Acute colonic inflammation triggers detrusor instability via activation of TRPV1 receptors in a rat model of pelvic organ cross-sensitization

Tirsit S. Asfaw,1 Joseph Hypolite,2 Gina M. Northington,1 Lily A. Arya,1 Alan J. Wein,2 and Anna P. Malykhina2

1Division of Urogynecology and Pelvic Reconstructive Surgery, Department of Obstetrics and Gynecology and 2Division of Urology, Department of Surgery, University of Pennsylvania, Glenolden, Pennsylvania

Submitted 7 December 2010; accepted in final form 3 April 2011

Asfaw TS, Hypolite J, Northington GM, Arya LA, Wein AJ, Malykhina AP. Acute colonic inflammation triggers detrusor instability via activation of TRPV1 receptors in a rat model of pelvic organ cross-sensitization. Am J Physiol Regul Integr Comp Physiol 300: R1392–R1400, 2011. First published April 6, 2011; doi:10.1152/ajpregu.00804.2010.—Chronic pelvic pain of unknown etiology is a common clinical condition and may develop as a result of cross-sensitization in the pelvis when pathological changes in one of the pelvic organs result in functional alterations in an adjacent structure. The aim of the current study was to compare transient receptor potential vanilloid 1 (TRPV1) activated pathways on detrusor contractility in vivo and in vitro using a rat model of pelvic organ cross-sensitization. Four groups of male Sprague-Dawley rats (N = 56) were included in the study. Animals received intracolonic saline (control), resiniferatoxin (RTX, TRPV1 agonist, 10−7 M), 2,4,6-trinitrobenzene sulfonic acid (TNBS, colonic irritant), or double treatment (RTX followed by TNBS). Detrusor muscle contractility was assessed under in vitro and in vivo conditions. Intracolonic RTX increased the contractility of the isolated detrusor in response to electric field stimulation (EFS) by twofold (P ≤ 0.001) and enhanced the contractile response of the bladder smooth muscle to carbachol (CCh). Acute colonic inflammation reduced detrusor contractility upon application of CCh in vitro, decreased bladder capacity by 28.1% (P ≤ 0.001), and reduced micturition volume by 60% (P ≤ 0.001). These changes were accompanied by an increased number of nonmicturition contractions from 3.7 ± 0.7 to 15 ± 2.7 (N = 6 in both groups, P ≤ 0.001 vs. control). Desensitization of intracolonic TRPV1 receptors before the induction of acute colitis restored the response of isolated detrusor strips to CCh but not to EFS stimulation. Cystometric parameters were significantly improved in animals with double treatment and approximated the control values. Our data suggest that acute colonic inflammation triggers the occurrence of detrusor instability via activation of TRPV1-related pathways. Comparison of the results obtained under in vitro vs. in vivo conditions provides evidence that intact neural pathways are critical for the development of an overactive bladder resulting from pelvic organ cross talk.

neurogenic inflammation; chronic pelvic pain; detrusor; contractility

Limited understanding of the causes, underlying mechanisms, and symptomatic complexity in patients with multidimensional pelvic pain make CPP disorders extremely difficult to cure. Clinical phenotyping of patients with CPP has been recently proposed in an attempt to clarify heterogeneity of these populations and to improve treatment by using individual targeted therapy for each patient (31).

One emerging concept for the coexistence of clinical disorders with functional CPP includes cross-sensitization within the pelvis due to shared neural pathways connecting different pelvic organs (5, 10, 26). Cross-sensitization develops as a result of noxious stimulus transmission from a diseased pelvic organ to an adjacent normal structure, resulting in neurogenic inflammation and the occurrence of functional changes in previously normal pelvic structures (26).

Several animal models have gone through the initial testing to mimic the comorbidity of human disorders and to investigate the mechanisms of multisystemic CPP (28, 36, 38). Previous animal studies have established a correlation between structural pathophysiological changes in the directly affected pelvic organs and secondary dysfunctions developed in adjacent pelvic structures (9, 27, 28, 36). It has been determined that colonic inflammation causes hyperactivity of bladder afferent fibers (44), hyperexcitability of bladder projecting sensory (27, 28) and spinal (38) neurons, increased release of proinflammatory neuropeptides in the urinary bladder (34, 45), and changes in the detrusor contractility (32). However, the exact pathways of transmission of nociceptive information from the inflamed colon to the urinary bladder as well as receptors and molecules activated during this transmission remain elusive.

