Sensitization of pelvic nerve afferents and mast cell infiltration in the urinary bladder following chronic colonic irritation is mediated by neuropeptides

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Ustinova EE, Gutkin DW, Pezzone MA. Sensitization of pelvic nerve afferents and mast cell infiltration in the urinary bladder following chronic colonic irritation is mediated by neuropeptides. Am J Physiol Renal Physiol 292: F123–F130, 2007. First published August 22, 2006; doi:10.1152/ajprenal.00162.2006.—Irritable bowel syndrome and interstitial cystitis frequently overlap. We have shown that acute colitis sensitizes urinary bladder afferents to both mechanical and chemical stimuli and that chronic colitis similarly produces neurogenic cystitis. We hypothesize that chronic irritation of the colon releases neuropeptides from bladder afferents, leading to receptor sensitization and neurogenic inflammation. Female Sprague-Dawley rats received intrarectal trinitrobenzenesulfonic acid (TNBS) or vehicle 3 days following either systemic capsaicin (CP) pretreatment or vehicle. Ten days later, action potentials of single-unit pelvic C-fiber afferents with receptive fields in the bladder were recorded under urethane anesthesia during graded bladder distensions (UBD) or intravesical capsaicin (vCP) administration. In controls, UBD increased bladder afferent firing in proportion to intravesical pressure. At intravesical pressures of 30 mmHg and above, the percent increase in afferent firing was significantly accentuated following TNBS compared with controls (1,222 ± 176 vs. 624 ± 54%, P < 0.01). The response to vCP was also enhanced (4,126 ± 775 vs. 1,979 ± 438%, P < 0.01). Systemic depletion of neuropeptides from sensory nerves abolished these effects. Histological examination of the bladders revealed an increase in mast cell density in TNBS-treated animals compared with controls (18.02 ± 1.25 vs. 3.11 ± 0.27 mast cells/100 field, P < 0.01). This effect was significantly ameliorated with CP (10.25 ± 0.95, P < 0.5 vs. TNBS-treated animals). In summary, chronic colonic irritation in the rat sensitizes urinary bladder afferents to noxious stimuli and causes mast cell infiltration in the bladder. Depletion of neuropeptides from sensory afferents diminishes these effects, suggesting they play an important role.

trinitrobenzenesulfonic acid; interstitial cystitis; irritable bowel syndrome; C fiber; capsaicin; mast cell

CHRONIC PELVIC PAIN (CPP) disorders such as irritable bowel syndrome (IBS), interstitial cystitis (IC), and chronic prostatitis (male CPP syndrome) affect both men and women and have a prevalence rate as high as 15% in both the United States and the United Kingdom (3, 6, 27, 30, 51). In the right setting, CPP can develop following acute or chronic irritation of individual pelvic visceral organs, their associated striated sphincters, striated muscular structures of the pelvic floor, and/or striated and cutaneous components of the pelvic abdominal wall and/or perineum (14). Because physiological activity of the colorectum and urinary bladder and their respective sensory input are a vital part of daily, conscious visceral pelvic activity (exclusive from other pelvic organs), it is not surprising that IBS and IC, analogous disorders of pelvic visceral pain and hypersensitivity, account for one-half of all cases of CPP (50).

Although the preponderance of IBS and IC in the spectrum of CPP disorders is not entirely unexpected, their propensity to overlap and concur with other CPP disorders is quite intriguing. As many as 40–60% of patients diagnosed with IBS also exhibit symptoms and fulfill diagnostic criteria for IC, while similarly as many as 50% of patients diagnosed with IC also have symptoms and fulfill diagnostic criteria for IBS (1, 33, 36, 49). Similarly, 26% of patients diagnosed with IC were also found to have concurrent pain of the vulva or vulvodynia (17), and 45% of males with chronic prostatitis or male CPP exhibited pain with bladder filling, a classic feature of IC (30). Neural “cross-talk” within the pelvis 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 (7–11, 22).

