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The sensitization of a broad spectrum of sensory nerve fibers in a rat model of acute postoperative pain and its response to intrathecal pharmacotherapy

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Abstract

Further understanding of pathophysiology of postoperative acute pain is necessary for its better management. The methodology of current threshold (CT) determination by using sine-wave stimuli at 3 frequencies has been used to selectively and quantitatively analyze the function of the subsets of fibers (i.e., frequency of 5, 250, and 2000 Hz recruits C-, Aδ-, and Aβ-fibers, respectively). The present study investigated how surgical incision would affect the CTs, and then assessed the efficacy of intrathecal pharmacotherapy. The CT required to evoke a paw withdrawal response was assessed over time at stimulus frequencies of 5 Hz (CT5), 250 Hz (CT250), and 2000 Hz (CT2000) in rats that had undergone surgical incision of the plantar skin and muscle. The CTs at all frequencies significantly decreased immediately after the incision. The decreased thresholds gradually recovered during the first week post-surgery. CT5 and CT250 (but not CT2000) remained significantly low even on day 7 post-surgery. Morphine at 5 μg/10 μL i.t. significantly reversed CT5 and CT250. NBQX (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid [AMPA]/kainate receptor antagonist) at 1.9 or 3.8 μg/10 μL i.t. significantly increased the thresholds over the pre-surgery threshold levels at all frequencies. MK-801 (*N*-methyl D-aspartate [NMDA] receptor antagonist) at up to 13.5 μg/10 μL i.t. did not significantly affect CTs at any frequencies. In conclusion, a broad spectrum of sensory fibers (Aβ, Aδ, and C) are sensitized at the spinal and/or peripheral level in the postoperative acute pain state. Spinal AMPA/kainate receptors but not NMDA receptors play a significant role in this sensitization.

1. Introduction

Pain is a common symptom in patients who underwent surgery. Systemic and intrathecal opioid medication has been commonly used to manage postoperative acute pain [24,25]. However, further understanding of pathophysiology of postoperative acute pain is necessary for its better management. In a preclinical model of postoperative acute pain, both evoked and non-evoked pain-related behaviors are caused by surgical incision of skin-muscle of a

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hind paw in rats [4]. Behavioral, histological, and electrophysiological studies have suggested that it is useful to elucidate the mechanism of postoperative acute pain [4,22,23,30,31]. Studies using this model so far have revealed an important role played by spinal excitatory amino acid (EAA) receptors in the generation of postoperative acute pain [23,29,33].

The methodology of current threshold (CT) determination by using sine-wave stimuli at 3 frequencies (2000, 250, and 5 Hz) has been used to selectively and quantitatively analyze the function of the subsets of fibers in animals [7,11,12,15,17,20] and humans [2,13,16,26,27]. Intracellular recording in isolated rat dorsal root ganglions (DRGs) has shown that large myelinated (Aβ) and small myelinated axons (Aδ) and small unmyelinated (C) axons are recruited at frequencies of 2000, 250, and 5 Hz, respectively [12]. *In vivo* rat studies have shown that the desensitization of C-fibers by intrathecal capsaicin treatment selectively increases the threshold at 5 Hz, and intrathecal morphine administration preferentially affects the thresholds at 5 and 250 Hz [17]. Another study in rats has shown that intrathecal administration of 5% lidocaine increases the thresholds at 250 and 5 Hz but not at 2000 Hz [20]. In the clinical setting, this methodology has been used for the diagnosis of small- and large-fiber neuropathies such as postherpetic neuralgia [26] and diabetic neuropathy [16]. In addition, the thresholds are significantly affected by drug treatments such as spinal anesthesia with lidocaine [13,27] and intravenous hydromorphone administration [2].

Intriguingly, studies using this methodology in rodent pain models suggest that the role of each of three fiber types varies according to types of disease which causes pain. The carrageenan injection into the rat hindpaw caused a decrease of paw withdrawal thresholds at 5 and 250 Hz stimulation and increase of the threshold at 2000 Hz stimulation, suggesting that Aδ- and C-sensory fibers were sensitized in the presence of inflammation [17]. In nerve-injured (partial sciatic nerve ligation) mice, paw-flexion thresholds at 250 and 2000 Hz stimulation markedly decreased, whereas threshold at 5 Hz significantly increased [15], suggesting that nociceptive responses mediated by Aβ/Aδ-fibers but not C-fibers were enhanced. There has so far been no study using the methodology in the rat model of postoperative acute pain. Given the hyperalgesic state observed after surgery and the utility of the sine-wave electrical stimulation paradigm, we sought to determine whether surgical incision differentially affects the thresholds assessed at different stimulation frequencies (2000, 250, and 5 Hz) in rats. We also examined whether the observed post-incision thresholds respond differentially to EAA receptor antagonists.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the Animal Care and Use Committee of the University of California, San Diego. Male Holzman Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) and housed in a 12:12-h day:night cycle with free access to food and water. They were used for experiments when their weight ranged between 280 to 400 g.

