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Role of the Rostral Medial Medulla in the Development of Primary and Secondary Hyperalgesia after Incision in the Rat

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Background: **Descending influences from the rostral medial medulla (RMM) contribute to secondary hyperalgesia in persistent inflammatory, neuropathic, and visceral pain models. The current study examined if descending inhibition or facilitation from the RMM modulates primary and secondary hyperalgesia after incision in the rat hind limb.**

Methods: **Bilateral RMM lesions were produced using the soma-selective neurotoxin ibotenic acid, and the effect of RMM lesion was examined on primary and secondary hyperalgesia 5 days after a plantar or gastrocnemius incision, respectively.**

Results: **Plantar incision reduced withdrawal thresholds to von Frey filaments applied adjacent to the incision (primary punctate hyperalgesia). The withdrawal thresholds were the same in RMM-lesioned and sham-operated rats. The response frequency to a blunt mechanical stimulus after plantar incision was increased (primary nonpunctate hyperalgesia) in both groups. Nonpunctate hyperalgesia was greater in lesioned rats on postoperative day 2 only; all other measures were not different. Primary heat hyperalgesia after plantar incision was not modulated by RMM lesion. Secondary punctate hyperalgesia after gastrocnemius incision was not affected by RMM lesion. Gastrocnemius incision did not produce secondary nonpunctate or heat hyperalgesia in either RMM lesion or sham rats.**

Conclusion: **Primary and secondary hyperalgesia after an incision were not modulated by descending influence from the RMM. The lack of contribution of descending facilitatory influences from the RMM to secondary hyperalgesia after gastrocnemius incision supports the notion that incision-induced pain involves dissimilar mechanisms compared with inflammatory and neuropathic pain.**

OUR understanding of the pathophysiologic mechanisms that contribute to enhanced nociceptive sensitivity has been greatly facilitated by the development of a variety of rodent persistent pain models. Inflammatory models of persistent pain, for example, typically involve peripheral injection of an inflammogen that results in nociceptor sensitization, enhanced excitability of spinal dorsal horn neurons, and enhanced behavioral nociceptive responses.[1–3] In addition to inflammatory pain models, a variety of rodent models of neuropathic pain have been developed that involve loose ligation of peripheral nerves, resulting in similar peripheral and central sensitization and behavioral hyperalgesia.[4]

Studies that have used persistent pain models to investigate mechanisms of behavioral hyperalgesia and central sensitization have focused primarily on afferent nociceptor sensitization and spinal cord neuroplasticity. Nociceptor sensitization after peripheral inflammation or neuropathy has been shown to be mediated by a variety of proinflammatory mediators and increased ion channel expression on peripheral nociceptors.[5,6] This sustained afferent input activates a variety of spinal intracellular signaling cascades (*e.g.*, *N*-methyl-D-aspartate receptors, nitric oxide, protein kinases, cyclic AMP response element binding protein), ultimately resulting in a synaptic plasticity in which spinal nociceptive neurons show enhanced excitability and expanded receptive fields to subsequent noxious stimuli.[7,8]

Although peripheral sensitization and spinal cord neuroplasticity clearly contribute to persistent pain states, more recent studies have identified activation of descending facilitatory influences from supraspinal sites as an additional important mechanism. A number of studies have demonstrated that spinal transection or, specifically, inactivation of the rostral medial medulla (RMM), significantly attenuates behavioral hyperalgesia and central sensitization in rodent models of persistent inflammatory and neuropathic pain.[9] Moreover, descending facilitatory influences appear to contribute primarily to enhanced nociceptive responses in uninjured tissue, distant from the site of insult (secondary hyperalgesia), but not to enhanced nociceptive responses recorded at the site of insult (primary hyperalgesia).[9,10]

Although inflammatory and neuropathic pain models have been most commonly used to investigate mechanisms of hyperalgesia and central sensitization, a recently developed rodent model of postoperative pain has found increased use in examining mechanisms of persistent pain after a surgical incision.[11] In this model,

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a surgical incision in the plantar aspect of the hind paw results in pain behaviors indicating primary mechanical (punctate and nonpunctate stimuli) and thermal hyperalgesia.[12] Similar to inflammatory and neuropathic pain models, mechanical hyperalgesia in this model appears to involve activation and sensitization of primary afferent nociceptors and neurons in the spinal dorsal horn.[13,14] However, pharmacologic studies suggest that certain novel mechanisms may contribute to primary hyperalgesia in this model.[15–18] More recently, a variation of this model has been developed in which a surgical incision in the gastrocnemius region of the rat hind limb results in reduced withdrawal thresholds to punctate mechanical stimuli applied to the plantar aspect of the heel remote to the incision, suggesting secondary punctate hyperalgesia.[19] The current series of experiments examined the role of descending influences from the RMM on primary and secondary hyperalgesia after a plantar and gastrocnemius incision, respectively. Because secondary hyperalgesia in a variety of persistent pain models involves facilitation from the RMM, we expect that secondary hyperalgesia after gastrocnemius incision will be blocked or attenuated in RMM-lesioned rats using the soma-selective neurotoxin ibotenic acid.

