Primary and Secondary Hyperalgesia in a Rat Model for Human Postoperative Pain

Peter K. Zahn, M.D.,* Timothy J. Brennan, M.D., Ph.D.†

Background: Previously, the authors developed and characterized a rat model for postoperative pain to learn more about pain produced by incisions. In this study, the responses to heat and mechanical stimuli were evaluated directly on or adjacent to the incision and at varying distances from the incision.

Methods: Rats were anesthetized with halothane and incisions were made at different locations in the plantar aspect of the foot. The response frequency to a blunt mechanical stimulus, the withdrawal threshold to von Frey filaments (15–522 mN), and the withdrawal latency to radiant heat were measured. Rats were tested before surgery, 2 h later, and then daily through postoperative day 9.

Results: After plantar incision, persistent hyperalgesia was observed immediately adjacent to or directly on the incision to punctate and blunt mechanical stimuli, respectively. The withdrawal threshold to punctate stimuli applied 1 cm from the incision was decreases through postoperative day 1. In a transitional area, between the distant and adjacent sites, the withdrawal threshold was intermediate and the duration of hyperalgesia was transient. Heat hyperalgesia was persistent but present when the stimulus was applied to the site of injury but not to a distant site.

Conclusion: Robust primary hyperalgesia to punctate and blunt mechanical stimuli was present. Hyperalgesia distant to the wound, or secondary hyperalgesia, occurred in response to punctate mechanical stimuli, was short-lived, and required greater forces. These results suggest that the most persistent pain behaviors in this model are largely primary hyperalgesia. (Key words: Animal model; central sensitization; incision; peripheral sensitization; thermal hyperalgesia.)

TISSUE injury, chemical irritation, and inflammation excite and sensitize nociceptors and central nervous system somatosensory pathways. This sensitization, evaluated in behavioral studies, causes hyperalgesia, decreases the nociceptive response threshold, and increases the response to suprathreshold stimuli. Early studies by Lewis and Hardy et al. distinguished two types of hyperalgesia by testing both the injured tissue and the surrounding uninjured areas: Primary hyperalgesia occurs when stimuli are applied within the site of injury; in contrast, secondary hyperalgesia is present when the stimuli are applied outside the injury in the surrounding area. Evidence indicates that primary hyperalgesia is caused by increased responsiveness of primary afferent nociceptors (peripheral sensitization), and secondary hyperalgesia is produced by enhanced responses of dorsal horn neurons to a given peripheral input (central sensitization).

Primary and secondary hyperalgesia to mechanical and heat stimuli have been evaluated after various injuries in humans, and different results have been obtained. Intradermal injection of capsaicin and burn injury in humans produced primary thermal and mechanical hyperalgesia; only secondary hyperalgesia to mechanical stimuli was present. Topical application of mustard oil produced primary and secondary mechanical hyperalgesia to punctate and stroking stimuli, whereas pressure hyperalgesia was observed only inside the injured area. In contrast, primary hyperalgesia to thermal but not mechanical stimuli was observed after intradermal injection of bradykinin in humans. Overall, these studies and others suggest that hyperalgesia depends on the type of injury, the stimulus modality tested, and the location of the testing site compared with the injured area.

A common cause of persistent pain and hyperalgesia in humans is postoperative pain. Because specific injuries cause unique patterns of hyperalgesia, we developed a rat model for human postoperative pain to learn more about the pain mechanisms caused by a surgical incision. A surgical incision in the plantar aspect of the rat hindpaw caused mechanical hyperalgesia to punctate and nonpunctate stimuli that closely parallels the postoperative course of patients. In preliminary observa-
tions, an enhanced withdrawal response to punctate stimuli in uninjured tissue 1 cm from the incision was observed, suggesting that secondary hyperalgesia occurs in this model.\textsuperscript{18} The purpose of this study was to evaluate further primary and secondary hyperalgesia to various stimuli after plantar incision. A preliminary report of some of these data has been made in abstract form.\textsuperscript{20}

