Colonic irritation in the rat sensitzes urinary bladder afferents to mechanical and chemical stimuli: an afferent origin of pelvic organ cross-sensitization

Elena E. Ustinova,1 Matthew O. Fraser,2 and Michael A. Pezzone1

1Department of Medicine, Division of Gastroenterology, Hepatology, and Nutrition, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; and 2Department of Surgery, Division of Urology, Duke University Medical Center and Durham Veterans Affairs Medical Center, Durham, North Carolina

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Ustinova, Elena E., Matthew O. Fraser, and Michael A. Pezzone. Colonic irritation in the rat sensitizes urinary bladder afferents to mechanical and chemical stimuli: an afferent origin of pelvic organ cross-sensitization. Am J Physiol Renal Physiol 290: F1478–F1487, 2006. First published January 10, 2006; doi:10.1152/ajprenal.00395.2005.—Chronic pelvic pain (CPP) disorders frequently overlap. We have demonstrated that acute and chronic colonic irritation can lead to neurogenic cystitis. We hypothesize that acute colonic irritation can sensitize urinary bladder afferents to mechanical and chemical stimuli. Single-unit afferent activity was recorded from fine filaments of the pelvic nerve in urethane-anesthetized Sprague-Dawley female rats before and 1 h after intracolonic administration of trinitrobenzenesulfonic acid (TNBS). Only spontaneously active afferents with receptive fields in the bladder and conduction velocities <2.5 m/s (unmyelinated C-fibers) were studied. Mechanical sensitivity was tested by bladder distension (BD) during saline infusion, whereas chemical sensitivity was tested with intravesical capsaicin, bradykinin, or substance P. Colonic irritation increased the resting firing rate of bladder afferents twofold (1.0 ± 0.2 vs. 0.49 ± 0.2 impulses/s, P < 0.05). Moreover, at low-pressure BDs (10–20 mmHg), a greater percentage of afferents exhibited increased activity following TNBS (73 vs. 27%, P < 0.05). Although the magnitude of the afferent response to BD was unchanged at low pressures, the response was greatly enhanced at pressures ≥30 mmHg and above (2.36 ± 0.56 vs. 8.55 ± 0.73 impulses/s, P < 0.05). Responses to capsaicin, bradykinin, and substance P were also significantly enhanced following TNBS, and all responses were blocked by bladder denervation. In rats, colonic irritation sensitizes urinary bladder afferents tonoxious mechanical and chemical stimuli. Interruption of the neural input to the bladder minimized this effect, suggesting a local afferent pathway from the colon. Thus, the overlap of CPP disorders may be a consequence of pelvic afferent cross-sensitization.

trinitrobenzenesulfonic acid; interstitial cystitis; irritable bowel syndrome; C-fiber; capsaicin

irritable bowel syndrome (IBS) and interstitial cystitis (IC), like other chronic pelvic pain (CPP) disorders, primarily affect women of reproductive age (44). Characterized by pain involving the pelvic cavity (IBS and IC) and/or the pelvic floor [levator ani syndrome, urethral syndrome, prostatodynia, vulvodynia, and orchialgia (42)], CPP affects as much as 15% of the population in both the United States and United Kingdom (28, 45). Because the colorectum and urinary bladder are two of the larger pelvic organs and because their functions are an integral part of daily, conscious, physiological pelvic activity, it is not surprising that IBS and IC, analogous disorders of pelvic visceral pain and urgency, account for 50% of CPP disorders (44).

It has become increasingly apparent that CPP disorders such as IBS and IC often overlap and occur concomitantly. As many as 40–60% of patients diagnosed with IBS also exhibit symptoms and fulfill diagnostic criteria for IC, while correspondingly, 38–50% of patients diagnosed with IC also have symptoms and fulfill diagnostic criteria for IBS (1, 33, 35, 43). Although the etiologies of both IBS and IC have been studied extensively, few have considered a common mechanism responsible for the development and the overlap of these and other causes of CPP.

