■ LABORATORY INVESTIGATIONS

Effect of Systemic and Intrathecal Morphine in a Rat Model of Postoperative Pain

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Background: To learn more about persistent pain after an incision, a rat model for postoperative pain has been developed. To further evaluate this model, the authors examined the effect of intrathecal (IT) and subcutaneous (SC) morphine, effective for postoperative pain relief in patients, on pain behaviors immediately after surgery and 1 day after surgery.

Methods: Rats were anesthetized with halothane, and a 1-cm incision was made in the plantar aspect of the foot and closed. After recovery, the rats were placed on an elevated plastic mesh floor, and withdrawal threshold was determined using calibrated von Frey filaments (15–522 mN) applied from beneath the test cage to an area adjacent to the wound at the heel. Pain behaviors also were assessed using the response frequency to a nonpunctate mechanical stimulus and a cumulative pain score.

Results: Mechanical hyperalgesia and nonevoked pain behaviors were present on the day of surgery and 1 day after surgery. Administration of either SC (0.3–3.0 mg/kg) or IT (0.16–5.0 μg) morphine reversibly increased the withdrawal threshold. The response frequency to the nonpunctate stimulus and the nonevoked pain scores also were decreased by 3 mg/kg of SC or 5 μg of IT morphine. Naloxone (1 mg/kg) reversed morphine-produced hypoalgesia.

Conclusion: This is the first study to demonstrate that mechanical hyperalgesia to a nonpunctate stimulus occurs after a surgical incision in the rat. This rat model of postoperative has several similarities to postoperative patients: mechanical hyperalgesia to punctate and nonpunctate stimuli, nonevoked pain, and pain behaviors inhibited by SC and IT morphine. This model also may be useful for predicting analgesia by investigational agents for postoperative pain. (Key words: Animal: rat. Morphine. Pain mechanisms: hyperalgesia; incision; postoperative.)

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BASIC and clinical research has increased our understanding of the pathophysiology of pain mechanisms, including postoperative pain; however, postoperative pain control continues to be a problem despite evidence that efficacious analgesia improves patient satisfaction, reduces morbidity, and perhaps decreases mortality after surgery. Most research in postoperative pain has focused primarily on the use of opioids, opioid dose reduction by nonsteroidal antiinflammatory agents, preemptive analgesia, and regional analgesia. Thus far, regional analgesia with combination therapies appears optimal but is not without risk and is not consistently proven to reduce costs.[1]

Pain from a surgical incision occurs at rest and is exacerbated by coughing, ambulation, and mechanical stimulation. The mechanical sensitivity of a surgical incision is an important property because the efficacy of postoperative analgesic treatments should be assessed using evoked responses during function such as movement if outcome is to improve with enhanced analgesia.[1] To advance our knowledge of postoperative pain and gain a better understanding of the mechanisms of pain from an incision, we developed and characterized a rat model of postoperative pain.[2] In this model, a surgical incision causes reproducible, quantifiable mechanical hyperalgesia that parallels the postoperative course of patients. Because opioids, administered systemically or intraspinally, are the most common agents used to manage postoperative pain, the effects of intrathecal (IT) and subcutaneous (SC) morphine were examined on pain behaviors to further evaluate this model of postoperative pain. The effects of morphine were tested immediately after surgery and again 24 h

later. Preliminary reports of these data in abstract form have been made.[3,4]

Methods and Materials

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 as issued by the International Association for the Study of Pain.[5]

Experiments were performed on 95 adult (weight, 300–350 g) male Sprague-Dawley rats (Harlan, Indianapolis, IN) housed in pairs before surgery. Food and water were available *ad libitum*. Postoperatively, the animals were housed individually with sterile bedding consisting of organic cellulose fiber (Cellu-Dri®, Shepherd Specialty Papers, Inc., Kalamazoo, MI). The incisions were checked daily, and any sign of wound infection or dehiscence excluded the animal from the study. Four 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 dilantin.

Foot Incision

All rats were anesthetized with halothane, 2%, delivered via a nose cone and were administered an intramuscular injection of penicillin (Flocillin®), 30,000 IU, in the triceps muscle. As described previously,[2] the plantar aspect of either hindpaw was prepared in a sterile manner with a 10% povidone-iodine solution and draped. A 1-cm longitudinal incision was made with a number 11 blade through skin and fascia of the plantar aspect of the foot, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally, and the muscle origin and insertion remained intact. After hemostasis with gentle pressure, 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.

Intrathecal Catheter Placement

For subarachnoid administration of morphine, IT catheters were placed in rats anesthetized by an intraperitoneal injection of chloral hydrate (250–300 mg/kg) and supplemented with halothane if needed. After sterile preparation of the posterior neck, a small PE-10 catheter (8.5 cm) was inserted through an opening in the atlantooccipital membrane to the lumbar spinal cord according to the method of Yaksh and Rudy.[6] 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. Experiments were begun not less than 4 days after IT catheter placement.

Behavioral Testing

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 x 27 x 15 cm) and allowed to acclimate. Baseline pain behaviors were measured as will be described.

Withdrawal responses to punctate mechanical stimulation were determined using calibrated von Frey filaments applied from underneath the cage through openings (12 x 12 mm) in the plastic mesh floor to an area adjacent to the wound (fig. 1). Each von Frey filament was applied once, starting with 15 mN and continuing until a withdrawal response occurred or 522 mN (the cut-off value) was reached. This was repeated three times with a 5- to 10-min test-free period between withdrawal responses. The lowest force from the three tests producing a response was considered the *withdrawal threshold*. The cut-off value, 522 mN, was recorded even if there was no withdrawal response to this force.

