

These data suggest that MK-801 to maintain the potentiation of spinal morphine requires a low concentration of naloxone. Thus, this potentiation may be mediated by the conversion of a "nonspecific" change in nociceptive processing to a "specific" change and/or a change in the ratio of receptor subtypes.

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Fig. 6. Dose response curves (log) of percent spinal morphine or saline in rats from 0 to 100 mg/kg of MK-801 and saline in rats from 0 to 100% of spinal morphine, or saline

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SPINAL OPIOID TOLERANCE AND NMDA RECEPTOR

Fig. 6. Dose response expressed as percent maximum effect (top) and percent area under concentration (bottom) of intrathecal morphine after chronic infusion for 7 days of intrathecal morphine, 10 mg/h MK801, 10 mg/h MK801 with morphine, or saline. Each dose-effect point represents the mean ± SE with four or more rats.

The apparent preservation of the effects of morphine in the presence of MK801 may reflect a concurrent increase in sensitivity to opioid receptor occupancy. Thus, in the current studies, we unexpectedly observed that the chronic delivery of MK801 alone resulted in a left shift of the morphine dose response that was present 24 h, but not 7 days after termination of chronic delivery. This has not, to our knowledge, been hitherto reported, and may have direct clinical implications should drugs of this class enter clinical practice. The persistence of this effect at a time in which MK801 was not present, emphasizes that the effect is unlikely to be due to altered kinetics, and most likely reflects a trophic change in spinal opioid action. We are not aware of any studies in which binding was examined with chronic NMDA antagonists exposure. Upregulation of receptor number with chronic antagonism has been reported for several systems, but not, to our knowledge, for NMDA sites. In any case, given the antagonistic effects we would expect from a more efficient NMDA receptor-linked system, increased NMDA sites or glutamate release would not likely account for the enhanced responsiveness to morphine, per se. These results thus raise the possibility that NMDA antagonists may not only prevent the processes that lead to a reduction in morphine sensitivity, but with chronic exposure result in a facilitation of the effects of receptor coupling.

Glutamate is a principal excitatory amino acid acting on the NMDA receptor. If glutamate receptor activity is necessary for, or facilitates development of the tolerant state, then delivery of an opioid should either increase spinal glutamate release or augment receptor effects of resting glutamate levels. In the unanesthetized rat, we have observed that spinal infusion of morphine for 6 days does not affect resting release of spinal excitatory amino acids in dialyse. However, at precipitation of withdrawal, there is a large increase in glutamate release, which is blocked by clonidine and MK801. These results are consistent with the effects observed with opioid tolerance on spinal C-fos activity where acute delivery of systemic morphine has little effect on resting C-fos, although it blocks stimulation evoked increases but during withdrawal there is a massive increase in C-fos activity. Alternatively, if spinal glutamate release is not augmented by morphine then it is possible that the postsynaptic effects are augmented. Activation of the NMDA receptor increases entry of extracellular calcium into the cell, which stimulates the production of nitric oxide. Inhibition of nitric oxide synthetase has been shown to attenuate some signs of withdrawal in morphine-tolerant rats, i.e., wet dog shakes and weight loss. Thus, it is possible that NMDA receptor antagonism could act to prevent tolerance and withdrawal by inhibition of nitric oxide synthetase. Activation of the NMDA receptor also evokes
a spinal release of glutamate, which can be blocked by the nitric oxide synthetase inhibitor L-nitro-L-arginine methyl ester.  

