Replicative Opioid Abstinence Causes Progressive Hyperalgesia Sensitive to N-Methyl-D-aspartate Receptor Blockade in the Rat

STUART A. DUNBAR and ISTVAN J. PULAI

Anesthesiology Research Laboratory, Department of Anesthesiology, Tufts University School of Medicine, Baystate Medical Center, Springfield, Massachusetts

Accepted for publication October 7, 1997 This paper is available online at http://www.jpnet.org

ABSTRACT

The opioid abstinence syndrome is a series of signs or symptoms that occur in the opioid-dependent state when abrupt drug withdrawal occurs. One of the signs of abstinence in the rat is hyperalgesia (Tilson et al., 1973; Johnson and Duggan, 1981; Ekblom et al., 1993; Feng and Kendig, 1995). This sign can be prevented by the preadministration of an NMDA receptor antagonist MK before opioid withdrawal (Dunbar and Yaksh, 1996a). Antagonism at this receptor also attenuates hyperalgesia in other models of pain in which secondary hyperalgesia and long-term potentiation occur (Collingridge and Bliss, 1987; Yamamoto and Yaksh, 1992; Sluka and Westlund, 1993). Abstinence is associated with dorsal horn cellular hyperexcitability (Johnson and Duggan, 1981; Zhao and Duggan, 1987), and isolated cell preparations have shown that this hyperexcitability is sensitive to NMDA receptor antagonism (Yukhananov and Larson, 1994). In vivo spinal release studies have shown that glutamate and tau-
rine are released during abstinence and also that this release can be prevented by NMDA receptor antagonism (Jhamandas et al., 1996). Conversely, the administration of glutamate (Ferreira and Lorenzetti, 1994; Okano et al., 1995) or NMDA (Raigorodsky and Urca, 1987; Sher and Mitchell, 1990) intrathecally induces hyperalgesia and will also antagonize the antinociceptive effects of spinal morphine (Srivastava et al., 1995). The hyperalgesia observed in behavioral studies (Gold et al., 1994) and the hyperexcitability observed in isolated cellular studies (Crain and Shen, 1995) can persist for periods long outlasting the usual rapid decay of tolerance that occurs with opioid withdrawal. Thus several lines of evidence show that, during abstinence, release of EAAs may lead to an EAA-mediated hyperexcitability of dorsal horn neurons which may last beyond the duration of the abstinence syndrome itself, so that repetitive episodes of abstinence might be expected to produce progressive excitation of the dorsal horn network with progressive facilitation of nociception. Although hyperexcitability and hyperalgesia associated with single episodes of abstinence have been demonstrated, to our

ABBREVIATIONS: %MPE, percentage maximal possible effect; EAA, excitatory amino acid; MK, (+) MK801 (dizocilpine hydrogen maleate); i.t., intrathecal; NMDA, N-methyl-D-aspartate; S.E., standard error of the mean; ANOVA, analysis of variance.
knowledge, the effect of repetitive abstinence on nociception has not been examined.

In addition to examining the effect of repetitive abstinence on nociceptive thresholds, the effect of repetitive abstinence on tolerance was also examined. Opioid withdrawal predictably will lead to a decline in tolerance. However, if release of EAAs during abstinence leads to NMDA receptor activation, and if tolerance is associated with activation of this receptor (Trujillo and Akil, 1991; Marek et al., 1991a, b; Ben Eliyahu et al., 1992; Gutstein et al., 1993; Dunbar and Yaksh, 1996a), then its blockade during abstinence should have a significant effect on tolerance to the analgesic effects of further opioid administration. Although coadministration of naloxone with morphine blocks tolerance (Yano and Takemori, 1977), intermittent antagonism with naloxone appears to increase the magnitude of spinal opioid tolerance (Ibuki et al., 1997), which supports the hypothesis that EAA release and NMDA receptor activation during abstinence may increase the degree of tolerance. To examine the hypothesis that abstinence-induced NMDA receptor activation may be a significant factor in the development of tolerance, a repetitive model of abstinence was used which allowed for NMDA receptor antagonism during abstinence. Previous studies have shown that coadministration of MK with morphine attenuates tolerance (Marek et al., 1991a, b; Ben Eliyahu et al., 1992; Gutstein et al., 1993; Dunbar and Yaksh, 1996a), but neither the preadministration of MK as a bolus dose (Marek et al., 1991b), nor the administration of MK in the established tolerant state (Trujillo and Akil, 1991; Dunbar and Yaksh, 1996a) has a significant effect on tolerance. None of these studies have examined the effect of NMDA receptor antagonism on the progression of tolerance when administered only during abstinence periods when maximal EAA release occurs (Jhamandas et al., 1996).

