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A Mouse Model of Incisional Pain

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OPTIMAL postoperative pain relief may facilitate patients' recovery.[1] However, identification of the optimal perioperative treatment remains elusive, in part because the specific mechanisms leading to enhanced sensitivity to pain (allodynia and hyperalgesia) in postoperative patients is unclear.[1,2] Recent studies using a rat model of postoperative pain[3] indicate that pain and hyperalgesia after an incision has unique characteristics.[4] For instance, ongoing input from the wound, not the afferent barrage during the injury, maintains sensitization of dorsal horn neurons and hyperalgesia after the incision.[5] Hence, peripheral sensitization, for example, sensitization of Aδ- and C-fibers,[6] might be necessary for mechanical hyperalgesia to occur after incision. The underlying molecular mechanisms involved in the sensitization processes after incision are, however, still unknown. Because transgenic mice lacking a single protein are potential instruments for further research, we created in the present study a mouse model of incision-induced pain behaviors.

Materials and Methods

Adult male 20- to 30-g C3H/He mice (Harlan, Indianapolis, IN) were used in the experiments in accordance with the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals.[7] Mice were maintained on a 12-h light–dark cycle with food and water available *ad libitum*. At the end of the experiments, all mice were euthanized with an overdose of pentobarbital. The Johns Hopkins Animal Care and Use Committee approved the protocols.

Plantar Incision

Mice were anesthetized with 1.5% to 2% isoflurane delivered *via* a nose cone. After antiseptic preparation of the right hind paw with 10% povidone–iodine solution (Betadine Solution, Purdue Frederick, Norwalk, CT), a 5-mm longitudinal incision was made with a no. 11 blade through the skin and fascia of the plantar foot. The incision was started 2 mm from the proximal edge of the heel and extended toward the toes. The underlying muscle was elevated with a curved forceps, leaving the muscle origin and insertion intact. The skin was apposed with a single mattress suture of 8-0 nylon on a TG175-8 needle (ophthalmic, 1716G; Ethicon, Somerville, NJ), and the wound was covered with antibiotic ointment (Bacitracin Zinc Ointment USP, Fougera & Co., New York, NY). The suture was removed at the end of postoperative day 2.

Control mice underwent a sham procedure that consisted of anesthesia, antiseptic preparation, and topical antibiotics without an incision.

Behavioral Testing

Response to von Frey Filaments. Individual mice were placed on an elevated plastic mesh floor and were covered with a clear Plexiglas chamber (5 × 5 × 8 cm). After acclimation for 20 to 30 min, withdrawal responses to punctate mechanical stimuli were determined using calibrated von Frey filaments (0.07-, 0.17-, 0.40-, 0.60-, 1.04-, 1.37-, and 2.0-g bending force; Stoelting, Wood Dale, IL). Each monofilament was applied five times to the plantar aspect of the right hind paw adjacent to the incision for approximately 1 s with a 10-s interval between stimulus presentations, starting with a force of 0.07 g and continuing in ascending order. A stimulus-related withdrawal of the tested paw was considered a withdrawal response. The paw withdrawal frequency (PWF) to each force was calculated from five applications (0–100%). The paw withdrawal threshold (PWT) was considered the force at which withdrawal occurred at least 3 times (response frequency ≥60%); 3.6 g was recorded as the PWT if less than 3 responses to all filaments occurred.

Response to a Radiant Heat Stimulus. Paw withdrawal latencies (PWL) to heat were determined in a manner similar to that described previously.[8] Each mouse was placed on a preheated glass platform (28–29.8°C) within the plastic chamber. After acclimation for 20 to 30 min, a radiant heat source was focused from underneath the glass to the middle of the incision area. The time required to cause withdrawal of the hind paw from the thermal stimulus was measured to the nearest 0.1 s (cutoff time 20 s). The results of three trials 5 to 10 min apart provided the average PWL.

