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Hind Paw Incision in the Rat Produces Long-Lasting Colon Hypersensitivity

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Abstract: Visceral injury has been shown to alter somatic sensitivity, but little is known about the effect of somatic insult on the viscera. In the present study, we examined (1) the effect of colon inflammation on somatic sensitivity and (2) the affect of hind paw incision on colon sensitivity. After intracolonic administration of trinitrobenzene sulfonic acid (TNBS) or zymosan, visceromotor responses to colorectal distension were increased to post-treatment day 8. Mechanical withdrawal thresholds in the hind paw were decreased in TNBS- and in zymosan-treated rats until post–intracolonic treatment day 2. There was no change in hind paw heat withdrawal latency in either group. Plantar incision of the hind paw resulted in a decrease in both hind paw mechanical withdrawal threshold and heat withdrawal latency and significantly increased the visceromotor response to colorectal distension from postincision days 1 to 8. The colon hypersensitivity was of longer duration than hyperalgesia at the site of hind paw incision. These results support the hypothesis that somatic injury and visceral inflammation can alter central processing of visceral and somatic inputs, respectively.

Perspective: ***Surgical procedures are common and typically associated with hyperalgesia at and around the site of incision. This report establishes in a model of postsurgical pain and hyperalgesia that a long-lasting visceral hypersensitivity may also accompany postsurgical hyperalgesia.***

Key words: *Colorectal distension, central sensitization, somatic pain, somatovisceral convergence, visceral pain, zymosan, incision.*

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Visceral pain is a common and typically difficult-to-manage form of pain, yet the majority of current pain research focuses on pain of nonvisceral origin. Visceral pain is characterized as being poorly localized, diffuse in nature, accompanied by exaggerated motor and autonomic reflexes, and typically referred to cutaneous and subcutaneous sites.[10,30] The poor localization, diffuse character, and referral of visceral pain is explained in large part by the anatomical organization of the visceral innervation and the convergence of visceral and nonvisceral inputs onto second-order spinal neurons. Accordingly, clinical visceral pain conditions, including irritable bowel syndrome (IBS), have been associated with cutaneous hyperalgesia in areas of referral.[25,35,40,41,44]

Changes in central processing are often implicated in cases of pain developing secondary to the primary site.[3,22,24,46-48] Staud et al[39] noted abnormalities in central nociceptive processing in subjects with fibromyalgia, including enhanced sensitivity to noxious cutaneous heat stimuli and enhanced temporal summation of repetitive C-fiber stimulation. The coexistence of several pain conditions may also have a peripheral component. Several studies have indicated that axons may send their peripheral terminals to anatomically distinct segments, causing the sensation of pain distant to the primary site.[11,23] Another anatomical explanation is that several primary sensory neurons converge onto second order neurons in the spinal cord and sensitization of 1 afferent

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pathway can alter input from other neurons at sites of convergence.[21,28,31,32]

If viscerosomatic convergence contributes to somatic hypersensitivity, then the converse should also be true. Somatic injury should enhance visceral sensitivity. Indeed, Miranda and coworkers[26] reported that experimental muscle hypersensitivity increases colon sensitivity. In this study, we used colon inflammation as a selective visceral insult and plantar incision as the somatic injury. As a model of postsurgical pain, the plantar incision model is particularly relevant because surgical procedures are relatively common and possible visceral hypersensitivity may also thus be a relatively common postsurgical event. This study addressed 2 questions: (1) Does colon inflammation in the rat sensitize responses to cutaneous/subcutaneous stimuli applied to the hind paw? (2) Does a plantar incision of the rat hind paw sensitize responses to colon distension?

Methods

Animals

All experiments were performed with male Sprague-Dawley rats (300–350 g; Harlan, Indianapolis, IN). Rats were housed 1 per cage, with food and water available ad libitum and maintained on a 12-hour light-dark cycle. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Iowa. After testing, all animals were euthanized by pentobarbital sodium overdose.

