Spinal Infusion of N-methyl-D-aspartate Antagonist MK801 Induces Hypersensitivity to the Spinal Alpha-2 Agonist ST91 in the Rat

STUART A. DUNBAR and TONY L. YAKSH

Department of Anesthesiology, University of California, San Diego, LaJolla, California
Accepted for publication February 12, 1997

ABSTRACT

MK801 (MK), an N-methyl-D-aspartate (NMDA) receptor antagonist, attenuates tolerance to spinal opioids. Whether this applies to other G-protein-coupled receptor systems is unknown. This study examines the effects of continuous spinal MK on tolerance to the antinociceptive effect of continuous spinal infusion of the alpha-2 agonist ST91 (ST). Intrathecal (i.t.) infusion pumps were implanted in rats which delivered for 7 days: saline (1 μl/h); ST (40 nmol/μl/h); MK (10 nmol/μl/h) + ST (40 nmol/μl/h); or MK (10 nmol/μl/h). Antinociception was measured daily on the hot plate. On day 8, groups received i.t. boluses of ST to generate dose-response curves. A separate ST-infused group received MK (10 nmol i.t.) on day 7. Each group received ST (40 nmol i.t.) 7 days after discontinuation of infusion. Co-infusion of MK with ST resulted in attenuation of the right shift in dose response seen in ST-infused rats and a small preservation of effect on daily testing. However, MK-infused rats showed a significant left shift in ST dose response. Acutely administered, MK did not restore ST sensitivity. One week after cessation of infusion, ST and ST + MK groups showed shorter duration of effect after i.t. ST bolus than controls. In conclusion, chronic spinal MK partially attenuates loss of sensitivity to chronic spinal ST. This supports the hypothesis that opioid- and adrenoceptor-induced tolerances are similarly modulated by the NMDA receptor. However, the increased sensitivity induced by MK alone suggests that NMDA receptor antagonism may not prevent the development of tolerance itself but may alter the expression of tolerance by inducing sensitivity via other alterations in cellular function.

The intrathecal delivery of opioid and alpha-2 agonists will yield a potent dose-dependent antinociception with a clearly defined agonist and antagonist pharmacology (Reddy et al., 1980; Yaksh and Reddy, 1981). Studies on the mechanisms of action of these families of agents have revealed similar underlying spinal mechanisms: 1) both exert a potent presynaptic effect on the release of transmitter from small primary afferents and 2) both are coupled by a G protein to increase potassium conductance which leads to a hyperpolarization of the membrane (Yaksh 1993; Yaksh et al., 1993). In addition to the mechanistic similarities of opioid and alpha-2 agonists, the continuous spinal infusion of each class of agent has been shown to result in a progressive decline in effect over several days (Russell et al., 1987; Loomis et al., 1988; Stevens et al., 1988; Stevens and Yaksh, 1989a,b; Sosnowski and Yaksh, 1990; Stevens and Yaksh, 1992; Takano and Yaksh, 1993). Systematic investigations have shown that this decline occurs in the face of continued steady-state drug exposure, does not depend on handling and shows minimal cross-tolerance between mu and alpha-2 agonists (Lameh et al., 1992). The use of a chronic infusion model has technical advantages over either systemic or intrathecal bolus delivery. First, chronic infusion produces a steady state of drug which avoids the peaks and valleys of drug exposure inherent in bolus delivery. Such daily troughs will lead to intermittent withdrawal in the intervening period before each injection, a factor that may contribute to the reported hyperalgesia observed during the course of tolerance induction in other models (Mao et al., 1994). Second, continuous infusion models preclude the need to repeatedly handle the animals, which creates a classical conditioning model (MacRae et al., 1987). Although the mechanism of tolerance development remains controversial, several studies have shown that the co-delivery of NMDA antagonists can attenuate the magnitude of opioid tolerance when the agents are delivered systematically by pellets or bolus injection (Trujillo and Akil, 1991; Marek et al., 1991a,b; Ben Eliyahu et al., 1992) and intrathecal by bolus delivery (Kest et al., 1993; Mao et al., 1993).

Received for publication July 30, 1996.

This work was supported by DA02110 (T.L.Y.) and by Anesthesiology Training grant NIH T32NS07329 (S.D.).