Neural “cross-talk” in the pelvis, which occurs when afferent activation of one pelvic structure influences afferent outflow to another, is necessary for the normal regulation of sexual, bladder, and bowel function and is likely mediated by the convergence of sensory pathways in the spinal cord (7, 8, 10–12, 21). For example, overlapping central projections of pelvic and pudendal afferents allow integration of somatic and parasymphathetic motor activity in the pelvis and facilitate the orchestration of sacral reflexes. Correspondingly, the convergence of afferents from the bladder and bowel is a common feature of visceral interneurons which are thought to mediate vesico- and colono-sphincteric reflexes and colono-vesical cross-inhibitory interactions (29). Because a neural substrate for pelvic organ cross-talk exists under normal conditions, alterations in these neural pathways by disease or injury may play a role in the development of overlapping CPP disorders and pelvic organ cross-sensitization.

Recently, in a novel experimental model of neural cross-talk and acute pelvic organ irritation, we demonstrated that acute cystitis can lower colorectal sensory thresholds to balloon distension and that acute colitis can produce acute irritative micturition patterns (34). Specifically, before bladder irritation, graded colorectal distensions (CRDs) to 40 cm H2O produced no notable changes in abdominal wall electromyographic (EMG) activity, a visceromotor measure of visceral pain. Following acute bladder irritation, however, dramatic increases in abdominal wall EMG activity in response to CRD were observed at much lower distension pressures, indicating colonic afferent sensitization. Analogously, following acute colonic irritation, bladder contraction frequency increased 66% suggesting sensitization of lower urinary tract afferents. The
development of pelvic organ cross-sensitization in the acute setting as seen in these studies suggested a role for and subsequent modulation of preexisting afferent pathways in the pelvis (34).

Likewise, in follow-up studies performed in our laboratory, we found that chronic colonic irritation can lead to neurogenic cystitis as manifested by irritative micturition patterns, the recruitment and activation of bladder mast cells, and the upregulation of neurotrophic and mast cell growth factors in the bladder and its supplying dorsal root ganglion (DRG) which is also known to contain convergent input from the chronically irritated distal colon. Thus, with continued irritation of a pelvic organ, neurotrophic factors produced by both smooth muscle and DRG neurons of the insulted organ (colon) may influence neurite outgrowth and axonal sprouting at the level of the spinal cord, resulting ultimately in motor and sensory changes in other nonirritated pelvic organs such as the bladder. Furthermore, upregulation of these same neurotrophic factors in both the nonirritated organ (bladder) and DRG-containing convergent pelvic input (bowel and bladder) may account for end-organ changes and afferent nerve cross-sensitivity influencing both neurite outgrowth at the level of the DRG and axonal sprouting in the nonirritated pelvic organ. Previously, no one has fully investigated the hypothesis that irritation of one pelvic organ may adversely influence and directly sensitize the afferents of another. Showing that acute colitis can also lead to hyperexcitability of DRG neurons supplying other pelvic organs, Malychina and colleagues (27) noted enhanced sodium currents in dispersed bladder DRG cells following acute colonic irritation with dextran sulfate sodium. To further these and our own studies and to directly test the hypothesis that colonic irritation can alter the mechanical and chemical sensitivity of urinary bladder afferents, we recorded single-unit C-fiber bladder activity from fine filaments of the pelvic nerve in urethane-anesthetized Sprague-Dawley female rats and assessed their responsiveness to mechanical and chemical sensitivity influencing both neurite outgrowth at the level of the DRG and axonal sprouting in the nonirritated pelvic organ.

Materials and Methods

**Animals.** Female Sprague-Dawley rats, 200–250 g in weight, were purchased from Hilltop Lab Animals (Scottsdale, PA) and were housed in standard polypropylene cages with ad libitum access to food and water in the University of Pittsburgh’s Central Animal Facility. All studies were approved by the University of Pittsburgh’s Institutional Animal Care and Use Committee and were found to meet the standards for humane animal care and use as set by the Animal Welfare Act and National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**In vivo physiological instrumentation.** All animals were anesthetized with urethane (1.2 g/kg sc; Sigma, St. Louis, MO) before undergoing in vivo physiological instrumentation. Following a midline laparotomy, a double-lumen transvesical catheter fashioned from PE-20 tubing (Fisher Scientific, Hanover Park, IL) was inserted through the bladder dome via a small cystotomy and ligated for urinary bladder filling and pressure recording while maintaining the bladder in its native position. One lumen of the catheter was used for introducing chemicals and draining the bladder, while the second lumen was connected to a blood pressure transducer (World Precision Instruments, Sarasota, FL) and a syringe pump (Harvard Apparatus, Holliston, MA) via three-way stopcocks for bladder filling and continuous measurement of intravesical pressure. Room temperature saline was infused into the bladder constantly at a rate of 0.05 ml/min during continuous open cystometry. A Transbridge transducer amplifier (World Precision Instruments) was used to amplify the signal from the pressure transducer which was processed using a PowerLab 8s unit data-acquisition system (ADInstruments, Mountain View, CA) connected to an Apple G5 computer. Cystometry catheters were calibrated with water-filled tubing attached to the transducer, the meniscus at 0 and 100 cm, relative to the height of the bladder.