**Methods**

**General**

These experiments were reviewed and approved by our institutional 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.\textsuperscript{21}

Experiments were performed on 60 adult (weight, 300–350 g) male Sprague-Dawley rats (Harlan, Indianapolis, IN) housed in pairs before surgery. Food and water were unrestricted. After operation, the animals were housed individually in clean bedding of organic cellulose fiber (Shepherd Specialty Papers, Kalamazoo, MI). The incisions were checked daily and any sign of wound infection or dehiscence excluded the animal from the study. Seven animals were excluded because of wound dehiscence. At the end of the protocol, all animals were killed with an overdose of a mixture of pentobarbital (200–400 mg) and phenytoin (25–50 mg) administered intraperitoneally.

**Surgery**

For foot incisions, all rats were anesthetized with 1.5 to 2% halothane delivered via a nose cone and then given an intramuscular injection of 30,000 IU penicillin in the triceps muscle. A group of rats was incised at the heel and tested there, another group was incised at the heel and tested 1 cm distally, and in a third group the test site and the incision were reversed. A final group was incised at the lateral heel area and tested at the medial heel area. Details of these experiments follow.

1. As described previously,\textsuperscript{18} a 1-cm longitudinal incision was made through the skin and fascia or the skin, fascia, and muscle of the plantar aspect of the right hindpaw, starting 0.5 cm from the proximal edge of the heel and extending toward the toes (figs. 1 and 2).

2. A 1-cm incision was performed through the skin and fascia starting from the distal tori and extending toward the heel (figs. 1 and 3).

3. On the lateral side of the foot near the heel, a 1-cm longitudinal incision was made through the skin and fascia starting 0.5 cm from the proximal edge and extending toward the toes (fig. 3). After all incisions, the skin was apposed with two mattress sutures of 5-0 nylon using 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. The person performing the behavioral tests was blinded to the type of incision performed (i.e., skin and fascia vs. skin, fascia, and muscle).

**Behavioral Tests**

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. Pain behaviors were measured as described.\textsuperscript{18,19}

For blunt mechanical stimulation, a circular plastic disk (5-mm diameter) attached to a von Frey filament (400 mN) was applied from underneath the cage directly on the intended incision or distant to the wound; a withdrawal response or lifting the foot off the mesh by the plastic disk without bending the filament was considered a response. Testing was repeated three times with approximately 5 min between measurements, and then the response frequency was calculated.

Withdrawal responses to punctate mechanical stimulation were determined using calibrated Semmes Weinstein von Frey filaments (Stoelting, Wood Dale, IL) applied from beneath the cage through openings (12 × 12 mm) in the plastic mesh floor to an area adjacent or distant to the wound. Each filament was applied once, starting with 15 mN and continuing until a withdrawal response occurred or 522 mN (the cutoff value) was reached. This was repeated three times with at least a 5-min test-free period between withdrawal responses. The lowest force from the three tests producing a response was considered the withdrawal threshold. The location of the testing site varied depending on the experimental group (for details see Experimental Protocols).

Withdrawal latencies to heat were assessed by applying a focused radiant heat source in unrestrained rats, as described by Meller et al.\textsuperscript{22} The heat stimulus was light from a 50-W projector lamp, with an aperture diameter of 6 mm, applied from beneath a heat-tempered glass floor (3-mm thick) on the middle of the incision, or the center of the beam was directed approximately 1.2 cm...
from the incision. Paw withdrawal latencies were measured to the nearest 0.1 s. Three trials 5–10 min apart were used to obtain an average paw withdrawal latency.

**Experimental Protocols**

Thermal and mechanical hyperalgesia observed directly on the wound or in an area within 1–3 mm (immediately adjacent) of the incision was considered primary hyperalgesia. Hyperalgesia occurring at test sites approximately 1–1.5 cm distant to the incision was considered secondary hyperalgesia (see Discussion). One intermediate site, approximately 0.5 cm from the incision, was used.