To assess responses to a nonpunctate mechanical stimulus, a 5-mm diameter, circular plastic disk attached to a von Frey filament (400 mN) was applied from underneath the cage through openings in the plastic mesh floor directly on the intended incision site (fig. 1E). The edges of the disk were flat. 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 determine the effect of morphine on withdrawal produced by a 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 each test period. The withdrawal frequency was calculated from the single test.
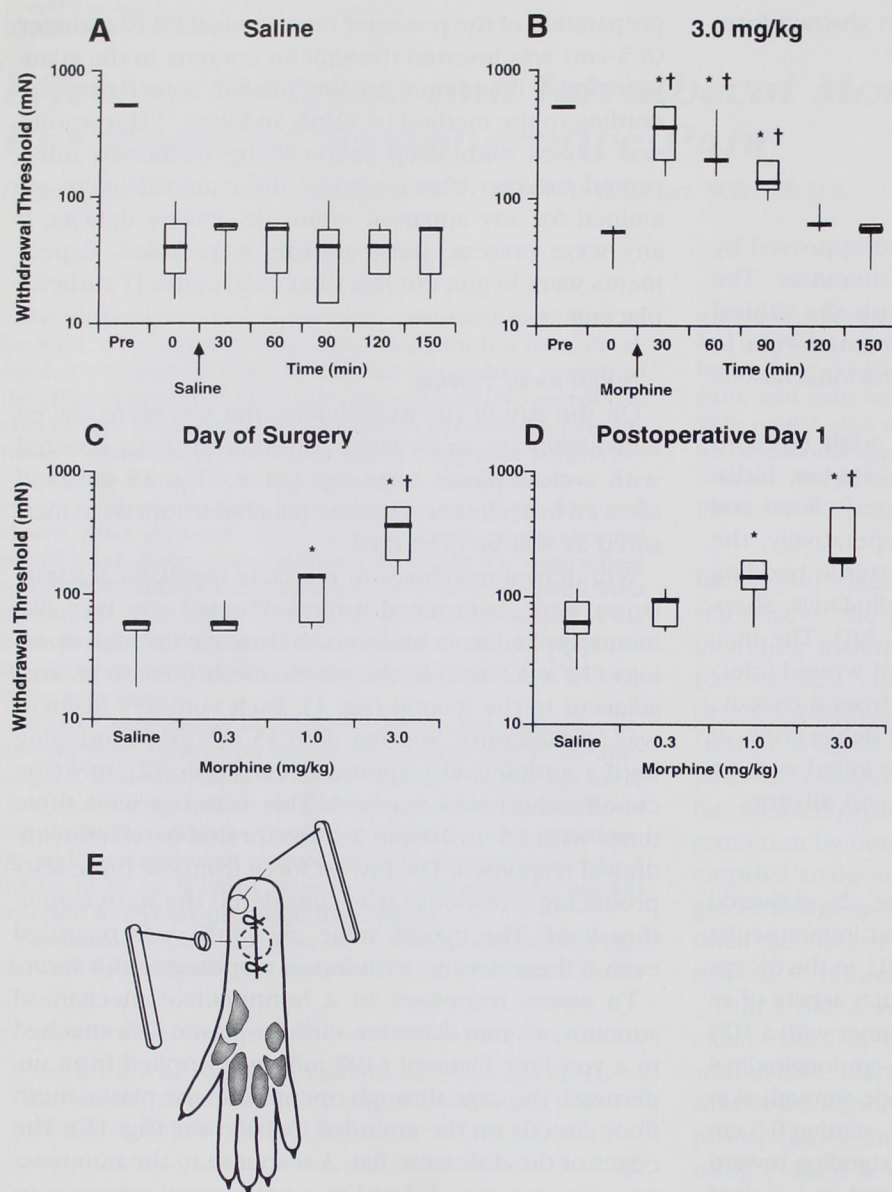
Fig. 1. Effect of subcutaneous (SC) morphine on punctate mechanical hyperalgesia caused by incision. The results are expressed as medians (horizontal line) with first and third quartiles (boxes), and tenth and ninetieth percentiles (vertical lines). A, Withdrawal thresholds after incision in saline-treated rats. B, Withdrawal threshold after incision in rats treated with 3 mg/kg of SC morphine on the day of surgery. C and D, Summary of withdrawal thresholds 30 min after administration of saline or morphine on the day of surgery and 1 day after surgery. E, Diagram of the plantar aspect of the rat foot showing site of application of von Frey filament (solid circle) and site of application of plastic disk (dashed circle). *$P < 0.05$ *vs.* 0 min by Friedman and Dunnett's test. †$P < 0.05$ *vs.* saline by Kruskal-Wallis and Dunnett's test.

A cumulative pain score, measured by a method adapted from Brennan *et al.*,[2] was used to evaluate the effect of morphine on nonevoked pain behaviors. Unrestrained rats were placed on a smaller plastic mesh floor (grid, 8 x 8 mm). 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 the majority of the 1-min scoring period, a 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 1 was given. The sum of the 12 scores (0–24) obtained during the 1-h session for each foot was calculated. 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.

Experimental Protocols

Morphine Administration. Twenty-four rats (300–350 g) were pretested for withdrawal threshold to von Frey filaments as described previously. 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 again tested. Saline, 0.3, 1, or 3 mg/kg of morphine was administered SC (n = 6 per group). Withdrawal threshold was measured every 30 min after morphine injection for the next 2 h. One day after surgery, the withdrawal threshold was again determined in the same rats. The same dose of morphine (or saline) was injected, and the effect of morphine on incision-induced hyperalgesia was measured.

Twenty-five additional rats with IT catheters were used to measure the effect of spinally administered morphine (0.16, 0.5, 1.7, or 5 μg; n = 5 per group) on punctate mechanical hyperalgesia caused by foot incision using a protocol similar to that described for SC morphine. Morphine was injected IT in a volume of 5 μl and flushed with 10 μl of normal saline.