Experimental Protocols

Punctate Mechanical and Heat Stimulation after Incision in Mice. For mechanical testing, 15 mice were habituated for 3 consecutive days by placing them on

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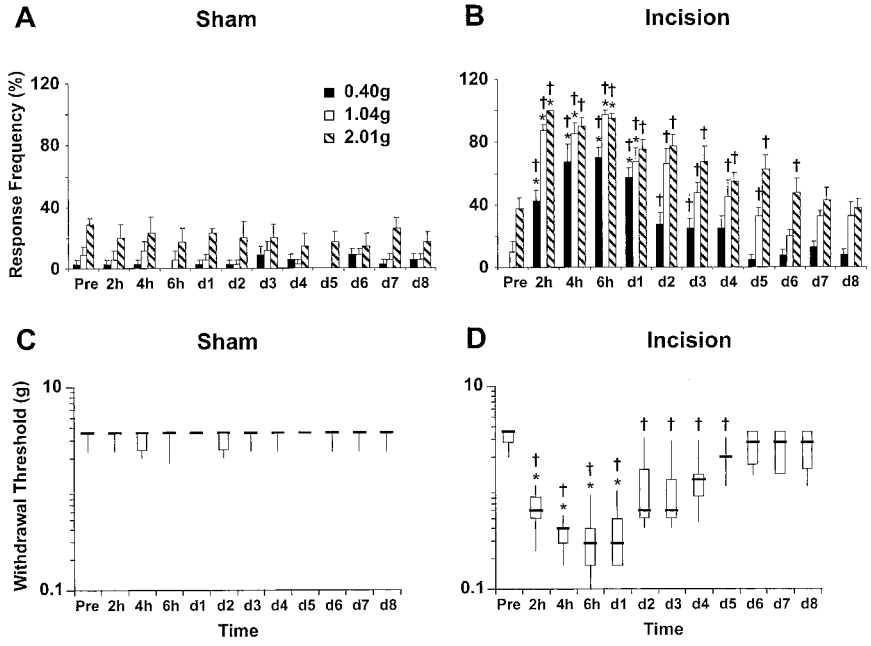

Fig. 1. Response to punctate mechanical stimuli (von Frey filaments) after a sham procedure and plantar incision in mice. (*A*) Paw withdrawal frequencies (PWFs) to von Frey filaments were stable before and after the sham procedure. Mean PWFs to filaments with a bending force of 0.40, 1.04, and 2.0 g before and after the sham procedure are shown. Results are expressed as mean and standard error of mean. (*B*) In mice with a plantar incision, mean response frequencies gradually increased. (*C*) After the sham procedure, paw withdrawal thresholds (PWTs) remained stable. The results are expressed as median (*horizontal line*) with first and third quartiles (*boxes*) and 10th and 90th percentiles. (*D*) After incision, median PWTs were reduced through postoperative day 1 (significant *vs.* pre and sham) and gradually returned to baseline. *P < 0.05 *versus* pre, †P < 0.05 *versus* sham.

the mesh within the test chamber. On the second and third habituation days, mechanical stimuli were applied using the sequence described previously; withdrawal responses assessed on the third habituation day were used to calculate baseline PWF and PWTs before incision/sham procedure. On the day of the experiment, mice were randomly assigned to receive a plantar incision (incision group, n = 8) or sham procedure (control group, n = 7); withdrawal responses were tested 2, 4, and 6 h after the incision/sham procedure and once a day until postoperative day 8.

To assess responses to radiant heat after the incision/sham procedure, 17 other mice were habituated on a heated platform for 3 days; pretesting 2 days and 1 day (pre) before a plantar incision (incision group, n = 8) or a sham procedure (control group, n = 9) was made. Thermal-evoked responses were tested 2 and 6 h after incision and once a day for the next 9 days.