2.2. Intrathecal catheter implantation

Intrathecal catheter implantation surgery was performed according to a previously reported method [14] for intrathecal drug administration. Under isoflurane anesthesia, a polyethylene catheter was inserted into the intrathecal space and advanced to the rostral edge of the lumbar enlargement through an incision in the atlanto-occipital membrane. Rats with normal

neurological functions and behavior were used for experiments no less than 5 days after surgery.

2.3. Plantar incision

Incision of the plantar skin and muscle was performed according to a previously reported method [4]. Surgery was performed under isoflurane anesthesia (4% for induction and 2-3 % for maintenance). After sterilizing the site for incision in the right hind paw, a 1-cm longitudinal incision was made through the skin and fascia, starting at 0.5 cm from the proximal edge of the heel and extending toward the toes. The underlying muscle was then elevated and incised longitudinally. The skin was apposed using two mattress sutures with 5-0 nylon. A separate group of rats received the isoflurane anesthesia but did not undergo the surgery and served as sham controls.

2.4. Current threshold measurement

Current thresholds (CTs) were measured according to a recently reported method [17]. Each rat was placed in a Ballman cage (Natsume, Tokyo, Japan) during the measurement. Animals were acclimated for at least 20 min after placement. A stimulation electrode (4 mm × 5 mm) was attached to the plantar surface of the ipsilateral (right) hind paw between the edge of the heel and the incision and secured with an adhesive tape. The skin patch dispersion electrode (44 mm × 44 mm) was attached to the ipsilateral side of the back where the hair had been shaved, and it was secured by lightly wrapping it with cotton gauze. In the present study, the CTs to be determined by electrical stimulation at frequencies of 2000, 250, and 5 Hz were termed CT2000, CT250, and CT5, respectively. Sine-wave stimuli were delivered via a constant current stimulator (Neurometer CPT/C; Neurotron Inc., Baltimore, MD). A 2-step strategy was used to define CTs: (i) initial intensity ranging and (ii) CT assessment. Initial intensity ranging was performed to approximate the threshold in order to facilitate CT determination. The stimulation intensity was gradually increased from 1 through a maximum of 2000 μA until a withdrawal response (lifting or flinching) of the right hindpaw was noted. The intensity at which the paw withdrawal response first occurred was recorded as the preliminary threshold intensity. Next, CT was assessed. Stimulation at the preliminary threshold intensity was delivered for 3 s, and the presence or absence of the withdrawal response of the hindpaw during the stimulation period was observed. In the presence or absence of a response, the stimulus intensity was decreased or increased, respectively. The step size of the increment or decrement was 10 μA at a ≤99 μA intensity range and 30 μA at an intensity range of 100 μA or more. The stimuli were repeatedly delivered until the withdrawal response disappeared (if the response was present at the first stimulation) or appeared (if the response was absent at the first stimulation), with 10-s intervals between each stimulation. CT was defined as the minimum current intensity (μA) that elicited the hindpaw withdrawal response. The baseline CTs (CT2000, CT250, and CT5) were first determined on each experimental day. For this, the CT measurement procedure mentioned above was repeated 3 times, and the third measurement value was employed as the baseline CT. After baseline CT measurement, the CT measurement procedure was conducted once at selected time points.

2.5. Time course of CTs associated with surgical incision

After the measurement of baseline CTs, rats (n=6/group) received plantar incision or sham treatment (anesthesia only). The animals were placed in the Ballman cage immediately after the treatment, and the CTs were measured at 30, 60, 90, 120, 180, and 240 min after the surgery, and again on days 1, 2, 3, 4, 7, 10, and 14 post-surgery.