This study thus examined the effect of repetitive opioid abstinence on nociception and tolerance in a model of repetitive opioid withdrawal. An intrathecal model provided the means to assess spinal tolerance at the termination of the infusion period. Although chronic, continuous infusion of intrathecal opioid does not limit exposure to the spinal portion of the central nervous system, a spinal bolus dose directed toward the lumbar area is believed to limit its antinociceptive effect mostly to that region allowing for the assessment of spinal tolerance (Stevens et al., 1988; Stevens and Yaksh, 1989a, b).

The following hypotheses were addressed specifically: (a) repetitive opioid abstinence causes progressive thermal hyperalgesia (progressively lowers nociceptive thresholds), (b) this hyperalgesia is sensitive to NMDA receptor blockade and (c) NMDA receptor antagonism during abstinence attenuates spinal opioid tolerance development.

**Methods**

**Animals.** Approval for this study was obtained from the Institutional Animal Care and Use Committee of Baystate Medical Center. Male Sprague-Dawley rats (350–400 g) were implanted at 4:00 P.M. and thereafter housed 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.. Each rat was implanted with a subarachnoid catheter system attached to a subcutaneous osmotic pump filled with saline, as described below. Animals had free access to food and water. Rats (four or more) were randomly assigned to one or the other group; experimental groups were run in tandem. All rats received a 7-day infusion followed by 16 h of saline infusion to clear the catheter, and after testing on the last (8th) day at 12:00 P.M. were sacrificed by an overdose of barbiturate.

**Preparation of the catheter with infusion pump and implantation.** The preparation of the catheter was as follows: A 5-mm piece of silastic tubing, previously soaked in chloroform to increase its internal diameter, was passed over both ends of a 120-mm length of PE-10 tubing to form a 10-mm loop at a distance of 15 mm from one free end and 95 mm from the other free end. The short end was then connected by heat fusing with a hot air jet to a 189-mm piece of PE-60 tubing which was coiled and emersed first in hot, then cold water to maintain this coil of 2 cm in diameter. Coiling enabled the catheter system to be inserted subcutaneously in the animal without the mechanical stress of uncoiling that would have otherwise occurred when implanted. This resulted in a coiled PE60 reservoir connected to an intrathecal PE-10 catheter similar to that described previously (Yaksh and Stevens, 1986). The whole catheter system was soaked in alcohol (70%) overnight, and then flushed with saline before priming on the day of implantation. Alzet osmotic minipumps (model 2002 delivering 0.5 μl/h; Alzet, Palo Alto, CA) were filled with saline in a sterile fashion. This pump is designed to deliver a constant infusion of 0.5 μl/h for 14 days after an initial activation period in the animal of 4 h. The catheter was filled with a sterile technique from the PE-60 end. A ruler was placed below a sterile transparent sheet to enable measurement of the catheter. Saline, drug or air bubble was carefully injected into the catheter by a 1-ml syringe as follows. Each 24-h period consisted of 27 mm of catheter. For each 24-h period the first 5 mm of the infusion was air (equivalent of approximately 4.4 h). This was followed by 22 mm (equivalent of approximately 19.6 h) of drug or saline. Thus 24 h of morphine was prepared by injecting 5 mm of air and then 22 mm of morphine solution. This was continued so that the pump was primed for a 7-day infusion period. The end of this period also had a 5-mm air bubble to separate it from the saline-filled pump solution, which hydraulically drove the system. The pumps are designed to run for 14 days, so the last day of the infusion (day 8) was saline which for 16 h flushed the catheter of any residual drug without needing to handle the animal. Pilot studies showed that daily latencies were unaffected by the addition of air bubbles.