ABBREVIATIONS: MK, (+)-MK801 (dizocilpine hydrogen maleate); ST, ST-91 HCL [2-[2,6-diethylphenylamino]-2-imidazolone]; intrathecal, i.t.; NMDA, N-methyl-D-aspartate; %MPE, percentage maximum effect; %AUC, percentage area under the dose-response curve; ANOVA, analysis of variance; GTP, guanine triphosphate.
1994). In recent work we used a well-defined spinal continuous infusion paradigm to demonstrate that co-infusion of MK with morphine over 7 days diminishes the daily reduction in effect otherwise observed with chronic administration, and also prevents the right shift in morphine bolus dose-response curves 1 day after termination of infusion (Dunbar and Yaksh, 1996). The mechanism of this effect of NMDA antagonism is uncertain. It has been hypothesized, however, that opiates may inhibit adenylate cyclase activity through their effect on a Gi protein. This serves to activate protein kinase C which in turn acts to phosphorylate the Gi protein and suppress its ability to inhibit adenylate cyclase. NMDA receptor occupancy has been shown to induce protein kinase C translocation during opiate exposure (Mao et al., 1994). To the degree that this is a viable hypothesis, it suggests that NMDA blockade may additionally serve to alter the development of tolerance to other agonists that act via Gi-coupled receptors. Given the apparent similarities between the mechanism whereby spinal alpha-2 receptors act to alter nociceptive transmission (Yaksh 1993; Yaksh et al., 1993), it seems reasonable to hypothesize that spinal NMDA blockade also prevents the loss of effect observed with chronic spinal delivery of an alpha-2 agonist.

The specific aims of this study were: 1) to assess the effects of NMDA receptor antagonism on an intrathecal model of continuous spinal alpha-2 agonist exposure with use of ST; 2) to quantify the magnitude of the development of tolerance induced by chronic ST 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 effect of an acute bolus of MK in ST-tolerant rats; and 4) to assess the effect of MK on ST tolerance 1 week after its discontinuation.

Methods

Animals. Approval for this study was obtained from the Institutional Animal Care and Use Committee of the University of California San Diego. Male Sprague-Dawley rats (350–400 g) were each implanted with an intrathecal catheter attached to a subcutaneous osmotic pump filled with saline or drug(s), as described below, and were housed thereafter in individual standard cages at room temperature on a 12-h light/12-h dark cycle (lights on 7:00 A.M.). Testing was performed during the light cycle at 12:00 P.M. Animals had free access to food and water. Rats were randomly assigned to one or the other group. All rats were sacrificed by overdose of barbiturate on the last day of testing, which was day 8 in most cases, or on day 14 when rats were redosed 1 week after cessation of infusion.

Preparation of the catheter with infusion pump and implantation. The preparation of the catheter has been described previously (Yaksh and Rudy, 1976). A 16-cm length of PE-10 tubing was connected to a 2-cm length of PE-60 tubing by heat with a hot-air jet. A 1-cm piece of silastic tubing previously soaked in chloroform to increase its internal diameter was then passed over both ends of the PE-10 tubing to form a loop at a distance of 3 cm from the end of the PE-10 tubing fused to the PE-60. The long end of the catheter was stretched to reduce diameter, soaked in alcohol (70%v) overnight and cut to a length of 9 cm from the silastic tubing. Alzet osmotic minipumps (model 2001 delivering 1 μl/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 μl/h for 7 days after an initial activation period of 4 h in the animal. The catheter and pump are then implanted between 9 A.M. and 12 P.M., according to the procedure originally described for chronic catheterization of the rat spinal cord (Yaksh and Hartly, 1988), with the additional modification of the subcutaneous osmotic pump. Animals were anesthetized with halothane and placed in a stereotaxic head holder. A midline incision was made to expose the atlanto-occipital membrane. The membrane was pierced, and the PE-10 end of the catheter passed intrathecally to a distance of 8.5 cm (caudal to the level of the thoracolumbar junction). The pump was 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. The loop end of the catheter was passed rostrally to exit percutaneously on the top of the skull. This PE-10 loop was cut at the end of the 7-day infusion period to discontinue infusion and used to administer external doses of drug. The wound was then sutured, including a loose ligature at the base of the loop to prevent it from moving. Animals fully recovered 15 to 30 min after implantation. Those rats showing any signs of motor impairment were sacrificed with an overdose of barbiturate.