2.6. Effect of intrathecal pharmacotherapy on decreased CTs

Drug evaluation was performed on the day of the surgery. After measuring baseline CTs, the plantar incision was performed. The animals were placed in Ballman cages, and pre-drug CTs were measured at 120 min after the surgery. Then, the drugs were intrathecally administered at 30 min after the pre-drug CT measurement (150 min after the surgery). The doses used were 0, 0.5, and 5 μg/rat for morphine; 0, 1.9, and 3.8 μg/rat (equivalent to 0, 5, and 10 nmol/rat) for NBQX; and 0, 4.7, and 13.5 μg/rat (equivalent to 0, 14, and 40 nmol/rat) for MK-801. Effects of these different doses on mechanical hyperalgesia had been previously examined [29,31,33] and were used to provide a reference for the relation between hyperalgesia and CTs. Next, the CTs were measured at 15, 30, 60, 90, 120, and 180 min after drug administration. A separate group of naive rats were used for the evaluation of drug (NBQX and MK-801) effects on the CTs in healthy condition. Assessment of CTs was carried out by an observer who was blinded to the drug treatment.

2.7. Drugs

Morphine sulfate was provided by Merck (Rahway, NJ). 1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium salt (NBQX) and (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate ((+)-MK-801) were purchased from Sigma Chemical Co. (St. Louis, MO). All drugs were dissolved in saline and administered intrathecally in a volume of 10 μL followed by a 10 μl saline flush. All drug concentrations were calculated in terms of the salt form of the drugs.

2.8. Statistics

Data were analyzed and plotted on graphs by using GraphPad Prism version 4 (GraphPad software Inc., CA). Data were expressed as the means ± SEM. CTs before and after surgery were compared using the paired *t* test to confirm the influence of the surgery. Two-way repeated-measures analysis of variance (ANOVA) with Bonferroni's post-tests was used when comparisons were made between incision and sham control or between drug treatments and vehicle control. A *P* value of <0.05 was defined to be significant.

3. Results

3.1. Time course of CTs associated with surgical incision

The means ± SEM. of the baseline CT in the incision group (n = 6) was 878 ± 58, 308 ± 45, and 217 ± 17 μA for CT2000, CT250, and CT5, respectively, and that in the control group (n = 6) was 860 ± 35, 287 ± 22, and 220 ± 28 μA. After the surgical incision, the CTs at all frequencies immediately (at the 30-min testing point) and significantly (vs. control group) decreased and remained reduced during the 4-h observation period (Fig. 1A–C). The decreased CTs gradually recovered in the first week, but CT250 and CT5 remained significantly low compared to the control even on day 7 post-surgery. The CTs were, however, not significantly low in comparison with those of the control group on days 10 and 14 post-surgery (Fig. 1D–F).

3.2. Efficacy of intrathecal morphine

The CTs after the surgical incision were significantly lower than the baseline CTs of any treatment group (0, 0.5, and 5 μg/10 μL of morphine). Intrathecal morphine (5 μg/10 μL) reversibly and significantly increased the reduced CT250 and CT5, although it did not significantly affect CT2000 (Fig. 2A–C). At the highest doses, there were no evident general behavior changes during the CT measurement in the Ballman cage.

3.3. Efficacy of intrathecal NBQX

The CTs after the surgical incision were significantly lower than the baseline CTs of any group (0, 1.9, and 3.8 μg/10 μL of NBQX). Intrathecal NBQX (1.9 and 3.8 μg/10 μL) reversibly and significantly increased CTs at all the tested frequencies. The CTs after NBQX administration increased over the baseline (pre-surgery) level (Fig. 3A–C). Intrathecal NBQX (3.8 μg/10 μL) significantly (vs. control) increased CTs at all the tested frequencies also in naive rats (Fig. 3D-F). At the highest doses, there were no evident general behavior changes during the CT measurement in the Ballman cage.

3.4. Efficacy of intrathecal MK-801

The CTs after the surgical incision were significantly lower than the baseline CTs of any group (0, 4.7, and 13.5 μg/rat of MK-801). Intrathecal MK-801 did not significantly increase the CTs at any tested frequency (Fig. 4A–C). Intrathecal MK-801 (13.5 μg/10 μL) did not significantly increase CTs at any frequencies in naive rats (data not shown). At the highest doses, there were no evident general behavior changes during the CT measurement in the Ballman cage.

