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Lack of Effect of Intrathecally Administered N-methyl-D-aspartate Receptor Antagonists in a Rat Model for Postoperative Pain

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Background: **Evidence from experiments by others indicates an important role for excitatory amino acids activating spinal n-methyl-d-aspartate (NMDA) receptors in models of persistent pain. The purpose of this study was to examine the effect of intrathecal (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801), a noncompetitive NMDA receptor antagonist, 2-amino-5-phosphonovaleric acid (AP5), a competitive NMDA receptor antagonist, and N-G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, on pain behaviors in a rat model of postoperative pain.**

Methods: **Rats with intrathecal catheters were anesthetized and underwent a plantar incision. Withdrawal threshold to punctate stimulation applied adjacent to the wound, response frequency to application of a nonpunctate stimulus applied directly to the wound, and nonevoked pain behaviors were measured before and after intrathecal administration of MK-801 or AP5. The effect of intrathecal L-NAME on mechanical hyperalgesia was also examined.**

Results: **Mechanical hyperalgesia increased and was persistent after plantar incision and was not decreased by intrathecal administration of 4, 14, or 40 nmol MK-801 or 10 nmol AP5. Only the greatest dose of AP5, 30 nmol, caused a small decrease in punctate and nonpunctate hyperalgesia. Intrathecal L-NAME had no effect. Neither intrathecal MK-801 nor intrathecal AP5 affected nonevoked pain behaviors. The greatest doses caused motor deficits.**

Conclusions: **Unlike intrathecal and systemic morphine, intrathecal NMDA receptor antagonists did not modify pain behaviors in this rat model of postoperative pain. These data suggest that NMDA receptors do not play an important role in the maintenance of postoperative pain behaviors and that NMDA receptor antagonists, administered spinally by themselves during the postoperative period, will not be useful for the treatment of postoperative pain in humans. (Key words: AP5; excitatory amino acids; incision; mechanical hyperalgesia; MK-801.)**

This article is highlighted in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

EXPERIMENTAL evidence from animal studies indicates that excitatory amino acids such as glutamate and aspartate contribute to the processing of nociceptive information in the dorsal horn of the spinal cord. These excitatory amino acids, contained in primary afferent fibers and interneurons of the dorsal horn,[1,2] activate N-methyl-D-aspartate (NMDA), non-NMDA, and metabotropic excitatory amino acids receptors to facilitate pain transmission.[3–5] It is hypothesized that the NMDA receptor complex in the dorsal horn of the spinal cord is inactive under normal conditions because little effect on normal nociception occurs after spinal administration of antagonists to this receptor. However, intense or repeated noxious stimuli result in the binding of excitatory amino acids in the dorsal horn; release of the voltage-dependent block on the cationic channel of the NMDA receptor complex; and entry of sodium, potassium, and calcium into postsynaptic cells facilitating dorsal horn neuron responsiveness. Consistent with this hypothesis is the discovery that intrathecal administration of NMDA receptor antagonists reduces hyperalgesia and decreases pain behaviors in animal models of persistent pain.[6–13] Because intrathecal administration of NMDA receptor antagonists do not greatly modify nociception, its potential role for specific clinical post-injury pain states has been proposed.[14]

A common cause of persistent pain and hyperalgesia

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in humans is postoperative pain. To learn more about the mechanisms of pain from a surgical incision and to study new therapies for postoperative pain treatment, we developed and characterized a rat model of postoperative pain.[15] A surgical incision in the plantar aspect of the rat hindpaw causes reproducible, quantifiable mechanical hyperalgesia and nonevoked pain behavior that parallels the postoperative course of patients well. Intrathecally and systemically administered morphine, used for postoperative pain relief in patients, inhibited pain behaviors in this rat model.[16] This model allows us to test potential new therapies for incisional pain that are not yet available for trials in humans after surgery. The purpose of the study was to assess the efficacy of intrathecal (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801), a noncompetitive NMDA receptor antagonist, and intrathecal 2-amino-5-phosphonovaleric acid (AP5), a competitive NMDA receptor antagonist, on pain behaviors after incision. Mechanical hyperalgesia to punctate and nonpunctate stimuli and nonevoked pain behaviors were measured. We also examined the effects of the nitrous oxide synthase inhibitor, N-G-nitro-L-arginine methyl ester (L-NAME), because hyperalgesia caused by NMDA receptor activation[17] appears to be in part mediated *via* nitrous oxide. A preliminary report of some of these data in abstract form has been made.[18]

Methods

General

These experiments were reviewed and approved by the institution's animal care and use committee. The animals were treated in accordance with the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals issued by the International Association for the Study of Pain.[19]

Experiments were performed on 138 adult (weight, 300–350 g) male Sprague-Dawley rats (Harlan, Indianapolis, IN) housed in pairs before surgery. Food and water were available *ad libitum*. After operation, the animals were housed individually with sterile bedding consisting of organic cellulose fiber (TREK; Shepherd Specialty Papers, Kalamazoo, MI). The incisions were checked daily and any sign of wound infection or dehiscence excluded the animal from the study. Eight animals were excluded for wound dehiscence; at the end of the protocol, all animals were killed with an overdose of a mixture of pentobarbital and phenytoin administered intraperitoneally.

Surgery

For subarachnoid drug administration, intrathecal catheters were placed in rats anesthetized by an intraperitoneal injection of chloral hydrate (250–300 mg/kg) and supplemented with halothane. After sterile preparation of the posterior neck, a small PE-10 catheter (8.5 cm) was inserted through an opening in the atlanto-occipital membrane to the lumbar spinal cord.[20] The wound was closed with deep followed by cutaneous interrupted sutures. After recovery, these animals were examined for any apparent motor or sensory deficits; if any were present, these animals were killed. Three days after catheter placement, 20 μl 2% lidocaine was administered intrathecally, and only rats with bilateral hindlimb paralysis were studied. Experiments were begun not less than 5 days after intrathecal catheter placement.

For foot incisions, all rats were anesthetized with 1.5–2% halothane delivered *via* a nose cone and were given an intramuscular injection of penicillin (Flo-Cillin, Fort Dodge Laboratories, Fort Dodge, Iowa), 30,000 IU, in the triceps muscle. A 1-cm longitudinal incision was made through skin and fascia of the plantar aspect of the foot, including the plantaris muscle.[15] The skin was apposed with two mattress sutures of 5-0 nylon on an FS-2 needle and the wound site was covered with a mixture of polymixin B, neomycin, and bacitracin ointment. After surgery, the animals were allowed to recover in their cages.

Pain Behaviors

On the day of the experiment, the rats were placed individually on an elevated plastic mesh floor covered with a clear plastic cage top (21 × 27 × 15 cm) and allowed to acclimate. Baseline pain behaviors were measured as described below.