The trachea was cannulated to facilitate respiration. Polyurethane catheters were inserted in a carotid artery and jugular vein for measurement of arterial pressure and administration of saline, respectively. An intrarectal catheter was inserted 6 cm into the anus for later administration of TNBS, the colonic irritant.

**Recording of nerve activity and identification of afferent endings.** The right pelvic nerve was isolated at the major pelvic ganglion (MPG), dissected free from surrounding tissue, and cut at a maximal distance from the ganglion. The cut end still contiguous with the bladder was positioned on a small platform and covered with mineral oil. Fine bundles were dissected and placed on one arm of a silver electrode, while a second arm was grounded. Impulses were amplified (Grass QP511; Grass, West Warwick, RI) and acquired with the PowerLab Software as above and counted by a rate meter in 1-s intervals. The rate meter threshold was set to count potentials of desired amplitude. A bundle that had one, or at most two, easily distinguishable active units was used.

Only spontaneously active afferents that had precise receptive fields in the bladder (i.e., afferents clearly responding to probing of the bladder surface with a fine-tipped rod) were studied. Conduction velocity was estimated by measuring the distance between the receptive field and recording electrode and dividing it by the latency between electrical stimulation of the receptive field and evoked potential. Afferent recording was limited to unmyelinated C-fibers as characterized by conduction velocities less than 2.5 m/s and capsaicin sensitivity. Fibers with high-conduction velocities and not responsive to capsaicin were discarded.

**Mechanical and chemical testing of afferents.** After identification of the sensory ending, the mechanical sensitivity of the afferent was tested by distension of the bladder with saline infusion at the rate of 0.25 ml/min to the maximal intravesical pressure of 60 mmHg. During the infusion, the external urethral sphincter was clamped to maintain pressure in the bladder. Afterward, the bladder was immediately emptied and returned to a baseline pressure of 4–6 mmHg. Distributions were repeated two to three times within 10- to 15-min intervals to ensure the stability of the response.

Chemical sensitivity of the afferent was tested with intravesical administration of bradykinin (1–10 μg) or substance P (1–100 μg) in 0.2-ml total volume (0.1 ml of capsaicin solution followed by 0.1 ml saline flush). Response of the afferent to capsaicin was compared with the response to administration of 0.2 ml of saline. Responses to capsaicin vehicle (10% ethanol in saline) were tested and found to be no different from responses to the same volume of saline.

Some afferents were also tested with intravesical administration of bradykinin (1–100 μg) or substance P (1–100 μg). All chemicals were purchased from Sigma. Although varying doses of capsaicin (0.1–100 μg), bradykinin (1–100 μg), and substance P (1–100 μg) were preliminarily studied, only doses that elicited stable pronounced responses in the majority of tested afferents were utilized for each respective compound. Responses of the afferents to chemical stimulation were measured as the average number of impulses (imp) per second over a period of 20 s during 1 min following chemical administration. All chemicals were instilled in a total volume of 0.2 ml which predictably increased intravesical pressure by 10–12 mmHg in all cases.

Although the urothelium is thought to be an impermeable barrier to intravesical agents, drugs with high lipophilicity such as capsaicin...
easily penetrate the urothelium and consequently exert their affects on C-fiber afferents. Less lipophilic drugs less easily penetrate the urothelium, but absorption still occurs. From our own experience, the required dose of such drugs needed to produce a response is at least 10 times higher than if applied on the serosal surface of the bladder.