Surgical Preparations

Rats were anesthetized with pentobarbital sodium (50 mg/kg; Nembutal; Abbott Labs, North Chicago, IL) administered intraperitoneally. Pairs of electrodes (Teflon-coated stainless steel wire; Cooner Wire Sales, Chatsworth, CA) were sutured into the external oblique musculature, just above the inguinal ligament, for electromyographic (EMG) recording as described previously.[29] Electrodes were tunneled subcutaneously and externalized through the dorsal aspect of the neck for future access. The wounds were closed with 4-0 silk and covered with a local anesthetic ointment. After surgery, rats were given 5.0 mL of 5% dextrose in normal saline subcutaneously. Animals were housed separately and allowed to recuperate for at least 3 days before testing. Any animals with apparent motor defects or that lost significant weight were excluded from behavioral testing.

Plantar Incision

A previously described plantar incision model was used.[8] Rats were anesthetized with 2% halothane, and the plantar aspect of the right hind paw was prepared in a sterile manner. A 1-cm longitudinal incision was made with a No. 11 blade through the skin and fascia of the plantar aspect of the hind paw beginning 0.5 cm from the end of the heel. The flexor muscle was elevated and a longitudinal incision was made. Skin was closed with 5-0 nylon sutures, and topical antibiotics were administered. Animals were allowed to recover in their cages, and sutures were removed on the third postoperative day. Rats were acclimated to behavioral testing 2 days before experiments began.

Hind Paw Mechanical Withdrawal Threshold

Rats were placed individually on an elevated plastic mesh grid, covered with clear plastic cages and allowed to acclimate for 1 hour. Withdrawal threshold was assessed by applying calibrated Semmes-Weinstein von Frey filaments (Stoelting, Wood Dale, IL) to the plantar aspect of the hind paw in inactive rats. Testing began with 10 mN, and increasing forces were applied until the hind paw was withdrawn. The maximum force applied was 250 mN. Rats were tested 3 times with a minimum of 10 minutes between tests. The lowest force required to induce withdrawal from the 3 tests was recorded as the mechanical withdrawal threshold. If no response was elicited by the 250 mN filament, the next highest filament, 586 mN, was recorded as the withdrawal threshold.

Heat Withdrawal Latency

Rats were placed individually on top of a 6.0-mm thick glass surface covered with a clear plastic cage and allowed to acclimate 45 to 60 minutes before testing. Withdrawal latencies to radiant heat were assessed by applying focused radiant heat from beneath the glass floor to the plantar aspect of the hind paw of an inactive rat. The heat source consisted of a 50-W projector lamp with an aperture diameter of 6 mm. Intensity of the heat was adjusted to produce a withdrawal latency in naive rats of 25 to 27 seconds. Each rat was tested 3 times, with at least 10 minutes between tests. The average of the 3 trials was used for paw withdrawal latency. To prevent tissue injury, the heat stimulus was cut off at 32 seconds. A slow heat test rather than a fast heat test (eg, 10 seconds) was used to detect subtle facilitation of the heat withdrawal latency by both incision and colorectal distension (CRD).

Visceromotor Response to Colorectal Distension

Contraction of the abdominal wall in response to a noxious visceral stimulus can be quantified through EMG recordings.[29] As previously described,[29] a flexible, lubricated latex balloon approximately 6 cm in length was attached to Tygon tubing (Akron, OH), inserted intraanally into the descending colon, and was kept in place by taping the catheter to the base of the tail. The colorectal balloon was distended for 20 seconds to 20, 40, 60, or 80 mm Hg, separated by 4-minute intervals. Balloon pressure was monitored via a pressure transducer and held constant during distension. EMG activity of the external oblique muscle was amplified, rectified, and recorded for 10 seconds before colon distension (baseline EMG activity), 20 seconds during distension (response = increase above baseline), and 10 seconds after termina-

tion of balloon inflation. The EMG signal was integrated and normalized as a change over baseline activity using Spike-2/CED 1401 data acquisition (CED 1401; Cambridge Electronic Design, Cambridge, UK).