Drugs and injection. The following drugs were used for continuous spinal infusion: ST, an alpha-2 agonist (Boehringer, Ingelheim, Germany) and MK, an NMDA receptor antagonist (Research Biochemicals International, Natick, MA). Drugs were dissolved in sterile normal saline. Drug doses, calculated as the free base, were expressed in nanomoles per hour for the infusion concentrations, or nanomoles per rat for the postinfusion dose-response studies. ST infusion concentration was 40 nmol/μl/h in all animals receiving ST, because this dose was found in pilot studies to have yielded a near-maximal increase in hot-plate latency on day 1 after implant without any attendant motor effects. The MK dose of 10 nmol/μl/h was found to have the maximal effect in attenuating tolerance in pilot studies with the least number of animals expressing side effects. In preliminary studies (Dunbar and Yaksh, 1996), we observed that MK doses exceeding 15 nmol/μl/h resulted in weight loss and motor weakness, whereas doses of 1 nmol/μl/h or less had little effect on tolerance.

Experimental paradigms. Animals were first tested on the hot plate and then implanted with an i.t. catheter. Testing was carried out daily on the hot plate between the hours of 10 A.M. and 12 P.M. from day 0, the day of implantation, to day 7 (at the end of 7 days of infusion). The groups examined were: MK (10 nmol/μl/h) + ST (40 nmol/μl/h); MK (10 nmol/μl/h); ST (40 nmol/μl/h); and normal saline (1 μl/h). A group infused with ST alone was given an i.t. bolus dose of 10 nmol MK in 10 μl of saline on day 7 to determine whether i.t. MK would restore ST sensitivity when administered as an acute bolus in ST-tolerant rats.

On day 7, after testing on the hot plate at approximately 12 P.M., the external loop of catheter was cut, and the i.t. part of the catheter was flushed with 10 μl of normal saline. On day 8 (24 h after stopping the infusion and flushing the catheter), a single probe dose of i.t. ST in 10 μl of saline was administered. Based on preliminary studies, rats were given one of the following i.t. probe doses of ST: those infused with saline were given either 4, 12 or 40 nmol of ST; those infused with ST alone were given 40, 120 or 400 nmol of ST; those infused with MK + ST were given either 12, 40 or 120 nmol of ST; those infused with MK alone were given either 0.4, 4 or 12 nmol of ST. Hot-plate latencies were measured at 0, 15, 30, 60 and 120 min. This assignment was continued until at least four or more rats in each group were obtained for each i.t. probe dose, which generated a dose-response curve for each group. Each rat was used only once.

Finally, four to eight rats from each of these groups were re-tested 1 week later, after cessation of infusion, to assess their response to an i.t. probe dose of 40 nmol of ST on the hot plate. Hot-plate latencies were measured at 0, 15, 30, 60, 120 and 180 min.

Antinociceptive testing and data analysis. The effects of i.t. infusions were assessed by the hot-plate test. The hot-plate 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. The endpoint for the hot plate was taken to be the licking of one the hind paws. In a small percentage of the animals, the rat did not show licking behavior but reliably displayed signs of agitation as evidenced by rapid stomping
of the foot, jumping and/or strong vocalization. In this event, the activity was taken to be the endpoint and that latency assigned. Post hoc inspection of the data revealed that this occurred in less than 5% of the cases and was distributed across treatment groups. A cutoff time of 60 sec was used to avoid tissue damage. Hot-plate data were expressed either as mean latencies for each group or as %MPE. %MPE was calculated as follows:

\[
\text{%MPE} = \frac{\text{Postdrug latency} - \text{base line} \times 100}{\text{Cutoff time} - \text{base line}}
\]

where postdrug latency is the response measured at the particular time after initiation of infusion or after i.t. dose of probe drug. Base line is the preinfusion or preprobe latency, and the cutoff time is 60 sec.

**Statistics.** Analysis of the dose-response curves and statistics were obtained with computer software programs (Abacus Concepts, Stat-View, Abacus Concepts, Inc., Berkeley, CA, 1992). Where applicable, data from hot-plate testing (absolute latencies or calculated %MPE) were analyzed by one- or two-way ANOVA to detect differences between groups. When differences were found, these findings were subjected to a Scheffe F-test (significant at 95%). Single points of comparison within the same group were made with a standard paired \( t \) test where applicable. Analysis of the dose-response curves by linear regression analysis was obtained with pharmacological software programs (Tallarida and Murray, 1981), by which calculation of the ED\(_{50}\) (95% confidence intervals) and tests for relative potency were performed. The tolerance ratio (the ratio of ED\(_{50}\) in drug-infused animals to ED\(_{50}\) of saline-infused animals) and 95% confidence intervals were calculated. Differences yielding critical values corresponding to P < .05 were considered statistically significant. Differences of P > .05 were considered not significant.