The catheter and pump were implanted according to the procedure originally described for chronic catheterization of the rat spinal cord (Yaksh and Rudy, 1976). Animals were anesthetized with halothane and placed in a stereotaxic head holder. A midline incision was made and a catheter was then implanted subcutaneously in a pouch to lie behind the shoulders. The loop end of the catheter was passed rostrally to exit percutaneously on the top of the skull. This PE-10 loop was cut on the 8th day, at 1200 hours, 16 hours after clearance of the catheter by saline from the pump and used to administer the probe dose of morphine. The wound was 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. Rats showing any signs of motor impairment were sacrificed with an overdose of barbiturate. Pilot studies in vivo, and in vitro (37°C water bath), with methylene blue, morphine and saline solutions were conducted to assess the accuracy of this system. These studies showed that minimal mixing occurred in vivo or in vitro, and that the pumps flowed at the stated rate so that at the end of the 7-day period all of the contents of the catheter had pumped through. A lag time of 4 h occurred after implantation before the pump started and the animal began to receive morphine. All pumps were examined when the experiments were terminated. Any systems found to be defective (in all cases either disconnected or blocked by blood) were eliminated from the study entirely. All animals were sacrificed by overdose of barbiturate after testing on day 8.
Drugs and injection. The following drugs were used for continuous spinal infusion: morphine sulfate (morphine) (Merck, Sharp and Dohme, West Point, PA), and (+)MK801 hydrogen maleate (MK) (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 post-infusion probe dose. The morphine infusion concentration, unless otherwise stated, was 40 nmol/0.5 µl/h in all animals, because in pilot studies this dose yielded a near-maximal increase in hot-plate latency on day 1 after implant without any attendant motor effects, and closely resembled the infusion concentration used in previous studies (Dunbar and Yaksh, 1996a). The MK infusion concentration was 10 nmol/0.5 µl/h as this was also found to cause the maximal effect in attenuating the hyperalgesia of withdrawal without any attendant side effects in this or in previous studies (Dunbar and Yaksh, 1996a). The probe dose of morphine administered on day 8 was 100 nmol in 10 µl per rat because based on preliminary studies this dose produced a sub maximal effect in the least tolerant group and a measurable effect in the most tolerant group.

Experimental paradigms. Animals were first tested on the hot plate and then implanted. Testing was carried out daily on the hot plate, at 4:00 p.m. on day 0 before implantation and at 12:00 p.m. subsequently. This allowed for the 4-h lag time to pass before the pump began to pump and ensured that animals were tested 16 h after the start of the infusion, close to the midcycle of each 24-h period. Abstinence was induced by infusion of saline solution [abstinence (saline)], or MK solution [abstinence (MK)]. The groups were: (1) saline for 7 days (SAL); (2) morphine for 7 days (MOR); (3) morphine with abstinence (saline) on day 6 (MORSAL6); (4) morphine with abstinence (saline) days 4 and 6 (MORSAL46); (5) morphine with abstinence (saline) on days 2, 4 and 6 (MORSAL246); (6) morphine except on days 2, 4 and 6 when morphine 8 nmol/h was infused (MORMOR(8)246); (7) morphine with abstinence (MK) on day 6 (MORMK6); (8) morphine with abstinence (MK) on days 2, 4 and 6 (MORMK246); and (9) saline with MK on days 2, 4 and 6 (SALMK246). On day 8, after testing on the hot plate at 12:00 p.m., 16 h after infusion of saline, the external loop of catheter was cut and the probe dose of morphine (100 nmol in 10 µl i.t.) was administered. Hot-plate latencies were measured at 0, 15, 30, 60 and 120 min. Rats were randomly assigned to each group and groups were run in tandem until four or more rats were obtained for each group. A summary of the experimental paradigms is provided in table 1.