Convergence of afferent pathways from the bladder and bowel is known to be a common feature of visceral interneurons, which are thought to mediate vesico- and colonicospincteric reflexes and colonovesical cross-inhibitory interactions (29). Fittingly, our recent studies implicated a role of preexisting neural pathways in the development of pelvic organ cross-sensitization by demonstrating that colonic hypersensitivity develops following the induction of acute cystitis and vice versa (35). Specifically, we demonstrated that acute cystitis can lower colorectal sensory thresholds to balloon distension and that acute colitis can produce acute irritative micturition patterns (35). Follow-up studies performed in our laboratory employing single unit C-fiber bladder afferent recording revealed that acute colonic irritation is capable of sensitizing urinary bladder afferents to mechanical and chemical stimuli, and interruption of the neural input to the bladder can ameliorate this effect, suggesting a direct afferent pathway from the colon (46).

Additionally, 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 (Liang R, Ustinova EE, Patnam R, Fraser MO, Pezzone MA, unpublished observations). 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 such as neurogenic inflammation, afferent nerve cross-sensitivity, and axonal sprouting in the nonirritated pelvic organ (Liang R, Ustinova EE, Patnam R, Fraser MO, Pezzone MA, unpublished observations).

Thus, taking into account and expounding on our prior findings, we hypothesize that chronic irritation of the colon releases neuropeptides from bladder afferent endings leading to receptor sensitization and neurogenic inflammation. To this end, 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 (bladder distension) and chemical [intravesical capsaicin (vCP), bradykinin, or substance P] stimulation 10 days following intracolonic administration of trinitrobenzenesulfonic acid (TNBS) or vehicle. To evaluate the role of C-fiber afferents and their associated neuropeptides, animals were pretreated with capsaicin (CP) or vehicle 3 days before TNBS administration.

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 (Prolab RMH 3000) 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 the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental group summary. Four groups of animals were utilized in these studies. Animals were first pretreated systemically with CP or CP vehicle over a 3-day period to chronically deplete C-fiber afferents and their associated neuropeptides, animals were pretreated with capsaicin (CP) or vehicle 3 days before TNBS administration.

Depletion of C-fiber afferents. Under 4% isoflurane anesthesia, rats received on 3 consecutive days daily injections of 0.2 ml sc of CP (20, 30, and 50 mg·kg⁻¹·day⁻¹) or CP vehicle (10% ethanol and 10% Tween 80 in saline) into the fat pad on the back of the neck. Induction of TNBS colitis. TNBS (1 M aqueous solution; Sigma) was instilled intrarectally under 4% isoflurane anesthesia as previously described by Morris et al. (31) and modified by Appleyard and Wallace (2) to induce acute colonic irritation under urethane anesthesia. Briefly, TNBS (30 mg in 50% ethanol, total volume 0.5 ml) was administered via a transanal approach using a PE-90 catheter whose tip was placed ~6 cm proximal to the anal verge. As an added precaution, Surgilube (Fougera, Melville, NY) was applied to the perineum to minimize any potential contaminant irritation due to anal leakage. This model of colitis is characterized by local areas of acute inflammation peaking at 4–7 days, followed by a chronic, mononuclear inflammatory cell infiltrate that persists up to 6 wk until it resolves without spontaneous relapse (15, 31).

In vivo physiological instrumentation. In vivo physiological instrumentation was performed as previously described (46) while the rats were under urethane anesthesia (1.2 g/kg sc, Sigma, St. Louis, MO). 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 the bladder was maintained 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. Cystometrig 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. After a 40-min equilibration period, cystometrograms were recorded during a 30-min period, and micturition intervals were calculated for each animal.

Recording of nerve activity and identification of afferent endings. Following completion of the cystometrogram, 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. 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 chemical intervention. Changes in afferent activity in response to interventions were expressed as percent change from the resting firing rate.

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 <2.5 m/s and capsaicin sensitivity (38). Fibers with high conduction velocities and not responsive to CP 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 (UBD) 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. UBDs were repeated two to three times within 10- to 15-min intervals to ensure the stability of the response. Afferent firing during bladder distension was averaged over 10-mmHg increments of intravesical pressure.

Chemical sensitivity of the afferent was tested with CP (0.1–10 μg in 0.2-ml total volume; 0.1 ml of capsaicin solution followed by 0.1-ml saline flush). The response of the afferent to CP was compared with the response to administration 0.2 ml of saline. Responses to CP vehicle (10% ethanol in saline) were tested and found to be no
different from responses to the same volume of saline. Due to the differences in baseline firing rate in the individual afferents, changes in afferent activity were also expressed as percent change from baseline.