Acute effect of intracolonic TNBS on bladder afferent activity. In 10 animals, bladder afferent responses to distension and capsaicin were tested before and 1 h after TNBS administration. TNBS (5% aqueous solution; Sigma) was instilled intrarectally as previously described by Morris et al. (32) and modified by Appleyard and Wallace (2) to induce acute colonic irritation under urethane anesthesia. Briefly, TNBS (50 mg/ml) dissolved in 50% ethanol (vol/vol) was administered via a transanal approach (total volume 0.5 ml) using a PE-90 catheter whose tip was placed ∼6 cm proximal to the anal verge. Because recordings were made with rats lying in the supine position, any potential leakage of the TNBS from the colon, although not observed, would not come in contact with the perineum and hence the urethral sphincter. As an added precaution, Surgilube (E. Fougera & Co., Melville, NY) was applied to the perineum to minimize any potential contaminant irritation due to anal leakage. In six animals (vehicle control), the protocol was repeated after giving TNBS vehicle, and results were compared with TNBS-treated animals.

Acute effect of intracolonic TNBS on bladder afferent activity following denervation. In seven rats, the left pelvic nerve and its adjacent vessel were ligated. The left MPG was lifted and separated by blunt dissection from the wall of the vagina, and the preganglionic nerve branches were transected. The left pudendal nerve was identified and transected. On the right side, hypogastric and pudendal nerves were identified and transected. The right pelvic nerve was transected at a maximal distance from the ganglion and used for recording of afferent activity as described above. Data obtained in this experimental group were compared with animals receiving intrarectal TNBS without bladder denervation.

Statistical analysis. Reported values represent means ± SE. Data were analyzed using GraphPad Prism 3.0 statistical software (San Diego, CA). Afferent firing rate was calculated as the average number of impulses per second over a period of 20 s. Resting activity represented maximal activity recorded 1 min before each intervention. Afferent firing during bladder distension was averaged over 10 mm of Hg increments of intravesical pressure. Afferent responses to capsaicin were measured during maximal activity 1 min following capsaicin administration. Differences between groups were determined by ANOVA, and differences between means were isolated by a Bonferroni correction for multiple t-tests. Contingency tables with χ² tests were used to determine the differences in proportions. Statistical significance was accepted at P < 0.05.

RESULTS

Effect of intracolonic TNBS on bladder afferent responses to mechanical bladder distension. Action potentials from 15 afferent nerve endings were recorded before and 1 h after intracolonic TNBS administration. Five of these recordings were obtained from filaments with only one active fiber. In the other 10 recordings, two active units were present, but differences in spike amplitude permitted the discriminate measurement of individual fiber activity. All afferent endings were located in the bladder and, correspondingly, responded with a burst of impulses on probing of the bladder wall. Afferent fibers used in this study were classified as C-fibers based on conduction velocities <2.5 m/s and capsaicin sensitivity (38). The average resting firing rate of the studied afferents was 0.53 ± 0.11 imp/s (range: 0.10 to 1.30 imp/s). Only a small percentage (<10%) of pelvic afferents with receptive fields on the bladder wall were discarded based on high-conduction velocities and insensitivity to capsaicin.

Figure 1A illustrates a representative bladder afferent response to bladder distension with saline. Note the spontaneous baseline activity and the increase in afferent firing with increasing intravesical pressure. One hour after intracolonic TNBS administration, the baseline firing rate of the afferent and its response to bladder distension were both increased compared with the controls (Fig. 1B).

Bladder afferent responses to saline distension for the 15 single-unit afferents are summarized and represented graphically in Fig. 2 both before and 1 h after intracolonic TNBS administration. The average afferent resting firing rate was 0.49 ± 0.10 imp/s before TNBS administration and 1.0 ± 0.2 imp/s 1 h afterwards (P < 0.05). As depicted in Fig. 2A, distension of the bladder during saline infusion led to an increase in the bladder afferent firing rate that was proportional to the intravesical pressure; this response, however, was more pronounced 1 h after TNBS. Although the firing rate at all vesical pressures tended to be higher after TNBS treatment, this difference was particularly evident and statistically significant at intravesical pressure levels of 30 mmHg and above (2.36 ± 0.56 vs. 8.55 ± 0.73 at 30 mmHg, P < 0.05).

Figure 2B demonstrates that in the control group of 12 afferent fibers, administration of TNBS vehicle alone had no effect on the baseline activity of the afferents or their response to bladder distension.

Figure 2C illustrates action potential data taken from 12 afferent nerve endings both before and 1 h after intracolonic TNBS in 7 animals in which bladder denervation was performed beforehand. Before administration of TNBS to denervated rats, average afferent resting activity and response to bladder distension were no different than controls with intact neural bladder input. In denervated rats, intracolonic TNBS did not increase baseline afferent resting activity or the afferent response to saline distension compared with pretreatment recordings. Specifically, the TNBS-induced increase in the bladder afferent response, which occurred at saline distension levels of 30 mmHg and above, was ameliorated by bladder denervation.