Another group (n = 10) 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 plastic disk and to the pinprick were again measured. Saline or 3 mg/kg of morphine was administered SC (n = 5 per group), and nonpunctate and pinprick responses were recorded. One day after surgery, the baseline response frequencies were again determined; the same dose of morphine (or saline) was injected. The effect of saline and 5 μg of IT morphine on nonpunctate mechanical hyperalgesia and pinprick after incision was measured in 10 rats.

A separate group of animals (n = 12) was pretested for nonevoked pain behavior as described previously. 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 or 3 mg/kg of SC morphine (n = 6 per group) was administered, and pain scores were recorded during the first, second, and fourth h after morphine on the day of surgery and one day after surgery. Similarly, the effect of 5 μg of IT morphine (n = 12) on nonevoked pain after incision was assessed.

Naloxone-treated Animals. In six rats, withdrawal thresholds to von Frey filaments (n = 3) and response frequencies (n = 3) to the plastic disc were measured, a foot incision was made, and after recovery responses were again determined. Morphine (3 mg/kg) was administered SC, and its effect on withdrawal threshold and response frequencies was established. Naloxone was administered 30 min later, and the effect of nalox-

one on morphine-induced hypoalgesia was assessed. Similarly, the effect of naloxone (1 mg/kg, SC) on IT morphine (5 μg)-induced hypoalgesia to punctate (n = 3) and nonpunctate (n = 3) stimuli was measured.

Drugs

Preservative-free morphine sulfate was purchased from Abbott Laboratories (Chicago, IL); the dose administered was based on mg or μg of morphine sulfate. Naloxone HCl was purchased from Astra Pharmaceuticals (Westborough, MA).

Statistical Analysis

The data were analyzed with nonparametric statistics. Friedman's test, the Kruskal-Wallis test, and Mann-Whitney rank-sum test were used. Multiple comparisons after Friedman's test or the Kruskal-Wallis test were performed by using Dunnett's test.[7] $P < 0.05$ was considered statistically significant.

Results

Throughout the experimental period, the animals remained well groomed and appeared to maintain normal food and water intake. Except for impaired weight-bearing on the area of the incision, gait appeared unaffected.

Subcutaneous Morphine

In the saline-treated group, the median withdrawal threshold to von Frey filaments decreased from 522 mN before surgery to 39 mN 2 h after incision. Hyperalgesia was persistent; the withdrawal thresholds were 58 mN or less throughout the day of surgery (fig. 1A). Greater doses of morphine produced higher withdrawal thresholds and longer lasting effects. The time course for the greatest dose of morphine, 3 mg/kg, is shown in figure 1B. Figure 1C summarizes the effect of morphine on withdrawal thresholds 30 min after drug administration on the day of surgery. This response at 30 min is shown because it was generally when the peak effect of morphine occurred. The next day baseline (predrug) withdrawal thresholds were measured and were similar to the day of surgery (data not shown). No drug effect from the previous treatment was apparent. The magnitude of hypoalgesia (fig. 1D) and the time course produced by morphine 1 day after surgery were similar to the response on the day of surgery (data for time course not shown).

For experiments in which the nonpunctate stimulus

was used, the mean response frequency increased from $0 \pm 0\%$ before surgery to $100 \pm 0\%$ 2 h later (fig. 2A) in the saline-treated group. Nonpunctate hyperalgesia remained consistent after saline vehicle injection. Morphine, 3 mg/kg (SC), significantly decreased the response frequency 30 and 60 min later ($P < 0.05$ *vs.* 0 min) on the day of surgery and similarly 1 day after surgery (fig. 2B). Morphine, 3 mg/kg SC, did not decrease the withdrawal frequency to pinprick on the nonincised foot on the day of surgery (fig. 2C) or 1 day after surgery (data not shown).

For nonevoked pain behavior, the median pain score increased from 0 before surgery to 19 2 h later in the saline-treated group (fig. 3A). During the first, second, and fourth h after vehicle injection, the median pain scores were 22.5, 20, and 19.5, respectively. Morphine (3 mg/kg, SC) decreased the median pain score during the first and second h on the day of surgery (fig. 3B) and 1 day after surgery (figs. 3C and 3D).

Intrathecal Morphine

Hyperalgesia was again persistent after incision in vehicle-treated animals (fig. 4A). The time course for the highest dose, 5 μg, is shown in figure 4B. The lowest dose of morphine produced no significant effect, but 0.5 μg or more of IT morphine increased the withdrawal threshold after incision ($P < 0.05$ *vs.* 0 min; fig. 4C). Again, greater doses caused higher withdrawal thresholds and more prolonged effects (data not shown). Similar inhibition was observed 1 day after surgery (fig. 4D).

Administration of 5 μg of IT morphine decreased the response frequency to the nonpunctate stimulus on the day of surgery (fig. 5A) and 1 day after surgery (fig. 5B). Morphine, 5 μg IT, inhibited the withdrawal response to pinprick on the nonincised foot from 30 to 90 min on the day of surgery (fig. 5C) and 1 day after surgery (data not shown). Morphine, administered IT, decreased the median pain score during the first and second h ($P < 0.05$) on both days of testing (fig. 6).