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. Licking of either hind paw was taken as the endpoint. A cutoff time of 60 sec was used to avoid tissue damage. Hot-plate data were expressed as %MPE, which was calculated as follows:

\[
\text{%MPE} = \frac{(\text{Post drug latency} - \text{base line})}{\text{Cut-off time} - \text{base line}} \times 100
\]

where post drug 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 pre-infusion on day 0 or preprobe latency on day 8, 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). Unless stated otherwise, data from daily hot-plate testing and the probe dose response were converted to %MPE and analyzed by one-way ANOVA to detect differences between groups. When differences were found, these findings were subjected to a Scheffe F-test (significance at 95%). Differences yielding critical values corresponding to \( P < .05 \) were considered significant.

Table 1: Groups and experimental paradigms:

<table>
<thead>
<tr>
<th>SAL</th>
<th>SALMK246</th>
<th>MOR</th>
<th>MORMK246</th>
<th>MORMK6</th>
<th>MORSAL246</th>
<th>MORMOR(8)246</th>
<th>MORSAL6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

(SAL=grey, MOR 40nmol/h =white, MOR 8nmol/h = hatched, MK=black)
Results

Time Course of the Effect of Spinal Infusions

The infusion of saline, morphine or MK 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 presented as %MPE ± S.E. are summarized in Table 2 and appear in figures 1 (a–f) and 2 (a–c). In all cases of significance, a post hoc Scheffe value was found to be significant also.

Base-line latencies. There was no significant difference in base-line latencies (seconds, unpaired t test, P > .05) on day 0 between the saline-infused group (13 ± 1 sec) and all other groups.

Saline and saline-MK controls. Saline-infused rats (SAL; n = 6) showed no significant difference in escape latencies from day to day during days 1 to 8, which demonstrates no significant effect of implantation, infusion of saline vehicle or daily testing (fig. 1a). Latencies in the SALK246 group (n = 4) were also not significantly different from the saline group on any days 1 to 8, which indicates that MK by itself did not alter daily latencies (fig. 2a).

Morphine Groups

MOR. This group (n = 6) had a maximal increase in latencies on day 1 that remained greater than those of the saline group from days 1 to 5. On days 6 and 7 latencies returned to values not significantly different from the saline group. There was no significant difference between this group on days 2 or 3 between this group and the MOR- and the SAL groups, and on day 4, the second period of abstinence, latencies were significantly less than the MOR group but not significantly less than the saline group. On days 5 and 6 and 7 and 8 (the third period of abstinence) and 7 and 8 (the fourth period of abstinence), latencies were significantly lower than both the MOR and saline groups. Latencies also decreased significantly on day 6, the third day of abstinence, compared with those on day 2, the first day of abstinence on (paired t test, P < .05) (fig.1e).

MORMK246. No significant difference occurred in latencies between this group (n = 7) and the MOR group on any of the days 1 to 8. However, compared with the MOR- and the SALK246 group, latencies on days 4 through 8 were significantly higher (fig. 1f). MORMK6. No significant difference occurred in latencies between this group (n = 4) and the MOR group on any day (fig. 2b). Latencies on day 6 were not significantly different from the MORGAL6 or SAL groups.

MORMK246. Latencies in this group (n = 7) showed an increase on day 1 that was not significantly different from any of the other morphine groups. There was no significant difference on days 2 or 3 between this group and the MOR- and the SAL groups. Furthermore, latencies on days 4 to 8 were significantly higher than the MORGAL6 group (fig. 2c). However, latencies declined during the infusion period so that there was no significant difference between this group and the MOR group on day 7. Furthermore, latencies on days 5 and 7 were significantly lower than day 1, and latencies on day 7 were not significantly different from base-line.

Assessment of Tolerance by Administration of Probe Dose of Morphine 100 nmol i.t. on Day 8

Effect of successive periods of abstinence on tolerance. The SAL and SALK246 groups achieved latencies that were not significantly different from each other after administration of the probe dose of 100 nmol of morphine. Latencies in the SAL group were significantly higher than MOR, MORSAL6 or MORGAL6 groups, but not significantly different from the MORMK246 group. Maximum latencies in the MOR group were also significantly less than those of the SAL and MORSAL6 groups, but not significantly different from those of the MOR group, but they were significantly less than the MORGAL6 group (fig. 3).

The effect of MK administration during abstinence on tolerance. No significant difference occurred in maximal
latencies obtained in the MORSAL6 group and the comparative MORMK6 group. There was no significant difference in maximal latencies between the MORSAL246 group and the comparative MORMK246 group (fig. 4).

