Changes in urinary bladder smooth muscle function in response to colonic inflammation

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¹Veterans Affairs Medical Center, ²Oklahoma Center for Neurosciences, ³Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; and ⁴Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia

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Noronha R, Akbarali H, Malykhina A, Foreman RD, Greenwood-Van Meerveld B. Changes in urinary bladder smooth muscle function in response to colonic inflammation. Am J Physiol Renal Physiol 293: F1461–F1467, 2007. First published August 22, 2007; doi:10.1152/ajprenal.00311.2007.—Visceral organ “cross talk” is suspected to contribute to multiorgan symptomatology found in conditions such as irritable bowel syndrome and interstitial cystitis. The goal of the present study was to investigate the short- and long-term effects of acute colitis on bladder detrusor muscle contractility. We hypothesized that inflammation of the colon leads to changes in bladder function via direct changes in detrusor smooth muscle contractility. In this study, colonic inflammation was induced in male rats via an enema of trinitrobenzenesulfonic acid (TNBS) (50 mg/kg, 0.5 ml, 25% ethanol). Colitis was confirmed using gross morphology, histology, and measurements of myeloperoxidase activity. Saline enema-treated rats served as controls. Three, 15, and 30 days post-enema treatment, bladder detrusor muscle contractility was investigated in response to electrical field stimulation (EFS), cholinergic agonism with carbachol (CCh), and KCl. During active colonic inflammation (day 3 post-TNBS enema), the bladder detrusor muscle appeared normal and showed no significant inflammation. However, abnormalities in bladder detrusor muscle contractility occurred in response to EFS and CCh but not KCl. During and after recovery from colonic inflammation (days 15 and 30 post-TNBS enema), changes in bladder detrusor muscle contractility in response to EFS and CCh returned to control levels. We found that a transient colonic inflammatory insult significantly attenuates the amplitude of bladder detrusor muscle contractions in vitro, at least in part, through changes in cholinergic innervation, which are reversible after recovery from the colitis.

trinitrobenzenesulfonic acid; colon; detrusor muscle; rat

CHRONIC PELVIC PAIN IS A COMMON condition affecting as many as 15% of women within the United States (14). Often, patients afflicted with chronic pelvic pain suffer from a variety of different conditions, including irritable bowel syndrome (IBS) and interstitial cystitis (IC). As many as 30–50% of patients diagnosed with IBS exhibit symptoms of IC, such as increased frequency and urgency to urinate, whereas as many as 40% of patients diagnosed with IC exhibit symptoms that fulfill the criteria for IBS (1, 3, 25). The mechanisms for the overlap of symptomatology expressed in patients with IBS and IC are unknown; however, it is suspected to arise from abnormalities in convergent neural pathways innervating the bladder and colon.

In experimental models, recent studies have attempted to address how pathology within one visceral organ can affect the function of another. Colonic irritation is capable of producing irregular micturition patterns, such as increased rate of micturition and enhanced urethral sphincter activity in rats (18). In addition, bladder irritation results in increased visceral sensitivity to colonic stimulation, an observation seen up to 7 days after induction of inflammation (12). Increased resting firing rates of bladder afferent fibers have also been reported during colonic irritation (21). Interestingly, increased afferent activity of these fibers in response to substance P, capsaicin, and bradykinin is abolished after bladder denervation (22). In addition, depletion of neuropeptides from sensory nerves has been found to restore bladder afferent activity and micturition rates during active colonic inflammation, suggesting that sensitization of afferent and efferent nerves is likely involved in visceral organ cross talk in pelvic organ disease (22).

Although the exact mechanism of cross talk between visceral organs in disease is unknown, sensitization of afferent and efferent nerve pathways innervating pelvic structures may occur at the level of the dorsal root ganglia (DRG), the spinal cord, and/or higher regions in the brain. Current evidence points to sensitization of afferent fibers, as well as DRG and spinal cord neurons. Specifically, dual-labeling studies have revealed that ∼3–15% of afferent nerve fibers innervating the bladder and colon are common to both structures (2, 10). In the lumbosacral region of the spinal cord, ∼30% of neurons respond to both bladder and colorectal stimulation (2, 19). In addition, lumbosacral bladder DRG and spinal neurons become hyperexcitable after colonic inflammation (13, 20).