Materials and Methods

General

All of the experimental procedures were approved by the Institutional Animal Care and Use Committee at The University of Iowa (Iowa City, IA). Adult male Sprague-Dawley rats (n = 42; weight, 250–300 g; Harlan, Indianapolis, IN) were used in all experiments 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.[20] Rats were individually housed on clean bedding of organic cellulose fiber (Shepard Specialty Papers, Inc., Kalamazoo, MI), where they had free access to food and water, and were maintained on a standard light–dark cycle. At the conclusion of the experiment, all rats were humanely killed with an overdose of a mixture of pentobarbital and phenytoin administered intraperitoneally.

Bilateral Rostral Medial Medulla Lesion

Three days before behavioral testing, rats received a bilateral RMM lesion using the soma-selective neurotoxin ibotenic acid as previously described.[21] Rats were initially anesthetized in a plastic chamber saturated with halothane (4%). After induction of anesthesia, rats were intubated, and anesthesia was maintained by ventilating with 1–2% halothane. Rats were mounted in a stereotaxic apparatus, a stainless steel guide cannula was lowered 2 mm dorsal to the target injection site, and ibotenic acid (0.25 μg/0.25 μl; pH 7.4) or vehicle

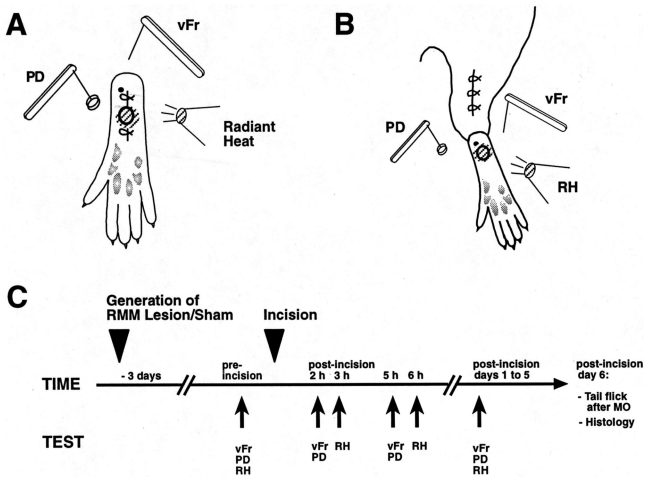

Fig. 1. Diagrams of the rat hind limb illustrating site of plantar (*A*) and gastrocnemius (*B*) incision, site of application of von Frey filaments, and site of application of blunt mechanical stimulus at the plantar aspect of the heel (filled circle). The thermal stimulus was focused near the same site on the glabrous skin (open circle). (*C*) The experimental protocol timeline is illustrated. Three days after generation of the sham or rostral medial medulla (RMM) lesion (triangle), pretest responses to von Frey filaments (withdrawal threshold), nonpunctate stimulus (response frequency), and heat (withdrawal latency) were determined. A plantar or gastrocnemius incision was performed (second triangle), and nociceptive responses were tested again at various times until day 5 after incision. For functional verification of RMM lesion, thermal tail-flick latencies were assessed at day 5 both before and after topical mustard oil application. At the conclusion of the experiment, RMM lesion was verified histologically. VFr = von Frey filaments; PD = plastic disk; RH = radiant heat; MO = mustard oil.

(phosphate-buffered saline) was microinjected bilaterally into the dorsal RMM (Gi) by lowering a 33-gauge needle through the guide cannula and delivering drug over a period of 30 s. The injection needle remained at the target site for an additional 2 min to allow for diffusion. The final coordinates for the lesion site relative to the interaural line were −2.0 mm (rostral–caudal), ± 0.5 mm (medial–lateral), and −9.0 mm (dorsal–ventral).[22] After injection, the injection needle and guide cannula were removed, and the surgical wound was closed using 4.0 silk sutures. All rats were allowed to recover in their cages, and animals displaying neurologic deficits or more than 10% weight loss over the next 3 days were excluded from the study.

Incisions

All rats received either a plantar or gastrocnemius incision during 1.5–2% halothane anesthesia delivered *via* a nose cone as previously described.[11,19]

Plantar Incision. After induction of anesthesia, the glabrous skin of the hind paw was prepared in a sterile manner. A 1-cm longitudinal incision was made through the skin and fascia with a number 11 scalpel blade starting 0.5 cm from the proximal edge of the heel and extending toward the toes (fig. 1A). The plantaris muscle was elevated and incised longitudinally, leaving the mus-

cle origin and insertion intact. The skin was apposed with two mattress sutures of 5–0 nylon on an FS-2 needle and the wound site was covered with antibiotic ointment. Sutures were removed at the end of postoperative day 2.