Withdrawal responses to punctate mechanical stimulation were determined using calibrated von Frey filaments (15–522 mN bending force) applied from underneath the cage through openings (12 × 12 mm) in the plastic mesh floor to an area adjacent to the wound (fig. 1A–F), as described previously.[15,16] Briefly, the lowest force from the three tests, separated by 5–10 min, producing a response was considered the withdrawal threshold. To measure responses to a nonpunctate mechanical stimulus, a circular plastic disk (5-mm diameter) attached to a von Frey filament (400 mN) was ap-

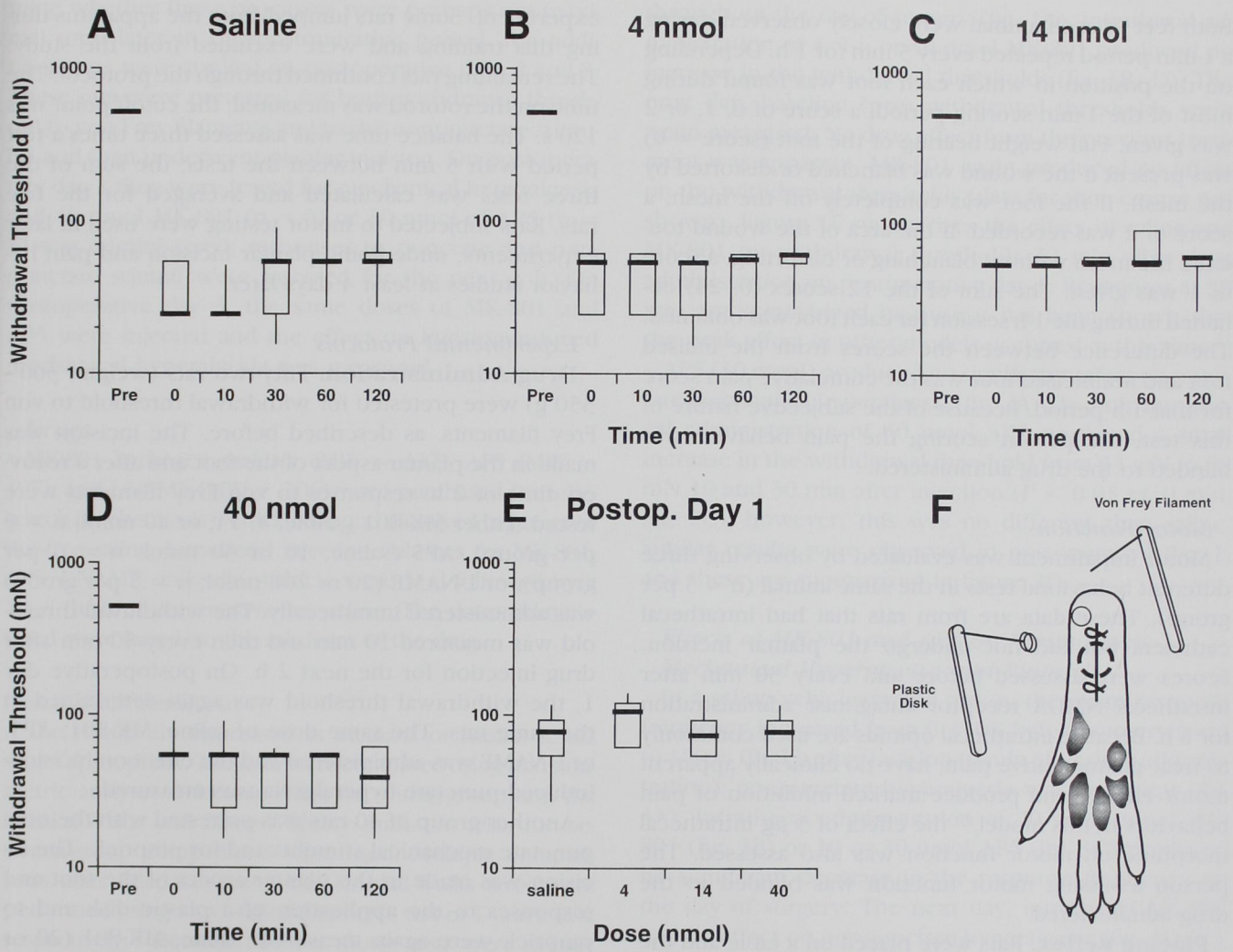

Fig. 1. Effect of intrathecal (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801) on punctate mechanical hyperalgesia caused by incision. The results are expressed as medians (*horizontal line*) with first and third quartiles (*boxes*), and 10th and 90th percentiles (vertical lines). (*A–D*) Withdrawal threshold after incision in rats treated with saline or 4, 14, or 40 nmol MK-801 on the day of surgery. (*E*) Summary of withdrawal thresholds 30 min after administration of saline or MK-801 on postoperative day 1. (*F*) Diagram of the plantar aspect of the rat foot showing the site of application of von Frey filament (*solid circle*) and the site of application of the plastic disc (*dashed circle*).

plied from underneath the cage through openings in the plastic mesh floor directly to the intended incision site. A response to the nonpunctate stimulus was defined as a withdrawal response or lifting of the foot by the plastic disk without bending the filament. This test was repeated three times with approximately 3–5 min between measurements; from these three trials the response frequency was calculated.

To test withdrawal produced by a suprathreshold nociceptive mechanical stimulus, a 5-mm-long tip of a safety pin attached to a von Frey filament (600 mN) was applied between the distal pads of the nonincised foot. This stimulus produced no noticeable tissue damage. The pinprick test was done only once during each test period. The withdrawal frequency was calculated from the single test.

A cumulative pain score, measured by a method adapted from Brennan *et al.*[15] and described previously,[16] was used to assess nonevoked pain behavior. Unrestrained rats were placed on a smaller plastic mesh floor (8 × 8 mm grid). Using an angled magnifying mirror, the incised and nonincised foot were viewed.

Both feet of each animal were closely observed during a 1-min period repeated every 5 min for 1 h. Depending on the position in which each foot was found during most of the 1-min scoring period, a score of 0, 1, or 2 was given. Full weight bearing of the foot (score = 0) was present if the wound was blanched or distorted by the mesh. If the foot was completely off the mesh, a score of 2 was recorded. If the area of the wound touched the mesh without blanching or distorting, a score of 1 was given. The sum of the 12 scores (0–24) obtained during the 1-h session for each foot was obtained. The difference between the scores from the incised foot and nonincised foot was the cumulative pain score for that 1-h period. Because of the subjective nature of this test, the person scoring the pain behavior was blinded to the drug administered.

Motor Function

Motor impairment was evaluated by observing three different behavioral tests in the same animal (n = 5 per group). These data are from rats that had intrathecal catheters but did not undergo the plantar incision. Scores were assessed before and every 30 min after intrathecal NMDA receptor antagonist administration for 4 h. Because intrathecal opioids are used commonly to treat postoperative pain, have no clinically apparent motor effects, and produce marked inhibition of pain behaviors in this model,[16] the effect of 5 μg intrathecal morphine on motor function was also assessed. The person assessing motor function was blinded to the drug administered.

Placing Reflex. Rats were placed on a table and the dorsum of either hindpaw was drawn across the edge of the table; the stimulus elicits a lifting of the paw onto the surface of the table (2 = normal; 1 = delay of 1–2 s; 0 = more than 2 s). Both hindpaws were scored three times during each test period with approximately 2–3 min between tests,[7] and the cumulative score was recorded.