Table 1 further characterizes the individual afferent response profiles and shows that resting activity increased in 9 of 15 afferents (60%) 1 h after intracolonic TNBS (P < 0.05). Furthermore, following intracolonic TNBS administration, a larger number of fibers became activated at lower intravesical pressures: 73% of the afferents increased their firing rate at an intravesical pressure of 10 mmHg (vs. 23% before TNBS, P < 0.05), while 100% became activated at 20 mmHg (vs. 53% before TNBS, P < 0.05). At the higher intravesical pressures (30–60 mmHg), all afferents exhibited increased activity irrespective of TNBS treatment, although the average afferent activity was significantly increased in TNBS-treated animals as illustrated in Fig. 2A. Following bladder denervation, the number of afferents activated in response to bladder distension was no different before or 1 h following intracolonic TNBS compared with intact controls (Table 1, Fig. 2C).

Effects of intracolonic TNBS on bladder afferent responses to capsaicin, bradykinin, and substance P. Figure 3 depicts a representative bladder afferent response to intravesical administration of 0.2 ml of normal saline or 1 μg of capsaicin in the same volume of saline both before (top) and 1 h after (bottom)
Fig. 1. Acute colonic irritation enhances bladder afferent responses to distension. This representative recording illustrates a bladder afferent response to distension before (A) and after (B) intracolonic trinitrobenzenesulfonic acid (TNBS) administration. Bladder pressure, bladder afferent action potentials, and rate meter output are depicted from top to bottom in each respective recording. Before TNBS administration, saline bladder distension led to a proportional increase in bladder afferent firing (A). After TNBS administration, the baseline firing rate of the afferent and its response to bladder distension were markedly increased (B). imp, Impulses.
intracolonic TNBS administration. Before TNBS administration, both intravesical saline and capsaicin (to a greater degree) led to increased afferent activity and increased bladder pressure. Following TNBS administration, the afferent response to saline was slightly increased, while the response to capsaicin was increased dramatically as seen in the tracing.

In Fig. 4, average baseline bladder afferent firing rates and bladder afferent responses to intravesical saline (control) and capsaicin are illustrated before and 1 h after intracolonic TNBS (A) or vehicle (B) in neurally intact and bladder-denervated rats (C). As was shown above, afferent responses to intravesical saline infusion (0.2 ml) (corresponding to low intravesical pressure) were no different following TNBS administration compared with baseline, although baseline activity itself was significantly increased in TNBS-treated animals (P < 0.05). In response to capsaicin, however, the maximal firing rate of the bladder afferents was 4.26 ± 0.58 imp/s before and 12.4 ± 2.8 imp/s after TNBS administration, nearly a threefold increase (P < 0.01). Administration of TNBS vehicle had no statistically significant effect on the responses of the afferents to saline or capsaicin. Following bladder denervation, the afferent responses to intravesical capsaicin were no different following TNBS administration compared with pretreatment controls. Likewise, the increases in baseline bladder afferent responses to intracolonic TNBS were ameliorated following bladder denervation.

Eight of the 15 tested afferents responded to intravesical administration of bradykinin (1–100 μg), whereas 12 were found to be activated with substance P (1–100 μg). Intracolonic administration of TNBS did not change the number of afferents responding to intravesical saline (0.2 ml) (corresponding to low intravesical pressure) and substance P.

Table 1. Acute colonic irritation lowers bladder afferent thresholds to bladder distension