Gastrocnemius Incision. During halothane anesthesia, the skin of the right hind limb was shaved distal to the knee and prepared with povidone iodine. A 3-cm longitudinal incision was made with a number 11 blade through the skin of the posterior hind limb, starting 1–1.5 cm from the edge of the heel extending to the popliteal region (fig. 1B). Using blunt dissection, the cutaneous tissue was separated from the gastocnemius muscle, and the two bodies of the gastrocnemius were split, leaving the origin and insertion of the muscles intact. The skin was apposed with three mattress sutures, and the wound site was covered with antibiotic ointment. Sutures were removed at the end of postoperative day 3.

Behavioral Nociceptive Testing

Responses to mechanical stimuli were measured as described previously.[15] Rats were individually placed on an elevated plastic mesh floor covered with a clear plastic cage top (21 × 27 × 15 cm) and allowed to acclimate. Withdrawal responses to punctate mechanical stimulation were assessed by applying calibrated Semmes Weinstein von Frey filaments (6, 11, 13, 28, 41, 61, 88, 116, and 198 mN bending force) from underneath the mesh (12 × 12 mm openings) to the plantar aspect of the proximal part of the heel. Each filament was applied once starting with a force of 6 mN and continuing until a withdrawal response occurred or 198 mN was reached. The lowest force from three trials that produced a response was considered the withdrawal threshold. If no withdrawal response occurred, the force of the next filament, 522 mN (the cutoff value), was assigned. Responses to a blunt mechanical stimulus were recorded by applying a circular plastic disk (5-mm diameter) attached to a von Frey filament (400 mN) from underneath the mesh to the plantar aspect of the heel. A withdrawal response or lifting the hind paw off of the mesh without bending the filament was considered a response. The response frequency was calculated from three stimulus applications 10 min apart.

Heat paw-withdrawal latencies were recorded in the same rats as previously described.[23] Briefly, after acclimating the rats for 15–20 min, a radiant heat source (50-W projector lamp, 6-mm aperture diameter) was focused from underneath a glass floor (3 mm thick) to the plantar aspect of the heel, and the time required to remove the hind paw from the noxious thermal stimulus was recorded. Paw withdrawal latencies were recorded to the nearest 0.1 s; the cutoff time was 20 s. Three trials, 5–10 min apart, were used to obtain an average withdrawal latency.

A plantar or gastrocnemius incision was generated as a measure of primary or secondary hyperalgesia, respectively. In both cases, mechanical or heat-evoked responses were elicited by stimulus application to the proximal part of the plantar aspect of the hind paw. Because the stimulus was applied directly on the wound or immediately adjacent to it in the case of the plantar incision, this was considered a measure of primary hyperalgesia. In the case of the gastrocnemius incision, because the stimulus was applied approximately 2 cm from the incision, this was considered a measure of secondary hyperalgesia.

Experimental Protocol

Thirty-four rats were used to examine the effect of bilateral chemical RMM lesion on primary and secondary hyperalgesia after incision. The experimenter was blinded as to whether an RMM-lesion or sham-lesion rat was being tested.

Three days after generation of the RMM lesion (n = 17) or sham lesion (n = 17), rats were pretested for response frequency to a nonpunctate mechanical stimulus, withdrawal threshold to von Frey filaments, and paw withdrawal latency to radiant heat (fig. 1C). After determination of pretest responses (baseline), a plantar incision (n = 16) or gastrocnemius incision (n = 18) was made in equal numbers of RMM- and sham-lesion rats as described above. Two hours after incision, response frequency to the nonpunctate mechanical stimulus and withdrawal threshold to von Frey filaments were tested; withdrawal latency to heat was tested 3 h after incision. Mechanical and thermal-evoked responses were then again tested 5 and 6 h after incision, respectively. Testing was repeated once a day in the morning until postoperative day 5 (fig. 1C).

In preliminary studies, bilateral RMM lesion was typically characterized as an area of neuronal degeneration in cresyl violet–stained tissue sections that was clearly identifiable at 3 and 5 days after generation, but less clearly identifiable 8 days after generation (unpublished observations). To verify that bilateral RMM lesion was still functional 8 days after generation, a group of sham- and RMM-lesion rats having undergone incision were tested in a model of secondary thermal hyperalgesia. In this model, facilitation of the tail-flick reflex after topical mustard oil application to the hind leg was previously shown to be prevented by bilateral RMM lesion.[21] Briefly, baseline thermal tail-flick latencies[24] were determined, and mustard oil (100%) was applied topically to the left hind leg, approximately 30 mm above the ankle.[25] After topical mustard oil application, tail-flick latencies were recorded at 5-min intervals for a period of 50 min. After tail-flick testing, rats were killed and brain sections stained for histologic analysis.