Ambulation. Walking behavior was observed for approximately 5 min (2 = normal; 1 = limping; 0 = paralyzed). Ambulation was scored once every test period.

Balance Time. In preliminary studies we observed that some rats with intrathecal catheters subjected to repeated rotorod (Ugo Basile Rota-Rod, Stoelting, Wood Dale, Illinois) testing over several days appeared ill and lost weight. To assess motor function without these problems, rats were placed on this rotorod, which was fixed and prevented from turning. They were trained for 15 min to balance on the rod on the day of the experiment. Some rats jumped from the apparatus during this training and were excluded from the study. The remaining rats continued through the protocol. The time on the rotorod was measured; the cutoff point was 120 s. The balance time was assessed three times a test period with 5 min between the tests; the sum of the three tests was calculated and averaged for the five rats. Rats subjected to motor testing were used in later experiments, undergoing plantar incision and pain behavior studies at least 4 days later.

Experimental Protocols

Drug Administration. Fifty-two rats (weight, 300–350 g) were pretested for withdrawal threshold to von Frey filaments, as described before. The incision was made in the plantar aspect of the foot and after a recovery time of 2 h, responses to von Frey filaments were tested. Either MK-801 (saline, 4, 14, or 40 nmol; n = 6 per group), AP5 (saline, 10 or 30 nmol; n = 6 per group), or L-NAME (20 or 200 nmol; n = 5 per group) was administered intrathecally. The withdrawal threshold was measured 10 min and then every 30 min after drug injection for the next 2 h. On postoperative day 1, the withdrawal threshold was again determined in the same rats. The same dose of saline, MK-801, AP5, or L-NAME was administered and the effect on incision-induced punctate hyperalgesia was measured.

Another group of 40 rats was pretested with the nonpunctate mechanical stimulus and for pinprick. The incision was made in the plantar aspect of the foot and responses to the application of a plastic disk and to pinprick were again measured. Saline, MK-801 (20 or 40 nmol; n = 6 each dose), AP5 (10 or 30 nmol; n = 6 each dose), or L-NAME (20 OR 200 nmol; n = 5 each dose) was administered intrathecally and nonpunctate and pinprick responses were recorded. On postoperative day 1, the baseline response frequencies were again determined; the same dose of MK-801, AP5, or L-NAME was injected and hyperalgesia was measured.

A separate group of rats (n = 15) was pretested for nonevoked pain behavior as described. An incision was made in the plantar aspect of the foot and, after a recovery time of 2 h, the cumulative pain score was measured. Saline, 40 nmol MK-801 or 30 nmol AP5 (n = 5 per group), was administered and pain scores were recorded during the first, second, and fourth hour after drug injection on the day of surgery and on postoperative day 1.

The NMDA receptor antagonists are thought to interfere with plasticity and central sensitization. To deter-

mine whether these processes were perhaps not maximal until later in the postoperative period, ten additional rats were studied on postoperative days 2 and 3. These rats were pretested for both withdrawal threshold to von Frey filaments and to the nonpunctate stimulus and then underwent plantar incision. On postoperative day 2 they were tested for mechanical hyperalgesia and 40 nmol MK-801 (n = 5) or 30 nmol of AP5 (n = 5) was administered; responses to punctate and nonpunctate stimuli were assessed for the next 4 h. On postoperative day 3, the same doses of MK-801 and AP5 were injected and the effect on incision-induced mechanical hyperalgesia was measured.

Drugs

MK-801 hydrogen maleate (MW = 337), AP5 (MW = 197), and L-NAME (MW = 306) were purchased from Research Biochemicals (Natick, MA) and dissolved in preservative-free saline. Intrathecal injection volumes for MK-801, AP5, L-NAME, and saline-vehicle were 5 μl followed by a 10-μl flush of preservative-free saline. The doses administered were based on the salt form of the drug.

Statistical Analysis

The results are expressed as medians or means ± SD when appropriate. The data were compared using nonparametric analyses. Friedman's test for within-group and the Kruskal-Wallis and Mann-Whitney rank-sum tests for between-group comparisons were used. Multiple comparisons after Friedman's and the Kruskal-Wallis tests were performed using a two-tailed Dunnett's test or Dunn's test, respectively.[21] Because baseline motor tests were already at the cutoff point and could only decrease after intrathecal drug administration, a one-tailed test was used. Probability values <0.05 were considered significant.

Results

Throughout the experimental period the animals remained well groomed and appeared to maintain normal food and water intake.

Effects of MK-801 and AP5 on Punctate Mechanical Hyperalgesia

In saline-treated animals, the median withdrawal threshold to von Frey filaments decreased from 522 mN (pre) before surgery to 24 mN 2 h (0 min before drug administration) after incision. Hyperalgesia was persistent; withdrawal thresholds were 54 mN or less throughout the day of surgery (fig. 1A). Intrathecal administration of 4, 14, or 40 nmol MK-801 produced no increase in the withdrawal thresholds (fig. 1B–D). The next day, baseline (pre) withdrawal thresholds were again measured. No drug effect from the previous treatment was apparent. MK-801 again produced no effect on the withdrawal thresholds (data for time course not shown). Figure 1E summarizes the effect of saline and MK-801 on withdrawal thresholds 30 min after drug administration on postoperative day 1. Responses at 30 min are summarized because it has been shown that the peak effect in other models occurred at this time.[11]

AP5 (10 nmol) produced no significant effect on punctate mechanical hyperalgesia (fig. 2A–D); only intrathecal administration of 30 nmol AP5 produced a small increase in the withdrawal threshold from 24 mN to 58 mN 10 and 30 min after injection ($P < 0.05$ *vs.* 0 min; fig. 2C); however, this was no different than saline. Similar results were observed in postoperative day 1, and these are summarized in figure 2D.

Effects of MK-801 and AP5 on Nonpunctate Mechanical Hyperalgesia and Pinprick

In a saline vehicle-treated group, the mean response frequency increased from 0 ± 0% before surgery (pre) to 100 ± 0% 2 h after incision (0 min after drug administration); nonpunctate hyperalgesia was persistent (fig. 3A). Intrathecal administration of 20 or 40 nmol MK-801 (fig. 3B) or 10 or 30 nmol AP5 (fig. 3C) produced no significant decrease in the response frequency on the day of surgery. The next day, intrathecal MK-801 had no effect on nonpunctate hyperalgesia (fig. 3D–F), but intrathecal administration of 30 nmol AP5 slightly decreased the response frequency compared with the saline vehicle group at 30 min ($P < 0.05$ *vs.* saline).

In all groups of animals, positive paw withdrawal responses (100 ± 0%) to pinprick were observed before and 2 h after foot incision. Intrathecal administration of saline, 40 nmol MK-801, or 30 nmol AP5 had no significant effect on the withdrawal frequency (100± 0%) on the day of surgery or on postoperative day 1 (data not shown).