4. Discussion

In the present study, the CTs at all frequencies decreased immediately after the surgical incision, suggesting that a broad spectrum of sensory fibers (Aβ, Aδ, and C) were sensitized by the surgery at the spinal and/or peripheral level. This rapid sensitization of a broad spectrum of sensory fibers could be a distinctive characteristic in postoperative acute pain state. The sensitization may occur at peripheral level (peripheral nerve terminal) or central level (the spinal cord level secondary to the peripheral surgery). There has been abundant evidence that hypersensitivity caused by different kinds of tissue injuries is based on distinct pathophysiological mechanisms. For example, each of inflammatory, neuropathic, and cancer pain states generates a unique set of neurochemical changes in sensory neurons and the spinal cord [8], and is best treated with different types of analgesic [9]. Furthermore, different sensitivities to intrathecal drugs including α_2-adrenoceptor antagonist and NMDA receptor antagonists suggest that mechanisms of hypersensitivity after incision differ from those after peripheral nerve injury [19]. Intriguingly, previous studies using the same methodology as the present study has suggested that influences on CTs vary according to types of disease which causes pain. Intra-plantar injection of carrageenan induces a decrease of CT5 and CT250 and an increase of CT2000 in rats [17]. Nerve injury (partial sciatic nerve ligation) decreases paw-flexion thresholds at 2000 and 250 Hz stimuli and increases the threshold at 5 Hz stimulation [15]. These could suggest differences in the contribution of each fiber to various pain states. Namely, Aδ-/C- fibers and Aβ-/Aδ-fibers seem to be preferentially sensitized in the inflammatory and neuropathic condition, respectively. Taken altogether, the rapid sensitization of a broad spectrum of sensory fibers observed in the present study adds to the increasing evidence that postoperative acute pain is distinct from other pain states, such as inflammatory and neuropathic pain, in pathophysiological mechanisms.

Various pain-related behaviors are present in the acute postoperative pain model, i.e., mechanical hyperalgesia (increase of response to blunt mechanical stimuli), mechanical allodynia (decrease of threshold to von Frey filaments), thermal hyperalgesia (decrease of latency to radiant heat), and spontaneous pain (increase of guarding behaviors) occur immediately after surgical incision [4,29,30,31]. The immediate development of those pain-related behaviors is consistent with the rapid decrease in the CTs observed in the present study. Based on this similarity in time-course, it is possible that the rapid sensitization of sensory fibers underlies development of the pain-related behaviors after surgical incision. It

has been shown that the mechanical allodynia and hyperalgesia are statistically significant (vs. sham) for a few days after the surgery but not on day 7 post-surgery [30]. This time-course is consistent with that for CT2000 which is not significantly low vs. sham on day 7 post-surgery in the present study. It is possible that the sensitization of Aβ-fibers underlies the mechanical allodynia and hyperalgesia after the surgery. On the other hand, the CT250 and CT5 remain significantly low even on day 7 post-surgery. Thus, it could be possible that the low CTs on day 7 post-surgery are related to other aspects of acute postoperative pain rather than mechanical allodynia and hyperalgesia, such as persistent thermal hyperalgesia (adjacent to the incision site) which is significant even on day 7 post-surgery [30]. In vitro electrophysiology study suggests that C-fibers isolated from the peri-incisional skin are sensitized to heat but not to mechanical stimuli [3], and i*n vivo* single-fiber recording from the left tibial nerve suggests that Aδ-, but not C-fibers are sensitized to mechanical stimuli after incision [22]. The results in the present study may, at least in part, reflect the evidences in those previous electrophysiology studies.

In the clinical setting, intrathecal morphine has been used in the management of acute pain after a wide range of surgical procedures [25]. In accordance with such clinical effectiveness, intrathecal morphine sufficiently inhibited mechanical hypersensitivity and spontaneous pain behaviors at a dose of 5 μg/10 μL in the acute postoperative pain model [31]. In the present study, the incision-evoked decrease in CT250 and CT5 was significantly reversed by the same dose (5 μg/10 μL) of morphine. In the previous neurophysiological studies, intrathecal morphine also inhibited spinal nociceptive transmissions [6,21]. Thus, the attenuation of the sensitization of Aδ- and C-fibers at the spinal level may underlie the beneficial effect of intrathecal morphine in the treatment of postoperative acute pain.

Intrathecal NBQX significantly increased the CTs at all frequencies, but MK-801 did not in the present study. This result suggests that the sensitization of a broad spectrum of sensory fibers after incision is mediated by spinal AMPA/kainate but not NMDA receptors. Accordingly, previous behavioral studies showed that incision-induced diverse pain-related behaviors (mechanical allodynia, mechanical hyperalgesia, thermal hyperalgesia and guarding behaviors) were significantly inhibited by AMPA/kainate receptor antagonists including NBQX, but not by NMDA receptor antagonists including MK-801 [23,29,33]. Furthermore, a recent *in vivo* electrophysiological study showed that spontaneous activities and sensitization in response to mechanical stimuli in the dorsal horn neurons were significantly inhibited by spinal administration of NBQX but not MK-801 in the acute postoperative pain model [32]. The result in the present study adds to the evidence that spinal AMPA/kainate receptors but not NMDA receptors play an important and exclusive role in the maintenance of the acute postoperative pain.