Histologic Verification of Rostral Medial Medulla Lesion

Cresyl violet staining of coronal brain sections was used to verify the extent of bilateral ibotenic acid RMM lesions as previously described.[21] Briefly, the brain was removed and placed in 10% formalin overnight followed by 30% sucrose for an additional 24 h. Thereafter, the tissue was frozen, cut into 40-μm sections using a cryostat, and mounted onto slides.

Statistics

In the case of thermal nociceptive testing, parametric tests were performed using a two-way analysis of variance for repeated measures and subsequent one-way analysis of variance. All other data were analyzed using nonparametric tests. The Friedman test was used for within-group comparisons, and the Kruskal-Wallis and Wilcoxon Mann–Whitney tests were used for between-group comparisons. Multiple comparisons after the Friedman and Kruskal-Wallis tests were performed using two-tailed Dunnett and Dunn tests, respectively. Data are expressed as median or mean ± SD when appropriate. $P < 0.05$ was considered statistically significant.

Results

Rostral Medial Medulla Lesion Histology

Bilateral microinjection of the soma-selective neurotoxin ibotenic acid (0.25 μg/0.25 μl) into the RMM resulted in bilateral RMM lesion characterized by an area of neuronal degeneration devoid of cell bodies that contained gliosis (fig. 2). The lesion was identifiable in cresyl violet–stained tissue sections obtained 3 days after generation of the lesion (fig. 2A). The lesion generally extended in a dorsal–ventral direction that included the gigantocellular and dorsal paragigantocellular nuclei. An area of neuronal degeneration in cresyl violet–stained tissue sections was visible 8 days after generation; however, the staining was less clearly identifiable in most sections made at this later time after RMM lesion (fig. 2B). No neuronal degeneration was observed in rats that had received sham lesion (phosphate-buffered saline; data not shown).

Effect of Rostral Medial Medulla Lesion on Primary Hyperalgesia to a Punctate Mechanical Stimulus

Baseline median withdrawal threshold to von Frey filaments (pretest) did not differ between the RMM lesion and sham groups before the plantar incision (figs. 3A and B). In the sham group, median withdrawal threshold decreased from 522 mN (before) to 12 mN or less after the plantar incision. Primary punctate hyperalgesia was apparent in the sham group for 2 days after plantar incision and then gradually returned to pretest values (fig. 3A). In the RMM lesion group, median withdrawal threshold decreased from 522 mN (before) to 13 mN or less after the plantar incision, and primary punctate hyperalgesia was apparent in the RMM lesion group for 3 days after the incision (fig. 3B). There was no difference between primary punctate mechanical hyperalgesia in lesioned *versus* sham rats ($P > 0.05$), indicating no modulation by descending influence from the RMM.

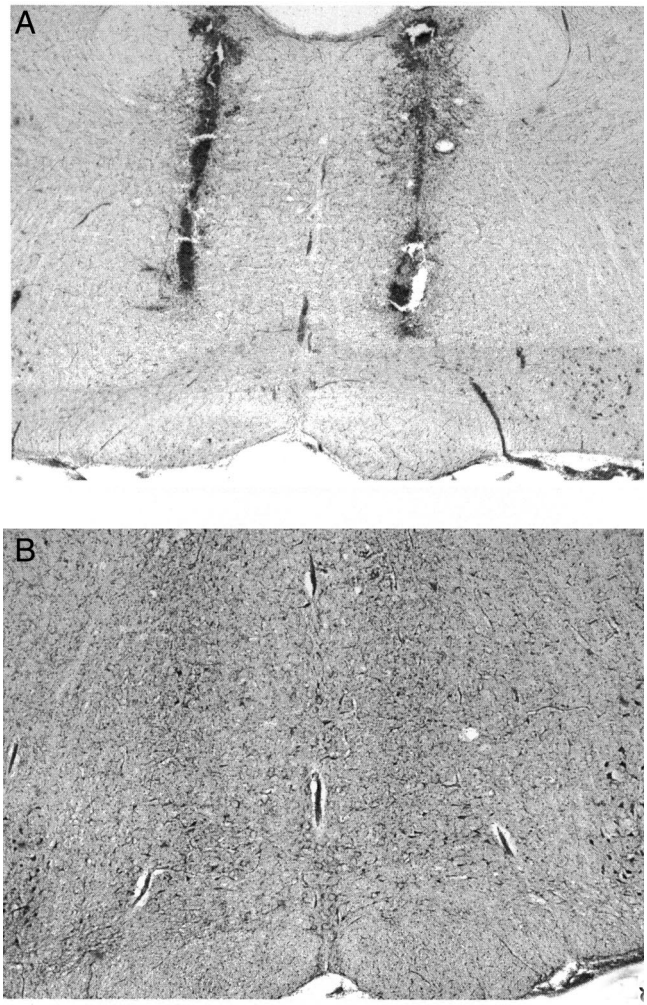

Fig. 2. Representative cresyl violet–stained coronal rostral medial medulla (RMM) section depicting a bilateral lesion of the RMM produced by ibotenic acid (0.25 μg/side) microinjection 3 days (*A*) and 8 days (*B*) after generation of the lesion.