Effect of AP5 and MK-801 on Mechanical Hyperalgesia 2 and 3 Days After Incision

Forty nanomoles of intrathecal MK-801 or 30 nmol AP5 was administered on postoperative days 2 and 3 to two other groups of animals (n = 5) after plantar incision (fig. 4A–D). Intrathecal MK-801 did not inhibit mechanical hyperalgesia. Similarly, AP5 produced a

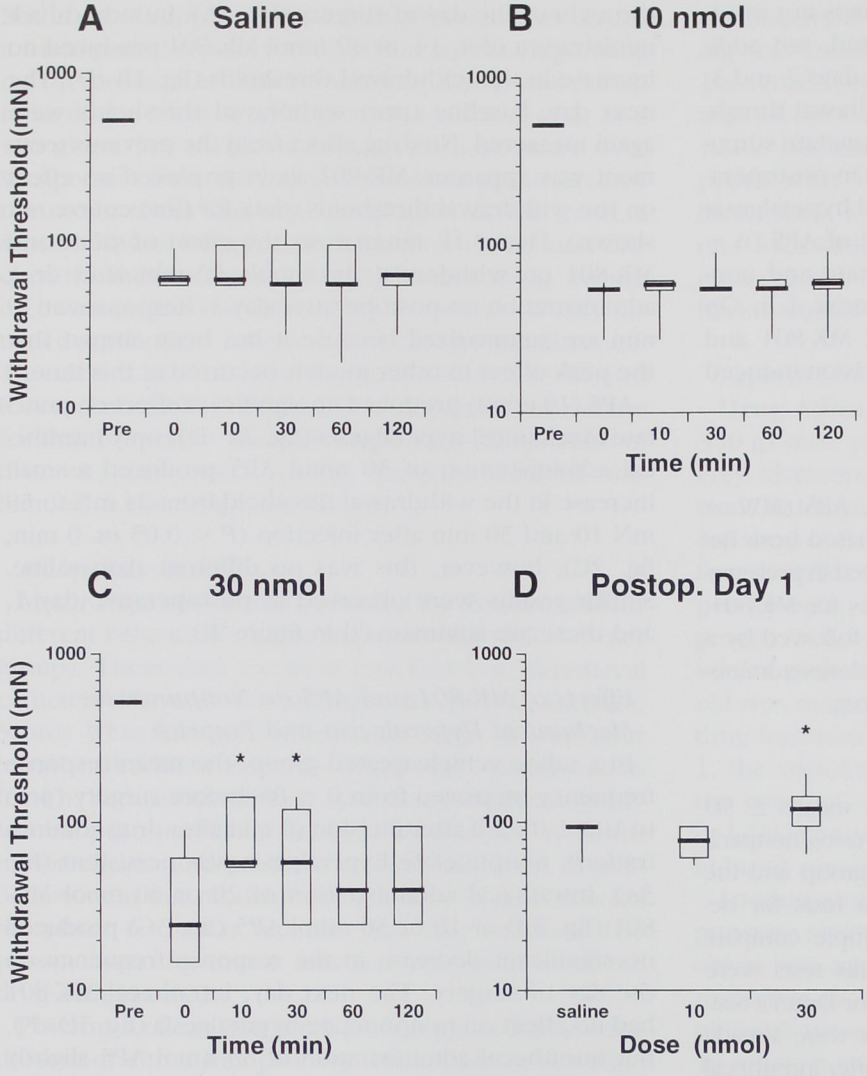

Fig. 2. Effect of intrathecal 2-amino-5-phosphonovaleric acid (AP5) on punctate mechanical hyperalgesia caused by incision. (*A–C*) Withdrawal threshold after incision in rats treated with saline or 10 or 30 nmol AP5 on the day of surgery. (*D*) Summary of withdrawal thresholds 30 min after administration of saline or AP5 on postoperative day 1. *$P < 0.05$ *versus* 0 min by the Friedman and Dunnett's tests. The box and whisker plots are described in figure 1.

small decrease in the response frequency to the nonpunctate stimulus on postoperative days 2 and 3 (fig. 4C-D); this was not statistically significant. These results are similar to our findings on the day of surgery and on postoperative day 1.

Effect of AP5 and MK-801 on Nonevoked Pain Behavior

In all groups of animals, similar nonevoked pain behavior was observed 2 h after surgery. During the first, second, and fourth hour after vehicle injection, the median pain scores were 22.5, 20, and 19.5, respectively (fig. 5A). Intrathecal administration of 40 nmol MK-801 or 30 nmol AP5 produced no significant decrease in the median pain score on either the day of surgery (fig. 5B-C) or on postoperative day 1 (fig. 5D-F).

Effect of AP5 and MK-801 on Motor Function

Stable, consistent measures of motor function occurred after saline vehicle injection and intrathecal administration of 5 μg morphine (table 1). The median cumulative placing score of the right hindpaw was significantly decreased 60 min after intrathecal administration of 40 nmol MK-801 ($P < 0.05$ *vs.* saline); the motor deficit of the left hindpaw was detectable but not statistically significant. The average cumulative balance time was decreased 30 and 60 min after MK-801, respectively ($P < 0.05$ *vs.* saline). The ambulation score was unaffected (table 1). Intrathecal administration