The nonselective reversal of CTs at all frequencies by NBQX is in accordance with the findings in previous electrophysiology studies. Single-unit extracellular recordings from the dorsal horn neurons indicated that intrathecal NBQX significantly inhibited the C-fiber mediated response [28]. Another study with a similar methodology indicated that the intrathecal administration of the AMPA/kainate receptor antagonist GYKI 52466 reduced the responses to both noxious and innocuous mechanical stimuli [5]. As AMPA/kainate but not NMDA receptor antagonists affected physiological nociceptive functions in previous behavioral studies [1,18], NBQX but not MK-801increased CTs in naïve rats in the present study. The nonselective effects in the present study may further raise the possibility that the inhibition of AMPA/kainate receptor causes the inhibition of physiological nociceptive functions. The high efficacy of the AMPA/kainate receptor antagonist study may lead to sufficient analgesia in patients with acute postoperative pain, although it seems likely that it may in fact lead to a broad sensory block. Thus, the therapeutic profile of AMPA/kainate

receptor antagonists in the treatment of postoperative acute pain in the clinical setting requires further investigation.

To conclude, the analysis of sensory nerve function by using sine-wave electrical stimuli in a rat model of postoperative acute pain has suggested that a broad spectrum of sensory fibers (Aβ, Aδ, and C) are sensitized by surgical incision at the spinal and/or peripheral level. This rapid sensitization of a broad spectrum of sensory fibers could be a distinctive characteristic in postoperative acute pain state. The distinct change of CTs from those in different pain models indicates that postoperative acute pain is distinct from other pain states in pathophysiological mechanisms. Results of intrathecal administration of EAA receptor antagonists suggest that spinal AMPA/kainate receptors but not NMDA receptors play a significant role in the sensitization of a broad spectrum of sensory nerve fibers.

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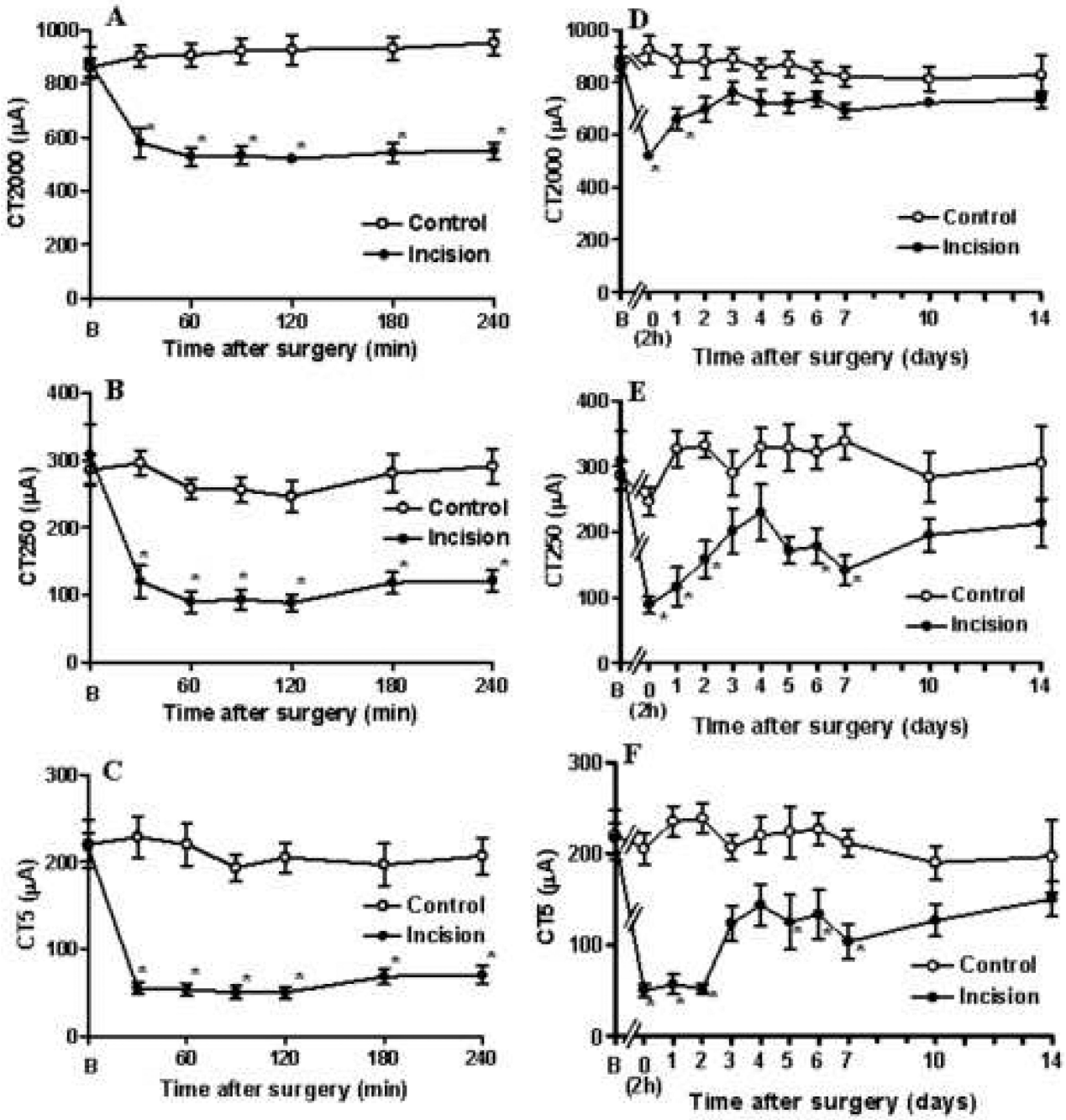