Effect of Rostral Medial Medulla Lesion on Secondary Hyperalgesia to a Punctate Mechanical Stimulus

Baseline median withdrawal threshold to von Frey filaments (pretest) did not differ between the RMM lesion and sham groups before the gastrocnemius incision (figs. 3C and D). After the gastrocnemius incision, median

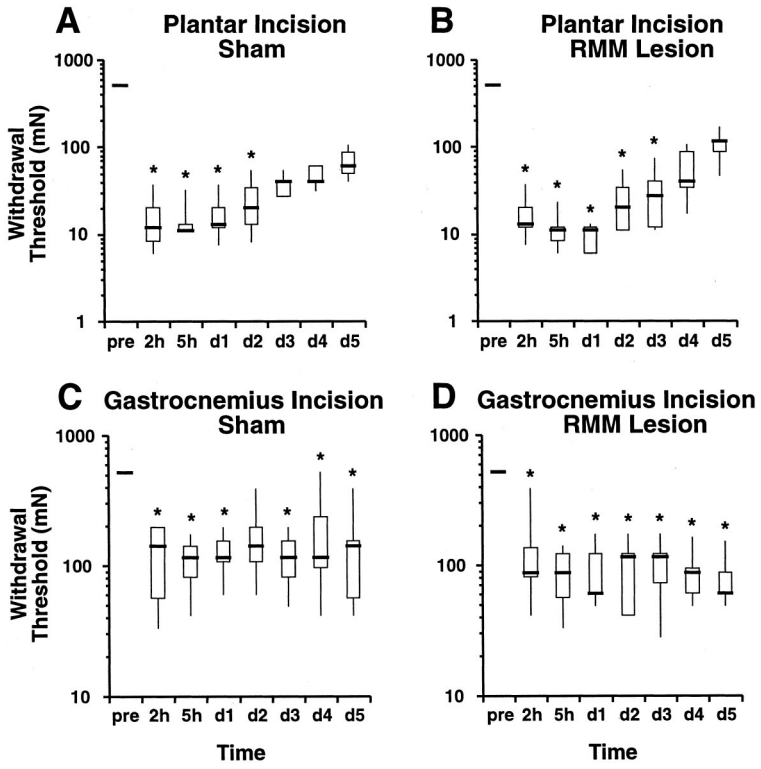

Fig. 3. Effect of neurotoxic rostral medial medulla (RMM) lesion on primary (*A*, *B*) and secondary (*C*, *D*) punctate hyperalgesia after a plantar or gastrocnemius incision, respectively. Primary punctate hyperalgesia was apparent until days 2 and 3 in sham and RMM lesion rats, respectively. In sham rats, secondary punctate hyperalgesia was apparent at the day of surgery and at days 1 and 3–5. In RMM lesion rats, secondary punctate hyperalgesia was apparent throughout the test period up to day 5. Data are represented as median (horizontal line) with first and third quartiles (boxes) and 10th and 90th percentiles (vertical lines). Pre = before incision. *Significant decrease in withdrawal threshold *versus* before incision ($P < 0.05$).

withdrawal threshold decreased from 522 mN (before) to 142 mN or less in the sham group. Secondary punctate hyperalgesia was apparent for the 5-day testing period after the gastrocnemius incision, with the exception of day 2 (fig. 3C). In the RMM lesion group, the median withdrawal threshold decreased from 522 mN (before) to 88 mN 2 h after the incision and was apparent throughout the 5-day testing period (fig. 3D). Similar to primary hyperalgesia, there was no difference between withdrawal thresholds in the RMM lesion and sham groups.

Effect of Rostral Medial Medulla Lesion on Primary Hyperalgesia to a Blunt Mechanical Stimulus

Baseline response frequency to a nonpunctate stimulus (pretest) did not differ between RMM lesion and sham groups before the plantar incision (fig. 4). After the plantar incision, mean response frequency in the sham group increased from 0 ± 0 to 75 ± 30% or greater at 2 and 5 h (fig. 4). Primary nonpunctate hyperalgesia was apparent for 2 days after the incision. In the RMM lesion group, mean response frequency after the incision increased from 0 ± 0 to 83 ± 11% or greater at 2 and 5 h (fig. 4) and was apparent for 3 days after the incision. The response frequency in the RMM lesion group was significantly ($P < 0.05$) greater than the RMM sham group at day 2 after the incision (fig. 4). Thus, primary nonpunctate hyperalgesia after plantar incision was only different on day 2 after incision in the RMM lesion group compared with the sham group.