**Results**

**Time Course of Effect of Spinal Infusions on Daily Hot-Plate Escape Latencies**

Chronic infusion of saline or agents had no observable effect on motor function. All rats entered into the study after implantation survived for the interval of the infusion without motor deficits. The daily hot-plate response latencies for all groups are presented in figure 1.

**Saline-infused rats.** There were no significant differences between latencies on day 0, 1 and 7 (day 0: 14 ± 1 sec vs. day 1: 14 ± 1 sec vs. day 7: 14 ± 1 sec, \( n = 12 \)) (fig. 1), demonstrating no significant effect of implantation, infusion of the saline vehicle or daily testing.

**MK-infused rats.** There were no significant differences between latencies in MK-infused rats (\( n = 12 \)) and saline-infused rats. There were no significant differences between latencies of MK-infused rats on day 0 compared with those on day 7 (day 0: 13 ± 1 sec vs. day 7: 15 ± 1 sec) (fig. 1). Thus there was no significant effect of intrathecal infusion of MK alone on daily hot-plate testing.

**ST-infused rats.** There were no significant differences between base-line latencies in ST-infused rats (\( n = 17 \)) and saline-infused rats (13 ± 1 sec vs. 14 ± 1 sec). All ST-infused rats showed a near-maximal increase in latencies on day 1 over base line (51 ± 3 sec), returning to base-line values on day 3 (18 ± 2 sec), which was not significantly different from saline-infused rats also on day 3 (15 ± 1 sec) (fig. 1). There were no significant differences between saline- and ST-infused rats on any day from day 3 until the end of the infusion.

**ST + MK co-infused rats.** Base-line latencies in ST + MK-infused rats (13 ± 1 sec, \( n = 12 \)) were not significantly different from saline-infused rats. They showed a similar increase in latencies on day 1 (48 ± 4 sec, \( n = 12 \)) that were not significantly different from ST-infused rats on the same day. Latencies in ST + MK-infused rats remained significantly elevated over saline- and MK-infused rats from day 1 until day 7 (P < .05, ANOVA, Scheffe). These latencies were also significantly elevated over those of rats infused with ST alone from day 3 to day 7 (P < .05, ANOVA, Scheffe) (fig. 1). However, there was a significant decrease in latencies during the infusion period, so that latencies on day 7 were significantly less than those on day 3 (day 3: 29 ± 3 vs. day 7: 19 ± 2 sec, P < .05, paired \( t \) test).

**Assessment of withdrawal on day 8.** On day 8 (24 h after infusions had finished), latencies of ST-infused rats were significantly lower than those on day 7 (8.9 ± 0.3 sec vs. 13 ± 1 sec, \( n = 17 \), P < .05, paired \( t \) test), and significantly lower than those of ST + MK-infused rats (15 ± 1 sec, \( n = 12 \)) and rats infused with saline (14 ± 1 sec, \( n = 12 \)) or MK alone (15 ± 1 sec, \( n = 12 \)) (P < .05, Scheffé, ANOVA) (fig. 2). Thus, increased thermal sensitivity, which was seen in ST-infused rats after discontinuation of infusion, was not seen in rats co-infused with ST and MK.

**Effect of acute administration of MK in ST-tolerant rats.** Rats infused with ST alone or saline, and given an i.t. bolus of MK (10 nmol) at 12:00 P.M. on day 7, the last day of the infusion (\( n = 4 \)), showed no significant increase in latencies over base line, demonstrating a lack of acute effect of MK in restoring sensitivity to ST (fig. 3).

**Probe Dose-Response Curves**

After severing the pump-catheter connection on day 7 and flushing the catheter with 10 \( \mu \)l of saline, 24 h was allowed to elapse before each rat was given one of several doses of i.t. ST to generate the probe dose-response curves on day 8.

**Saline- and MK-infused rats.** In saline-infused rats, 24 h after terminating infusion, bolus doses of i.t. ST revealed a monotonic dose-response (%) curve (ED\(_{50}\) = 12 nmol). In MK-infused rats, the i.t. ST probe dose-response (%) curve was not significantly different from saline-infused rats. They showed a similar increase in latencies on day 1 (48 ± 4 sec, \( n = 12 \)) that were not significantly different from ST-infused rats on the same day. Latencies in ST + MK-infused rats remained significantly elevated over saline- and MK-infused rats from day 1 until day 7 (P < .05, ANOVA, Scheffe). These latencies were also significantly elevated over those of rats infused with ST alone from day 3 to day 7 (P < .05, ANOVA, Scheffe) (fig. 1). However, there was a significant decrease in latencies during the infusion period, so that latencies on day 7 were significantly less than those on day 3 (day 3: 29 ± 3 vs. day 7: 19 ± 2 sec, P < .05, paired \( t \) test).