In the present study, we hypothesized that hyperexcitability of bladder afferent neurons induced by colonic inflammation is capable of producing changes in bladder detrusor muscle contractility. Given that IBS is characterized by the presence of minimal inflammation and that heightened visceral sensitivity and altered colonic motility are still observed after recovery from colonic inflammation (7, 8, 11, 23), we also sought to determine whether any changes observed within bladder detrusor muscle contractility during active colonic inflammation would persist after recovery of the colonic inflammatory response.

METHODS

Animals. Experiments were performed on male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing ∼200–300 g. Rats were caged in pairs with free access to food and water and were kept under a 12:12-h light-dark cycle. Rats were housed in an animal care
facility under environmentally controlled conditions (21°C) and were allowed to acclimatize to the facility 1 wk before experiments were performed. Experiments were performed 3, 15, or 30 days after administration of a trinitrobenzenesulfonic acid (TNBS) or saline enema. The Oklahoma City Veterans Affairs Medical Center Animal Care Subcommittee and the University of Oklahoma Health Sciences Center Institutional Animal Care and Use Committee approved all experimental protocols.

**Induction of colonic inflammation.** After an acclimation period, rats were fasted overnight for ~12–18 h with free access to water. Rats were then removed from the animal facility and brought to the laboratory where they were briefly anesthetized with isoflurane (3%) for a period of ~10 min. While sedated, a flexible tube ~3 mm in diameter was inserted 8.0 cm from the anus; this tube contained a 0.5-ml solution of 50 mg/kg TNBS (Sigma, St. Louis, MO) diluted in 25% ethanol and 25% deionized water. Saline enema-treated rats served as controls. To minimize the loss of TNBS or saline solution, rats were inverted with their rear elevated until they regained consciousness. After they regained consciousness, rats were returned to their home cages, were observed for ~1 h, and were then returned to the animal facility. Stools were graded daily for a period of 3 days to determine the severity of TNBS-induced colonic inflammation (5). Scores were based on stool formation, consistency, and color. Scores ranged from 0 to 5, in which 0 was given for normal pellet formation and 5 for severe diarrhea. The presence of blood within the feces was also tested with an occult blood indicator test (Beckman Coulter, Fullerton, CA). Animals displaying a high fecal occult score were chosen for the study.

**Colonic damage assessment.** After euthanasia, the entire colon was removed from the cecum to the rectum. The distal section was then cut open, cleaned with Krebs, and removed for analysis. A qualified blinded observer using a grading scheme modified from Morris et al. (16) graded the morphological characteristics of the colon. Scores ranged from 0 to 6 in which a score of 0 was given for no colonic damage, 1 for the presence of minimal inflammation, 2 for the presence of one ulcer and minimal inflammation, 3 for the presence of 1 or 2 ulcers and inflammation, 4 for the presence of more than 2 ulcers, 5 for the presence of more than 2 ulcers, and 6 for the presence of severe necrosis.

**Myeloperoxidase activity.** Myeloperoxidase (MPO) activity, an indicator of neutrophil infiltration, was measured in colon and bladder samples obtained from each experimental group. Colon and bladder tissues were weighed and homogenized in ice-cold 0.5% hexadecyltrimethylammonium bromide (pH 6) buffer at a dilution of 1 ml/20 tissues were weighed and homogenized in ice-cold 0.5% hexadecyltrimethylammonium bromide (pH 6) buffer at a dilution of 1 ml/20. Aliquots of 1 ml were then removed from the animal facility and brought to the experimental protocols.

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produced by TNBS, we found that there was recovery of the colon. Measurements taken on days 15 and 30 revealed that the colonic damage assessment, histological appearance, and MPO content in the colon from TNBS-treated rats were different from those in saline enema-treated controls.

**Effect of an intracolonic TNBS enema on the bladder.** During active colonic inflammation (day 3 post-TNBS enema), no changes in bladder morphology were observed, and the appearance of the bladder resembled that from the saline enema-treated control rats. In addition, no changes in MPO levels within the bladder (Fig. 1B) were found, and a careful histological characterization of the bladder revealed no changes in bladder morphology during active colonic inflammation (Fig. 2 and Table 1). These levels remained consistent and comparable on recovery from the colitis (data not shown).