Effect of Rostral Medial Medulla Lesion on Responses to a Nonpunctate Mechanical Stimulus after a Gastrocnemius Incision

In the RMM lesion and sham groups, response frequency to a nonpunctate stimulus did not differ before

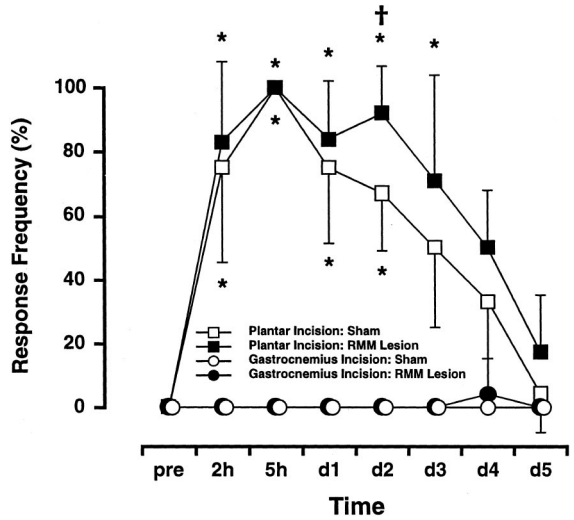

Fig. 4. Effect of neurotoxic rostral medial medulla (RMM) lesion on responses to a nonpunctate mechanical stimulus after a plantar or gastrocnemius incision. Primary nonpunctate hyperalgesia in sham (open squares) or RMM lesion (filled squares) rats was present until day 2 or 3 after plantar incision, respectively. Secondary nonpunctate hyperalgesia was not observed in sham (open circles) or RMM lesion (closed circles) rats. Data are mean ± SD. Pre = before incision. *Significant increase in response frequency *versus* before incision ($P < 0.05$, Friedman and Dunnett test). †Significant increase in response frequency *versus* sham ($P < 0.05$, Kruskal-Wallis and Dunnett test).

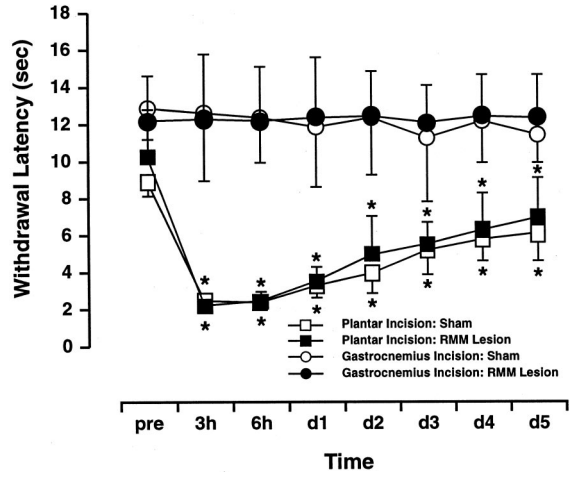

Fig. 5. Effect of neurotoxic rostral medial medulla (RMM) lesion on thermal hyperalgesia after incision. Primary thermal hyperalgesia after plantar incision was apparent in sham and RMM lesion rats throughout the test period up to day 5. Secondary thermal hyperalgesia after a gastrocnemius incision was not observed in either sham or RMM lesion rats. Data are mean ± SD. Pre = before incision. *Significant decrease in thermal paw withdrawal latency *versus* before incision ($P < 0.05$).

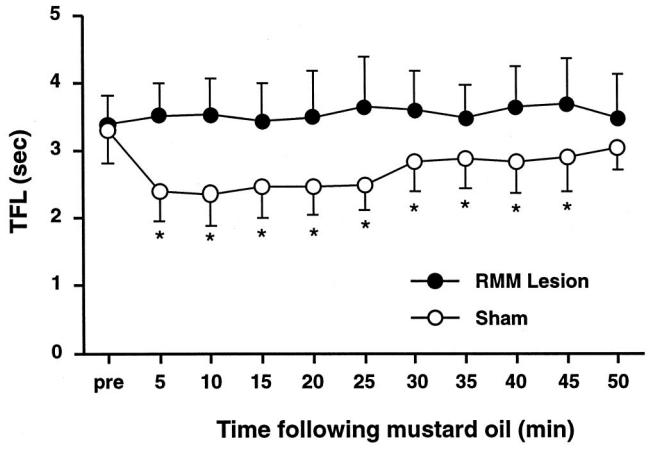

Fig. 6. Effect of neurotoxic rostral medial medulla (RMM) lesion on secondary thermal hyperalgesia after topical mustard oil. Topical application of mustard oil produced a decreased thermal tail-flick latency (TFL) in sham rats that was completely prevented in RMM lesion rats. Data are mean ± SD of the tail-flick latency (seconds) before and after topical mustard oil application. Pre = before application. *Significant decrease in tail-flick latency *versus* before incision ($P < 0.05$, Dunnett test).

the gastrocnemius incision (0 ± 0%; fig. 4). Generation of the gastrocnemius incision did not result in secondary blunt mechanical hyperalgesia in either the RMM lesion or sham group, and responses between these groups after the incision did not differ throughout the 5-day testing period (fig. 4).