**Assessment of withdrawal on day 8.** On day 8 (24 h after infusions had finished), latencies of ST-infused rats were significantly lower than those on day 7 (8.9 ± 0.3 sec vs. 13 ± 1 sec, \( n = 17 \), P < .05, paired \( t \) test), and significantly lower than those of ST + MK-infused rats (15 ± 1 sec, \( n = 12 \)) and rats infused with saline (14 ± 1 sec, \( n = 12 \)) or MK alone (15 ± 1 sec, \( n = 12 \)) (P < .05, Scheffé, ANOVA) (fig. 2). Thus, increased thermal sensitivity, which was seen in ST-infused rats after discontinuation of infusion, was not seen in rats co-infused with ST and MK.

**Effect of acute administration of MK in ST-tolerant rats.** Rats infused with ST alone or saline, and given an i.t. bolus of MK (10 nmol) at 12:00 P.M. on day 7, the last day of the infusion (\( n = 4 \)), showed no significant increase in latencies over base line, demonstrating a lack of acute effect of MK in restoring sensitivity to ST (fig. 3).

**Probe Dose-Response Curves**

After severing the pump-catheter connection on day 7 and flushing the catheter with 10 \( \mu \)l of saline, 24 h was allowed to elapse before each rat was given one of several doses of i.t. ST to generate the probe dose-response curves on day 8.

**Saline- and MK-infused rats.** In saline-infused rats, 24 h after terminating infusion, bolus doses of i.t. ST revealed a monotonic dose-response (%) curve (ED\(_{50}\) = 12 nmol). In MK-infused rats, the i.t. ST probe dose-response (%) curve was not significantly different from saline-infused rats. They showed a similar increase in latencies on day 1 (48 ± 4 sec, \( n = 12 \)) that were not significantly different from ST-infused rats on the same day. Latencies in ST + MK-infused rats remained significantly elevated over saline- and MK-infused rats from day 1 until day 7 (P < .05, ANOVA, Scheffe). These latencies were also significantly elevated over those of rats infused with ST alone from day 3 to day 7 (P < .05, ANOVA, Scheffe) (fig. 1). However, there was a significant decrease in latencies during the infusion period, so that latencies on day 7 were significantly less than those on day 3 (day 3: 29 ± 3 vs. day 7: 19 ± 2 sec, P < .05, paired \( t \) test).

**Assessment of withdrawal on day 8.** On day 8 (24 h after infusions had finished), latencies of ST-infused rats were significantly lower than those on day 7 (8.9 ± 0.3 sec vs. 13 ± 1 sec, \( n = 17 \), P < .05, paired \( t \) test), and significantly lower than those of ST + MK-infused rats (15 ± 1 sec, \( n = 12 \)) and rats infused with saline (14 ± 1 sec, \( n = 12 \)) or MK alone (15 ± 1 sec, \( n = 12 \)) (P < .05, Scheffé, ANOVA) (fig. 2). Thus, increased thermal sensitivity, which was seen in ST-infused rats after discontinuation of infusion, was not seen in rats co-infused with ST and MK.

**Effect of acute administration of MK in ST-tolerant rats.** Rats infused with ST alone or saline, and given an i.t. bolus of MK (10 nmol) at 12:00 P.M. on day 7, the last day of the infusion (\( n = 4 \)), showed no significant increase in latencies over base line, demonstrating a lack of acute effect of MK in restoring sensitivity to ST (fig. 3).

**Probe Dose-Response Curves**

After severing the pump-catheter connection on day 7 and flushing the catheter with 10 \( \mu \)l of saline, 24 h was allowed to elapse before each rat was given one of several doses of i.t. ST to generate the probe dose-response curves on day 8.
curve showed a significant 6-fold parallel left shift as compared with saline-infused rats (ED$_{50}$ = 2.1 nmol) (P < .05). The ED$_{50}$ as measured by %AUC was also significantly decreased by 4-fold, which showed an increased duration of effect as well as peak effect (fig. 4, table 1).