Experimental Protocols

Colorectal Distension in Unincised Rats

Baseline CRD responses, heat withdrawal latencies, and mechanical withdrawal thresholds were established. The next day, colonic inflammation was induced by 1 of 2 different inflamogens that produce different magnitudes of monocyte infiltration into inflamed tissue. Rats were anesthetized with halothane and either trinitrobenzene sulfonic acid (TNBS, 0.5 mL, 30 mg/kg in 50% ethanol; Sigma, St. Louis, MO), a hapten that produces transmucosal ulceration and inflammation, or zymosan (1 mL, 25 mg/mL), which is derived from a yeast cell wall and produces less ulceration, was instilled into the distal colon of different groups of rats (n = 9), using a 16-gauge stainless steel feeding needle connected to a 1-mL syringe. Rats in control groups (n = 9) received an equal volume of sterile saline. Six hours after administration of TNBS, zymosan or saline, the visceromotor response (VMR) was recorded in response to CRD. After CRD testing, rats were allowed to acclimate for 60 minutes before heat withdrawal latencies, and mechanical withdrawal thresholds were determined. The same sequence and methods of testing responses to colon distension, heat withdrawal latencies, and mechanical withdrawal thresholds was repeated 1, 2, 4, and 8 days after colonic inflammation.

Colorectal Distension After Plantar Incision

EMG electrodes were implanted in rats as described above, and baseline responses to colon distension, heat withdrawal latencies, and mechanical withdrawal thresholds were established. The next day, a plantar incision was made; 6 hours later, the VMR was recorded in response to CRD, as described above. Rats were allowed to acclimate 1 hour after CRD testing; hind paw heat withdrawal latencies and mechanical withdrawal thresholds were then measured. The same sequence and methods of testing responses to colon distension, heat withdrawal latencies, and mechanical withdrawal thresholds was repeated 1, 2, 4, and 8 days after hind paw incision.

Data Analysis

All changes in responses after incision and induced colon hypersensitivity are compared versus baseline. Statistical analysis was preformed using SigmaStat (Version 3.01; SPSS Inc., Chicago, IL) and Prizm 4.0 (Graphpad Software, San Diego, CA). Results were analyzed by using parametric 2-way ANOVAs for repeated measures. Data were further analyzed by parametric and nonparametric ANOVA when indicated. The F values are reported. The treatment effects were compared versus the baseline immediately before intervention. When the 1-way ANOVA was significant, post hoc Dunnett's or Dunn's tests was performed versus baseline for parametric and nonparametric analyses, respectively. A value of $P < .05$ was considered statistically significant. All data are given as mean ± standard error or median and percentiles.

Results

Hind Paw and Visceromotor Responses After Colon Inflammation

When tested 2 days after intracolonic treatment, the stimulus-response functions to colorectal distension were significantly increased (F = 166.8) in rats treated with TNBS (n = 9) or with zymosan (F = 157.82, n = 9) (both $P < .01$, repeated-measures ANOVAs) relative to saline-treated counterparts (Fig 1). Thus, both intracolonic TNBS and zymosan produced significant colon hypersensitivity.

Before intracolonic administration of TNBS, the median hind paw withdrawal threshold was 98 mN, which decreased significantly to 11 mN when first tested 6 hours after TNBS (F = 24.8, $P < .05$ vs baseline; Fig 2B).