Effect of Rostral Medial Medulla Lesion on Incision-induced Responses to a Noxious Thermal Stimulus

Baseline mean withdrawal latency to radiant heat did not differ between RMM lesion and sham groups before plantar incision (fig. 5). After the plantar incision, mean withdrawal latency decreased from 8.9 ± 0.8 s to 2.5 ± 0.4 s at 3 h in the sham group and remained decreased for the 5-day test period. Similarly, mean thermal paw withdrawal latency decreased in the RMM lesion group from 10.2 ± 2.6 s to 2.2 ± 0.3 s 2 h after the plantar incision. Heat hyperalgesia was apparent for the 5-day test period. No significant differences in primary heat hyperalgesia after a plantar incision were observed between the RMM lesion and sham groups throughout the test period (fig. 5).

Thermal paw withdrawal latency was also assessed after a gastrocnemius incision; no secondary heat hyperalgesia was observed in either the RMM lesion or sham group (fig. 5).

Effect of Rostral Medial Medulla Lesion on Mustard Oil–induced Secondary Thermal Hyperalgesia

To confirm that bilateral RMM lesion was functional 5 days after incision (8 days after lesion), a group of sham and RMM lesion rats that had been tested for incision-induced primary or secondary mechanical hyperalgesia were subsequently tested for secondary thermal hyperalgesia produced by topical mustard oil. Facilitation of the thermal tail-flick reflex after topical mustard oil application has previously been shown to be prevented in RMM lesion rats.[21] Because pretest and post–mustard oil tail-flick latencies did not differ in rats that had received a plantar or gastrocnemius incision (data not shown), they were represented as one group. Pretest tail-flick latencies for sham (n = 11) and RMM lesion (n = 13) groups did not differ; however, facilitation of the tail-flick reflex after topical mustard oil was completely prevented in the RMM lesion group compared with the sham group (fig. 6).

Discussion

This is the first study to examine the influence of brainstem facilitatory systems on the control of mechanical and thermal hyperalgesia caused by incision injury. Most important, secondary punctate mechanical hyperalgesia after gastrocnemius incision was not altered by a bilateral RMM lesion, indicating that a descending facilitatory influence from the RMM is not involved in secondary hyperalgesia after incision. After plantar incision, primary punctate hyperalgesia was similar in lesioned and sham rats; nonpunctate mechanical hyperalgesia was greater on the second day. Finally, primary hyperalgesia to heat after plantar incision was unaffected by RMM lesion.

The Rostral Medial Medulla Is an Important Supraspinal Brain Site for Modulating Persistent Pain States

The main result of the current study, a lack of descending influence from the RMM to hyperalgesia after incision, was an unexpected finding. From studies performed by us and other investigators, it is well established that the RMM is an important supraspinal site involved in both inhibiting and facilitating spinal behavioral and dorsal horn neuron responses to noxious stimulation *via* descending funicular projections that terminate in the dorsal horn.[26] A variety of stimuli have been shown to activate descending inhibitory or facilitatory influences from the RMM, including environmental cues, illness-inducing agents, acute noxious stimuli, and direct chemical– electrical RMM stimulation.[10,27–30] In addition, studies involving RMM inactivation have demonstrated a significant contribution of descending facilitatory influences from the RMM to hyperalgesia in persistent pain models of inflammatory, neuropathic, neurogenic, and visceral pain.[21,25,31–37]

Rostral Medial Medulla Lesion Using the Soma-selective Neurotoxin Ibotenic Acid

In the current study, RMM lesion using the soma-selective neurotoxin ibotenic acid was used to examine a potential role for descending influences from the RMM in modulating primary or secondary hyperalgesia after an incision. We and other investigators previously used ibotenic acid–induced RMM lesion to demonstrate descending inhibitory and facilitatory modulation of inflammatory hyperalgesia.[21,38]

Given the ineffectiveness of RMM lesion on hyperalgesia caused by plantar incision, we retested a group of sham and RMM lesion rats in an additional model of mustard oil–induced hyperalgesia. We previously showed that RMM lesion prevents secondary thermal hyperalgesia after topical application of mustard oil.[36] Confirmation that the RMM lesion was functional was important given that histologic analysis revealed the lesion to be difficult to identify 8 days after generation, at the conclusion of behavioral testing (data not shown). In the current study, however, mustard oil–induced thermal hyperalgesia was completely prevented in the RMM lesion group compared with the sham lesion group. These results demonstrate that the RMM lesion was functional when tested 8 days after generation.