**Discussion**

We hypothesized that abstinence associated with EAA release and NMDA receptor activation, if repetitive, would cause progressive hyperalgesia in a manner similar to the progressive nociceptive sensitization seen in other models of chronic pain in which activation of this receptor occurs. In addition we hypothesized that NMDA receptor blockade during abstinence should have a significant effect on tolerance to the antinociceptive effects of further opioid administration.

**Repetitive abstinence progressively lowered nociceptive pain thresholds.** Continuous infusion of morphine was associated with rapid onset of tolerance without the development of hyperalgesia even after cessation of the infusion period (fig. 1b). An additional preceding period of absti-
ence was also not associated with hyperalgesia (fig. 1c), but when two preceding periods of abstinence were introduced before the end of the infusion period, significant hyperalgesia developed (fig. 1d). When a third preceding period of abstinence was introduced, hyperalgesia increased within this same group so that latencies decreased significantly between the first and third periods of abstinence (fig. 1e). This shows that progressive hyperalgesia developed with repetitive drug withdrawal in conjunction with repetitive periods of opioid administration. If hyperalgesia was secondary to tolerance alone, then it would have been most pronounced after termination of the infusion period in the continuously infused group, but this was not so (day 8 latencies; fig. 1b vs. fig. 1e). This shows that progressive hyperalgesia developed with repetitive drug withdrawal in conjunction with repetitive periods of opioid administration. If hyperalgesia was secondary to tolerance alone, then it would have been most pronounced after termination of the infusion period in the continuously infused group, but this was not so (day 8 latencies; fig. 1b vs. fig. 1e). Furthermore, infusion of a lower maintenance concentration of morphine in lieu of complete drug withdrawal prevented hyperalgesia entirely (fig. 1f). Conversely, as successive periods of abstinence were introduced, each group showed greater sensitivity (or less tolerance) to the probe bolus dose of morphine administered on day 8 (fig. 3). The greater preservation of sensitivity to the probe dose of morphine observed on day 8 in groups in which drug withdrawal occurred is consistent with previous studies which have shown that the concentration of agonist and the duration of agonist receptor occupancy are the predominant requirements for development of tolerance (Orarovats et al., 1953; Bharagava, 1978; Yano and Takemori, 1977; Crain et al., 1979). Thus we found, as predicted, a direct relationship between the degree of tolerance and the total duration of opioid exposure during the infusion period. Previous studies have indicated that the severity of the opioid abstinence syndrome is proportional to the underlying tolerant state of the animal, so that the ED50 of naloxone required to precipitate abstinence decreases as the animal is exposed to more intensive pretreatment with morphine (Tilson et al., 1973). In other studies a close temporal relationship between tolerance and dependence has been observed (Cox et al., 1975), which led investigators to propose that these two phenomena are part of the same process. However in this study, hyperalgesia, a manifestation of opioid dependence, and tolerance seem to develop as separate entities. This observation contrasts with previous studies which have reported hyperalgesia as a direct manifestation of opioid tolerance (Mao et al., 1994). These studies used intermittent dosing of opioid as a model of tolerance, a factor which we believe may have led to this conclusion.

The observation that hyperalgesic thresholds persisted despite continuing exposure to morphine during nonabstinence periods (day 6, fig. 1d or days 4 or 6, fig. 1e) may be explained by the effect of spinal EAA release on the antinociceptive effects of morphine. Opioids are believed to produce analgesia by hyperpolarization of the pre- and postsynaptic cell membrane of small nociceptive afferents as well as preventing presynaptic neurotransmitter release (Jessell and
Iversen, 1977; Sastry, 1979; Carstens et al., 1979). Abstinence can cause enhanced sensitivity to a variety of excitatory neurotransmitters (Collier, 1966; Satoh et al., 1976). Thus with drug withdrawal, persistent hyperexcitability of the dorsal horn network may occur, resulting in insensitivity of spinal afferents to the hyperpolarizing antinociceptive effect of morphine. The loss of effect of morphine cannot be explained simply by opioid tolerance alone, because when abstinence was introduced, greater sensitivity to the higher bolus dose of morphine occurred at the end of the infusion period (fig. 3). We thus propose that there may be two factors that contribute to the loss of antinociceptive effect of morphine during abstinence-induced hyperalgesia: (1) abstinence-induced hyperexcitability in dorsal horn afferents rendering these neurons less susceptible to the hyperpolarization usually produced by morphine, and (2) tolerance secondary to a variety of other possible causes (G protein uncoupling, etc.). In support of this proposal are the observations described in the next section that NMDA receptor antagonism during abstinence blocked hyperalgesia but did not prevent the progressive development of tolerance.