**Spinal N-methyl-D-aspartate Antagonism and Withdrawal**

As previously reported with systemic drug delivery, after chronic exposure to morphine, rats displayed a clear hyperalgesia and signs of behavioral agitation directed at the caudal dermatomes of the body during withdrawal. This suggested a hyperalgesic component in the tolerant state. Such hyperpathia has been recently reported by other investigators. With systemic delivery, acutely delivered spinal MK801 prevented some but not all of these signs, suggesting that during withdrawal there is a particular increase in glutamate release. As noted earlier, we recently showed that, using spinal dialysis in unanesthetized rats, naloxone treatment results in a prominent release of glutamate in animals rendered tolerant to morphine concentrations and treatment schedules identical to those employed here. This occurs with a time course that precisely parallels the withdrawal signs associated with the precipitated abstinence syndrome. Importantly, the observed hyperalgesia during withdrawal and its sensitivity to spinal NMDA antagonism also is consistent with the ability of intrathecal NMDA to evoke hyperalgesia in the non tolerant rat.  

Intracellular Mechanisms of Opioid Tolerance and the N-methyl-D-aspartate Receptor  

Several changes in the receptor-effector mechanism have been proposed as a cause of tolerance. Morphine by its action at the µ opioid receptor produces an inhibition of adenylyl cyclase activity and thus a resultant decrease in cyclic adenosine monophosphate formation.  

**Spinal Receptor Tolerance and Glutamate**

The current studies demonstrate that in the face of stable morphine concentrations, the loss of effect produced by spinal delivery is prevented by antagonism of the NMDA ionophore. As reviewed earlier, this emphasizes the role of glutamate release in the tolerance process. This simple consideration leads to the speculation that increased glutamate release may exacerbate the loss of response to the opioid. Such a consideration has surprising theoretical implications regarding two clinically relevant issues. First, considerable evidence suggests that a variety of pain states, such as observed after the injection of formalin into the paw or carrageen into the knee, may lead to a hyperalgesia that is reversed by NMDA antagonism. Consistent with this pharmacology, such stimuli have been shown to be associated with an increase in spinal glutamate release.

References

1. Trujillo KA, Abbraccio MP. Effects of NMDA receptor antagonists (ketamine, MK801) on the withdrawal syndrome in the rat. J Pharmacol Exp Ther 1989; 250: 146-152.
2. Mark P, Ben- opposing evidence. The authors thank the following for their contributions: MPS, SP, and ASSB.
3. Mark P, Ben- opposing evidence. The authors thank the following for their contributions: MPS, SP, and ASSB.
5. Mark P, Ben- opposing evidence. The authors thank the following for their contributions: MPS, SP, and ASSB.
6. Mark P, Ben- opposing evidence. The authors thank the following for their contributions: MPS, SP, and ASSB.
7. Mark P, Ben- opposing evidence. The authors thank the following for their contributions: MPS, SP, and ASSB.
be associated with a marked stimulus-dependent increase in spinal glutamate, which is prevented by opioids. It thus seems appropriate to consider that such pain states might in fact exaggerate the evolution of tolerance. This hypothesis is in direct conflict with previous comments that pain may diminish tolerance development and that this may somehow account for the difference in apparent rates of tolerance development observed in preclinical studies (using acute experimental pain models) and chronic clinical pain states. Second, as noted, in the face of transient opioid withdrawal, glutamate may show prominent increases. We thus speculate that in the case of bolus opioid dosing, such peaks and valleys of effect may in itself be associated with an enhanced evolution of the tolerant state. Such consideration would suggest that steady-state drug concentrations may under certain circumstances be superior to bolus delivery. These considerations provide a testable hypothesis with relevance both to our understanding of the neurobiology of drug tolerance as well as to the clinical importance of drug delivery paradigms.

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References
S. DUNBAR AND T. L. YAKSH

42. Chen L, Huang LY: Protein kinase C reduces Mg2+ block of NMDA-receptor channels as a mechanism of modulation. Nature 1992; 356:251–3
43. Rane SG, Dunlap K, Kinase C activator 1,2-oleoyl acetylglycerol attenuates voltage-dependent calcium current in sensory neurons. Proc Nat Acad Sci USA 1986; 83:184–8
47. Neugebauer V, Lucke T, Schaible HG: N-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists block the hyperexcitability of dorsal horn neurons during development of acute arthitis in rat’s knee joint. J Neurophysiol 1993; 70:1355–77

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