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<th>Denervated Before TNBS</th>
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<tr>
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<td>15 (100%)</td>
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<td>Number of afferents with increased baseline activity after TNBS</td>
<td>9 (60%)</td>
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<td>Number of afferents with increased activity during bladder distention to</td>
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<td>10 mmHg</td>
<td>4 (27%)</td>
<td>11 (73%)*</td>
<td>3 (25%)</td>
<td>2 (17%)†</td>
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<td>20 mmHg</td>
<td>8 (53%)</td>
<td>15 (100%)*</td>
<td>6 (50%)</td>
<td>7 (58%)†</td>
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After intracolonic trinitrobenzenesulfonic acid (TNBS) administration, the resting activity increased in 60% of bladder afferents. Following TNBS, a significantly larger number of afferents increased their firing rates at the lower bladder distension (BD) pressures (10–20 mmHg). At the higher BD pressures (30–60 mmHg), there was no difference between the numbers of fibers activated before and after TNBS (postthreshold). After bladder denervation, the TNBS-induced increase in baseline bladder afferent activity was ameliorated as was the increase in the number of afferents exhibiting enhanced activity in response to low-pressure BD. *P < 0.05 after TNBS vs. before TNBS. †P < 0.05 denervated vs. intact.
afferents responding to each of the chemicals; however, TNBS did dramatically increase their respective activity (Fig. 5A). Specifically, the response of the afferents to bradykinin was significantly increased from 1.9 ± 0.6 to 11.1 ± 3.1 imp/s ($P < 0.01$), whereas the response to substance P increased from 2.4 ± 1.3 to 6.4 ± 1.4 imp/s ($P < 0.05$). Administration of TNBS vehicle to six additional animals had no effect on the responses of eight bradykinin- and substance P-sensitive afferents (Fig. 5B).

DISCUSSION

Providing compelling evidence that acute colonic irritation with TNBS directly sensitizes the mecano- and chemoreceptive properties of urinary bladder C-fibers traveling within the pelvic nerve, these studies shed further light on our previous work demonstrating that acute bladder irritation can lower colorectal sensory thresholds to balloon distension and that acute colitis can similarly produce acute irritative micturition patterns (34). In our previous studies, cross-organ pelvic reflexes and acute cross-organ irritative alterations in physiological functioning and sensation (bladder-to-bowel and vice versa) were described, and the development of cross-sensitization in this setting suggested a role for, and subsequent modulation of, preexisting afferent pathways in the pelvis (34). Such cross-organ afferent pathways may originate centrally via spinal or supraspinal circuits (including spinal antidromic dorsal root reflexes) and/or peripherally, directly from the colon via antidromic axon reflexes from a single dichotomizing primary afferent supplying two structures (prespinal convergence).

In our current studies, evidence supporting the sensitization of urinary bladder mechanoreceptive C-fibers following intra-colonic TNBS is demonstrated in Figs. 1 and 2. As shown by the representative tracing in Fig. 1, acute colonic irritation led to enhanced firing of bladder C-fibers in response to saline bladder distension. Figure 2A depicts these afferent responses...
as a function of intravesical distension pressure and shows that although basal bladder afferent activity is increased following TNBS, the magnitude of the response to bladder distension is no different from controls until relatively high intravesical pressures are reached (30–60 mmHg). Because bladder C-fiber afferents typically respond to “noxious” intravesical pressures (30–50 mmHg) under normal conditions (20), it’s not unexpected that their greatest increase in magnitude following cross-organ sensitization would occur in that same pressure range. Although such differences in the magnitude of bladder afferent activity were not appreciable until intravesical pressures of 30 mmHg and above were reached, there was evidence that individual afferent mechanical thresholds were decreased following TNBS as shown in Table 1. Saline distension of the bladder to 10 mmHg activated 27% of bladder C-fiber afferents, whereas 73% of these same afferents were responsive 1 h after TNBS treatment. Likewise, bladder distension to 20 mmHg activated 53% of control afferents before TNBS, while 100% were responsive 1 h afterwards (Table 1). At 30 mmHg and above, afferents were maximally activated regardless of TNBS exposure, although, as mentioned above (Fig. 2A), the magnitude of the response in this pressure range was significantly enhanced following TNBS treatment. These results suggest that colonic irritation (and presumably activation of colonic afferent pathways) directly or indirectly lowered the mechanosensitive thresholds of the bladder C-fibers as has been shown previously for the bladder following direct intraluminal bladder irritation (20). The decrease in bladder afferent thresholds to mechanical distension following intracolonic TNBS could account for increases in micturition rate and decreases in micturition volume as observed in our acute and chronic studies of pelvic organ cross-sensitization and which are also thought to occur clinically in IC (34).