Transient receptor potential vanilloid receptor 1 (TRPV1) has been of interest in studies of pain and noxious stimulation for many years (11, 12, 13). TRPV1 agonists are well known for causing prolonged desensitization of sensory neurons and fibers followed by an extended refractory period and reduced sensitivity to subsequent noxious stimuli (4). The ability of vanilloids to reduce pelvic pain was tested in patients with painful bladder syndrome/interstitial cystitis (15, 35) and in patients with urge incontinence associated with refractory detrusor overactivity (25, 41).

In this study, we investigated the role of TRPV1-activated pathways on the detrusor contractility in vivo and in vitro using a rat model of pelvic organ cross-sensitization induced by acute colonic inflammation. The goals of the present investigation included: 1) determination of the effects of intracolonic TRPV1 receptor desensitization before the induction of colonic inflammation on the contractility of isolated detrusor muscle; 2) in vivo assessment of bladder function using cystometry in conscious animals; and 3) comparison of the effects of TRPV1...
receptor activation and/or experimental colitis on the detrusor function under in vitro vs. in vivo conditions.

MATERIALS AND METHODS

Animals and experimental groups. Sprague-Dawley male rats (N = 56, 200–250 g; Charles River Laboratories, Malvern, PA) were used in this study. All protocols were approved by the University of Pennsylvania Institutional Animal Care and Use Committee and adhered to the guidelines for experimental pain in animals published by the International Association for the Study of Pain. For in vitro studies of detrusor contractility, rats (N = 32) were housed two per cage with free access to food and water and maintained on a 12:12-h light-dark cycle. Rats scheduled to undergo in vivo cystometry were precatheterized (urinary bladder) from the vendor (200–250 g; Charles River Laboratories) and delivered 3 days postsurgery. These animals were kept in individual cages to avoid possible damage to catheters by their cage mates. Baseline cystometric parameters were recorded 5–7 days after the arrival of rats to the animal facility. That period was given for acclimation and relief of stress due to surgery and transportation.

For both in vitro and in vivo studies, animals were divided into four experimental groups. The control group of animals received intracolonic enema with saline. The resiniferatoxin (RTX, TRPV1 agonist) group had intracolonic administration of RTX (10–7 M, 0.7–0.8 ml). RTX was dissolved in a mixture of 50% ethanol and 50% Tween 80 to make a 10–3 M stock solution. To achieve the final concentration of RTX (10–7 M) for intracolonic instillation, 1 μl of RTX stock solution was diluted in 10 ml of saline (1:10,000 times). The third experimental group received 2,4,6-trinitrobenzene sulfonic acid (TNBS, 0.7–0.8 ml, 12.5 mg in 50% ethanol/50% H2O), a chemical irritant that causes inflammation in the colon. Rats in the fourth experimental group were pretreated with RTX followed by TNBS instillation 2 days later. All animals were killed 3 days after the last treatment. Schematic presentation of the experimental design is shown in Fig. 1. Intracolonic administration of drugs was performed under light isoflurane (VEDCO, St. Joseph, MO) anesthesia via a flexible catheter made of polyethylene tubing (6 cm long, inside diameter 1.6 mm, outside diameter 2.1 mm) and attached to a 1-ml syringe. Rats were fasted for 24 h before the instillation procedure.

To assess the severity of induced inflammation, the daily Disease Activity Index (DAI) was calculated as previously described (28). Briefly, the DAI was determined by scoring changes in animal weight, occult blood positivity, gross bleeding, and stool consistency. We used three grades of weight loss (0 = no weight change; 1 = up to 10–12% of weight loss; 2 = >20% loss), three grades of stool consistency (0 = normal; 1 = loose; and 2 = diarrhea), and three grades of occult blood (0 = negative; 1 = occult blood-positive; and 2 = gross bleeding). DAI was measured only in the group with TNBS treatment because DAI is a scoring system validated to grade specifically the severity of colonic inflammation. Out of 10 animals in the TNBS group, 8 developed inflammation in the colon with cumulative DAI of 4 and were included in the study. Our previous studies showed that animals with the DAI score of four and above developed distinct histological changes in the colon (thickening of the colonic wall, signs of hemorrhage, etc.) without noticeable histological or biochemical changes in the urinary bladder in this model of colon-bladder cross-sensitization (28, 34). Because RTX treatment does not lead to the development of hemorrhage, wall thickening, and/or gastrointestinal bleeding, two groups treated either with RTX alone or with RTX-TNBS were not assessed using DAI. No data have been published so far to show whether double RTX-TNBS treatment causes the same severity of colonic inflammation compared with TNBS alone; therefore, we could not select only animals that had some signs of colonic inflammation over those with no obvious signs of inflammation. All animals in both RTX and RTX-TNBS groups were included in the data analysis.