**Effect of TNBS-induced colitis on bladder detrusor muscle contractility in response to EFS.** In saline enema-treated control rats, bladder detrusor smooth muscle contractility was characterized by a graded increase in the amplitude of the contraction in response to EFS that was dependent on the frequency of the stimulation (Fig. 3). However, the amplitude of the detrusor smooth muscle contraction in response to EFS was visibly depressed in rats with active colonic inflammation produced by TNBS. Although this attenuation of the muscle response was statistically significant at higher frequencies of stimulation (16 and 32 Hz), a clear trend in depression was observed at all frequencies of EFS (P < 0.05). The reduction of the bladder muscle contractility in response to EFS was still observed at day 15 post-TNBS-induced colonic inflammation, although it was not found to be statistically significant compared with the saline controls. On day 30 post-TNBS enema, the levels of bladder detrusor muscle contractility in response to EFS, although slightly elevated compared with the saline controls group, were not statistically significant.

A series of experiments was performed to investigate the nature of the neurotransmitters released by enteric nerve terminals in response to EFS that may account for the changes of bladder detrusor muscle contractility associated with colitis. Detrusor muscles were isolated from rats during acute colonic inflammation (day 3 post-TNBS enema) or from control animals (day 3 post-saline enema), and the contractile responses to increasing frequency of EFS (0.5–16 Hz) were studied in the absence and presence of atropine (1 μM) in the bathing solution. The atropine-sensitive and atropine-resistant components of the responses were measured for each frequency of stimulation and expressed as percentage of the responses obtained in the absence of atropine. The summarized data (Fig. 4) show that, during colonic inflammation, the cholinergically mediated component of the responses of the detrusor muscle was decreased. In the context of this experimental paradigm, this finding implies that colonic inflammation is associated with an impairment of enteric cholinergic neurotransmission and an increased role of noncholinergic neurotransmitter mechanisms.

**Effect of TNBS-induced colitis on detrusor muscle contractility in response to CCh.** To further investigate whether the colitis-induced changes in detrusor muscle contractility involve changes in cholinergic receptors, we investigated the concentration-effect relationship for the cholinergic agonist CCh. In saline enema-treated control rats, addition of CCh to the organ bath resulted in a concentration-dependent increase in bladder detrusor muscle contractility. However, we observed that, during the period of active colonic inflammation, the amplitude of the bladder detrusor muscle contractility in response to CCh was significantly reduced compared with saline-treated controls (Fig. 5). The E\textsubscript{max} of bladder detrusor muscle to CCh was significantly reduced (P < 0.001) during active colonic inflammation (E\textsubscript{max} for saline = 0.38 ± 0.02 g/mg, E\textsubscript{max} for TNBS = 0.19 ± 0.03 g/mg). After recovery from colonic inflammation, the contractility responses of the detrusor muscle to CCh returned to levels not statistically different from those observed in saline-treated animals. Moreover, a detailed pharmacological characterization revealed no changes in the EC\textsubscript{50} between the saline- and TNBS-treated animals (saline: 1.33 × 10^{-6} ± 0.2 M, TNBS: 1.80 × 10^{-6} ± 0.1 M) at any of the three time points (days 3, 15, or 30). The lack of a significant shift in the concentration-effect curves to CCh suggests that, despite the decrease in the maximal contractile response, the sensitivity of muscarinic receptors in the bladder had not changed as a result of active colonic inflammation.

**Effect of TNBS-induced colitis on bladder detrusor muscle contractility in response to KCl.** Contractions were induced by high KCl to investigate whether colonic inflammation may alter the ability on the smooth muscle to contract in response to receptor-independent membrane depolarization. Although the amplitude of bladder detrusor muscle contractions in response to KCl was slightly diminished on day 3 in TNBS-treated rats compared with saline-treated rats, the difference did not reach...
statistical significance ($P > 0.05$) (0.22 ± 0.04 g in 6 TNBS-treated rats, 0.27 ± 0.03 g in 8 saline-treated rats). After recovery from colonic inflammation 15 and 30 days after TNBS enema, the response to high KCl obtained in the bladder muscle remained unchanged (data not shown).