Fig. 1.

Time course of current thresholds (CTs) associated with the plantar incision. Panels A, B, and C depicted the time courses of CT2000, CT250, and CT5, respectively, for 4 h on the day of surgery. Panels D, E, and F depicted the time courses of CT2000, CT250, and CT5, respectively, for 14 days post-surgery. Baseline (B) CTs were measured just before surgical incision. The threshold determined 2 h after the surgery was plotted as the representative value of day 0 post-surgery in panels D, E, and F. Data were expressed as the means ± SEM. for 6 rats. $*P < 0.05$, compared with the control (sham) group, as determined by two-way repeated-measures ANOVA with Bonferroni's post-tests (A–F).

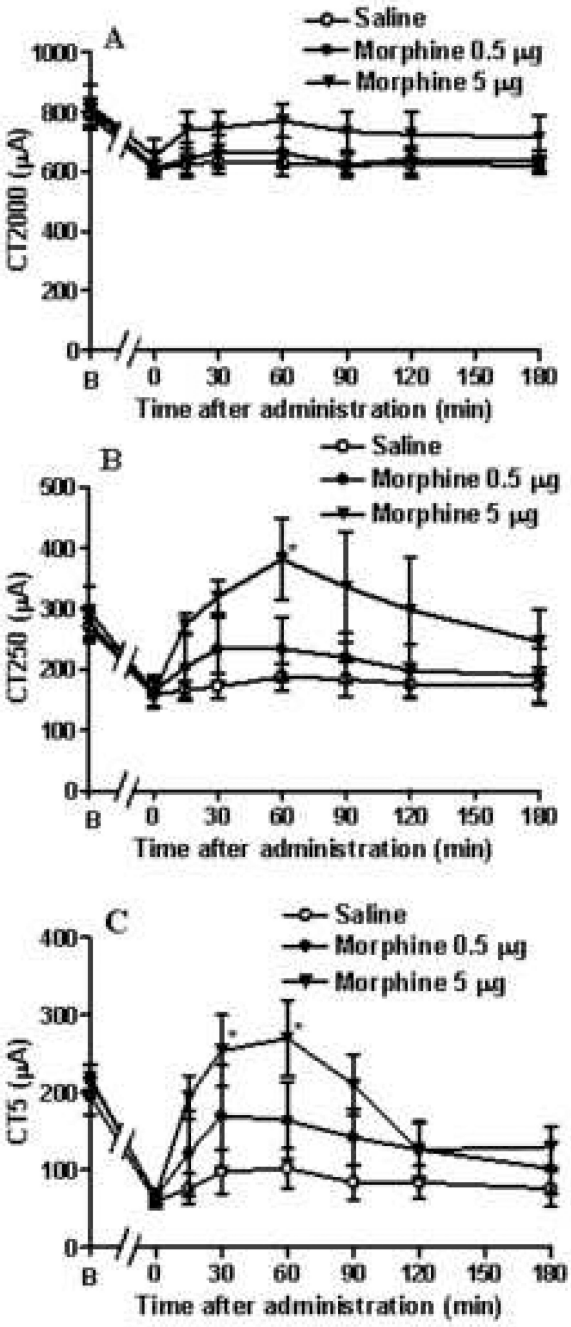

Fig. 2.