**Stimuli Applied Immediately Adjacent to or Directly on the Incision.** Separate groups (n = 6 per group) were pretested for responses to the blunt mechanical stimulus, withdrawal to von Frey filaments, and withdrawal latency to radiant heat at the proximal part of the hindpaw near the heel. An incision near the proximal edge of the heel was made through the skin and fascia of the plantar aspect of the right hindpaw, including the plantaris muscle (n = 6), or through skin
and fascia (n = 6) without incising the plantaris muscle. A sham operation (anesthesia, antibiotics, and surgical preparation) was performed in six rats. After a recovery time of 2 h, responses to the blunt mechanical stimulus, punctate stimuli, and radiant heat were tested. The responses to these stimuli were determined for the next 9 days in all groups.

**Stimuli Applied outside the Area of the Incision.**

Eighteen rats were pretested with von Frey filaments and a radiant heat stimulus between the distal tori of the plantar aspect of the foot, approximately 1.2 cm away from the end of the intended incision at the heel. Responses to the blunt mechanical stimulus applied 1 cm from the incision were not assessed because the toes tended to wrap around the disk when it was applied to the distal tori, preventing it from being released after application. The incision was made near the heel through skin, fascia, and the plantaris muscle (n = 6) or through skin and fascia only (n = 6). A sham operation was performed in six rats. Responses to punctate mechanical stimuli and to radiant heat were measured again 2 h after incision and for 9 days thereafter.

A separate group of rats (n = 12) was pretested by applying the blunt mechanical stimulus, von Frey filaments, and radiant heat to an area near the heel on the plantar aspect of the foot and 1 cm away from the intended incision. A 1-cm incision was made through the skin starting at the distal torus and extending toward the heel. Because there is no muscle underlying the skin at the torus, an incision extending into deeper tissues was not performed. Six rats underwent a sham operation. Responses to the blunt mechanical stimulus, von Frey filaments, and radiant heat were determined at this proximal test site.

Twelve rats were pretested for withdrawal threshold to von Frey filaments on an area near the heel on the
medial side of the foot. An incision was made through skin and fascia on the lateral side of the plantar aspect of the hindpaw (n = 6). A sham operation was performed in a separate group of animals (n = 6) and responses to punctate stimuli were determined.

Statistical Analysis
The data were compared using nonparametric tests to analyze results of the mechanical stimuli and parametric analyses for the data obtained for thermal responses.

For data regarding mechanical stimuli, Friedman’s test for within groups, the Kruskal–Wallis test, and Wilcoxon–Mann–Whitney rank-sum test for between-group comparisons were used. For withdrawal latencies to heat, a two-way analysis of variance for repeated measures and subsequent one-way analysis of variance were performed. Multiple comparisons after Friedman’s test and Kruskal–Wallis test and one-way analysis of variance were performed using a two-tailed Dunnett’s and Dunn’s tests, respectively. The results are expressed as the median or mean ± SD when appropriate. P < 0.05 was considered significant.

Results

Hyperalgesia to a Blunt Mechanical Stimulus
In sham-operated rats, the mean response frequency was 6 ± 6% or less before and after sham surgery (fig. 1A). After incision to the skin and fascia, the mean response frequency was increased from 2 h to 2 days after incision (P < 0.05 vs. sham). Hyperalgesia to the blunt mechanical stimulus was present for 4 days after incision to the skin, fascia, and muscle (P < 0.05 vs. sham). There was no difference between the groups with skin and fascia compared with skin, fascia, and...
muscle incision. Distant hyperalgesia to the blunt mechanical stimulus was not observed (fig. 1B).

**Primary and Secondary Hyperalgesia to Punctate Mechanical Stimulation**

In sham-operated rats, the median withdrawal threshold to von Frey filaments was 522 mN before and 198 mN or more 2 h to 9 days after sham treatment (figs. 2A–D). The median withdrawal threshold immediately adjacent to the incision was 94 mN or less from 2 h to 3 days after incision to the skin and fascia only (P < 0.05 vs. sham). Incision to the skin, fascia, and muscle similarly decreased the withdrawal threshold from 2 h to 3 days after surgery (P < 0.05 vs. sham). There were no significant differences between the incised groups (skin and fascia vs. skin, fascia, and muscle); a gradual return toward preincision values occurred in both groups.