Fig. 1. Schematic presentation of the experimental design. A: time scale of the treatments and experimental end points during in vitro contractility studies. B: sequence of recordings of urodynamic parameters during cystometry in awake and freely moving rats. RTX, resiniferatoxin; TNBS, 2,4,6-trinitrobenzene sulfonic acid.
In vitro measurements of detrusor contractility. For in vitro recordings of detrusor contractility, animals were killed 3 days after the last treatment (Fig. 1). Midline laparotomy was performed to remove the bladders and distal colon. The colon was evaluated for the signs of hemorrhage and wall thickening in all groups as described in our previous studies (28, 34). The results of histological evaluation of the bladder and colon cross sections are presented in Fig. 2. Each bladder was divided in two halves longitudinally and weighed. Full-thickness strips of the bladder wall were tied to silk threads and suspended to L-shaped hooks in 15-mL organ bath chambers. The chambers were filled with the Tyrode buffer (in mM: 125 NaCl, 2.7 KCl, 23.8 NaHCO3, 0.5 MgCl2·6H2O, 0.4 NaH2PO4·H2O, 1.8 CaCl2, and 5.5 dextrose), maintained at 37°C, and perfused continuously with a mixture of 95% O2 and 5% CO2. After a 30-min equilibration period, the length of optimal force development (L0) was determined by increasing the length of each strip in 1.5-mm increments until maximal contractile response to the electrical field stimulation (EFS; 70 V, 32 Hz, train duration of 1 ms) was achieved. The tissues were washed three times with Tyrode buffer (10 min each) to reequilibrate the muscle. After determination of L0, depolarization with high-potassium chloride solution (KCl, 125 mM) was carried out to evaluate the tonic and phasic properties of the detrusor muscle. The tissues were washed three times (10 min each) with Tyrode buffer before the application of the muscarinic receptor agonist carbachol (CCh). Cumulative doses of CCh (10–7 to 10–4 M) were added to each organ bath to trigger the muscle contractions and assess the contractile response to muscarinic receptor activation. Contraction parameters were measured using PowerLab Lab-Chart version 7.1.2 software (ADInstruments, Colorado Springs, CO).

Cystometry studies. Conscious rats were placed in cystometry cages (16 cm width, 12 cm height, and 24 cm length) without any restraint and allowed to acclimate for 30 min. The tip of the exteriorized bladder catheter located at the base of the rat neck was connected to a pressure transducer and an infusion pump of the cystometry station (Small Animal Laboratory Cystometry, Catamount Research and Development, St. Albans, VT) using a T-shaped valve. Room temperature saline solution (0.9% NaCl) was infused in the bladder at a rate of 120 μL/min. Cystometric parameters were recorded synchronously and continuously. Urine volumes were measured by means of a fluid collector connected to a force displacement transducer integrated into the data acquisition system. Micturition events were recorded after each voiding episode, which allowed the volume peak to return to baseline. Each animal was observed for up to four to six voiding cycles. Cystometry parameters [bladder capacity (BC), maximum micturition pressure, micturition rate, intravesicular pressure, intermicturition interval, and number of micturition and nonmicturition contractions (NMC)] were recorded continuously using data acquisition software (Small Animal Laboratory Cystometry, Catamount Research and Development). NMC were defined as increased values in detrusor pressure from baseline at filling (nonvoiding) phases. Each animal underwent baseline cystometric evaluation before any treatment was administered. Second cystometric assessment was done at day 3 after the last assigned treatment for each animal in the manner described above (Fig. 1). After the final evaluation, the rats were killed by the overdose of isoflurane.