Effect of Systemic Morphine Administration on Enhanced Responses to Mechanical Stimuli and Radiant Heat after Incision. Twenty other mice were habituated similarly as described; to randomize the effects of previous testing, 10 mice were tested for their response to radiant heat and the other 10 were tested by applying mechanical stimuli first. On the day of the experiment, a plantar incision was made in the right hind paw. After a recovery period of 2 h, mechanical and heat testing were performed (time 0) in the order used to determine baseline in each mouse. At 3 h after the incision, the mice were randomly assigned to receive morphine (3 or 10 mg per kg body weight; Abbott Laboratories, Abbott Park, IL) or a saline vehicle subcutaneously. Responses to mechanical and heat stimuli were determined 30, 90, 150, 210, and 270 min after administration of morphine. The experimenter was blinded to the substance (vehicle, morphine) and dose that was injected.

Statistical Analysis

PWLs to heat were compared by a two-way analysis of variance (ANOVA). PWTs and PWFs to von Frey filaments were analyzed using nonparametric tests. We used Friedman's test for within-group analysis and the Kruskal-Wallis and Wilcoxon-Mann-Whitney tests for between-group comparisons. In addition, we used a two-tailed Dunnett's test to make multiple comparisons following Friedman's test and a two-tailed Dunn's test for these comparisons following the Kruskal-Wallis test. Data in the text are expressed as median or mean ± standard deviation when appropriate. $P < 0.05$ was considered statistically significant.

Results

Throughout the experimental period, all mice remained well-groomed and maintained normal food and water intake. Signs of spontaneous pain behaviors such as licking, biting, and flinching after plantar incision in mice were not obvious. Guarding behavior and impaired weight bearing of the incised hind limb occurred only initially in mice after incision.

Protocol A

In control mice, PWFs to Frey filaments before and after the sham procedure were stable (fig. 1A). In the incision group, mean PWFs to the 0.4-g, 1.04-g, and 2.0-g filaments increased from 0 ± 0%, 10 ± 19%, and 38 ±

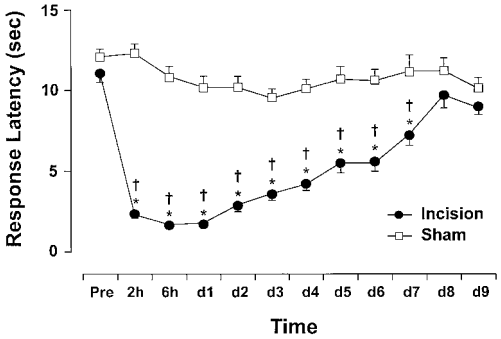

Fig. 2. Paw withdrawal latencies (PWLs) to radiant heat after a sham procedure and after plantar incision in mice. Mean PWLs were reduced in mice after incision until postincision day 7 (significant *vs.* pre and sham). The symbols represent the mean ± standard error of mean. *$P < 0.05$ *versus* pre; †$P < 0.05$ *versus* sham.

20% before to 43 ± 20%, 88 ± 19%, and 100 ± 0% 2 h after incision ($P < 0.05$ *vs.* pre and sham) and remained increased for 6 h (2.0 g) and throughout postincision day 1 (0.4 g, 1.04; fig. 1B).

Median PWTs in control mice were 3.6 g before and after the sham procedure (fig. 1C). In the incision group, median PWTs decreased from 3.6 g before to ≤ 0.6 g 2 h to 1 day after incision ($P < 0.05$ *vs.* pre and sham; fig. 1D).

Mean PWLs to radiant heat were stable before and after the sham procedure (fig. 2). Mean PWLs to radiant heat decreased from 11.1 ± 1.8 s before to 2.3 ± 0.5 s and 1.7 ± 0.5 s 2 and 6 h after incision and remained decreased for 7 days ($P < 0.05$ *vs.* pre and sham; fig. 2).