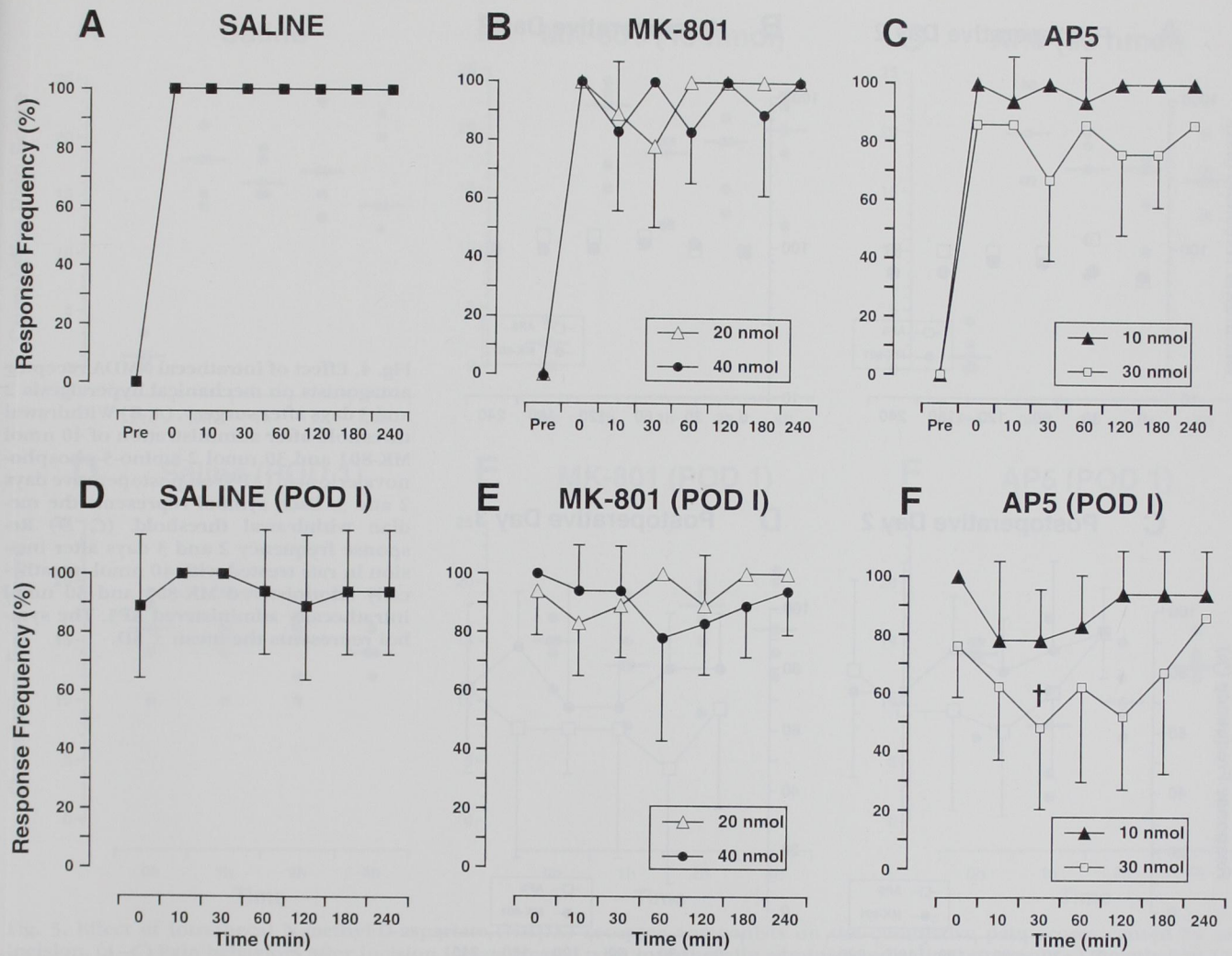

Fig. 3. Effect of intrathecal N-methyl-D-aspartate (NMDA) receptor antagonists on nonpunctate mechanical hyperalgesia after incision. (*A–C*) Response frequency after intrathecal administration of saline, (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801; 20 and 40 nmol), or 2-amino-5-phosphonovaleric acid (AP5; 10 and 30 nmol) on the day of surgery. (*D–F*) Response frequency after drug administration on postoperative day 1. †$P < 0.05$ *versus* saline by the Kruskal-Wallis and Dunnett's tests. The symbols represent the means ± SD. POD1 = postoperative day 1.

of 30 nmol AP5 decreased the median placing score at 30 min ($P < 0.05$ *vs.* 0 min); the right and left hindpaw were similarly impaired. The average cumulative balance time was decreased 30 min after intrathecal AP5 administration ($P < 0.05$ *vs.* saline). AP5 did not affect the ambulation score.

Effect of L-NAME on Punctate and Nonpunctate Hyperalgesia

Intrathecal administration of 20 or 200 nmol L-NAME produced no decrease in punctate mechanical hyperalgesia on the day of surgery (fig. 6A–B) and on postoperative day 1 (data not shown). No effect on nonpunctate hyperalgesia was observed either (fig. 6C). Two rats developed motor deficits the day after receiving the 200-nmol dose of L-NAME; data from these rats was not reported. Other signs were suggestive of toxicity with the highest dose of L-NAME; rats vocalized during application of van Frey filaments and the plastic disk in this group, although mechanical hyperalgesia was unaffected. We have not observed this vocalization in other studies.

Discussion

The most important finding of the present study is that intrathecal administration of either a competitive or

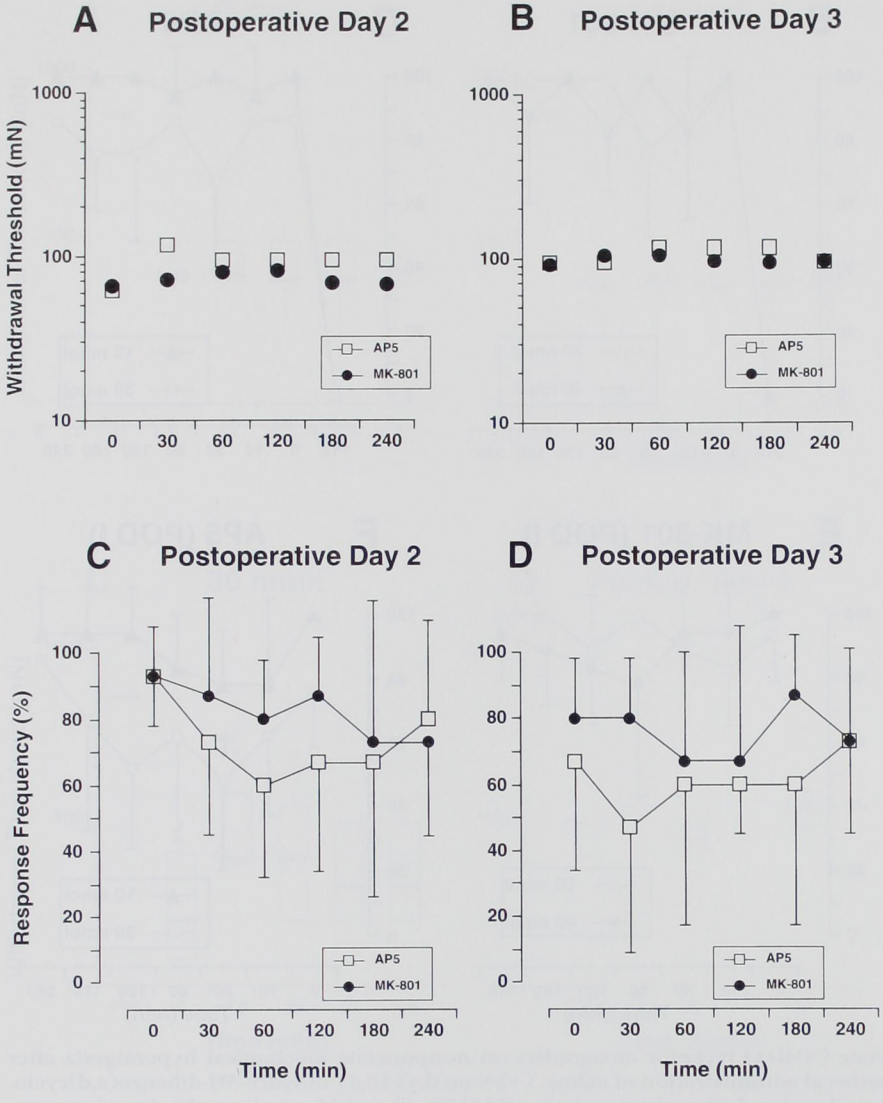

Fig. 4. Effect of intrathecal NMDA receptor antagonists on mechanical hyperalgesia 2 and 3 days after surgery. (*A, B*) Withdrawal threshold after administration of 40 nmol MK-801 and 30 nmol 2-amino-5-phosphonovaleric acid (AP5) on postoperative days 2 and 3. Each symbol represents the median withdrawal threshold. (*C, D*) Response frequency 2 and 3 days after incision in rats treated with 40 nmol intrathecally administered MK-801 and 30 nmol intrathecally administered AP5. The symbol represents the mean ± SD.

noncompetitive NMDA receptor antagonist produced minimal effects on mechanical hyperalgesia in this rat model of incisional pain. In addition, no decrease in nonevoked pain behaviors was observed. The greatest dose tested of each NMDA receptor antagonist caused motor impairment; greater doses would likely increase the motor deficit, making these assessments of pain behaviors difficult. Consistent with these observations, intrathecal administration of L-NAME caused no decrease in mechanical hyperalgesia.