**ST-infused rats.** The i.t. ST probe dose-response (%MPE) curve in ST-infused rats displayed a significant right shift, such that the ED$_{50}$ (121 nmol) was significantly increased by a factor of 10 as compared with saline-infused rats (P < .05). The ED$_{50}$ as measured by %AUC was also significantly increased by a factor of 20 as compared with saline-infused rats (fig. 4, table 1) (P < .05). This shows that in ST-tolerant animals, the peak as well as the duration of effect of a probe dose of ST was substantially decreased.

**ST + MK-infused rats.** ST + MK-infused rats showed a significant decrease in the expected right shift in the dose-response (%MPE) curve such that the ED$_{50}$ (34 nmol) was significantly increased by a factor of 3 as compared with saline-infused rats, and significantly decreased by a factor of 3 when compared with ST-infused rats (P < .05). The ED$_{50}$ as measured by %AUC was significantly increased by a factor of 6 as compared with saline-infused rats, and significantly

---

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>ED$_{50}$ %MPE</th>
<th>ED$_{50}$ %AUC</th>
<th>Tolerance Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>12 (11-14)</td>
<td>18 (15-24)</td>
<td>1.0</td>
</tr>
<tr>
<td>ST</td>
<td>121 (93-158)</td>
<td>199 (139-384)</td>
<td>10 ± 1.4</td>
</tr>
<tr>
<td>ST + MK</td>
<td>34 (27-44)</td>
<td>72 (47-111)</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>MK</td>
<td>2.1 (1.1-3.7)</td>
<td>3.9 (1.7-6.6)</td>
<td>0.16 ± 0.05</td>
</tr>
</tbody>
</table>

---

*a* Values expressed are nanomoles with 95% confidence intervals in parentheses.

*b* ED$_{50}$ drug/saline ± S.E.
decreased by a factor of 3 as compared with ST-infused rats (P < .05) (fig. 4, table 1). This indicates that in ST + MK-infused rats, sensitivity to peak effect and duration of ST was significantly increased compared with rats infused with ST alone.

**Response to single i.t. probe dose of ST 7 days after cessation of infusion.** Five or more rats in each group infused with saline, ST, ST + MK and MK alone were given a 40 nmol i.t. probe dose of ST 7 days after discontinuation of infusion (on day 15). ST- and ST + MK-infused rats showed a significant and abrupt decrease in duration of effect as compared with saline and MK infusion groups (latencies at 120 min: ST, 12 ± 1; ST + MK, 13 ± 2 vs. saline, 32 ± 8; MK, 32 ± 6 sec, P < .05, ANOVA, Scheffe). This occurred despite all groups having achieved the same peak effect (%MPE) at 60 min. There was no significant difference between groups infused with ST (ST or ST + MK), however; thus prior administration of MK had no effect on attenuation of tolerance in either of the two ST-infused groups when tolerance was reassessed at this time.

**Discussion**

**Spinal analgesic tolerance.** Spinal delivery of mu, delta, kappa and alpha-2 adrenoreceptor agonists produces a powerful antinociceptive effect. The spinal effect of each receptor is believed to reflect a common regulatory organization: 1) a presynaptic action on small primary afferents blocking transmitter release and 2) a postsynaptic hyperpolarization mediated by increased potassium conductance through Gi protein coupling (Yaksh, et al., 1993; Yaksh, 1993). Continued agonist receptor occupancy uniformly results in a concentration-dependent increase in nociceptive response latency, followed over several days by a reliable decline in this effect, as well as a right shift in agonist dose response, the magnitude of which is proportional to the concentration of the tolerogen and inversely proportional to the efficacy of the agonist (Stevens and Yaksh, 1989a; Takano and Yaksh, 1993). This decline is believed to reflect a pharmacological effect of continued drug-receptor interaction and occurs without animal handling (Stevens et al., 1988; Stevens and Yaksh, 1989a,b, 1992; Sosnowski and Yaksh, 1990). The mechanism for this reduction effect is controversial, but functionally resembles a down-regulation of receptor number or a loss of second messenger coupling. Although no specific studies exist for the spinal alpha-2 receptor, binding studies have shown only a modest and inconsistent reduction in opioid binding with chronic opiate exposure (Nishino et al., 1990; Bhardwaj and Gulati, 1990; Wong et al., 1992; Gourarderes et al., 1993), and this has led to a focus on changes in second messenger coupling. There are similarities in the second messenger systems of opioid and alpha-2 agonists (both suppress adenyl cyclase and act via G protein coupling); however, minimal cross-tolerance of one to the other is seen after chronic administration (Lamah et al., 1992), which suggests fundamental differences in cellular function during tolerance development.