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. Responses of the afferents to chemical stimulation were measured as the average number of impulses 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 CP easily penetrate the urothelium and consequently exert their effects 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.

Morphology and mast cell histology. Four animals from each experimental group were euthanized using pentobarbital sodium (100 mg/kg ip, Abbott Laboratories, North Chicago, IL). The distal colon was dissected from the anus to the splenic flexure, longitudinally opened, and examined macroscopically. Representative cross-sectional colonic tissue samples located 6 cm from the anus (i.e., the site of TNBS administration) were embedded in OCT medium (Miles Laboratories, Elkhart, IN) and frozen. The urinary bladders were excised and dissected longitudinally along their midline. Both halves were embedded in OCT medium and frozen. Using a cryostat (Microm HM 505 E, Mikron Instruments, San Marcos, CA), multiple sections from both organs were obtained at a thickness of 5 μm. Serial sections were mounted and stained with hematoxylin/eosin and Giemsa stains (25). Mast cells were counted independently by two investigators under a ×100-power field as previously described (39). Ten to twenty fields were counted for colon and bladder sections from each animal, and the average number of mast cells per field was calculated.

Statistical analysis. Reported values represent mean ± SE. Data were analyzed using GraphPad Prism 3.0 statistical software (San Diego, CA). Differences between groups were determined by ANOVA, and differences between means were isolated by a Bonferroni correction for multiple t-tests. Statistical significance was accepted at P < 0.05.

RESULTS

Effects of CP pretreatment and TNBS-induced colitis on micturition rates. As shown in Fig. 1 and corresponding to a 42% increase in micturition rate, voiding intervals were significantly decreased 10 days following intrarectal TNBS treatment (CP+/TNBS+). The average micturition interval was 307 ± 28 s in control animals (CP-/TNBS-) vs. 177 ± 21 s in TNBS-treated animals (CP-/TNBS+) (P < 0.01). CP pretreatment alone (CP+/TNBS−) had no effect on voiding frequency; however, CP pretreatment completely abolished the decrease in the voiding interval induced by TNBS (CP+/TNBS+). There were no cystometric abnormalities noted during the filling phases of the micturition curves to suggest detrusor overactivity in the TNBS-treated animals.

Effects of CP pretreatment and TNBS-induced colitis on bladder afferent responses to UBD. Action potentials were recorded from 26 single-unit bladder afferents in 15 control rats (CP-/TNBS−) and from 18 single-unit bladder afferents fibers in 9 rats with TNBS-induced colitis (CP-/TNBS+). No significant differences in the afferent resting firing rates were noted between these two groups (0.44 ± 0.08 impulses/s for controls and 0.57 ± 0.19 impulses/s following TNBS). Similarly, neither CP pretreatment alone (CP+/TNBS−; 12 afferents from 7 rats) nor CP pretreatment followed by TNBS administration (CP+/TNBS+; 15 afferents from 8 rats) changed the resting firing rate of bladder afferents (i.e., 10 days post-TNBS or -TNBS vehicle administration; 0.37 ± 0.17 impulses/s for CP+/TNBS− vs. 0.46 ± 0.19 impulses/s for CP+/TNBS+). Bladder afferent responses to UBD with saline are summarized in Fig. 2 for all four groups. As seen in Fig. 2 in all four groups, UBD increased bladder afferent activity in proportion to intravesical pressure. This distension-induced enhancement in bladder afferent firing was more pronounced in the TNBS-treated rats (CP-/TNBS+) as evidenced by the greater slope (Fig. 2). At intravesical pressures of 10–20 mmHg (nonnoxious range), bladder afferent activity in all groups was equivalent; however, at UBD pressures of 30 mmHg and above, bladder afferent activity was substantially increased in the CP-/TNBS+ group (1,222 ± 176 vs. 624 ± 54% in CP-/TNBS− at 30 mmHg, P < 0.05).

CP pretreatment (CP+/TNBS−) had no significant effects on the afferent responses to UBD compared with controls. In the group of animals that received TNBS after pretreatment with capsaicin (CP+/TNBS+), the responses of the afferents to UBD were significantly attenuated compared with the TNBS alone group (CP-/TNBS+) and equivalent to controls (CP-/TNBS− and CP+/TNBS−).