Reversal of Morphine-induced Hypoalgesia by Naloxone

Injection of 3 mg/kg of SC morphine increased the median withdrawal threshold to 198 mN after plantar incision (fig. 7A). After naloxone (1 mg/kg, SC) was administered, the median withdrawal threshold decreased to 24 mN or less. In a separate group of animals, morphine decreased the response frequency after incision (fig. 7B). One mg/kg of SC naloxone increased the
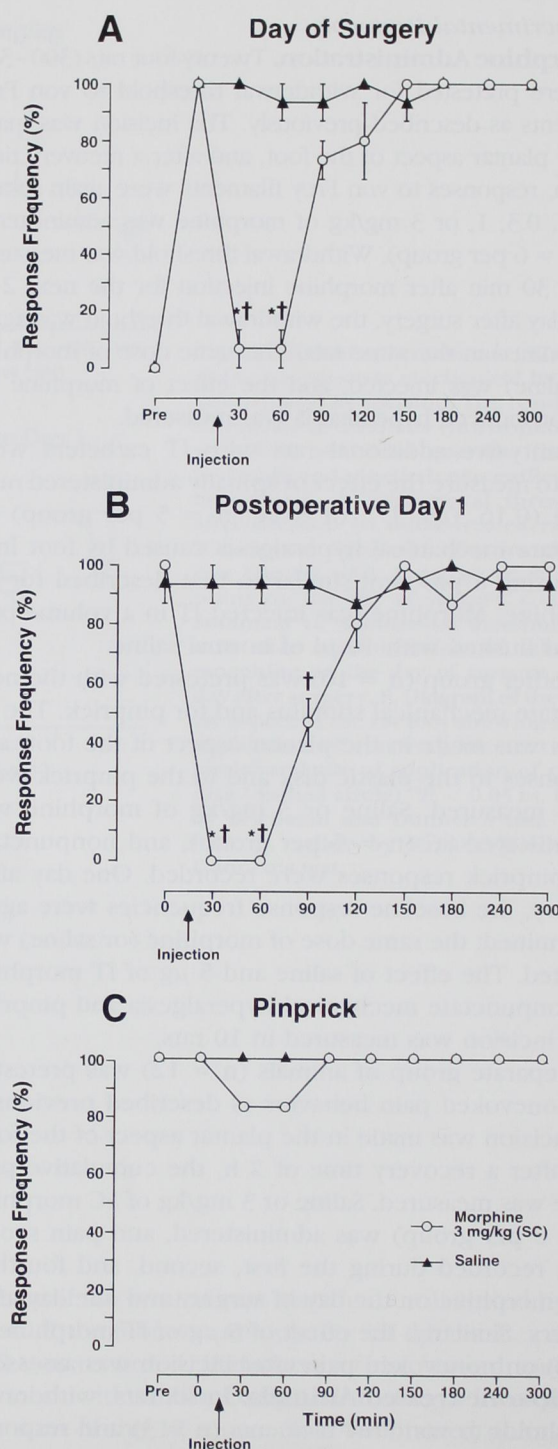
Fig. 2. Effect of subcutaneous (SC) morphine on nonpunctate mechanical hyperalgesia. *A,* Response frequency in saline- and morphine-treated animals (given at arrow) on the day of surgery. *B,* One day after surgery. *C,* Effect of SC morphine on withdrawal responses to pinprick. *$P < 0.05$ *vs.* 0 min by Friedman and Dunnett's test. †$P < 0.05$ *vs.* saline by Mann-Whitney rank sum test. The symbol represent the mean ± SEM.
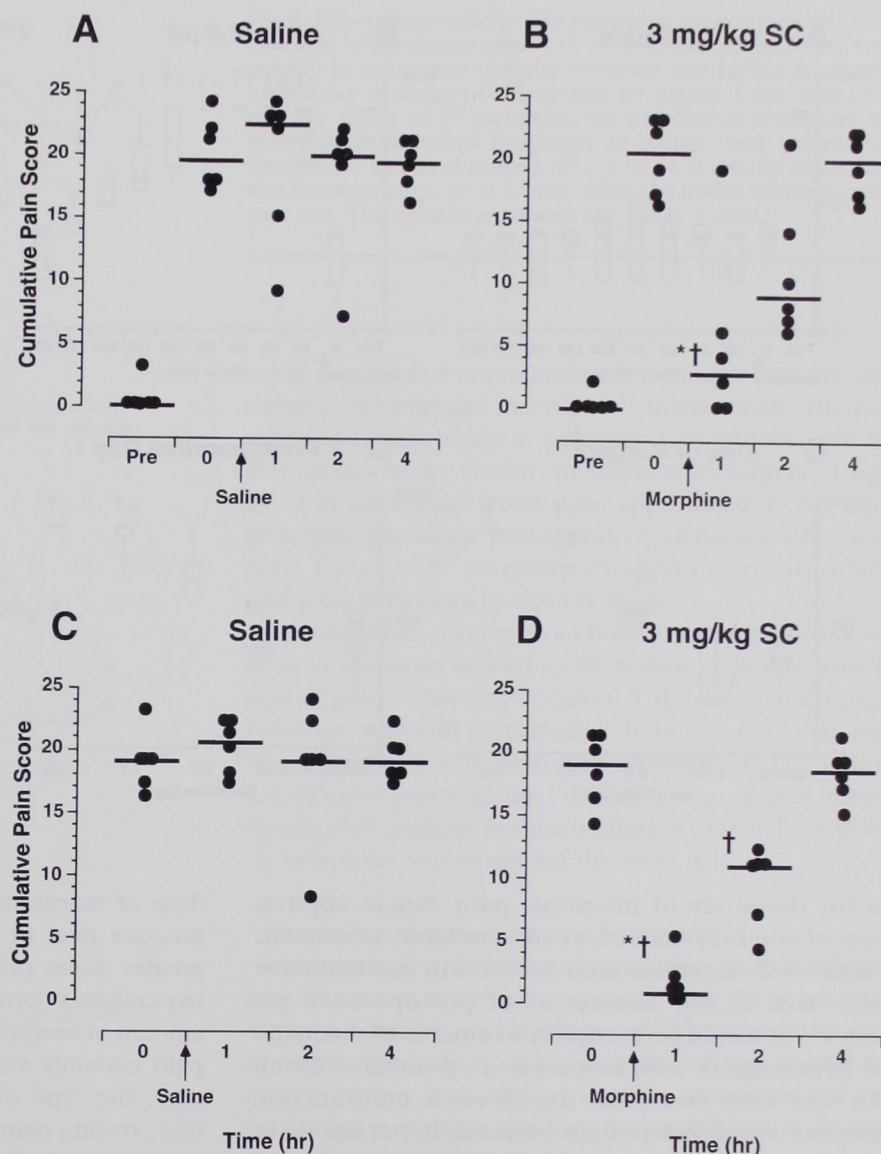
Fig. 3. Effect of subcutaneous morphine on cumulative pain scores caused by an incision. In saline-treated rats (*A*) and in morphine-treated rats (*B*) on the day of surgery. *C* and *D* are of 1 day after surgery. *$P < 0.05$ *vs.* 0 min by Friedman and Dunnett's test. †$P < 0.05$ *vs.* saline by Mann-Whitney rank sum test. Each dot is one score; the horizontal line represents the median.

mean response frequencies to 89 ± 11% or greater. Administration of 1 mg/kg of SC naloxone reversed inhibition of mechanical hyperalgesia by IT morphine to punctate (fig. 7C) and nonpunctate stimuli (fig. 7D).