Statistical analysis. All data are expressed as means ± SE. The raw traces of detrusor contractility from in vitro experiments were analyzed manually and then exported into SigmaPlot 11 Software (Systat Software, San Jose, CA). The peak amplitude of each contraction curve upon applied stimulation was normalized to the wet tissue weight for each bladder strip. Concentration-response curves after application of CCh and EC50 values were obtained using the fitting analysis function in the pharmacology section of the SigmaPlot software. Cystometric parameters were uploaded from the acquisition software into analysis software (SOF-552 Cystometry Data Analysis, Version 1.4; Catamount Research and Development). Maximum pressure at micturition, BC, micturition volume (MV), number of NMC, intermicturition interval, and micturition rate indexes were calculated. Data were statistically analyzed using one-way repeated-measures ANOVA between control and experimental groups fol-

Fig. 2. Hematoxylin and eosin (H&E) staining of the distal colon and urinary bladder from control and experimental animals. A: cross sections (10 μm) of the distal colon from control, RTX-treated, TNBS-treated, and RTX + TNBS-treated groups. In the group with TNBS treatment, please note the signs of colonic inflammation and tissue damage, including sites of hemorrhage and infiltration (arrow a), thickening of the muscle layer (arrow b), and disruption of the colonic crypts (arrow c). In the group with combined treatment (last panel), no significant tissue damage was observed except for mild thickening of the muscle layer (arrow d). B: cross sections of the urinary bladder from control, RTX-treated, TNBS-treated, and RTX + TNBS-treated groups. No visible alterations in the cytoarchitecture of the urinary bladder were observed after intracolonic treatments. All images were taken at X10 magnification. C: concentration of myeloperoxidase (MPO) enzyme in the distal colon from control and experimental groups. *P ≤ 0.05 vs. control.
allowed by Bonferroni’s posttest, as appropriate (Systat Software). Differences between the groups were considered statistically significant at $P$ values $\leq 0.05$.

Drugs. TNBS, RTX, and CCh were purchased from Sigma Aldrich (St. Louis, MO). All chemicals used for Tyrode buffer were purchased from Thermo Fisher Scientific (Waltham, MA). Isoflurane was received from VEDCO.

RESULTS

Effects of EFS on the contractility of the detrusor muscle after desensitization of colonic TRPV1 receptors. To assess the role of TRPV1 receptors in the development of cross-sensitization between the inflamed colon and urinary bladder, we performed a series of in vitro experiments using isolated urinary bladder strips. Isometric contractions were studied in response to EFS at 32 Hz. This frequency was chosen based on our previous studies that showed the alterations in detrusor contractility at higher frequencies (32). Acute colonic inflammation (TNBS group) did not lead to significant changes in detrusor contractility after EFS. However, intracolonic application of RTX caused a twofold increase of maximal amplitude ($n = 16$, $P \leq 0.001$ vs. control; Fig. 3A) and of contractile force slope (velocity of the contraction, $n = 16$, $P \leq 0.01$; Fig. 3B). Desensitization of intracolonic TRPV1 receptors with RTX before the induction of experimental colitis had the same effect on the contractile response of the detrusor muscle as the one observed in the RTX group (Fig. 3). We previously tested if TNBS could also trigger minor bladder inflammation by using MPO assay and H&E staining (34). Analysis of the H&E-stained cross sections of the urinary bladder performed in this study showed that neither RTX nor TNBS (alone or in combination) caused detectable structural changes in the urinary bladder, confirming that the urinary bladder did not develop any signs of acute inflammatory reaction (Fig. 2, B and C).

Response of the detrusor muscle to stimulation by KCl. Stimulation of the detrusor muscle strips in vitro with KCl during active colonic inflammation (3 days post-TNBS enema) did not reveal any changes compared with the control group (Fig. 4). There was no significant difference in the amplitude or velocity of the detrusor contractile response to KCl after intracolonic RTX either. However, the amplitude of urinary
bladder contractions was increased significantly by 121% in the group with double treatment (RTX followed by TBNS, n = 16, P ≤ 0.001; Fig. 4A). Contractile force slope was not affected by any treatment upon KCl stimulation (Fig. 4B).