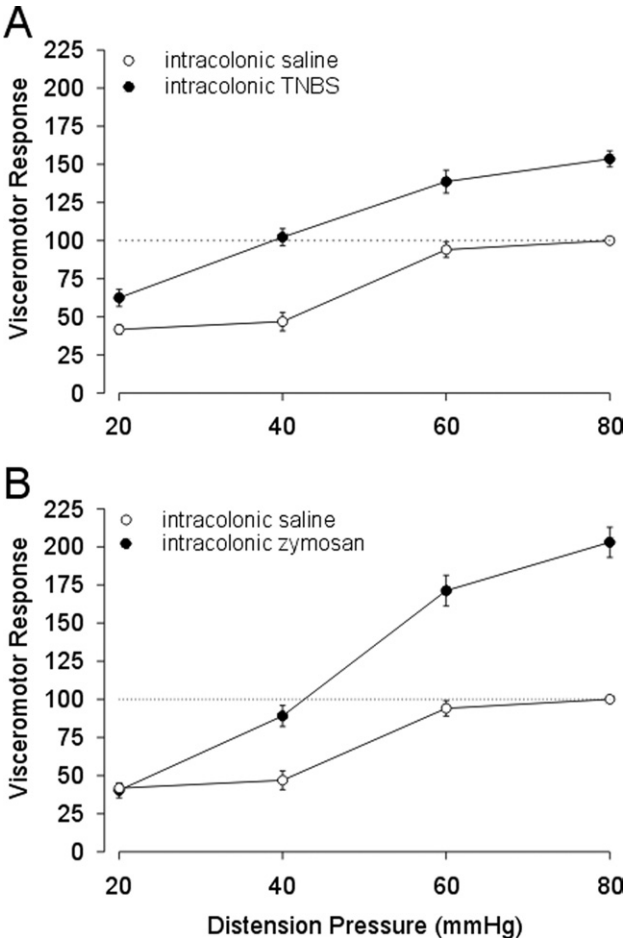

Figure 1. Mean visceromotor response (as percent of baseline) to graded CRD (20–80 mm Hg) in rats receiving intracolonic administration of trinitrobenzene sulfonic acid (TNBS) or saline vehicle **(A)**. Data of mean visceromotor response (as percent of baseline) to graded colorectal distension (20–80 mm Hg) in rats receiving intracolonic administration of zymosan or saline vehicle **(B)**. Intracolonic treatments were given 2 days before testing.

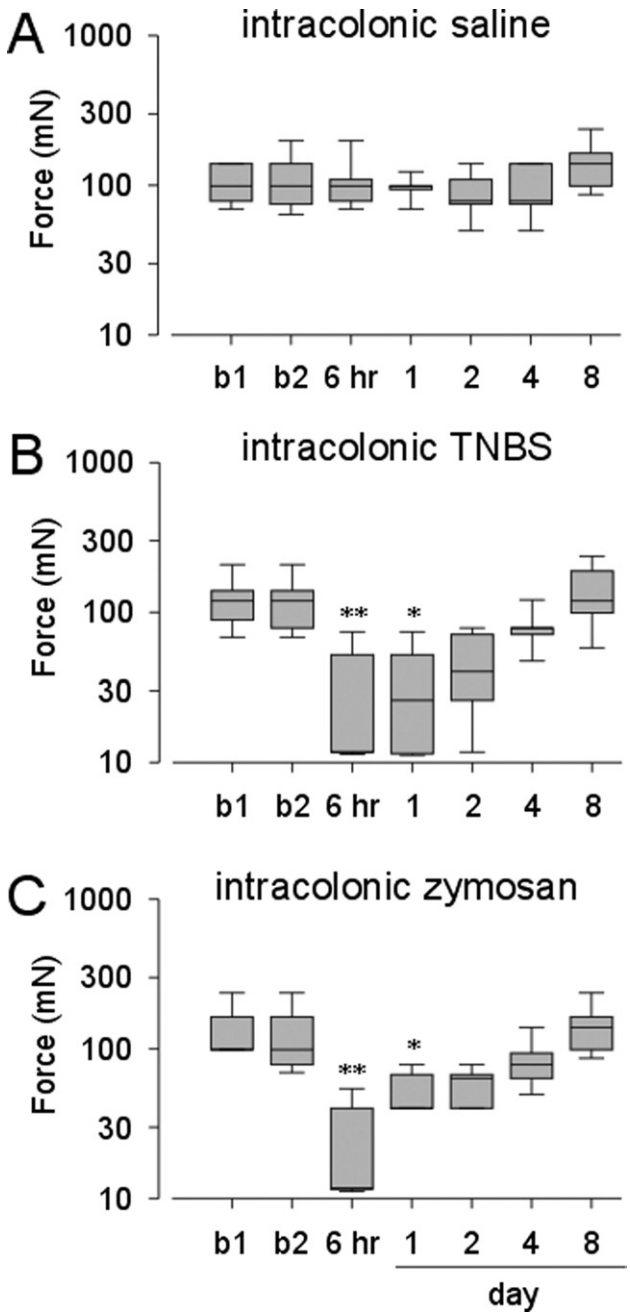

Figure 2. Hind paw mechanical withdrawal threshold in rats treated with intracolonic administration of saline **(A)** or trinitrobenzene sulfonic acid (TNBS) **(B)**. Hind paw mechanical withdrawal threshold in rats treated with intracolonic administration of zymosan **(C)**. Results are expressed as median (horizontal line) with 1st and 3rd quartiles (boxes) and 10th and 90th percentiles denoted by error bars. b1 = baseline 1; b2 = baseline 2 (*$P <$ .05 vs b2; **$P <$ .01 vs b2).