Discussion

An important finding of the present study is documentation of mechanical hyperalgesia to a nonpunctate stimulus after a surgical incision in the rat, a circumstance analogous to pressure applied to a patient's incision. Morphine was used to modify pain behaviors in

this rat model because it is almost always used for postoperative pain relief in patients. Because neuraxial opioids are highly effective for postoperative pain in patients, this model may be useful for predicting the response of spinally administered investigational agents for postoperative pain.

This rat model of incision-induced hyperalgesia was developed because no pain models of hyperalgesia paralleled the operative and postoperative state well. Because postoperative pain is exacerbated by coughing, movement, and pressure applied to the wound, we have emphasized mechanical hyperalge-
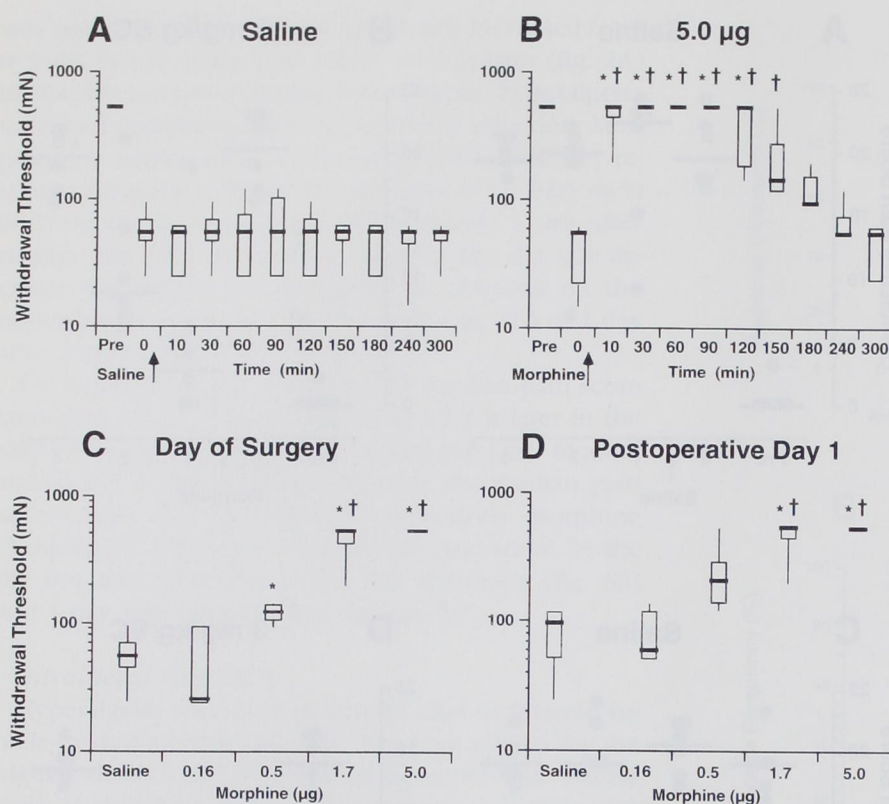
Fig. 4. Effect of intrathecal (IT) morphine on punctate mechanical hyperalgesia caused by an incision. *A*, Withdrawal threshold in saline-treated rats on the day of surgery. *B*, Withdrawal threshold after incision in rats treated with 5 μg of IT morphine on the day of surgery. *C* and *D*, Summary of withdrawal thresholds 30 min after administration of saline or IT morphine on the day of surgery and 1 day after surgery. *$P < 0.05$ *vs.* 0 min by Friedman and Dunnett's test. †$P < 0.05$ *vs.* saline by Kruskal-Wallis and Dunnett's test. The box and whisker plots are described in Fig. 2.

sia for the study of incisional pain. Single applications of von Frey filaments evoke reliable, consistent withdrawal responses after incision in rats and have been used in the assessment of postoperative patients.[8,9] It would be incorrect to equate all mechanical hyperalgesia with responses to punctate stimuli like von Frey filaments; therefore, a nonpunctate stimulus was developed. In patients, hyperalgesia to nonpunctate stimuli applied directly to the wound is present after several types of surgery.[10-12] It is possible that responses from a punctate stimulus applied adjacent to the incision may be affected by drug treatments differently than responses caused by a nonpunctate stimulus activating a large area and placed directly on the wound. Similarly, ongoing, nonevoked pain may be modified in a distinct way. In the present study, similar inhibition of all pain behaviors by morphine was observed.

Comparisons to Clinical Studies

Much lower doses of systemically administered morphine than those used in rats are typically required to produce antinociception in humans. The minimum dose of morphine used to decrease the pain rating to noxious heat in healthy volunteers was 0.08 mg/kg; greater doses produce more pronounced effects.[13] In hyperalgesia produced by a surgical incision, the amount of morphine required to decrease postoperative pain certainly varies depending on the extent of surgery, the type of surgery and the pain measurement (*e.g.*, resting pain or provoked pain). In general, single doses of 0.05–0.3 mg/kg of morphine reduced visual analog pain scores in patients after most surgeries.[14,15] Provoked stimuli, like cough or movement, appear less sensitive to systemic opioids in patients after surgery.[16] The dose of morphine producing antinociception in patients and postoperative pain relief are generally similar; yet, compared with humans, antinociception and hypoalgesia by morphine require greater doses in rats.