The effect of NMDA receptor antagonism during abstinence on nociception and tolerance. NMDA receptor antagonism blocked hyperalgesia when administered during periods of abstinence (fig. 2c), which demonstrates that NMDA receptor activation is an integral part of abstinence-induced hyperalgesia. This did not significantly prevent the progressive development of tolerance, however, as indicated by (1) the progressive loss of effect observed during the infusion period as shown by (a) latencies on days 5 and 7 in the MORMK246 group, which were significantly less than latencies on day 1, and (b) latencies on day 7 in this same group, which were not significantly different from base line (fig. 2c, table 2); (2) the response to the probe bolus dose of morphine administered at the end of the infusion period, which was not significantly different from that of the comparative MOR-SAL246 group, which did not receive MK during periods of abstinence (fig. 4); and (3) the failure of MK to reverse tolerance already established as indicated by comparison of latencies on day 7 in groups MORSAL6 (fig. 1c) and MORMK6 (fig. 1b). Although latencies were significantly high in the MORMK246 group from day 4 until the end of the infusion period when compared with the MORSAL246 group. This does not necessarily represent an attenuation of tolerance in the MORMK246 group, because it may simply reflect the ability of MK to block hyperalgesia in this group.

The effect of MK on tolerance when administered only during periods of abstinence contrasts with chronic spinal infusion studies in which continuous co-infusion of the same concentration of MK with morphine for 7 days appeared to block tolerance entirely (Dunbar and Yaksh, 1996a). In these studies, however, an apparent state of hypersensitivity to morphine (as well as to another G protein-coupled agonist ST91) developed when MK was infused alone, i.e., in the morphine or ST91 naive rat (Dunbar and Yaksh, 1996b). This gives rise to the possibility that other, as yet unknown effects may result from chronic infusion of MK leading to altered sensitivity to these agents. In this study preadministration of MK for 24-h periods did not cause this hypersensitivity as indicated by an equivalent response to the probe dose of morphine on day 8 in groups infused with MK and similar groups that were not infused with MK (fig. 4). However, it is possible that full dose-response curves in these groups at the end of the infusion period may reveal more subtle differences between them that was not apparent from this single probe dose study.

If EAA release and NMDA receptor activation are primary factors in the development of tolerance, their blockade would also be expected to attenuate tolerance, but this was not observed. Thus these observations would not support the
hypothesis outlined in the introduction that EAA release during abstinence is a substantive factor in opioid tolerance. Periodic drug withdrawal and the disassociation of agonist and receptor appears to have been the predominant effect in determining the magnitude of tolerance.

**Association between abstinence, nociception and tolerance.** The findings above show that the manner in which tolerance is induced (intermittent dosing vs. continuous infusion) may have a substantial effect on thermal escape tests of antinociception. Thus daily dosing regimens with morphine may alter nociceptive thresholds negatively and potentially lead to an erroneous interpretation of thermal hyperalgesia as an indication of the animals’ underlying tolerant state. It is conceivable that compounds other than MK, which also block hyperalgesia, may alter daily escape latencies in the same fashion, giving rise to the erroneous impression of having “attenuated tolerance.” The administration of a probe dose of opioid to assess tolerance may circumvent this problem providing that the hyperalgesia itself does not significantly alter the magnitude at which tolerance develops. In this study we found that hyperalgesia was not a substantive factor in the development of tolerance as compared with the effect of continuous opioid exposure.