As shown in Fig. 2B, the bladder afferent response to TNBS vehicle was no different than saline-treated controls signifying that TNBS itself played a direct role in the cross-sensitization process. Moreover, amelioration of the effects of TNBS by bladder denervation (Fig. 2C) confirms that local diffusion or systemic effects of the intracolonic irritant do not account for our findings. Correspondingly, macroscopic and microscopic evaluation of the bladder in this setting, as well as in our chronic studies and those of others (27), revealed the absence of gross or histological bladder damage as contributing factors. In our chronic TNBS studies, there were no apparent adhesions or any evidence of a locally spread inflammatory mass between the bladder and other pelvic organs such as the colon (i.e.,

Fig. 4. Bladder denervation ameliorates TNBS-induced chemical bladder sensitization. A: in response to intravesical capsaicin injection (30 μmol in 0.2 ml), afferent firing rates increased from 0.31 ± 0.14 to 4.26 ± 0.58 imp/s before and from 1.42 ± 0.2 to 12.4 ± 2.8 imp/s after TNBS administration. B: administration of TNBS vehicle had no effect on afferent responses to capsaicin. C: denervation of the bladder abolished the sensitizing effects of TNBS on bladder afferent responses to capsaicin. *Statistically significant after vs. before TNBS.

Fig. 5. Acute colonic irritation sensitizes bladder afferent responses to bradykinin and substance P. Intravesical administration of bradykinin (10 μg in 0.2 ml) increased bladder afferent firing rates from 0.5 ± 1 to 1.85 ± 0.5 imp/s, and administration of substance P (10 μg in 0.2 ml) increased bladder afferent firing rates from 0.5 ± 1 to 2.32 ± 1.2 imp/s (NS). After TNBS administration, bladder afferent responses to bradykinin and substance P increased by 500% and 180%, respectively. B: TNBS vehicle had no effect. *Statistically significant after vs. before TNBS.
nonneurogenic or transmural irritation). The bladder, a relatively small pelvic organ lying low and anterior in the pelvis and shielded from the colon by the uterus, was at least 2–3 cm away from the foci of the treated colonic segment, which was 6 cm from the anal verge and, by default, very dorsal in the abdominal cavity. Reassuringly, after bladder denervation, bladder distension in the noxious range (30–60 mmHg) did lead to increasing afferent activity whose magnitude was no different from sham controls (pre-TNBS; Table 1). Similarly, at lower intravesical pressures (10–20 mmHg), the TNBS-induced sensitization of bladder afferents (as manifested by lowered sensory thresholds) was ameliorated following denervation as were changes in basal afferent activity. Although pelvic organ denervation was not performed in our previous studies of pelvic organ cross-sensitization (34), neurogenic cystitis induced in the central nervous system by the pseudorabies virus was similarly preventable by bladder denervation, again implicating neural pathways (22).

Like bladder mechanoreceptors, bladder chemoreceptors were also sensitized 1 h following intracolonic TNBS. Specifically, acute irritation of the colon increased the magnitude of the bladder afferent responses to intravesical administration of capsaicin, bradykinin, and substance P (Figs. 3–5). As shown in Figs. 3 and 4A, the response to intravesical capsaicin was dramatically increased (nearly 3-fold) 1 h following intracolonic TNBS. Because the intravesical volume was low (0.2 ml) during capsaicin infusion, the majority of this effect can be attributed to the direct chemical effect of capsaicin itself on the bladder afferents rather than that due to mechanical distension. These findings are in accord with those in the literature showing that chemically induced cystitis in animals is associated with sensitization of chemosensitive afferents and/or recruitment of afferents normally unresponsive to mechanical stimulation (14, 15, 20). Inflammatory agents, such as prostaglandin E2, serotonin, histamine, and adenosine, as well as nerve growth factor (NGF), can induce functional changes in C-fiber afferents that can lead to their hyperactivity and increased excitability (4, 14, 16, 19), and these changes in C-fiber afferent properties likely translate into increased pain sensation (18, 20). In our studies of chronic pelvic organ cross-sensitization, NGF and histamine release in the lower urinary tract has been implicated. As was the case in the mechanical testing of the bladder afferents, TNBS vehicle had no effect on the afferent responses to capsaicin (Fig. 4B). Furthermore, the effects of capsaicin were also ameliorated by bladder denervation (Fig. 4C), strongly suggesting that acute colonic irritation sensitizes bladder afferents via a neurogenic process, thereby enhancing their responsiveness to mechanical and chemical stimulation.