Comparisons with Clinical Studies

There is little information on the role of NMDA receptor antagonists in postoperative pain. Nevertheless, the analgesic properties of systemically administered ketamine, a noncompetitive NMDA receptor antagonist, are well known. Ketamine has been used for postoperative pain treatment[22–24]; however, analgesic doses were associated with side effects such as nightmares and dissociative states. Lower doses of ketamine alone had no dysphoric side effects but produced only marginal analgesia.[25] Ketamine, in combination with morphine, provided better postoperative pain relief and reduced the requirement for morphine as well.[26]

Several investigators administered ketamine epidurally to produce analgesia in patients after operations. These early studies indicated that epidural ketamine reduced pain in patients after lower abdominal and ex-

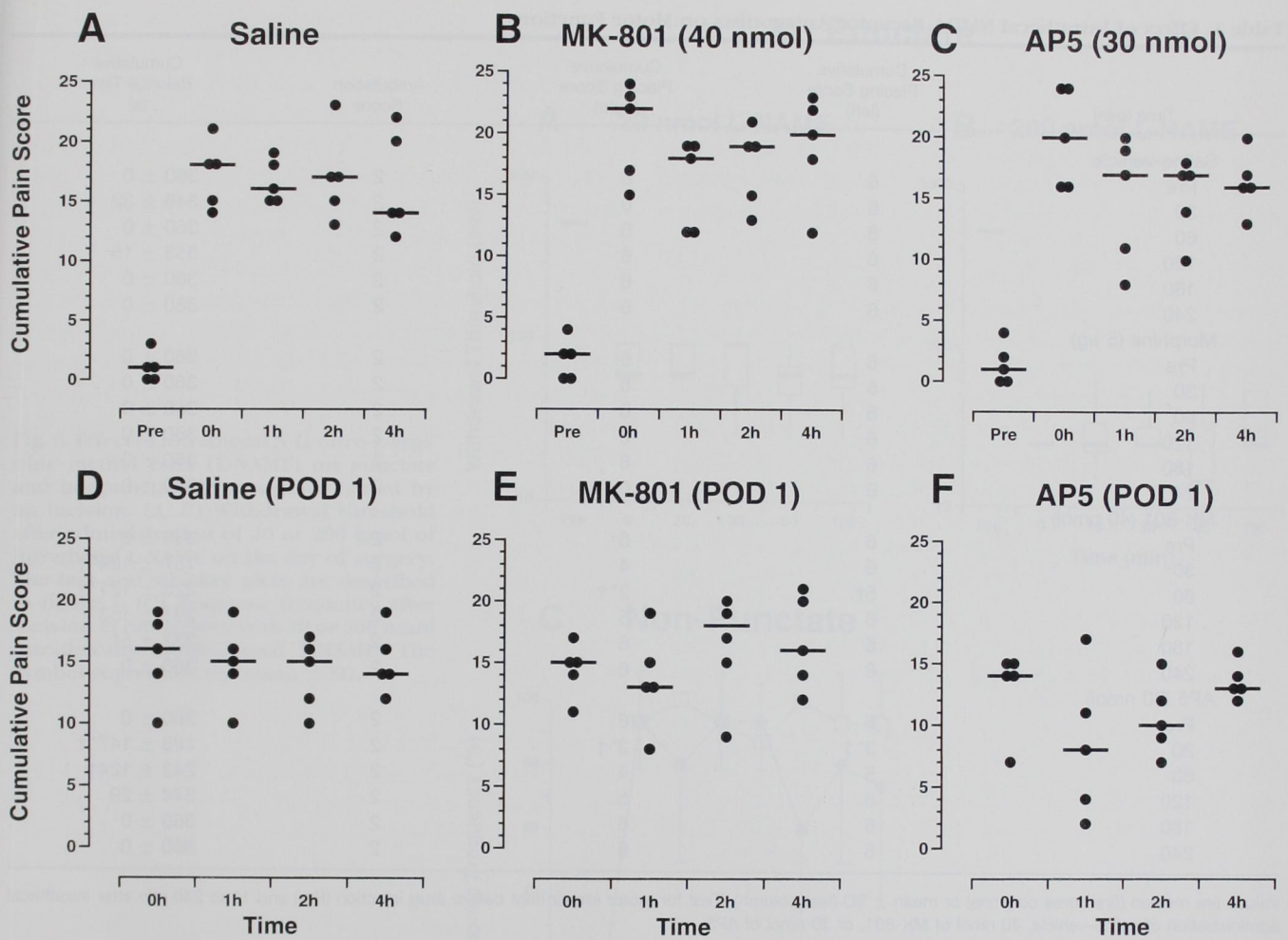

Fig. 5. Effect of intrathecal N-methyl-D-aspartate (NMDA) receptor antagonists on the cumulative pain scores caused by an incision. (*A–C*) Pain behaviors after incision in rats treated with intrathecally administered saline, 40 nmol (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801), or 30 nmol 2-amino-5-phosphonovaleric acid (AP5) on the day of surgery. (*D–F*) Pain scores after incision in rats treated with these drugs on postoperative day 1. Each dot is one score; the horizontal line represents the median.

tremity procedures[27] and after cholecystectomy.[28] A problem with these preliminary reports is that a systemic effect of epidurally injected ketamine could not be excluded. In contrast, several later studies noted no postoperative pain relief after epidural administration of ketamine in patients after thoracotomy and major surgical procedures,[29] gynecologic operations,[30] and orthopedic surgeries.[31,32] In three of these studies,[30–32] epidurally administered ketamine alone provided marginal postoperative pain relief, whereas epidural morphine was highly effective. Similarly, marked reduction in pain behaviors was observed after intrathecal administration of morphine in this rat model,[16] and only minimal effects on pain behaviors were observed after intrathecal injection of NMDA receptor antagonists.