**Spinal NMDA receptor antagonism and spinal alpha-2 tolerance.** NMDA receptor antagonism by both non-competitive (MK) or competitive [LY274614, (+)-6-phosphonomethyl-decahydroisoquinolin-3-carboxylic acid] antagonists can attenuate tolerance to opioids in both systemic and intrathecal models of drug delivery (Trujillo and Akil, 1991; Marek et al., 1991a,b; Ben Eliyahyu et al., 1992; Mao et al., 1994; Kest et al., 1993; Dunbar and Yaksh, 1996). These studies have been largely carried out with the mu opioid agonist morphine, and it seems plausible that this effect should occur also with agents believed to act through similar cellular mechanisms. In the present work, co-infusion of intrathecal MK for 1 week along with exposure to the alpha-2 agonist ST partially prevented the expected loss of antinociception produced by chronic alpha-2 agonist administration and attenuated the expected right shift in the probe dose-response curve to ST. This reduction in right shift did not appear to be secondary to a direct effect of intrathecal MK, because 1) MK had no effect on hot-plate latencies either acutely or chronically administered in this or in previous studies with this model (Dunbar and Yaksh, 1996); 2) there were no significant differences in latencies on the first day of infusion (day 1) between ST- and ST + MK-infused rats; 3) acute delivery of MK after 7 days of ST exposure did not alter base-line latencies; and 4) in the probe dose studies, despite termination of infusions for 24 h (which we believe permitted clearance of any infused agent), ST + MK-infused rats failed to show the expected right shift observed in animals exposed to ST alone, which indicates that prior exposure to MK altered responsiveness to the agonist ST in the absence of MK. Although pharmacokinetic data for elimination of drug after cessation of infusion were not obtained, previous studies with higher single-bolus doses have shown an effective half-life of 2 h (Yaksh et al., 1993), far greater than the 12 half-lives that were permitted to pass before testing was carried out on day 8.

**Facilitation evoked by chronic spinal NMDA antagonism.** An important observation was that exposure to the NMDA antagonist alone produced a significant left shift in the ST probe dose-response curve. This was not caused by alteration in base-line latencies because these were unchanged by chronic MK exposure. Additionally, there was no effect seen with acutely delivered MK in ST-tolerant rats; so if any residual effect of MK had persisted 24 h after cessation of infusion, it would be unlikely to account for this observation. Thus, although isobolographic analysis of drug combination was not performed, we do not think that this effect reflects a simple synergic interaction between receptor systems. Moreover, previous studies have failed to show a synergistic action of opioids and NMDA receptor antagonists during chronic exposure (Marek et al., 1991a,b, Trujillo and Akil, 1991). Accordingly, we believe that continued NMDA receptor antagonism produces a change in system function which leads to a sensitization of the system. These effects were similarly observed with spinal morphine (Dunbar and Yaksh, 1996). To our knowledge this facilitation with chronic alpha-2 agonist exposure has not been reported previously.

**Hypothesized mechanisms of interaction of NMDA antagonism and ST activity.** One possible mechanism proposed for tolerance to ST and morphine is the down-regulation of receptor number. NMDA receptor antagonism does not alter the affinity or density of mu, delta, kappa-1 or kappa-3 opioid binding sites in rat brain homogenates (Tiseo et al., 1994). Thus it appears unlikely that this mechanism accounts for the ability of MK to attenuate tolerance to ST. With regard to intracellular coupling, alpha-2 adrenoceptor agonists inhibit adenylate cyclase activity via a G inhibitory
GTP membrane-bound protein decrease in cAMP formation. This activates protein kinase C, which phosphorylates the G inhibitory protein, and suppresses its ability to inhibit adenylyl cyclase, which leads to a compensatory increase in adenylyl cyclase activity. Protein kinase C translocation by NMDA receptor activation during opioid exposure has been shown to occur (Etoh et al., 1992), and spinal opiate tolerance has been blocked by GMI ganglioside which prevents this translocation (Mayer et al., 1993). Such increases in protein kinase C activity could modulate K channel activity (Alkon and Naito, 1987) or reduce the Mg\(^{2+}\) block of the NMDA receptor (Chen and Huang, 1992). The opioid [D-Ala\(^2\),N-Me-Phe\(^{3}\),Gly-ol]enkephalin, via protein kinase C activation, diminishes the inhibitory effects of the mu agonist on Ca\(^{2+}\) channel opening (Rane and Dunlap, 1986; Ewald et al., 1988, 1989). Thus chronic NMDA receptor antagonism may interfere with this process and, as in the case of opioids, may also attenuate alpha-2 agonist tolerance in the same manner.