Cholinergic regulation of detrusor contractility in vitro. To further investigate whether TRPV1- and/or colitis-activated pathways involve changes in cholinergic regulation, we studied the concentration-response relationship of detrusor muscle strips upon application of the cholinergic agonist CCh. Cumulative addition of 10⁻⁷ to 10⁻⁴ M of CCh to the bath solution resulted in concentration-dependent contractions of isolated detrusor strips in saline-treated animals (n = 16; Fig. 5A). Acute colonic inflammation significantly diminished the response of the detrusor muscle to stimulation with CCh, whereas intracolonic RTX had the opposite effect (Fig. 5B). Incubation of the muscle strips with CCh in the RTX group led to an increased amplitude of contractile response; however, the results from the double treatment (RTX followed by TNBS) were not different from the control values. CCh concentration-response curves for all experimental groups are presented in Fig. 5C and reflect a rightward shift of EC₅₀ in the TBNS group (EC₅₀ = 10.4 × 10⁻⁶ M, n = 16) and a leftward shift in the RTX group (EC₅₀ = 0.96 × 10⁻⁶ M, n = 16) compared with the saline-treated animals (EC₅₀ = 4.8 × 10⁻⁶ M, n = 16). Differences in concentration-contraction curves in RTX and TBNS groups in response to CCh may suggest that these treatments not only affect the contractility of the detrusor muscle but also the sensitivity or binding affinity of the muscarinic receptors in the bladder wall. Additional studies are warranted to clarify the underlying mechanisms of muscarinic receptor activation in this model of colon-bladder cross talk.

Cystometric analysis of bladder function in awake unrestrained rats. To evaluate the effects of intact neural connections existing between the colon and urinary bladder on the detrusor contractility in vivo and compare them with results in vitro, we studied the bladder activity using cystometric measurements in conscious rats. Each experimental group consisted of six animals. Cystometric evaluation was performed in all rats before any treatment and served as a baseline. Differences in parameters were first compared with the baseline for each rat followed by further comparisons among the groups. Intracolonic saline did not change the cystometric profile in the control group of animals (Fig. 6A shows raw recordings at baseline and Fig. 6B 3 days postsaline). However, substantial changes were observed at day 3 post-TNBS treatment (Fig. 6, C and D). Acute colonic inflammation reduced BC from 1.96 ± 0.15 to 1.41 ± 0.14 ml (N = 6, P ≤ 0.001; Fig. 7A) and MV by 60% (N = 6, P ≤ 0.001 vs. control; Fig. 7A) and increased the number of NMC from 3.7 ± 0.7 to 15 ± 2.7 during a 2-h cystometric evaluation (N = 6, P ≤ 0.001; Fig. 7B). Neither intracolonic RTX alone nor in combination with subsequent TNBS treatment caused substantial alterations of cystometric parameters. The only exception was observed in the RTX/TNBS group and included a decreased value of BC (1.56 ± 0.18 ml, N = 6, P ≤ 0.001; Fig. 7A) compared with the control group. Other cystometric parameters such as maximal pressure at micturition, intermicturition pressure interval, threshold pressure, and basal pressure were unaltered in all experimental groups.

**DISCUSSION**

The results of our study revealed that desensitization of colonic TRPV1 receptors with and without subsequent colonic inflammation leads to changes in the contractility of detrusor muscle studied under in vitro and in vivo conditions. Previous results from our laboratory have established a correlation between activation of bladder projecting afferent pathways by colonic inflammation at the level of primary sensory ganglia and lumbosacral spinal cord (28, 38). However, the role of intact nerve connections between the colon and urinary bladder and molecular mechanisms in the peripheral and central nervous systems were not specified. In this study, we compared detrusor contractility in vitro in the absence of extrinsic neural, hormonal, or immunological factors with in vivo bladder function using dynamic cystometric evaluation.
TRPV1 receptors are broadly expressed in the gastrointestinal tract and genitourinary system (3, 4, 17, 18, 29). In humans, TRPV1 mRNA was detected in the prostate, testis, penis, bladder, and extrinsic sensory neurons innervating these organs (42). In the urinary bladder, TRPV1 is expressed mostly in the detrusor smooth muscle, urothelium, interstitial cells within lamina propria (8, 33, 48), and on the blood vessels in the bladder wall (19). TRPV1 is also abundantly expressed in primary sensory neurons projecting to the pelvic viscera. In a rat, 69% of dorsal root ganglion (DRG) neurons innervating the urinary bladder express TRPV1 (24). Pathological conditions in the pelvis (trauma, inflammation, nerve injury, etc.) in most cases lead to upregulation of TRPV1 signaling pathways (17, 43). Thus TRPV1 was shown to be upregulated in colonic afferent fibers of IBS patients and associated with symptom severity (1). Intraluminal application of capsaicin evoked painful sensations in the human gut because of activation of intestinal chemoreceptors (40). Studies using animal models of acute and chronic inflammation in different organs of gastrointestinal (6, 7, 20, 30, 39) and genitourinary (14, 16, 47) systems showed an increased excitability of DRG neurons receiving direct afferent input from the inflamed organs.