Effect of Intrathecal MK-801 and AP5 on Hyperalgesia in Animal Models

It is generally accepted that intrathecal NMDA receptor antagonists have little antinociceptive effect in normal animals.[10,33,34] Several investigators have examined the effect of spinally administered NMDA receptor antagonists in models of persistent pain and hyperalgesia. Intrathecal administration of 6 nmol MK-801[6] or 10–18 nmol AP5[6,7,35] significantly attenuated the development of pain behaviors caused by intraplantar injection of

Table 1. Effect of Intrathecal NMDA Receptor Antagonists on Motor Function

Time (min)	Cumulative Placing Score (left)	Cumulative Placing Score (right)	Ambulation Score	Cumulative Balance Time (s)
Saline-vehicle				
Pre	6	6	2	360 ± 0
30	6	6	2	346 ± 32
60	6	6	2	360 ± 0
120	6	6	2	353 ± 15
180	6	6	2	360 ± 0
240	6	6	2	360 ± 0
Morphine (5 μg)				
Pre	6	6	2	360 ± 0
30	6	6	2	360 ± 0
60	6	6	2	360 ± 0
120	6	6	2	360 ± 0
180	6	6	2	360 ± 0
240	6	6	2	360 ± 0
MK-801 (40 nmol)				
Pre	6	6	2	360 ± 0
30	6	4	2	187 ± 124*†
60	5†	2*†	2	223 ± 121*†
120	6	5	2	291 ± 153
180	6	6	2	337 ± 51
240	6	6	2	360 ± 0
AP5 (30 nmol)				
Pre	6	6	2	360 ± 0
30	3*†	2*†	2	198 ± 147*†
60	5	4	2	243 ± 124†
120	6	5	2	344 ± 29
180	6	6	2	360 ± 0
240	6	6	2	360 ± 0

Values are median (first three columns) or mean ± SD (last column). Test for motor impairment before drug injection (Pre) and 10 to 240 min after intrathecal administration of saline-vehicle, 40 nmol of MK-801, or 30 nmol of AP5.

* $P < 0.05$ *versus* 0 min by Friedman and Dunnett's test.

† $P < 0.05$ *versus* saline by Kruskal-Wallis and Dunnett's test.

formalin, a model of chemical irritation. Ren *et al.*[10] and Yamamoto *et al.*[33] examined the effect of these drugs in rats made hyperalgesic to radiant heat by intraplantar injection of carrageenan; thermal hyperalgesia was attenuated or reversed after intrathecal administration of 8–10 nmol MK-801[10,33] or 20 nmol AP5.[10] Thermal hyperalgesia to radiant heat was also reduced by intrathecal injection of 2.5 to 20 nmol MK-801[8,13,34,36] or 15 nmol AP5[13] in experimental peripheral neuropathy.

Despite the large number of trials of these drugs in the formalin model and thermal hyperalgesia, the effect of intrathecal NMDA receptor antagonists on mechanical hyperalgesia has been studied less. Ren and Dubner[11] examined the effect of intrathecal MK-801 and AP5 on mechanical hyperalgesia after inflammation caused by intraplantar injection of Freund's adjuvant; mechanical hyperalgesia was assessed by multiple applications (one to five times) of von Frey filaments and paw withdrawal threshold was determined. Intrathecal administration of much greater doses (30 nmol MK-801 or 203 nmol AP5) than those used in studies of thermal hyperalgesia reduced mechanical hyperalgesia by 54% and 62%, respectively. Recently, Chaplan *et al.*[35] examined the effect of NMDA receptor antagonists for suppression of allodynia caused by tight ligation of the L5 and L6 spinal nerves, as described by Kim and Chung.[37] Intrathecal AP5 produced inhibition in doses ranging from 6–60 nmol; MK-801, administered intrathecally in doses of 3–30 nmol, had little effect. Others observed that 10 nmol intrathecal MK-801 had no effect on mechanical hyperalgesia after nerve injury.[34]

Because intrathecal administration of these drugs re-

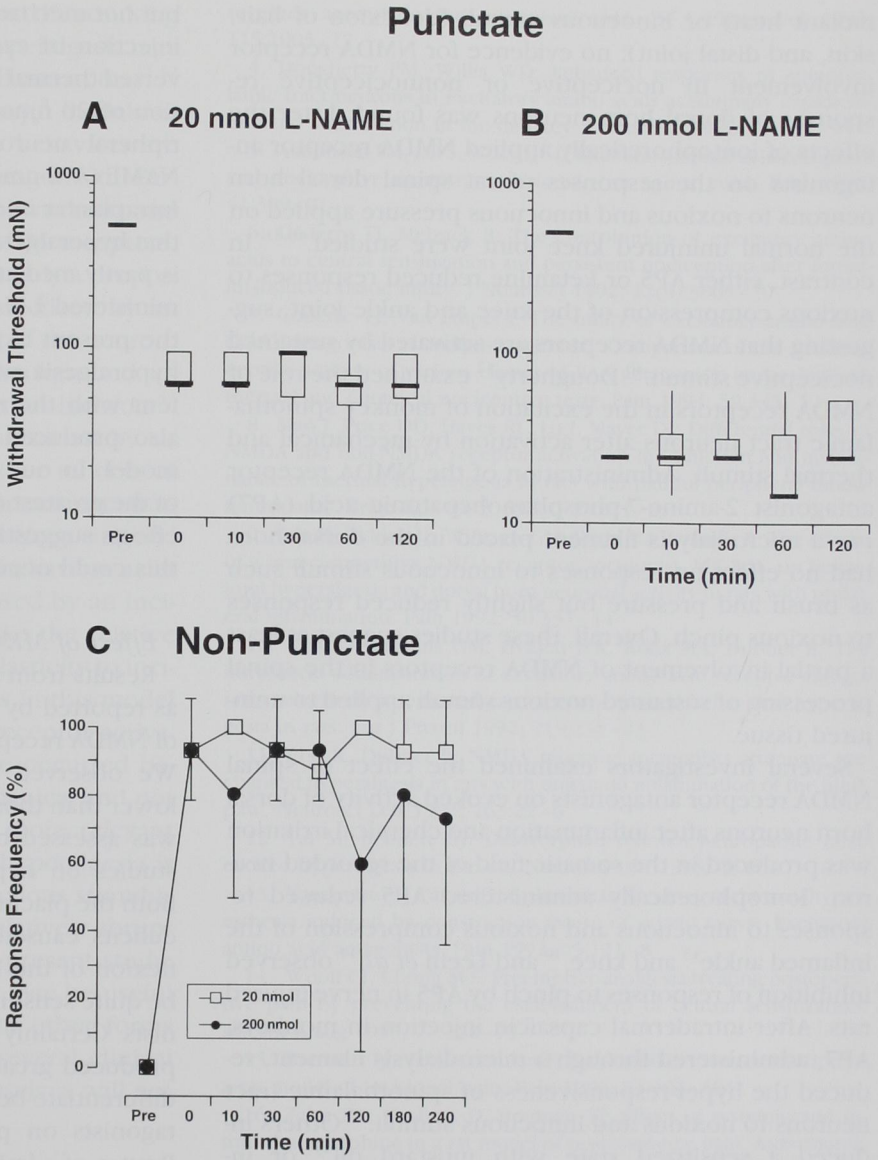

Fig. 6. Effect of intrathecal N-G-nitro-L-arginine methyl ester (L-NAME) on punctate and nonpunctate hyperalgesia caused by an incision. (*A, B*) Withdrawal threshold after administration of 20 or 200 nmol of intrathecal L-NAME on the day of surgery. The box and whisker plots are described in figure 1. (*C*) Response frequency after incision in rats treated with 20 or 200 nmol intrathecally administered L-NAME. The symbol represents the mean ± SD.

duces responses in rats made hyperalgesic to radiant heat and radiant heat is typically a stimulus that is 6–8 mm in diameter on the rat hindpaw, we examined the effect of a nonpunctate mechanical stimulus 5 mm in diameter. Perhaps punctate mechanical stimuli are insensitive to NMDA receptor antagonists because of the small area of tissue stimulated. In the present study, little inhibition of responses to the nonpunctate stimulus was observed, suggesting that the area of the stimulus (spatial summation) is not critical for inhibition of mechanical hyperalgesia by intrathecal NMDA receptor antagonists. In addition, others have shown that nonevoked, spontaneous behaviors are inhibited by these drugs[7,35,38]; we also studied nonevoked pain and again found no effect.