**DISCUSSION**

Although previous studies have shown that colonic inflammation is associated with abnormalities in bladder function in vivo (12, 18, 21, 22), in the present study, we examined in a rodent model whether active colonic inflammation produced by TNBS induces abnormalities in bladder detrusor smooth muscle contractility measured in vitro. The advantage of this technique in understanding the potential mechanism(s) of visceral organ cross talk is that it allows for a detailed pharmacological characterization of the smooth muscle contractility in the absence of extrinsic neural, hormonal, and immunologic factors. Furthermore, TNBS-induced colitis causes long-term colonic hypersensitivity, despite recovery from the active inflammatory response in the colon (7).

During active TNBS-induced inflammation, we observed increased ulceration, tissue injury, and elevated MPO activity in the colon, which is in agreement with previous findings (6, 16, 23). Despite the active inflammatory insult in the colon, no changes in bladder histology or MPO content were observed in tissue taken from TNBS-treated rats. These findings are also in agreement with previous studies that found minimal changes in the histological appearance of the bladder during active colonic inflammation (12, 22). Although no visible signs of inflammation were found in the bladder during active colonic inflammation, a recent study has noted a change in the population of mast cells, suggesting a possible role of mast cells in afferent nerve sensitization (22). In contrast, active colonic inflammation has been found to hinder the development of the endometrium and myometrium of the uterus, yet this was only observed during severe colitis (9). In addition, diminished MPO activity has been found within the uterus during active colonic inflammation, indicating that pathologies within the visceral organ cross talk is that it allows for a detailed pharmacological characterization of the smooth muscle contractility in the absence of extrinsic neural, hormonal, and immunologic factors. Furthermore, TNBS-induced colitis causes long-term colonic hypersensitivity, despite recovery from the active inflammatory response in the colon (7).

**Table 1. Histological lesion scores in the colon and bladder of rats 3 days post-TNBS enema treatment**

<table>
<thead>
<tr>
<th>Region</th>
<th>Ulceration</th>
<th>Inflammation</th>
<th>Depth of Lesion</th>
<th>Fibrosis</th>
<th>Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>1.7±1.2</td>
<td>2.7±0.9</td>
<td>1.7±1.2</td>
<td>2.3±0.7</td>
<td>8.3±2.3</td>
</tr>
<tr>
<td>Bladder</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are mean ± SE. See text for description of scoring system. TNBS, trinitrobenzensulfonic acid.
colon are capable of affecting estrous cycle-dependent changes in uterine neutrophil levels (9). This is compelling because chronic pelvic pain conditions are commonly reported in women, suggesting that ovarian hormonal changes may be a key component influencing pelvic organ cross talk within these conditions.

Our findings are consistent with other studies demonstrating that active colonic inflammation results in abnormalities in bladder motility (12, 18, 21). The reduction in bladder detrusor muscle contractility in response to EFS and CCh during TNBS-induced active colonic inflammation suggests that alterations in the bladder detrusor muscle contractile response to EFS returned to levels not significantly different from controls at 15 and 30 days postcolonic inflammation.

Fig. 3. Changes in the amplitude of the bladder detrusor muscle contractility to electrical field stimulation (EFS) 3 days after saline (n = 12) or TNBS (n = 10) enema treatment (A), 15 days after saline (n = 6) or TNBS (n = 6) enema treatment (B), and 30 days after saline (n = 6) or TNBS (n = 9) enema treatment (C); n = no. of rats. A significant diminution in contractility was found at higher frequencies (16 and 32 Hz) during active (3 days) colonic inflammation (*P < 0.05; **P < 0.01). Bladder detrusor muscle contractile response to EFS returned to levels not significantly different from controls at 15 and 30 days postcolonic inflammation.

Fig. 4. Effect of active colonic inflammation on the proportion between cholinergically (atropine-sensitive) and noncholinergically (atropine-resistant) mediated components of contractions induced by EFS. Responses to EFS (0.5–32 Hz) in detrusor muscle strips isolated from rats with active colonic inflammation (day 3 post-TNBS enema) (A) or control animals (day 3 post-saline enema) (B) were studied in the absence and presence of atropine (1 μM) in the bathing solution. Note the decrease in the cholinergic component of the responses in detrusor muscles isolated from TNBS-treated rats compared with saline-treated animals.