Withdrawal thresholds increased until they were indistinguishable from baseline values at day 2. There was no change from baseline in hind paw heat withdrawal latency in rats treated with intracolonic TNBS ($F = 3.6$, $P >$ .05 vs baseline; Fig 3B).

Similarly, in rats treated with intracolonic zymosan, the hind paw withdrawal threshold significantly decreased from a median of 98 mN before zymosan administration to 11 mN 6 hours after zymosan administration ($F = 32.5$, $P <$ .05 vs baseline; Fig 2C). Withdrawal thresholds returned to baseline by day 2. As found after intracolonic TNBS treatment, there was no change in hind paw heat withdrawal latency after intracolonic zymosan administration ($F = 1.2$, $P >$ .05 vs baseline; Fig 3C). There were no changes relative to baseline in either mechanical threshold ($F = 9.7$, $P >$ .05) or heat withdrawal latency ($F = 3.7$, $P >$ .05) after intracolonic saline treatment (Figs 2A and 3A).

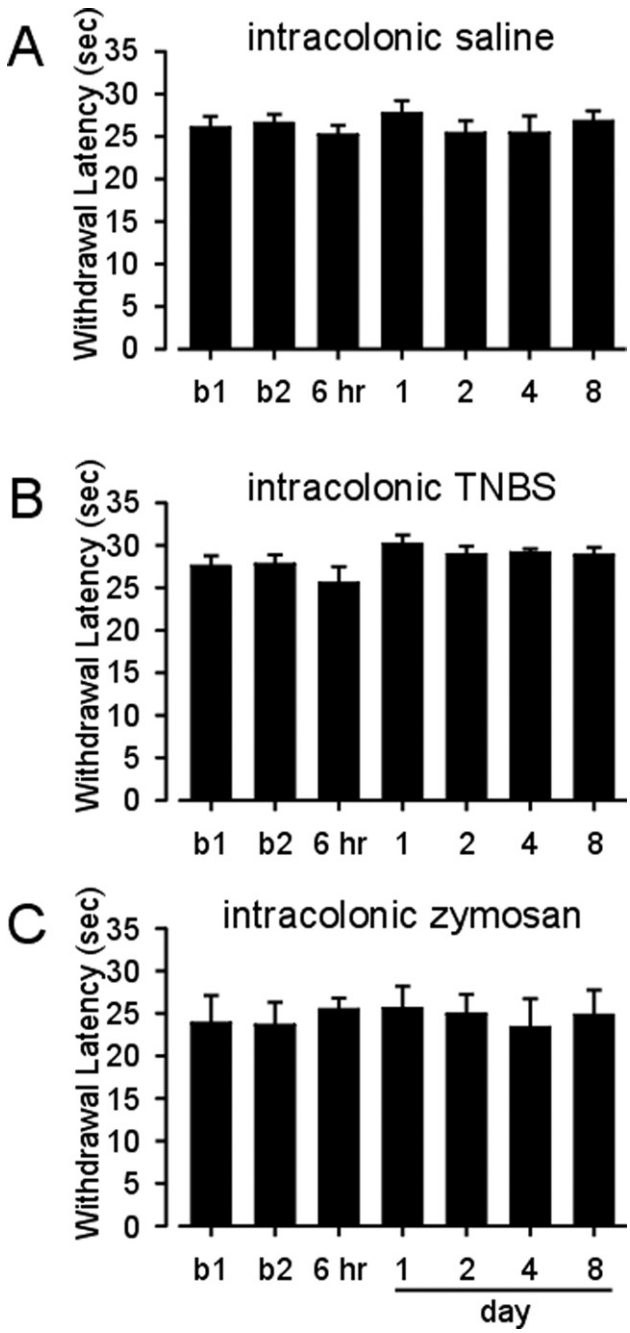

Figure 3. Mean hind paw heat withdrawal latency of rats treated with intracolonic saline **(A)** or intracolonic trinitrobenzene sulfonic acid (TNBS) **(B)**. Mean hind paw heat withdrawal latency of rats treated with intracolonic zymosan **(C)**. Vertical lines are standard error of the mean. b1 = baseline 1; b2 = baseline 2.