Effect of intrathecal morphine on CTs in rats that underwent surgical incision. Panels A, B, and C depicted the time courses of CT2000, CT250, and CT5, respectively. Baseline (B) CTs were measured just before surgical incision. Pre-drug CTs were measured at 120 min after the surgery, and morphine or saline was administered at time 0 (150 min after the surgery). Data were expressed as the means ± SEM. for 5 rats. The threshold after the surgical incision (time 0) was significantly lower than the baseline (pre-surgery) threshold in all the groups (0, 0.5, and 5 μg/rat), as determined by the paired t test. *$P < 0.05$, compared with the control (saline) group, as determined by two-way repeated-measures ANOVA with Bonferroni's post-tests.

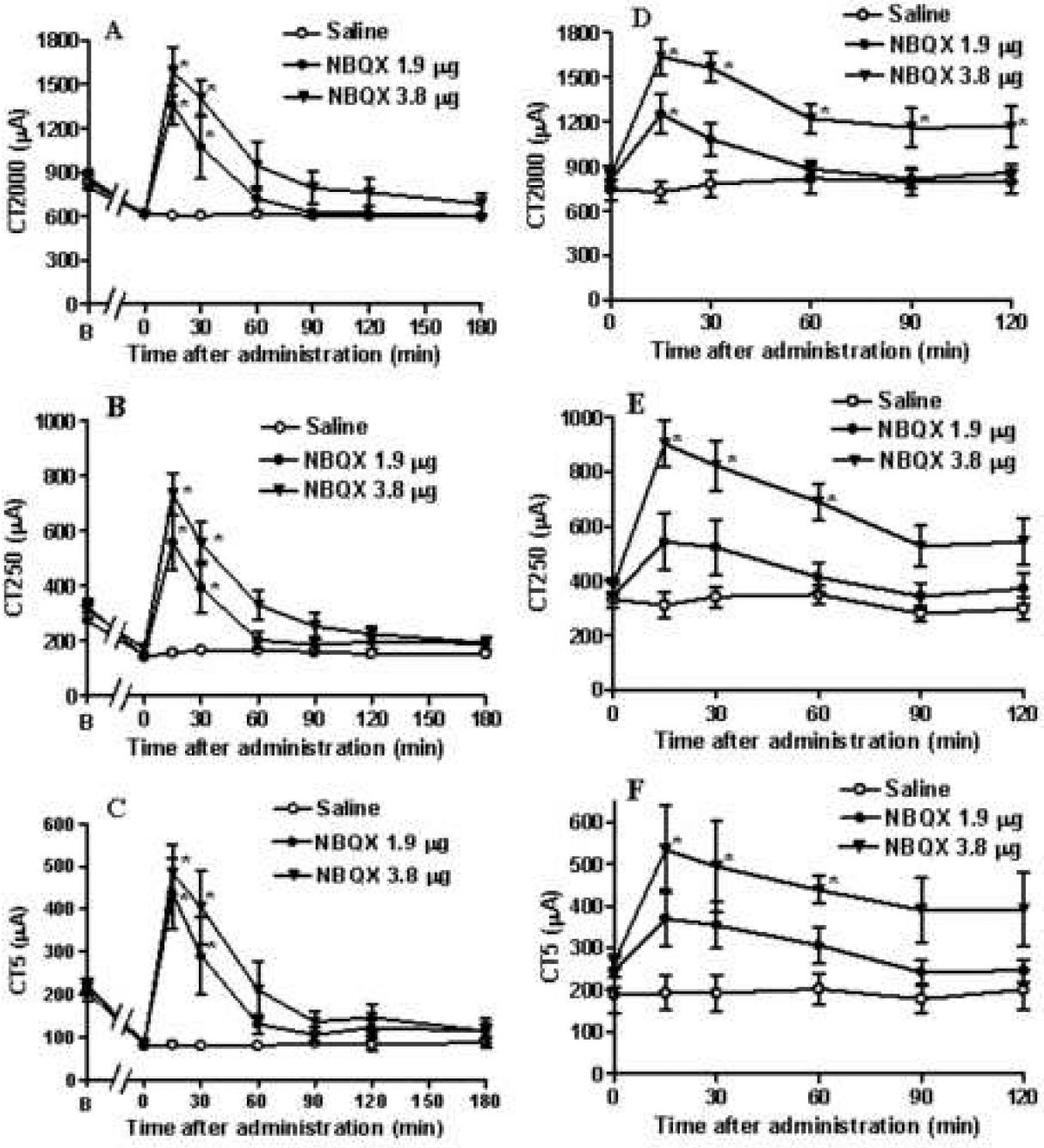

Fig. 3.