These comments are in accord with the processes by which NMDA receptor blockade prevents loss of the receptor-mediated effect of the agonist. However, we have observed with both morphine and ST, that continued exposure to MK alone resulted in a left shift in agonist dose response. In this regard, we must also hypothesize that there may be one or more parallel processes involved: 1) NMDA antagonism may serve to prevent the evolution of processes which reduce the effect produced by agonist-receptor coupling (“prevents” tolerance and dependence); and/or 2) NMDA antagonism may result in a facilitation of the effect produced by the agonist-receptor coupling, thereby producing a left shift in the dose-response curve of the agonist-naive rat, which functionally antagonizes the desensitization otherwise produced by chronic agonist exposure. Although the mechanism of such sensitization is unknown, it represents a fundamentally different alternative to the currently popular hypothesis that NMDA antagonism “prevents tolerance.” Both agonists behave as if they possessed greater intrinsic activity. Increasing the intrinsic activity of the agonist in the face of a given degree of receptor down-regulation would be expected to yield a smaller right shift (as was observed in these studies). This has been shown for mu and alpha-2 agonists, in which agents with higher intrinsic activity show a lesser right shift after chronic administration (Stevens and Yaksh, 1989; Sosnowski and Yaksh, 1990; Takano and Yaksh, 1993).

Whatever effect MK had on tolerance or sensitivity to ST, it was not evident 1 week after termination of the infusion period when both ST-infused groups demonstrated a shorter duration of effect to a bolus of ST than rats infused with saline or MK alone, which had not received ST previously (fig. 5). This shows that residual underlying changes in sensitivity to ST consistent with tolerance still existed 1 week after infusion, regardless of whether animals were exposed to MK or not. Thus NMDA receptor-induced changes in ST sensitivity decayed sooner and at a different rate than the decay of those changes in cellular function associated with ST tolerance itself.

**Alpha-2 withdrawal evoked hyperalgesia.** After discontinuation of ST infusion, a significant reduction in thermal escape latency was noted in ST-infused rats. Similar results were noted in opioid-infused rats (Dunbar and Yaksh, 1996). This hyperalgesia is believed to reflect a withdrawal sign and was not observed in rats infused with ST + MK, perhaps reflecting the ability of MK to prevent the evolution of a dependent state or to prevent the expression of this state during abstinence, as has been seen in spinal morphine-tolerant rats (Dunbar and Yaksh, 1996). The origin of this hyperalgesia is uncertain; but naloxone delivery in spinal morphine-tolerant rats will evoke release of spinal glutamate (Ibuki et al., 1995). Intrathecal glutamate receptor agonists also produce thermal hyperalgesia (Malmberg and Yaksh, 1992). Accordingly, we now speculate that withdrawal from chronic spinal alpha-2 exposure may result in a hyperalgesic state because of the rebound release of spinal glutamate and that this process may be blocked by NMDA receptor blockade.

**Conclusion.** This study shows that chronic, but not acute, antagonism of the spinal NMDA receptor with spinal MK attenuates the development of spinal tolerance to the alpha-2 adrenoreceptor agonist ST, and importantly produces sensitization to ST in the nontolerant state. MK also was found to prevent withdrawal hyperalgesia normally observed in ST-tolerant rats. The observation of sensitization by NMDA antagonism alone raises the possibility that the attenuation of the agonist-receptor-mediated effects associated with chronic exposure may have several mechanisms. Thus NMDA receptor antagonism may not prevent the development of tolerance itself but may alter the expression of tolerance by inducing sensitivity via other nonspecific alterations in cellular function.

**References**


---

*Fig. 5. Time course of the antinociceptive effect, expressed as %MPE, of a single bolus of i.t. ST (40 nmol) 1 week after discontinuation of a chronic 7-day infusion of i.t. ST (40 nmol/h), MK (10 nmol/h), ST (40 nmol/h) with MK (10 nmol/h) and saline. Each line presents the mean ± S.E. of five or more rats. Rats infused with ST or ST + MK showed a similar shorter duration of effect than saline controls or rats infused with MK alone (%saline or MK vs. ST or ST + MK, at 120 min, P < .05).*


Send reprint requests to: Stuart Dunbar, MB. Research Fellow, Department of Anesthesiology 0818, University of California, San Diego, 9500 Gilman Drive, LaJolla, CA 92103.