Effects of NMDA Receptor Antagonists on Dorsal Horn Neurons

Further information about the importance of spinal NMDA receptors in nociceptive transmission can be gained from studies on individual spinal dorsal horn neurons. Headley *et al.*[39] examined the effect of iontophoretically administered ketamine on responses of dorsal horn neurons of cats and rats to noxious (pinch and

radiant heat) or innocuous stimuli (deflexion of hair, skin, and distal joint); no evidence for NMDA receptor involvement in nociceptive or nonnociceptive responses of dorsal horn neurons was found. Later the effects of iontophoretically applied NMDA receptor antagonists on the responses of rat spinal dorsal horn neurons to noxious and innocuous pressure applied on the normal uninjured knee joint were studied.[40,41] In contrast, either AP5 or ketamine reduced responses to noxious compression of the knee and ankle joint, suggesting that NMDA receptors are activated by sustained nociceptive stimuli.[40] Dougherty[42] examined the role of NMDA receptors in the excitation of monkey spinothalamic tract neurons after activation by mechanical and thermal stimuli. Administration of the NMDA receptor antagonist 2-amino-7-phosphonohepatonic acid (AP7) *via* a microdialysis filament placed in the dorsal horn had no effect on responses to innocuous stimuli such as brush and pressure but slightly reduced responses to noxious pinch. Overall, these studies suggest at least a partial involvement of NMDA receptors in the spinal processing of sustained noxious stimuli applied to uninjured tissue.

Several investigators examined the effect of spinal NMDA receptor antagonists on evoked activity of dorsal horn neurons after inflammation and chemical irritation was produced in the somatic field of the recorded neuron. Iontophoretically administered AP5 reduced responses to innocuous and noxious compression of the inflamed ankle[43] and knee,[40] and Leem *et al.*[44] observed inhibition of responses to pinch by AP5 in nerve-injured rats. After intradermal capsaicin injection in monkeys, AP7, administered through a microdialysis filament, reduced the hyper-responsiveness of spinothalamic tract neurons to noxious and innocuous stimuli.[42] Others induced a sensitized state with mustard oil[45] or intraplantar injection of formalin[46] and observed that NMDA receptor antagonists reduced hyperreflexia and hyperresponsiveness of dorsal horn neurons, respectively. Overall, these experiments indicate that NMDA receptors are important in part for the transmission of information in spinal dorsal horn neurons sensitized by inflammation or chemical irritation.

Effect of Intrathecal L-NAME on Hyperalgesia in Animal Models

Several investigators have used inhibition of spinal NO synthase by intrathecal injection of L-NAME to assess the role of NO in pain behaviors. Intrathecal doses as low as 20 nmol L-NAME markedly reduced thermal but not mechanical hyperalgesia caused by intraplantar injection of carrageenan.[47] These same low doses reversed thermal hyperalgesia after intrathecal administration of 20 nmol L-NAME in rats with experimental peripheral neuropathy.[48] Intrathecally administered L-NAME (370 nmol) diminished late pain behaviors[49] after intraplantar injection of formalin. It has been suggested that hyperalgesia produced by NMDA in the dorsal horn is partly mediated *via* nitrous oxide.[17] Intrathecally administered L-NAME (20 and 200 nmol) was studied in the present experiments and no effect on mechanical hyperalgesia was observed; these findings are consistent with the results that NMDA receptor antagonists also produced little effect on pain behaviors in this model. In our experiments, intrathecal administration of the greatest dose of L-NAME produced untoward side effects suggestive of toxicity. Data from others indicate this could occur.[50]

Effect of MK-801 and AP5 on Motor Function

Results from tests of motor dysfunction demonstrate, as reported by others,[51] that intrathecal administration of NMDA receptor antagonists produced motor deficits. We observed decreases in motor function at doses lower than those reported by others.[7,35] Motor function was assessed because the endpoint of the behavioral studies on hyperalgesia was a withdrawal response. Both the placing reflex and the balancing time detected deficits caused by these drugs. Both tests examined flexion of the lower extremities, and this appeared to be quite sensitive to intrathecal NMDA receptor antagonists. Certainly greater doses of these drugs would have produced greater motor deficits, making it difficult to differentiate between the effects of NMDA receptor antagonists on pain behaviors and motor impairment. Routes of administration concentrating the drug to the dorsal horn and reducing ventral horn (motor) effects may show different results. The present study indicates that intrathecally injected NMDA receptor antagonists produce more motor dysfunction than inhibition of pain behaviors caused by an incision.

Conclusions

These studies suggest that spinal NMDA receptors are not critical for the maintenance of pain behaviors in this rat model of postoperative pain. Results from this study are in contrast to findings from experiments in other animal models; there are several important impli-

cations of this finding. First, an incision and the resulting nociceptive behaviors and mechanical hyperalgesia may be less intense than that caused by inflammation,[11,52] chemical irritation,[35] and the nerve injury,[8] models in which sensitization processes and pain behaviors are inhibited by spinal NMDA receptor antagonism. Perhaps tissue injury by incision may lack the intensity and severity to sustain activation of spinal NMDA receptors. In support of this, we have observed that only a proportion of dorsal horn neurons become sensitized by an incision placed within the receptive field of the neuron; approximately 50% exhibit increases in background activity and expanded receptive fields after an incision.[53] This degree of dorsal horn neuron sensitization by an incision is less than that observed in other models of persistent pain.[40] Second, perhaps a pretreatment strategy, such as intrathecal administration before surgery, would modify the development of pain behaviors as in other models. Third, pain behaviors caused by an incision may be largely primary hyperalgesia; the relative importance of central sensitization and plasticity to incisional pain is not known but may be less in this model and perhaps less dependent on NMDA receptor activation. Fourth, these differences may have occurred because our studies were focused on mechanical and not thermal stimuli. Mechanical stimuli are more relevant to clinical postoperative pain. Thermal hyperalgesia is a measure of withdrawal latency to a noxious stimulus of greater duration (3–6 s) than single, relatively abrupt stimuli used in some of the tests in the present study. Thus spinal NMDA receptor antagonists may be useful for the treatment of neuropathic pain or other forms of persistent pain, but our results and several clinical studies suggest that these drugs by themselves will not be effective for pain relief after surgery.

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