Effect of intrathecal NBQX on CTs. Panels A, B, and C depicted the time courses of CT2000, CT250, and CT5, respectively, in rats that underwent surgical incision. Panels D, E, and F depicted the time courses of CT2000, CT250, and CT5, respectively, in naive rats. In panels A, B, and C, baseline (B) CTs were measured just before surgical incision. Pre-drug CTs were measured at 120 min after the surgery, and NBQX or saline was administered at time 0 (150 min after the surgery). In panels D, E, and F, NBQX or saline was administered at time 0 (just after baseline measurement). Data were expressed as the means ± SEM. for 5 rats. The threshold after the surgical incision (time 0) was significantly lower than the baseline (pre-surgery) threshold in all the groups (0, 1.9 and 3.8 μg/rat), as

determined by the paired t test (panels A-C). *$P < 0.05$, compared with the control (saline) group, as determined by two-way repeated-measures ANOVA with Bonferroni's post-tests (panels A-F).

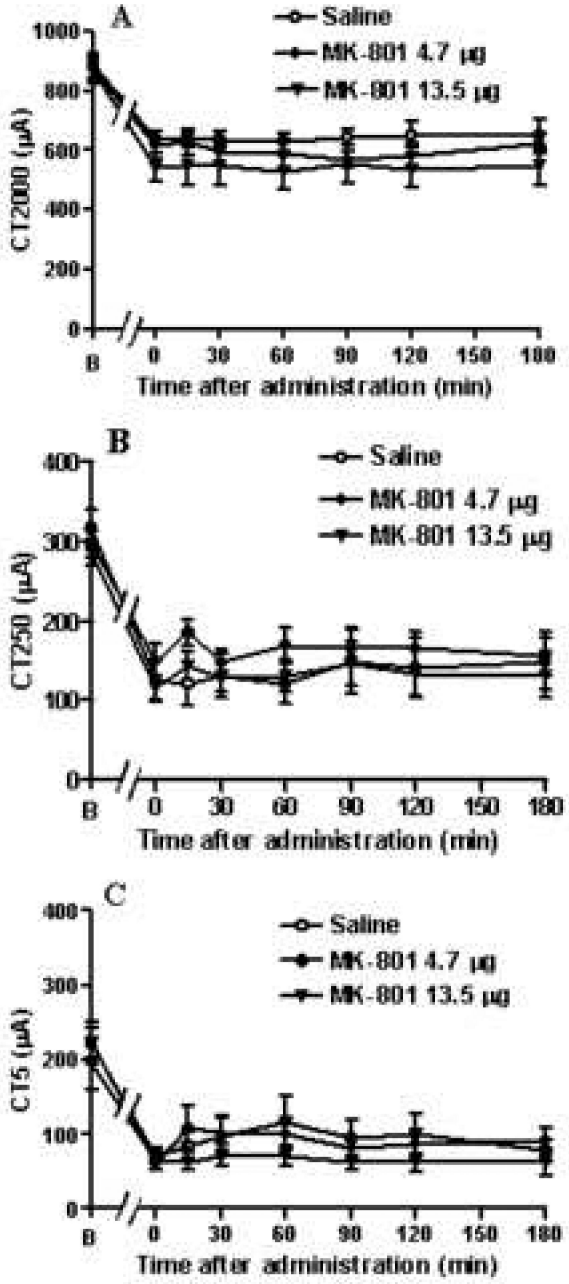

Fig. 4.

Effect of intrathecal MK-801 on CTs in rats that underwent surgical incision. Panels A, B, and C depicted the time courses of CT2000, CT250, and CT5, respectively. Baseline (B) CTs were measured just before surgical incision. Pre-drug CTs were measured at 120 min after the surgery, and MK-801 or saline was administered at time 0 (150 min after the surgery). Data were expressed as the means ± SEM. for 5 rats. The threshold after the surgical incision (time 0) was significantly lower than the baseline (pre-surgery) threshold in all the groups (0, 4.7 and 13.5 μg/rat), as determined by the paired t test. Statistical analysis (two-way repeated-measures ANOVA with Bonferroni's post-tests) showed no significant difference between control (saline) and MK-801 groups.