Median withdrawal thresholds to von Frey filaments applied between the distal tori, an area approximately 1 cm from the intended incision at the heel, were 360 mN before and 157 mN or more 2 h to 9 days after sham operation (fig. 2E–H). Withdrawal thresholds at this distal test site tended to be lower compared with those of the test site at the heel, which corresponds to findings of a previous study. Punctate hyperalgesia 1 cm from the incision to the skin and fascia was not different than that in the sham group. Withdrawal thresholds to stimuli applied 1 cm from an incision to the skin, fascia, and muscle were decreased 2 h and 1 day after surgery (P < 0.05 vs. sham). Again, there was no difference between groups undergoing skin and fascia versus skin, fascia, and muscle incision.

Stimuli applied to the distal part of the foot could stretch the wound by dorsiflexion of the foot and activate sensitized nociceptors in the wound. After incision to the skin and fascia between the distal aspect of the foot, punctate mechanical hyperalgesia at the heel was transient (P < 0.05 vs. sham), in this case observed only on the day of surgery (figs. 3A–C). After skin and fascia incision to the lateral side of heel, testing 0.5 cm medially revealed punctate mechanical hyperalgesia 2 h and 1 day (P < 0.05 vs. sham) after surgery (figs. 3D–F). Thus, punctate hyperalgesia was also observed at two other positions 0.5 and 1 cm distant to the incision, indicating that this remote hyperalgesia is not specific for a particular location. In addition, it is unlikely that application of filaments to the heel distorts afferents in the distal part of the foot, because the filaments applied at this location do not cause dorsiflexion of the foot.

**Hyperlgesia to Radiant Heat**

After an incision to the skin and fascia, the withdrawal latency to radiant heat applied directly to the wound decreased (P < 0.05 vs. sham) for 7 days (fig. 4A); similar results were observed in a separate group of animals after an incision to the skin, fascia, and muscle. There were no significant differences between the incision groups; a gradual return toward preincision values occurred in both groups. Withdrawal latency to radiant heat was tested at two areas remote from the incision, and no secondary hyperalgesia was observed (Figs. 4B and C).

**Discussion**

This study evaluated the effect of various mechanical and thermal stimuli on hyperalgesia caused by an incision. Accordingly, this model is characterized by robust and persistent hyperalgesia to punctate and blunt mechanical stimuli and radiant heat applied directed on or immediately adjacent to the incision. Secondary hyperalgesia in this model is apparent only with punctate mechanical stimulation and is of shorter duration than primary hyperalgesia. Overall, an incision through the skin, fascia, and muscle is not markedly different than an incision through the skin and fascia. In previous studies, a difference between withdrawal thresholds in groups undergoing incision to the skin and fascia and the skin, fascia, and muscle occurred only 2 h after incision; results of later tests were not different. This difference at 2 h was not present in this study, suggesting, as discussed before, that, for the tests of hyperalgesia used, few differences can be detected between groups with skin and fascia and skin, fascia, and muscle incisions.