Although the effects of TNBS colitis on other pelvic organ afferents have not been previously evaluated, changes in nociceptive DRG neurons supplying the bowel have been noted (31). Specifically, the action potential threshold in neurons from TNBS-treated animals was reduced by >70%, and the TNBS-induced hyperexcitability in nociceptive DRG neurons and changes in the properties of sodium and potassium channels at the soma persisted after removal from the inflamed environment (31). Showing that chronic colitis can also lead to hyperexcitability of DRG neurons supplying other pelvic organs, Malychina and colleagues (27) noted increased excitability in bladder DRG neurons following colonic irritation with dextran sulfate sodium. The effects of bladder denervation in this setting were not assessed nor were the putative afferent pathways in the pelvis. Although neither study was performed in the acute setting, perhaps acute modulation of these same afferent pathways may have led to acute cross-organ afferent sensitization (had it been assessed) as observed in our own studies.

First, to describe bowel-bladder neural interactions, Denny-Brown and Robertson (13) documented in 1933 that during elimination, micturition and defecation normally alternated. Kock and Pompeius (24) later demonstrated (1963) that urinary bladder motility was inhibited by stimulation of the anal canal, rectum, or perineal skin, and these effects were thought to be mediated by a peripheral adrenergic mechanism via the hypogastric nerves (40) and by a central mechanism involving pelvic nerve afferents (6, 17, 40). Further shedding light on such bowel-bladder neural interactions, McMahon and Morris (29) showed that the convergence of afferent inputs from the bowel and bladder was a common feature of visceral interneurons in the sacral cord, and those interneurons identified with type II compound-receptive fields were proposed as mediators of the reciprocal inhibitory reflexes between the bladder and bowel (29) and may be an example of the convergence-projection theory proposed by Ruch (37). Further implicating spinal convergence as a mechanism of pelvic organ cross-sensitization, Qin and Foreman (36) recently reported that 32% of L6-S2 spinal neurons received convergent inputs from both the urinary bladder and colon. Thus, in our current studies, colonic irritation and activation of colonic afferents may acutely sensitize bladder afferents (at least those traveling in the pelvic nerve) via convergent spinal pathways likely involving spinal interneurons. Similarly, antidromic dorsal root reflexes may also play a role (41) and could also account for the neurogenic inflammation seen in our studies of chronic pelvic organ cross-sensitization.

As an alternative or contributing mechanism, pelvic organ cross-afferent sensitization could develop by means of dichotomizing pelvic afferents whereby irritation of one pelvic organ sensitizes another via afferent terminals emanating from the same DRG neuron. The presence of 2.5 times as many fibers in the dorsal root and mixed nerve as there are cell bodies in the appropriate DRG (25) is consistent with this hypothesis. DRG neurons with dichotomizing axons were first proposed by Sinclair et al. (39) and have been reported in several species and range from 0.5 to 15% of all afferents (30). Dichotomizing afferents, which can be identified by both dual retrograde labeling studies and/or electrophysiological recordings, have been previously identified in pelvic organs by de Groat and colleagues (9, 23). Using retrograde tracers, de Groat found that 3–6% of afferents innervating the colon and urogenital organs in both the Wistar rat and the cat were dual labeled and thus possibly dichotomizing. Our recent studies confirm that dual-labeled or potentially dichotomizing afferents exist in the Hilltop strain of Sprague-Dawley rat and were as high as 15% of all bladder and colon afferents (5). Similarly, Bahns and colleagues (3), studying responses of sacral visceral afferents from the lower urinary tract and colon, found single units that were indeed excited by stimulation of two pelvic organs. Although such hardwiring (dichotomization) may exist and such neurons may play a physiological role, further studies are...
needed to clarify their role in pelvic organ cross-sensitization and the overlap of pelvic pain syndromes.

In conclusion, in rats, intrarectal administration of TNBS sensitizes urinary bladder afferents to mechanical and chemical stimuli. Interruption of extrinsic input to the bladder ameliorated this effect, suggesting a neural pathway which may originate directly from the colon or via spinal or supraspinal pathways. These cross-organ pelvic afferent interactions likely represent components of a complex neural network involving sensory pathways in the pelvis which are likely important for the normal pelvic regulation and integration of sexual, bowel, and bladder function. Thus these data provide further support for neural processes in mediating cross-sensitization of pelvic organs and the overlap of IC, IBS, and other CPP disorders. Ongoing studies are attempting to address these neural mechanisms in greater detail with a specific focus on the role of hypogastric and other central inputs and pathways.

REFERENCES