Recent studies have suggested that there are similarities between the cellular adaptations that occur in nociception and tolerance (for review, see Basbaum, 1995). Nociception and tolerance may share the same NMDA receptor mechanism (Neugebauer et al., 1993) and several spinal postsynaptic second messengers, such as intracellular calcium, protein kinase and nitric oxide. Thus nitric oxide synthetase inhibition (Adams et al., 1993; Cappendijk et al., 1993; Sor-kin, 1993; Elliott et al., 1994; Bhargava, 1995), protein kinase inhibition (Vaccarino et al., 1987; Codere and Melzack, 1992; Mayer et al., 1993) and NMDA receptor antagonism have been reported to attenuate the development of both hyperalgesia and opioid tolerance. It is possible, and it has been proposed, that these similarities account for the relative failure of opioids to provide analgesia in some studies of chronic pain (Mao et al., 1995; Dickenson, 1994), although this has been disputed (Backonja et al., 1995; Neil et al., 1990).

From the observations made herein, it is possible that the effects of NMDA receptor antagonists in “preventing” tolerance may in part be secondary to their ability to block the hyperexcitability and hyperalgesia seen with daily thermal testing when models of tolerance are used that include repetitive abstinence. In addition, other effects, perhaps neurotoxic (Nakki et al., 1995; Furber et al., 1995), behavioral (Hargreaves and Cain, 1995) or unexplained sensitization (to opioid and alpha-2 agonists) (Dubar and Yaksh, 1996a, b), may account for reports of their ability to attenuate tolerance. Furthermore, their ability to block dependence is also unclear. NMDA receptor antagonists are reported in several studies to block only some aspects of the abstinence syndrome (Thorat et al., 1994; Ben Elyahu et al., 1992; Dunbar and Yaksh, 1996a). This discrepancy between attenuation of tolerance and attenuation of only some signs of dependence may reflect their ability to block manifestations of abstinence which are spinal in origin (hypersensitivity to thermal and other non-noxious stimuli such as light touch) and relate to activation of the NMDA receptor during abstinence at this level, whereas other signs such as head shaking and teeth chattering remain unaffected.

This study suggests that abstinence, especially when repetitive, leads to long-term changes in the central nervous system which may enhance nociception. These changes most likely result from the effects of EAA release and NMDA receptor activation which, as discussed above, occur during abstinence. It would appear that these effects outlast the duration of opioid drug withdrawal and evolve independently from the process of tolerance itself. This effect of abstinence may resemble the “wind-up phenomenon” or long-term potentiation of hyperalgesia and chronic pain models (Woolf and Thompson, 1991). The site, or sites, of these changes leading to this hyperexcitability, or hyperalgesia, could be spinal or could involve more rostral sites. However, although glutamatergic projections play a role in activation of the locus ceruleus during abstinence, this site is not susceptible to NMDA receptor blockade (Rasmussen, 1995), which perhaps suggests that the hyperalgesia of abstinence may be a predominantly spinal cord phenomenon. The above-mentioned study is an intrathecal study in the rat, and these findings may be peculiar to the rat in this model. Furthermore, these involve relatively high doses of spinally infused morphine and as such, may have more relevance to the spinal administration of opioids in such a potent fashion. However, these observations made in these experiments that repetitive periodic opioid abstinence can lower pain thresholds may have significance for chronic opioid therapy in the management of chronic pain.

**Conclusion.** This study shows that repetitive opioid abstinence progressively lowers nociceptive thresholds in the rat. This effect can be blocked by NMDA receptor antagonism and prevented by the administration of a continuous lower maintenance concentration of morphine providing for constant receptor agonist occupancy. Although known to prevent EAA release, NMDA receptor antagonism during abstinence did not prevent progressive tolerance development, which suggests that mechanisms other than amino acid release are responsible for opioid tolerance.

**Acknowledgment.** We thank Dr. Tony Yaksh for his advice in designing the spinal infusion system.

**References**


Cunninghod GF and Bliss TVP (1987) NMDA receptors; their role in long-term potentiation. Trends Neurosci 10:288–294.

Send reprint requests to: Stuart Dunbar, M.B., Assistant Professor, Dept. of Anesthesiology, Tufts University School of Medicine, Baystate Medical Center, Springfield, MA 01199.