Neuraxial opioids produce antinociception in humans[17] and have become an important technique in the management of postoperative pain. Site, extent, and kind of surgery have an impact on the neuraxial morphine dose required; in general, 3–5 mg of epidural morphine or 0.2–0.5 mg of IT morphine reduce pain at rest from most major surgeries. Evoked pain responses

A Day of Surgery
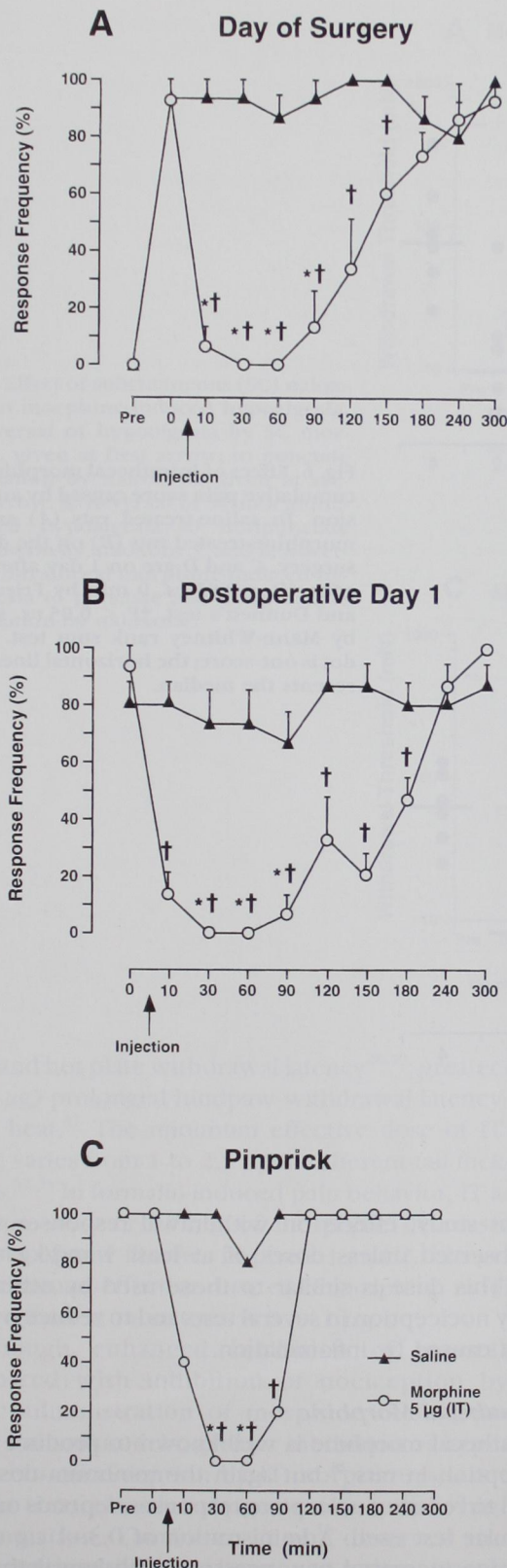

B Postoperative Day 1

C Pinprick

Fig. 5. Effect of intrathecal (IT) morphine on nonpunctate mechanical hyperalgesia caused by an incision. *A,* Response frequency in saline-and morphine-treated rats (given at arrow) on the day of surgery. *B,* Response frequency 1 day after surgery. *C,* Effect of IT morphine on withdrawal responses to pinprick. Withdrawal frequency in saline- and morphine-treated rats (given at arrow). *$P < 0.05$ vs. 0 min by Friedman and Dunnett's test. †$P < 0.05$ vs. saline by Mann-Whitney rank sum test. The symbol represent the mean ± SEM.

using mechanical stimulation after surgery also are decreased by neuraxial morphine.[18] Interestingly, the potency of IT morphine in postoperative patients and in this rat model are similar. In adults after surgery, 4 μg/kg of IT morphine (total dose approximately 0.3 mg) generally produces postoperative analgesia after surgery; 1.7 μg of IT morphine (5 μg/kg) markedly inhibited pain behaviors in this rat model.

One day after surgery, we chose to examine the same drug in the same animal to determine if similar inhibition of pain behaviors occurred 1 day later when pain behavior was still remarkable. It is possible that the hyperalgesia after incision may be maintained by different receptor mechanisms 1 day after surgery than immediately after surgery. Similar inhibition of pain behaviors by morphine was observed the next day.

Systemically Administered Morphine

Comparison of the dose of morphine used to inhibit pain behaviors in the present study with those used by others to modify nociception and hyperalgesia in rats is useful in assessing the sensitivity of this model to opioids. Others have shown that the dose of morphine producing a significant change in nociception in rats depends on the particular test used. Low doses of morphine (0.1–1.0 mg/kg) increased the hot plate latency[19] and vocalization threshold to paw pressure,[20] but these same doses did not change withdrawal thresholds to hindpaw pressure[21] or pain behavior rating after formalin injection.[22] At least 1.25 mg/kg of morphine was required. Two studies required 2.5 mg/kg or more to prolong tail flick latency[23,24] or the hindpaw withdrawal latency to noxious radiant heat.[25] Overall, except for hot plate latency and vocalization thresholds, significant effects in most tests are usually observed with doses greater than 1 mg/kg; in some cases, greater doses are required.