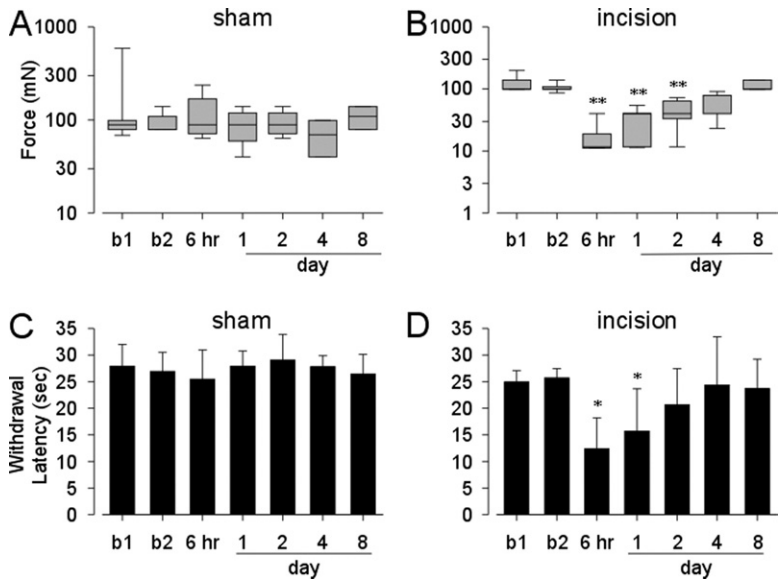

Figure 4. Withdrawal responses to punctuate mechanical stimuli in rats after sham incision or plantar incision (**A** and **B**). Results are expressed as median (horizontal line) with 1st and 3rd quartiles (boxes) and 10th and 90th percentiles. Mean hind paw heat withdrawal latency in rats after plantar incision (**C** and **D**). Vertical lines are standard error of the mean. Mean visceromotor response (as percent of baseline) to graded colorectal distension (20–80 mm Hg) in rats undergoing plantar incision in the hind paw or sham surgery **(E)**. Vertical lines are the standard error of the mean. b1 = baseline 1; b2 = baseline 2 (*$P < .05$ vs b2; **$P < .01$ vs. b2).

Hind Paw and Visceromotor Responses After Plantar Incision

In the control group, the median withdrawal threshold (98 mN) was unchanged after the sham incision ($F = 9.5$, $P > .05$; Fig 4A). After a plantar incision, however, the median withdrawal threshold was reduced from 98 mN to 11 mN when first tested 6 hours after the incision (Fig 4B). The withdrawal threshold remained decreased through postoperative day 2 ($F = 38.2$, $P < .05$ vs baseline) and was indistinguishable from baseline thereafter. Hind paw withdrawal latencies to heat were unchanged after the sham procedure (27.9 ± 4.1–25.4 ± 5.5; Fig 4C), whereas hind paw withdrawal latency was significantly decreased to 12.3 ± 5.9 6 hours after incision ($F = 8.2$, $P < .05$ vs baseline) and remained significantly different from baseline through postoperative day 1 (Fig 4D). By 4 days after incision, the mean paw withdrawal latency returned to baseline values.

These same rats were tested for responses to graded CRD before and after either a sham incision or incision of the hind paw. The VMR to distension was significantly increased at all times tested after hind paw incision except when tested 6 hours after incision ($F = 106.27$, $P < .05$ vs baseline; Fig 5A). Neither sham incision nor repetitive CRD testing was associated with colon hypersensitivity ($F = 0.92$, $P > .05$; Fig 5B). Accordingly, hind paw incision produced colon hypersensitivity that persisted after mechanical and thermal hind paw hyperalgesia had resolved (Fig 4).

Discussion

The principal findings of this study contribute to a growing literature supporting the importance of cross-sensitization between visceral and nonvisceral structures after experimental injury. Thus, colon inflammation not only produces colon hypersensitivity to balloon distension but also hind paw hyperalgesia to mechanical stimuli. Conversely, hind paw incision, a model of postsurgical nociception and hypersensitivity, not only produces hind paw hyperalgesia to both thermal and mechanical stimuli but increases responses to colon distension, an indication of colon hypersensitivity.