**Primary and Secondary Hyperalgesia**

Few investigators have studied how incisions cause pain and sensitization of nociceptors. Primary mechanical hyperalgesia after various tissue injuries has been observed by applying punctate and nonpunctate mechanical stimuli adjacent to or directly on the area of injury. As reviewed by Treede et al., nociceptors sensitized to mechanical stimuli have been difficult to identify, leading some to suggest that primary mechanical hyperalgesia may be caused by sensitization of mechanically insensitive afferents that become responsive to mechanical stimuli after injury or inflammation. In addition, primary mechanical hyperalgesia may be a result, in part, of central sensitization.
Hyperalgesia caused by stimuli applied 1 cm from the incision is probably secondary hyperalgesia. Receptive fields of primary afferents do not expand outside the area of injury. At most, expansion of primary afferent receptive fields has been shown to occur up to 5 mm after a pressure injury in the rat tail. Further evidence that distant hyperalgesia is secondary hyperalgesia comes from studies by Campbell et al. in the hairy skin of the monkey. The effect of thermal stimuli on primary afferent nociceptors after a cut injury, specifically C fibers sensitive to mechanical and thermal stimuli (CMH fibers), was evaluated. An incision adjacent to the receptive field increased the background activity and responses to thermal stimuli of CMH nociceptors. No heat
sensitization was present when the cut was placed outside the receptive field of the CMH nociceptors. The observation that incisions did not cause sensitization to thermal stimuli in uninjured regions helps to distinguish between zones of primary and secondary hyperalgesia. From this, it is suggested that distant punctate hyperalgesia is not caused by the spread of inflammation to afferents; if this occurred in the current study, then distant thermal hyperalgesia also would be expected.

Responses of afferent fibers in the glabrous skin of the plantar aspect of the rat foot to von Frey filaments have been evaluated previously. Results from these experiments show that low-threshold mechanoreceptors (Aβ fibers), which are not normally involved directly in the transmission of nociceptive information, had mechanical thresholds ranging from 6–14 mN; nociceptors (Aδ or C fibers) were activated by filaments ranging from 45–187 mN. In the current study, after skin incision, punctate primary hyperalgesia occurred at median mechanical thresholds of 30 mN (range, 15–41 mN; fig. 2B). From this, hyperalgesia immediately adjacent to the incision may be a result of nociceptors sensitized by surgery to punctate mechanical stimuli (peripheral sensitization). A second explanation is enhanced central synaptic efficacy to Aβ-mechanoreceptor stimulation in central neurons sensitized by activated nociceptors from the incision (central sensitization). Third, both processes may occur; that is, peripheral sensitization of nociceptors and responses to mechanical stimulation could be amplified by central sensitization.

In the current study, after skin incision, punctate secondary hyperalgesia occurs at median thresholds of 200 mN (range, 61–522 mN and 116–522 mN; figs. 2F and 3B). These results suggest that secondary hyperalgesia, hyperalgesia in uninjured surrounding tissue, requires nociceptor activation. Low-threshold mechanoreceptors, ranging from 6–14 mN, by themselves do not evoke pain behaviors. Similar results were reported for secondary punctate hyperalgesia after capsaicin injection in humans.

An interesting comparison of primary and secondary hyperalgesia can be made by evaluating the withdrawal responses in rats shown in figures 2B, 3B, and 3E. All rats underwent skin and fascia incisions and were tested at the same site, adjacent to the heel. As noted before, punctate hyperalgesia is lower immediately adjacent to the wound (fig. 2B, median 30 mN) compared with uninjured sites surrounding the incision (fig. 3B, median 200 mN 2 h after surgery; \( P < 0.05 \) vs. 30 mN). In addition, primary hyperalgesia immediately adjacent to the wound in this model persists longer than does secondary hyperalgesia (\( P < 0.05 \) days 1 through 4). At a transitional zone, 0.5 cm from the incision (fig. 3E), the withdrawal threshold is intermediate (median, 90 mN) and hyperalgesia is sustained for 2 days.

Results from the current study suggest that a combination of processes creates a decreasing gradient for mechanical hyperalgesia away from the incision (fig. 5). The area immediately adjacent to the incision (primary zone) is characterized by the lowest withdrawal thresholds (range, 15–41 mN) and persistent hyperalgesia. Primary afferent nociceptor sensitization is probably present and central sensitization may amplify the responses to mechanical stimuli. In the area distant from the incision (secondary zone), where hyperalgesia results from central sensitization, withdrawal thresholds are greatest and relatively short-lived compared with the primary zone. The intermediate area (gray area) has greater thresholds than does the primary zone, and hyperalgesia is relatively short-lived.