Effect of Bilateral Rostral Medial Medulla Lesion on Primary Hyperalgesia after Incision

Bilateral RMM lesion had no effect on primary thermal hyperalgesia after a plantar incision. This result is consistent with a previous study demonstrating RMM lesion to be ineffective in preventing primary thermal hyperalgesia, which supports the general notion that descending facilitatory influences do not significantly contribute to primary heat hyperalgesia.[10,21,25] In the current study, the magnitude of primary punctate mechanical hyperalgesia was not modulated in RMM-lesioned rats.

Rostral medial medulla lesion resulted in a modest enhancement of primary nonpunctate hyperalgesia. Because responses to the blunt mechanical stimulus did not differ in sham and RMM lesion groups before the incision, these results suggest that plantar incision may activate descending inhibitory influences from the RMM. Although it has been appreciated that acute noxious stimuli can activate descending inhibitory influences,[39] several studies have demonstrated enhanced descending inhibitory modulation in persistent pain states. Schaible *et al.*[40] found that, after knee joint inflammation, spinal cold block resulted in a further enhancement of spontaneous activity and stimulation-evoked responses of spinal dorsal horn neurons. In a similar study involving persistent hind-paw inflammation, Ren and Dubner[41] reported that reversible spinal block resulted in an enhanced increase in responses to noxious stimuli, spontaneous activity, and expansion of spinal dorsal horn neuron receptive fields. Activation of descending inhibitory influences has been demonstrated in additional persistent pain models of neurogenic, visceral, and illness-induced hyperalgesia.[31,36,37,42] That persistent nociceptive input results in enhanced descending inhibitory modulation is consistent with the notion that descending modulation serves an adaptive role to maintain a state of basal nociceptive responsivity.[10] However, because only primary nonpunctate hyperalgesia was greater in RMM-lesioned rats, activation of descending inhibition plays only a modest role for primary mechanical hyperalgesia after incision.

Effect of Bilateral Rostral Medial Medulla Lesion on Secondary Hyperalgesia after Incision

In addition to primary hyperalgesia, we also examined a potential role for descending modulatory influences from the RMM in a novel model of secondary hyperalgesia involving a gastrocnemius incision.[19] Because behavioral responses were measured in uninjured tissue (plantar) distant from the site of injury (gastrocnemius), we considered this a model of secondary hyperalgesia. The results from the current study demonstrate that RMM lesion was ineffective in preventing secondary punctate hyperalgesia.

We previously showed that secondary hyperalgesia to heat does not occur after gastrocnemius incision.[19] However, the RMM is a brainstem site that has been implicated in modulating responses to thermal stimuli applied remote to a tissue injury.[9,10,21] Thus, it is possible that the absence of heat hyperalgesia after gastrocnemius incision is partly caused by induction of descending inhibitory input from the RMM. However, because withdrawal latencies to radiant heat after gastrocnemius incision were similar in sham and lesion rats, enhanced descending control from the RMM does not affect spinal nociception to heat after incision.

It is somewhat surprising that descending facilitatory influences from the RMM do not contribute to secondary

hyperalgesia in this model, given the growing body of evidence demonstrating that supraspinal influences significantly contribute to secondary hyperalgesia–allodynia in a variety of persistent pain models.[9,21,33,34] One possible explanation for the lack of involvement of descending facilitatory influences after an incision may be unique characteristics of this model. For example, although nearly all rodent persistent pain models involve either peripheral inflammation or nerve injury, this model involves neither. Therefore, unique peripheral mechanisms probably contribute to nociceptor sensitization after an incision. In addition, secondary hyperalgesia after an incision is elicited only by punctate, but not nonpunctate or thermal stimuli. Tissue injuries that cause hyperalgesia to heat, even in an uninjured area (secondary hyperalgesia), usually involve descending facilitatory influences from the RMM.[21] Furthermore, distinct spinal excitatory amino acid receptors appear to contribute to hyperalgesia and central sensitization in this model, *i.e.*, non–*N*-methyl-D-aspartate receptors, but not *N*-methyl-D-aspartate or metabotrophic glutamate receptors, mediate incision-induced hyperalgesia.[15–18] Thus, incision-induced hyperalgesia has distinct characteristics and appears to have unique peripheral, spinal, and supraspinal mechanisms in comparison to inflammatory and neuropathic pain models.

In conclusion, although studies involving neurogenic and neuropathic pain models have found that descending facilitatory influences from the RMM contribute to secondary hyperalgesia, this was not observed after a gastrocnemius incision. Except for a transient effect on primary nonpunctate responses, descending influences from the RMM do not modulate primary punctate or thermal hyperalgesia after plantar incision. In particular, the lack of contribution of descending facilitation to secondary hyperalgesia after an incision probably reflects the unique mechanisms inherent to incision-induced pain.

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