Referred hypersensitivity from the viscera is associated with increased tenderness to palpation and increased areas of referral and is attributed to sensitization in the central nervous system.[12,16,33,37] Central sensitization results from increased input from sensitized and previously silent visceral afferents, which increases the excitability of central neurons on which the afferents terminate.[6] Spinal visceral afferent input terminates principally on neurons in superficial spinal laminae, laminae V and X around the central canal, but, unlike nonvisceral afferent input, visceral afferent input extends and arborizes significantly within the spinal cord to include multiple spinal segments both rostral and caudal to the segment of entry. In contrast, somatic inputs are generally limited to the segment of entry and immediately adjacent segments. Accordingly, the number of spinal neurons affected after visceral insult increases significantly[17] and contributes to increased excitability of spinal neurons that also receive convergent nonvisceral inputs. Similarly, altered afferent input after injury of nonvisceral tissues can cause heterosynaptic facilitation, sensitizing input from other areas of the body. Several studies have suggested that this central sensitization is N-methyl-D-aspartic acid (NMDA) dependent.[26,49]

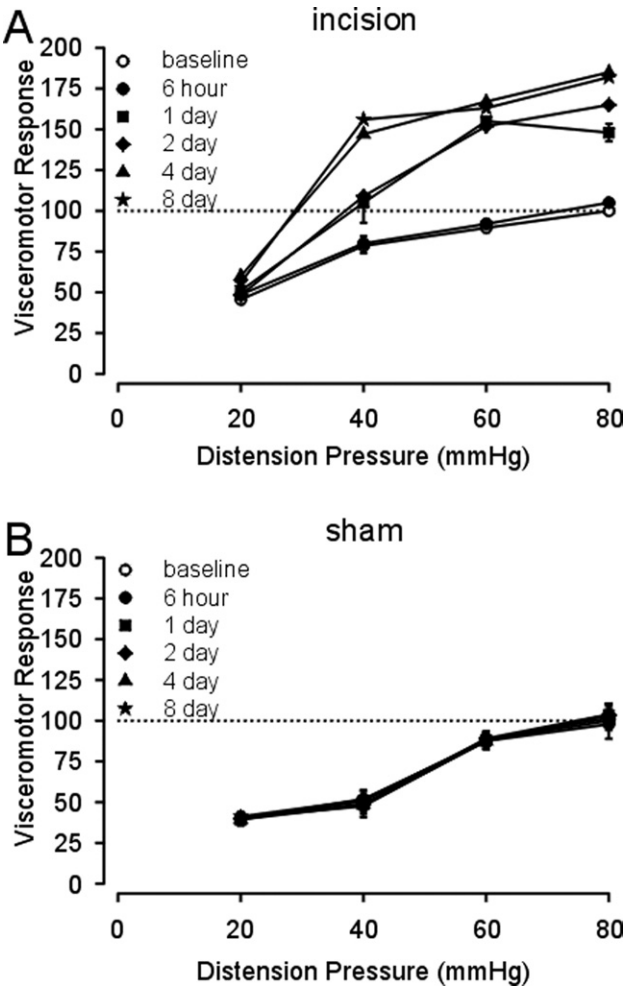

Figure 5. Mean visceromotor response (as percent of baseline) to graded colorectal distension (20–80 mm Hg) in rats after plantar incision **(A)** or sham incision **(B)**. Vertical lines are standard error of the mean. In **A**, $P < .05$ vs baseline control for all times of testing except 6 hours after hind paw incision (see text).

Viscerosomatic Convergence

The present study confirms previous reports of TNBS- and zymosan-induced colon hypersensitivity in the rat[9,14,15,27,38] and incision-induced thermal and mechanical hyperalgesia after a hind paw incision.[50] We also confirmed the development of nonvisceral hyperalgesia after a visceral insult (see below) and, importantly, established a robust and long-lasting colon hypersensitivity after a distant nonvisceral insult, hind paw incision. The clinical implication of this finding relates to likely changes in visceral sensitivity and perhaps function after nonvisceral surgery (or indeed other nonvisceral insults).