**Comparison with Clinical Studies**

Several investigators have performed sensory testing in humans after surgery. Mechanical pain thresholds were reduced using a pressure algometer applied directly to the surgical incision in patients after herniorrhaphy.
PRIMARY AND SECONDARY HYPERALGESIA—INCISIONAL PAIN

abdominal hysterectomy, and cholecystectomy and immediately adjacent to the incision in patients after gastrectomy and abdominal hysterectomy. Results from these studies indicate that pressure delivered by a 0.25- to 4-cm² blunt mechanical stimulus directly on or adjacent to abdominal and pelvic incisions in patients causes pain that is probably primary hyperalgesia. This pressure hyperalgesia persists for at least 4 days after surgery and correlates with pain at rest and with movement.

A few investigators have studied secondary hyperalgesia to a pressure stimulus at locations distant to incisions in patients and have reported disparate results. Kavanagh et al. showed a decreased pain threshold by applying a pressure stimulus on the chest wall contralateral to the site of injury in patients after thoracotomy. In another study, pressure hyperalgesia was observed as much as 15 cm from the incision in patients after hysterectomy, but not when the pressure stimulus was applied to the legs, an area remote to the injury. A pressure probe may have distorted an abdominal or pelvic incision even from a 15-cm distance and may not necessarily indicate secondary hyperalgesia. In the rat foot, a distant nonpuncture pressure stimulus does not appear to distort the injured tissue or cause pain behavior.

Applying von Frey filaments outside the apparent area of injury in patients after abdominal hysterectomy and nephrectomy was not painful before surgery but caused pain after operation. Results from these studies show that hyperalgesia to punctate mechanical stimuli occurs in uninjured areas surrounding incisions and indicates that secondary hyperalgesia (and therefore central sensitization) occurs after surgery.

Although it is accepted that hyperalgesia from the area of injury after a surgical incision contributes to postoperative pain in patients, the importance of secondary hyperalgesia in postoperative pain is not known. Stuhlaug et al. recently reported that parenteral administration of ketamine, a noncompetitive N-methyl-D-aspar-tate receptor antagonist, greatly reduced punctate mechanical hyperalgesia in uninjured areas surrounding the incision in patients after nephrectomy. Resting and evoked pain scores, the intensity of primary hyperalgesia to a pressure stimulus, and cumulative morphine consumption were not different compared with those indices in the placebo-treated group. These results begin to distinguish clinical postoperative pain from punctate secondary hyperalgesia and suggest that secondary hyperalgesia requires further study before its role in clinical postoperative pain is understood fully.

Heat Hyperalgesia

Heat hyperalgesia in the area of injury occurs in various persistent pain states. It is agreed that primary heat hyperalgesia is caused by nociceptors sensitized to heat. However, heat hyperalgesia in surrounding uninjured tissue has been observed in some models of inflammation. These results reinforce the concept that different injuries produce unique patterns of hyperalgesia. The clinical importance of heat hyperalgesia in postoperative pain in patients is not yet understood. However, it has been proposed that perhaps fever, local inflammation, and warmth from vasodilation may enhance nociceptor activity in surgical wounds.

Conclusion

After plantar incision, a pressure stimulus caused only primary hyperalgesia. Application of von Frey filaments caused primary and secondary punctate hyperalgesia; secondary hyperalgesia is comparatively short-lived and requires nociceptor activation. Only primary heat hyperalgesia occurs. Because secondary hyperalgesia is short-lived, persistent pain after surgery in large part may be primary hyperalgesia, perhaps amplified by central sensitization.

The authors thank David Kramer for technical assistance during these experiments and Drs. Corey L. Cleland and Gerald F. Gebhart for reviewing the manuscript.

References

5. Lewis T. Pain. New York, MacMillan, 1942
7. Baumann TK, Simone DA, Shain CN, LaMotte RH. Neurogenic hyperalgesia: The search for the primary cutaneous afferent fibers that