Effects of CP pretreatment and TNBS-induced colitis on bladder afferent responses to chemical irritation with vCP. Figure 3 illustrates bladder afferent responses to intravesical infusion of saline and vCP compared with baseline activity. Increases in bladder afferent activity in response to intravesical saline infusion (0.2 ml; corresponding to low intravesical pressure) were no different across experimental groups including those receiving TNBS. Although animals pretreated with CP-pretreated tended to have less of an increase in afferent...
firing in response to saline infusion, this trend was not significant.

In response to vCP, the bladder afferent firing rate was markedly increased in the CP−/TNBS+ group (4,126 ± 775 vs. 1,979 ± 438% for CP−/TNBS−, P < 0.01). Pretreatment with CP alone (CP+/TNBS−) had no statistically significant effect on the responses of the afferents to vCP compared with controls (CP−/TNBS−), although the increase in afferent responses to vCP in animals with TNBS-induced colitis was abolished (1,450 ± 357% in CP+/TNBS+ vs. 4,126 ± 775% in CP−/TNBS−, P < 0.01).

Effects of CP pretreatment and TNBS-induced colitis on bladder afferent responses to chemical irritation with intravesical administration of bradykinin and substance P. Fifteen bladder afferents from the control group (CP−/TNBS−) were further assessed for bradykinin and substance P responsiveness. Eight of the 15 bladder afferents tested from the control group responded to intravesical administration of bradykinin (1–100 μg), and 12 of 15 were found to be activated with substance P (1–100 μg). Intrarectal administration of TNBS (CP−/TNBS−) did not significantly change the proportion of afferents responding to either of the chemicals: 12 of 19 tested afferents responded to bradykinin, and 14 of 15 were activated with substance P. TNBS, however, did dramatically increase the magnitude of the responses to both chemicals (Fig. 4). Specifically, the response of the afferents to bradykinin increased from 1,013 ± 159 to 1,813 ± 419 (P < 0.05), while the response to substance P increased from 665 ± 75 to 1,987 ± 338% (P < 0.05).

Pretreatment with CP completely abolished the responses of bladder afferents to intravesical administration of bradykinin and substance P in both CP pretreatment groups (CP+/TNBS− and CP+/TNBS+).

Morphology and mast cell counts in distal colon and urinary bladder. In the colon, none of the animals in control (CP−/TNBS−) or CP pretreatment (CP+/TNBS−) groups showed macroscopic abnormalities (Fig. 5). In contrast, animals in both groups that received TNBS (CP−/TNBS+ and CP+/TNBS+) showed marked macroscopic alterations in the distal colon (5–7 cm from the anus). These alterations included mucosal edema, bowel wall thickening, erosions, and ulcerations. All of these changes were more pronounced in the CP+/TNBS+ group and, in three of four rats, were accompanied by an incomplete bowel obstruction with proximal colonic dilatation. There were no cases of gross bowel perforation.

Microscopically, distal colonic sections from control and CP-pretreated groups showed normal a colonic wall with focal mild submucosal edema. Occasional mast cells were present (0.8 ± 0.3 and 1.5 ± 0.5 cells/100-field in CP−/TNBS− and CP+/TNBS− groups, respectively, P = 0.124, not significant) (Fig. 6).
Microscopic changes in the colons of the animals treated with TNBS (CP−/TNBS+ and CP+/TNBS+ groups) consisted of severe mucosal damage, characterized by focal ulcerations with associated polymorphonuclear and histiocytic infiltrates. The mucosa adjacent to ulcers revealed edema, hemorrhage, and distortion of crypt architecture. Mast cell infiltration was present in the lamina propria and muscularis propria (Fig. 5). The number of mast cells per ×100-power field in CP−/TNBS+ and CP+/TNBS+ groups was significantly higher than in the control group (3.0 ± 1.1 and 5.9 ± 1.0, respectively, vs. 0.8 ± 0.3, \( P < 0.05 \)) (Fig. 6).

Fig. 5. Distal colon histological sections stained with Giemsa. Representative images were taken from CP−/TNBS−, CP+/TNBS−, CP−/TNBS+, and CP+/TNBS− treated animals (A–D, respectively). The densely staining granular cells (arrowheads) represent mast cells (×100 power).