Protocol B

In saline-treated mice, median PWTs to von Frey filaments decreased from 3.6 g before to 0.6 g 2 h after incision and remained decreased throughout the day of surgery (fig. 3A). Subcutaneous morphine attenuated the reduction in PWTs after incision in mice; after 10 mg morphine, median PWTs increased from 0.4 g 2 h after incision (time 0) to 3.6 g 30 min after injection ($P < 0.05$ *vs.* vehicle and time 0; fig. 3B and C). Morphine dose-dependently reversed the reduced PWLs to heat after plantar incision in mice. PWLs increased for 30 min after 3 mg morphine and for 90 min after 10 mg morphine ($P < 0.05$ *vs.* vehicle and time 0; fig. 3D).

Discussion

Plantar incision in mice enhances responsiveness to mechanical and thermal stimuli that are indicative of primary mechanical and thermal hyperalgesia. Alterations are characterized by increased PWFs and reduced PWTs to punctate mechanical stimuli for 2 days and by reduced PWLs to radiant heat for 7 days. Primary mechanical and heat hyperalgesia after incision in mice is blocked by systemic morphine, a common analgesic to manage postoperative pain. Our observations indicate that the mouse model of incision-induced pain could be a reliable, useful tool to investigate the mechanisms of postoperative pain in genetically modified mice.

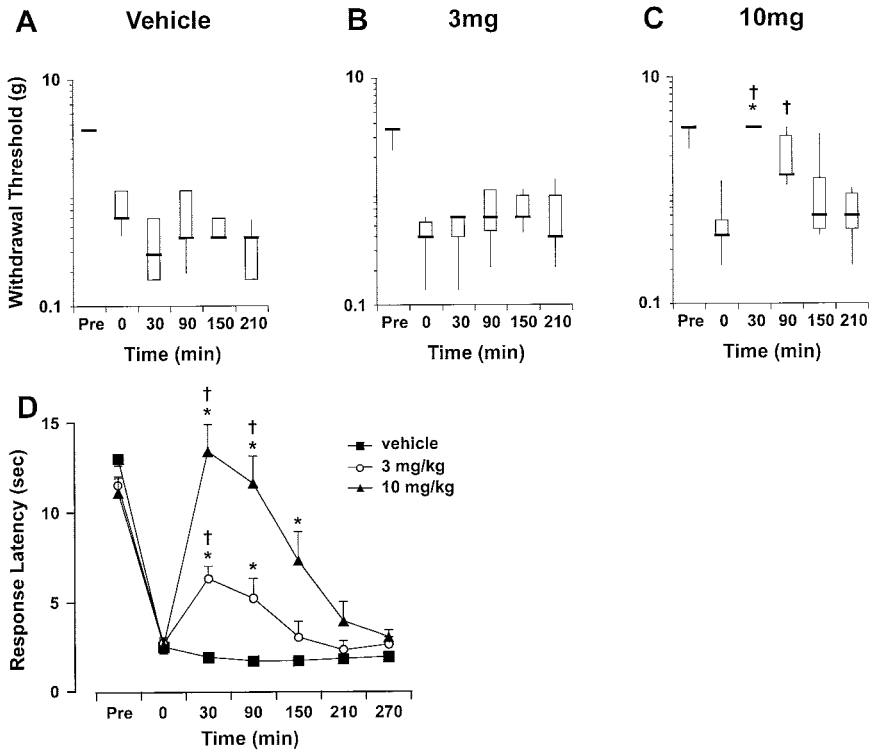

Fig. 3. Effect of subcutaneous morphine on paw withdrawal thresholds (PWTs) to mechanical stimuli (*A–C*) and paw withdrawal latencies (PWLs) to radiant heat (*D*) after plantar incision in mice. (*A*) PWTs after incision in saline-treated mice. The results are expressed as median (*horizontal line*) with first and third quartiles (*boxes*) and 10th and 90th percentiles (*vertical lines*). (*B*) PWTs after incision in mice treated with 3 mg/kg of morphine on the day of surgery. (*C*) PWTs after incision in mice treated with 10 mg/kg of morphine on the day of surgery. (*D*) PWLs to radiant heat in saline- and morphine-treated mice after incision. The symbols represent the mean ± standard error of mean. *$P < 0.05$ *versus* pre; †$P < 0.05$ *versus* sham.