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Our findings are consistent with other studies demonstrating that active colonic inflammation results in abnormalities in bladder motility (12, 18, 21). The reduction in bladder detrusor muscle contractility in response to EFS and CCh during TNBS-induced active colonic inflammation suggests that alterations in
cholinergic neurotransmission occur within the bladder detrusor muscle and also confirms our hypothesis that pathologies within one visceral organ are capable of causing abnormal changes in another visceral organ. Previous studies performed in an in vivo rodent model have shown that colonic inflammation is associated with increased frequency of micturition, a characteristic also found in patients with bladder detrusor muscle instability and IC. However, in vitro, we observed an attenuation of the muscle responses to EFS and CCh. Interestingly, the same diminution in detrusor muscle contractility has been observed in patients with either detrusor instability or IC (15, 17) and in an animal model of IC (4). The observation that there was no significant changes within the EC50 value of the CCh concentration-response curve in response to active colonic inflammation suggests that the cross-talk between visceral organs in pathological states is not capable of altering the properties of the muscarinic receptor within the detrusor muscle. Similar observations were also observed within circular and longitudinal muscle of the uterus during active colonic inflammation (9). Uterine muscle contractile responses to both CCh and oxytocin, although significantly depressed during active colonic inflammation, have no effect on the underlying sensitivities of both receptors. Furthermore, the finding that active colonic inflammation had no significant effect on the response of detrusor muscle to KCl suggests that the decreased contractility to EFS and CCh is not directly through functional changes of the bladder smooth muscle contractile apparatus. However, some changes resulting in a decreased response to CCh may be due to alteration of the intracellular smooth muscle mechanisms coupling the activation of muscarinic receptors to the contractile response. Together, because no distinguishing signs of inflammation and no inflammatory infiltrate were found within the bladder detrusor muscle during the active colonic inflammatory insult, the results from our study suggest that altered neuronal signaling, likely resulting from afferent nerve sensitization, may be the underlying mechanism of visceral organ cross talk. Although not investigated directly in the present study, our findings support the notion that neurogenic inflammation is involved in the pathogenesis of various chronic pelvic pain syndromes, including IBS and IC (24). Our future investigations will test the hypothesis that abnormal afferent signaling associated with an active inflammation within the colon produces bladder detrusor dysmotility.

In the present study, we designed experiments to investigate whether the abnormalities in bladder detrusor muscle contractility persist after resolution of acute colonic inflammatory response. The rationale for these experiments is that an acute inflammation has been shown to induce colonic hypersensitivity to luminal distension, which persists despite recovery of the colonic mucosa (7, 11). On recovery from TNBS-induced colitis, as demonstrated morphologically, histologically, and biochemically, we observed that the diminished detrusor muscle contractility measured in response to EFS and CCh was no longer apparent and that the contractile responses of the bladder detrusor muscle returned to levels that resembled those measured in saline enema-treated controls.

In conclusion, we have shown in a rodent model that a transient colonic inflammatory insult significantly attenuates the amplitude of bladder detrusor muscle contractions in vitro, at least in part, through changes in the balance between cholinergic and noncholinergic regulation of bladder contrac-

Fig. 5. Changes in the amplitude of bladder detrusor muscle contractility to carbachol (CCh) 3 days after saline (n = 12) or TNBS (n = 10) enema treatment (A), 15 days after saline (n = 6) or TNBS (n = 6) enema treatment (B), and 30 days after saline (n = 6) or TNBS (n = 9) enema treatment (C); n = no. of rats. Bladder detrusor muscle contractility in response to CCh was significantly decreased during active colonic inflammation (*p < 0.05, **p < 0.01) and recovered to levels not significantly different from controls on day 15 and day 30 post-TNBS enema. No differences in the EC50 between treatment groups were observed.
tility. These changes however are not permanent, as they are reversed after recovery from the colitis.

GRANTS
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REFERENCES