Fig. 6. Distal colon mast cell quantitation. Mast cells were quantitated in CP−/TNBS−, CP+/TNBS−, CP−/TNBS+, and CP+/TNBS+ treatment groups under ×100 magnification. Colonic mast cell numbers were significantly elevated in CP−/TNBS+ and CP+/TNBS+ treatment groups compared with controls. The number of mast cells in the CP+/TNBS+ group was significantly higher (2-fold) than those of the CP−/TNBS+ group. * \( P < 0.05 \) vs. CP−/TNBS+. † \( P < 0.05 \) vs. CP+/TNBS−.

Gross examination of the urinary bladders did not reveal macroscopic abnormalities in any of the experimental groups. Microscopically, in CP−/TNBS− and CP+/TNBS− groups, bladder morphology was normal, whereas in CP−/TNBS+ and CP+/TNBS+ groups there was mild to moderate submucosal edema. No epithelial damage or polymorphonuclear infiltrate was seen. Mast cells were predominantly located around submucosal and adventitial blood vessels with occasional infiltration of the lamina propria and muscularis propria (Fig. 7). The principal microscopic alteration was an increase in the number of mast cells in the bladder in the animals with TNBS-induced colitis (Fig. 8). In TNBS-treated animals, the number of mast cells per ×100-power field was significantly higher (18.02 ± 1.25 and 10.25 ± 0.95 in CP−/TNBS+ and CP+/TNBS+ groups, respectively) than in groups not treated with TNBS (3.11 ± 0.27 and 5.4 ± 0.50 cells in CP−/TNBS− and CP+/TNBS− groups, respectively) (Fig. 8).

Contrary to the mast cell counts in the colon, the number of mast cells in the bladder was significantly higher in the CP−/TNBS+ group than in the CP+/TNBS− group: 18.02 ± 1.25 vs. 10.25 ± 0.95, \( P = 0.036 \) (Fig. 8).

DISCUSSION

Corroborating our acute studies of pelvic organ cross-sensitization (35, 46), our current findings provide compelling evidence that chronic colonic irritation (10 days post-intrarectal TNBS administration) directly sensitizes the mechano- and chemoreceptive properties of urinary bladder C fibers traveling within the pelvic nerve. In our original 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 (35). This role of preexisting afferent pathways was further supported in our acute studies (1 h post-TNBS administration), where we first showed that colonic irritation is capable of sensitizing urinary bladder afferents to mechanical and chemical stimuli, a mechanism found to be directly dependent on neural input to the bladder (46). Correspondingly, in the chronic setting, by showing that CP pretreatment completely ameliorates these effects (both for mechanical and chemical urinary bladder stimulation), a role of C-fiber afferents and the release of their active neuropeptides in this pelvic cross-sensitization process are further substantiated. Such afferent cross-sensitization pathways (both acute and chronic) 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) (5).

Although bladder C-fiber sensitization in response to intrarectal TNBS was noted in both our acute (46) and our current chronic studies, unlike in the acute setting, basal bladder afferent firing 10 days after intrarectal TNBS administration had completely returned to control levels (equivalent to TNBS vehicle). The normalization of basal bladder afferent activity during this 10-day interval adds further credence to this model of chronic bladder hyperalgesia and provides further experimental support for the postinfectious or postirritative disease models of IBS, IC, and other CPP disorders (40, 47), where often an initial pelvic organ insult leads to visceral hypersensitivity that only becomes apparent when physiological stimuli exceed the diminished visceral afferent sensory thresholds. Similarly, in our chronic studies the associated development of neurogenic cystitis as evidenced by increased mast cell counts in the absence of macroscopic bladder damage is also supportive of our disease hypothesis as it also reflects human correlational studies for IBS, IC, and other pain syndromes (34, 37, 43, 45, 48).