The Mouse Model of Incision-induced Pain

The mouse incision model described here was adapted from a well-established rat model of incisional pain developed by Brennan et al.[3] Because mice are physiologically and behaviorally distinct from rats,[9,10] some modifications were required to establish the model in mice. The length of the incision, for example, was 5 mm in the mouse *versus* 10 mm in the rat, and the incision was closed with one mattress suture (size 8-0) instead of the two used in the rats. Furthermore, the investigation of responses to punctate mechanical stimuli after incision in the mouse was modified from studies in rats after incision. In a preliminary experiment, we tested several different methods, including the one used in rats after incision[3,8] and the Dixon up and down method modified for mice.[11] However, a new sequence similar to one recently used by us and others in mice to study mechanical hyperalgesia was the most reliable and produced reproducible responses in mice after incision.[12–16] As described in Materials and Methods, seven von Frey filaments were applied five times each in an ascending sequence after incision in mice. Testing was continued regardless of whether withdrawal occurred. This could limit reduced withdrawal thresholds as a result of rewarding and learning after repeated testing in mice. Because withdrawal to the monofilaments was not increased in mice after the sham procedure, increased response frequencies and threshold reduction in mice after plantar incision is most likely the result of the incision injury. This method also allowed us to calculate PWFs to each filament force and PWTs in mice after incision as two reliable ways to quantify primary mechanical hyperalgesia after incision in mice.

The absolute PWTs before and after incision are considerably lower in mice as compared with rats.[3,8] However, this corresponds with the substantially lower force needed to excite nociceptors in uninjured glabrous skin of mice (1 g for A-delta and 2.4 g for C-fiber nociceptors[17] *vs.* rats (approximately 5 g for A-delta and 8 g for C-fiber nociceptors).[18] In fact, the relative magnitude of primary mechanical hyperalgesia after plantar incision in mice and in rats is very similar; PWTs dropped well below the response threshold of nociceptors in glabrous skin of mice and rats.[17,18] PWTs to punctate mechanical stimuli are reduced for 2 days in mice; this is similar to the primary punctate mechanical hyperalgesia after plantar incision in rats that usually lasts 3 days after plantar incision.[3,8]

Responses to heat stimulus in mice were assessed using methods similar to those described in rats.[8] In mice, a marked reduction in PWLs to radiant heat (from 11.1 s before incision to 1.7 s at 6 h after incision) occurs, indicating the development of a profound primary heat hyperalgesia after incision. Primary hyperalgesia to heat lasts until postincision day 7 and, therefore, longer than primary hyperalgesia to mechanical stimuli after incision. In conclusion, the magnitude and time course of primary mechanical and heat hyperalgesia after plantar incision in mice and rats are similar[3,8] but differ from that of other animal pain models,[10,11,19,20] indicating either a quantitative or qualitative difference in the underlying mechanisms.

Clinical Implications

Punctate mechanical hyperalgesia around a surgical incision in postoperative patients has been demonstrated. Furthermore, pain exacerbated by coughing or movement, which could reflect mechanical hyperalgesia, is very common in patients after surgery and could impair postoperative recovery.[1,2,21–23] Therefore, mechanical evoked pain after surgery is important for postoperative pain, and the underlying mechanisms must be studied to improve postoperative analgesia and the outcome of patients after surgery.

Only few studies have investigated changes in the responsiveness to heat around a surgical incision in postoperative patients. In one study, pain thresholds to a heat stimulus applied adjacent to the wound are reduced in patients after cholecystectomy and inguinal herniaplasty.[24] Because cooling a surgical wound reduces spontaneous pain after surgery,[25] changes in heat sensitivity after a surgical incision might be important for postoperative pain. However, the role and underlying mechanisms of thermal hyperalgesia for postoperative pain is not yet known.

In conclusion, the mouse incision model could be a useful tool to study the neurobiologic mechanisms of pain after surgery, for example, the role of single proteins/receptors in postoperative pain using genetically modified mice.

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