Some models of hyperalgesia respond differently to systemic opioids than tests of nociception. Several stud-
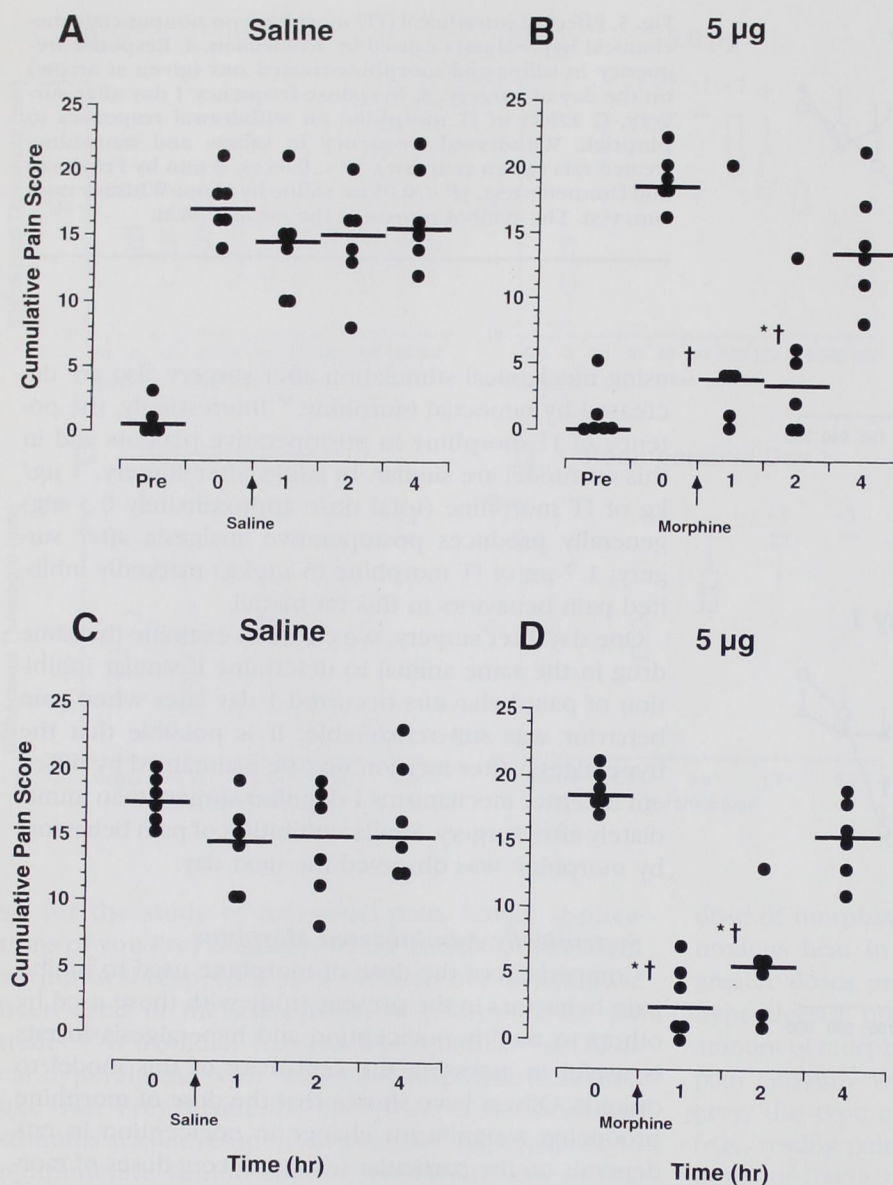
A **Saline**

B **5 µg**

C **Saline**

D **5 µg**

Fig. 6. Effect of intrathecal morphine on cumulative pain score caused by an incision. In saline-treated rats (*A*) and in morphine-treated rats (*B*) on the day of surgery. *C* and *D* are on 1 day after surgery. $*P < 0.05$ *vs.* 0 min by Friedman and Dunnett's test. $†P < 0.05$ *vs.* saline by Mann-Whitney rank sum test. Each dot is one score; the horizontal lines represents the median.

ies have demonstrated enhanced responses to systemic morphine in hyperalgesia induced by inflammation.[20,21,25,26] Because rats treated to induce hyperalgesia have lower baseline nociceptive thresholds (or latencies), a higher percent inhibition of pain behaviors would be produced by the same absolute increase in threshold (or latency).[27] Therefore, these results should be interpreted with caution. This comparison cannot be made using von Frey filaments because the rats usually do not withdraw to forces lower than the cutoff filament (522 mN). Thus, increases produced by morphine in nonincised animals cannot be detected. In the present study, effects on withdrawal responses were not observed unless doses of at least 1 mg/kg were used. This dose is similar to those used by others to modify nociception in several tests and to reduce hyperalgesia caused by inflammation.