As indicated above, the present data are consistent with previous studies demonstrating an increased sensitivity in nonvisceral tissue after irritation or inflammation of the viscera in rodent models.[5,7,18,20] For example, turpentine-induced inflammation of the bladder produced heat hyperalgesia in the hind limb for at least 24 hours.[19] In another study,[18] bladder inflammation was associated with a referred mechanical and thermal hyperalgesia of the abdominal wall. Referral of visceral pain is not unique to animal models. In most interpretations of such experimental outcomes, convergence of inputs on the same or nearby spinal segments are considered to be the cause of cross-sensitization between visceral and nonvisceral tissues. Afferent fibers from the colon project to thoracolumbar (T13-L2) and lumbosacral (L6-S1) segments of the rat spinal cord.[10,28] Electrical stimulation of spinal nerves has shown that the plantar aspect of the hind paw is predominately innervated by nerves entering spinal segments L4 and L5.[42] Supraspinal sites are also likely to be involved[6,43] but are not commonly studied.

A specific effect of colon inflammation and hypersensitivity on hind paw mechanical withdrawal threshold was noted. There was no effect of colon inflammation on responses to hind paw heating, however. These results are consistent with other studies on central sensitization in which injury facilitates remote mechanical but not heat responses. For example, subcutaneous capsaicin injection produces remote secondary hyperalgesia to mechanical but not to heat stimuli in humans.[4] Both hind paw and gastrocnemius incision produce secondary mechanical but not heat hypersensitivity.[34] This is consistent with enhanced responses of A-fibers but not C-fibers after injury.[51] A mechanistic explanation for these outcomes is not obvious. Virtually all visceral afferent axons are either Aδ- or C-fibers and terminate centrally on neurons principally in the superficial laminae of the spinal dorsal horn. One mechanistic explanation that merits consideration and examination is that mechanosensitive hind paw afferents converge on spinal neurons that receive visceral afferent input, whereas thermosensitive hind paw afferents synapse onto spinal neurons that do not receive a principal visceral convergent input. This remains to be directly tested, however.

Somatic referred hypersensitivity in humans also has been documented, for example, in ureteric colic, renal infection, interstitial cystitis, and IBS.[3,48] Verne et al[44] found visceral hyperalgesia and cutaneous hyperalgesia in IBS patients that was greatest in lumbosacral dermatomes, corresponding to likely spinal hyperexcitability in nearby spinal segments. Somatic hypersensitivity, however, has not been universally demonstrated in patients with IBS when examining hypersensitivity to electrical stimulation of somatic structures or immersion of the hand in ice water.[39] However, patients with IBS and fibromyalgia had lower pain thresholds than IBS patients or healthy control subjects, suggesting the possible role of changes in central nervous system processing.[1,13,52]

Somatovisceral Convergence

Consistent with the convergence of visceral and nonvisceral inputs onto spinal dorsal horn neurons, the visceromotor response to CRD was elevated 1 day after plantar incision (but not when tested at 6 hours) and remained elevated for 8 days; we did not continue testing beyond this time. Elevated responses to noxious pressures were highest on postoperative days 4 and 8 when compared with baseline values, suggesting that colon

sensitization peaks several days after hind paw insult. Responses to distension were increased only within the noxious range of distending pressures (40, 60, and 80 mm Hg). Although we did not test beyond day 8 after incision, unpublished results suggest that colon sensitization is present for up to 2 weeks. One interpretation of these findings is that neurons in supraspinal sites, principally those associated with descending modulation of spinal sensory input, have been sensitized and maintain visceral hypersensitivity after hind paw insult. Irritable bowel syndrome and other "functional" gastrointestinal disorders are characterized by discomfort and pain in the absence of visceral tissue inflammation or injury. It has become increasingly evident that supraspinal neurons and networks are important to maintenance of such functional disorders, but it remains to be determined what leads to these changes supraspinally. It has been shown that visceral insult in neonatal and young animals[2,36] leads to visceral hypersensitivity in the adult long after the neonatal insult has resolved. Similarly, neonatal paw injury alters visceromotor response in adult rats.[45] Thus, functional visceral disorders characterized by hypersensitivity need not require a prior visceral insult but could develop from an apparently unrelated nonvisceral insult. The experimental design used here examined insults that influenced spinal neurons in nearby or overlapping spinal segments, and, given the known anatomical distribution of visceral and nonvisceral afferent terminations in the spinal cord, are understandable. What is unknown is whether and to what extent a distant nonvisceral insult (eg, to a forepaw, thoracic incision, etc) might lead to colon hypersensitivity as described here.

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