As shown in Fig. 2, at intravesical pressures of 10–20 mmHg (nonnoxious range), bladder afferent activity in all groups was equivalent; however, at UBD pressures of 30 mmHg and above, bladder afferent activity was substantially increased in the animals treated with TNBS alone (CP+/TNBS+). Because bladder C-fiber afferents typically respond to “noxious” intravesical pressures (30–50 mmHg) under normal conditions (20), it is not unexpected that their greatest increase in magnitude following cross-organ sensitization would occur in that same pressure range as we have shown previously (46).
Evidence for chemical sensitization of bladder afferents was not limited to intravesical CP irritation. Responses to bradykinin and substance P were similarly accentuated 10 days following TNBS. These findings support 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 (12, 13, 20). Inflammatory mediators, such as prostaglandin E2, serotonin, histamine, and adenosine, as well as nerve growth factor, can modulate the functional properties of C-fiber afferents, leading to their hyperactivity and increased excitability (4, 12, 16, 19), and these changes in C-fiber afferent properties likely translate into increased pain sensation (18, 20). The modulation of these responses with systemic CP pretreatment further substantiates the role of C-fiber afferents in our model of pelvic organ bladder cross-sensitization. Although such afferent cross-sensitization pathways may involve central and/or peripheral afferent circuits (5) and although systemic CP does not preferentially affect individual pelvic organs, a direct role of bladder afferent endings was previously substantiated in our acute studies in which selective bladder denervation completely ameliorated these cross-sensitization effects (46). Moreover, amelioration of the effects of TNBS by bladder denervation confirmed that local diffusion or systemic effects of the intracolonic irritant did not account for our findings (46).

As expected, marked macroscopic and microscopic alterations including mastocytosis were noted in the distal colon 10 days following intrarectal TNBS administration. All of these changes were more pronounced in the CP+/TNBS+ treatment group, suggesting a protective role of CP-sensitive afferents in colonic mucosal injury, a phenomenon described previously in both the fore- (42) and hindgut (28). Gross examination of the urinary bladder, however, did not reveal macroscopic abnormalities in any of the experimental groups. Furthermore, microscopically, no epithelial damage or polymorphonuclear infiltrate was seen at 10 days, and this lack of bladder injury is in agreement with our acute studies of colonic irritation (46) as well as those of others (26). Interestingly, as noted in the colon (although at the site of direct mucosal injury), the number of bladder mast cells was significantly increased following TNBS-induced colitis (Fig. 7). In contrast to the colon, bladder mast cells were predominantly located around submucosal and adventitial blood vessels, with occasional infiltration of the lamina propria and muscularis propria, suggesting a neurohumoral pattern of chemotaxis.

The observed urinary bladder mastocytosis in this setting is not unexpected as a contributing role of mast cells in IC (45) and IBS (34, 37, 48) and in other disorders characterized by hyperalgesia and neurogenic inflammation has been previously implicated (43). There is a preponderance of evidence implicating potential pathophysiological mast cell–nerve interactions in the sensitization of visceral afferent nerves as the mast cell possesses a formidable armamentarium of nociceptive molecules including adenosine phosphates, bradykinin, histamine, leukotrienes, potassium, lymphokines, tumor necrosis factor (TNF), and prostaglandins (44). Anatomic evidence further supporting this mast cell-mediated modulation of afferent nerve function is apparent in the close apposition of mast cells to nerve fibers in both the human gastrointestinal tract (32, 41) and the urinary bladder (23).

Further supporting a neurogenic pathogenesis of the observed bladder mastocytosis, we previously demonstrated increased urinary bladder expression of stem cell factor and nerve growth factor, two potent mast cell growth and sensitizing factors following both acute and chronic colonic irritation with TNBS (Liang R, Ustinova EE, Patnam R, Fraser MO, unpublished observations). Furthermore, the differential effects of CP pretreatment on bladder and colonic mastocytosis in response to intrarectal TNBS likely also reflect differing mechanisms of mast cell stimulation. Accordingly, the near normalization of bladder mast cells in animals pretreated with CP before TNBS is consistent with a neurogenic mechanism of stimulation, while in the colon, the direct tissue insult and impairment of protective mechanisms following CP pretreatment were sufficient stimuli to enhance the mast cell response (42).

In summary, chronic colonic irritation in the rat with TNBS sensitizes urinary bladder afferents to mechanical and chemical stimuli and induces bladder mastocytosis. A role of C-fiber afferents was further substantiated in this model of neurogenic cystitis as CP pretreatment significantly ameliorated these effects. 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.

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