Intrathecal Morphine

Intrathecal morphine is well known to produce antinociception in rats,[28] but again the minimum dose required to reduce nociceptive responses depends on the particular test used. Administration of 0.3 - 1 µg of IT morphine increased paw pressure withdrawal thresh-
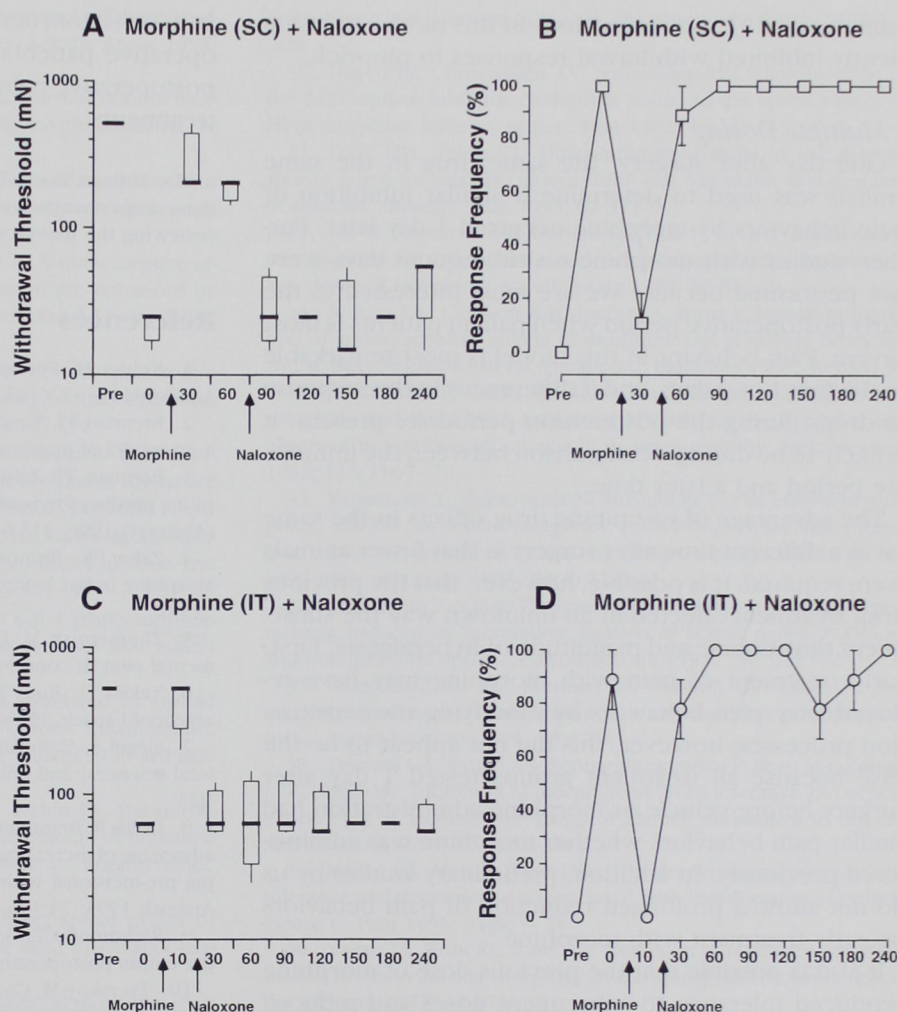
Fig. 7. Effect of subcutaneous (SC) naloxone on morphine-induced hypoalgesia. *A,* Reversal of hypoalgesia by SC morphine, given at first arrow, to punctate stimulation by naloxone, given at second arrow. *B,* Reversal of SC morphine-induced hypoalgesia to nonpunctate stimulation by naloxone. *C* and *D,* Reversal of intrathecal morphine-induced hypoalgesia to punctate and nonpunctate stimulation by naloxone.

old[29] and hot plate withdrawal latency[28,30]; greater doses (1–3 μg) prolonged hindpaw withdrawal latency to radiant heat.[31] The minimum effective dose of IT morphine varies from 1 to 2.5 μg in different tail flick paradigms.[32,33] In formalin-induced pain behavior, IT administration of 1 or 10 μg of morphine decreased the number of paw flinches in phase 1 and 2.[34] In general, the minimum effective dose of IT morphine modifying nociception in most tests is 0.3–1 μg.

Although enhanced inhibition of hyperalgesia compared with inhibition of nociception by systemic administration of morphine is suggested in some animal models, greater inhibition of hyperalgesia *versus* nociception by IT morphine is less clear. In rats treated with carrageenan to induce hindpaw inflammation, Yamamoto *et al.*[35] observed an equal increase in withdrawal latency to radiant heat on the

inflamed and on the untreated hindpaw after the IT administration of 1 or 10 μg of morphine. An equal increase in withdrawal latency after IT morphine also was noted in a model of neuropathic pain.[36] In general, similar doses of IT morphine used by others reduced pain behaviors in this postoperative pain model.

Responses to Pinprick

A response to pinprick was used to measure the effect of these doses of morphine on withdrawal to a suprathreshold mechanical nociceptive stimulus. As discussed previously, the doses of systemic morphine that modify pain behaviors in this postoperative pain model modify nociception in some tests but in the present study did not inhibit responses to pinprick. Morphine,

administered IT, was efficacious in this model and transiently inhibited withdrawal responses to pinprick.

Multiple Dosing

One day after surgery, the same drug in the same animal was used to determine if similar inhibition of pain behaviors by morphine occurred 1 day later. Further studies with morphine on subsequent days were not performed because we are most interested in the early postoperative period when pain in patients is most severe. Pain behavior in this model is most remarkable in the first 1 or 2 days, and if differences in the response to drugs during the postincision period are present, it is likely to be during a comparison between the immediate period and a later time.

The advantage of examining drug effects in the same rat at a different time after surgery is that fewer animals were required. It is possible, however, that the previous drug treatment affected in an unknown way the subsequent time course and magnitude of hyperalgesia. First, early treatment of pain with morphine may have reduced later pain behaviors by modifying the sensitization processes; however, this did not appear to be the case because all treatment groups tested 1 day after surgery before vehicle or morphine administration had similar pain behaviors whether morphine was administered previously. In addition, preliminary studies by us do not show a prolonged reduction of pain behaviors by early treatment with morphine.[37]

It also is possible that the previous dose of morphine produced tolerance to subsequent doses and reduced its inhibition of pain behaviors. This was not observed. Studies in normal rats reported acute opiate tolerance after administration of 10 mg/kg of morphine for 5–7 days.[38,39] Neuraxial opioids also require a longer exposure to produce tolerance. Using the tail flick test, Yaksh et al.[40] demonstrated that twice daily administration of 15 μg of IT morphine caused tolerance to develop after approximately 3 days. Thus, in the short treatment period in the present study, tolerance is unlikely.

Conclusion

As we develop a better understanding of pain mechanisms and hyperalgesia, specific models for specific pain syndromes are necessary. In addition, the measures of pain behaviors studied in animals should be relevant to the measure of clinical pain and clinical outcome in patients. This novel incisional model with quantifiable pain behaviors occurring on a time scale similar to postoperative patients will improve our understanding of postoperative pain and facilitate investigations of novel treatments.

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