Intracolonic application of the TRPV1 agonist RTX increased the contractility of the isolated detrusor in response to EFS and CCh without changes in response to KCl and cystometric parameters. It is known that EFS causes contractions of the smooth muscle via stimulation of the release of neurotransmitters and neuropeptides from the nerve terminals coursing in the muscle layer while contractions in response to KCl occur due to direct effects on the muscle contractile apparatus. An increased response to EFS after RTX application suggests that RTX stimulated an extra synthesis/release of neuropeptides from bladder nerve supply and also affected the cholinergic regulation of bladder smooth muscle. Our previous findings showed that intracolonic application of RTX can cause massive release of substance P and calcitonin gene-related peptide from the bladder afferent terminals leading to the development of a neurogenic bladder over a period of time (34). Ustinova and colleagues (45) determined that chronic colonic irritation sensitized urinary bladder afferents to noxious stimuli and caused mast cell infiltration in the bladder. The latter effects were reversed by systemic administration of the TRPV1 agonist capsaicin, suggesting the involvement of TRPV1 receptors in colon-bladder cross talk. Systemic RTX treatment was also
effects of TRPV1 activation on the expression of muscarinic receptors were shown in experiments with subcutaneous capsaicin injections in neonatal rats, which observed an enhanced expression of muscarinic receptors in various regions of the adult brain (49). Several lines of evidence also suggest that TRPV1 channel is involved in the CCh-induced influx of extracellular Ca\(^{2+}\) (46). These studies determined that CCh-induced extracellular Ca\(^{2+}\) influx was significantly more pronounced in HEK 293 cells transfected with human TRPV1, and desensitization of TRPV1 before stimulation with CCh reduced extracellular Ca\(^{2+}\) influx to the level observed in nontransfected cells.

Acute colonic inflammation had no substantial effect on the detrusor contractility in vitro in our experiments (except a reduced contractile response to CCh), whereas cystometric studies showed a decrease in BC and MV accompanied by an increased number of NMC. These data are consistent with previously published results that showed a reduced response of the detrusor muscle to CCh after acute experimental colitis without changes upon stimulation by KCl (32). The reduction in bladder detrusor muscle contractility in response to CCh during TNBS-induced active colonic inflammation suggests that alterations in cholinergic neurotransmission occur within the bladder detrusor muscle. The same study (32) observed a reduction in the detrusor response to high-frequency EFS (16 and 32 Hz), whereas we did not reach statistical significance in our experiments. We believe this discrepancy in findings could be because of some differences in methodological approaches, including a lower concentration of TNBS used for the induction of colonic inflammation in our experiments and application of only a single frequency of 32 Hz, which stimulated the maximal release of neuropeptides from nerve terminals. Additionally, our animal model is a model of mild rather than severe colonic inflammation, which makes it more relevant to the human conditions observed in the clinical setting. Patients with functional pelvic pain and diagnosis of irritable bowel syndrome or interstitial cystitis/painful bladder syndrome do not develop severe inflammation in the pelvis. However, they complain of increased visceral sensitivity, abdominal discomfort, and pelvic pain (1). Our results provide evidence that smooth muscle itself is not severely affected in this model because cross-sensitization develops predominantly via neural pathways, and effects on smooth muscle contractility are secondary.

Despite modest effects of TNBS-induced colitis on detrusor contractility in vitro, cystometric parameters measured in freely moving rats were significantly changed in this group of animals. Specifically, acute colonic inflammation substantially reduced BC and urine volume at micturition and increased the number of NMC by almost fivefold. Previous studies have identified an increased number of electromyographic contractions in rats with acute colonic inflammation caused by chemical irritants (36); however, this is the first report of the direct cystometric evaluation of the detrusor function under in vivo conditions using a rat model of colon-bladder cross-sensitization. Our results also suggest that intact nerve connections are important for detrusor instability to develop, and these changes can be recorded by cystometry in conscious animals.

Desensitization of intracolonic TRPV1 receptors before the induction of acute colonic inflammation had limited protective effect on the contractility of the isolated detrusor. It restored
the response of bladder smooth muscle to CCh but not to EFS stimulation. Additionally, cystometric parameters were improved significantly in the group with double treatment and became indistinguishable from the control values during the cystometric evaluation. Histological assessment and the results of MPO assay showed that animals in the group with double treatment developed mild inflammation compared with the control group. In the group with double treatment, the contractile response of the detrusor strips to KCl was increased compared with single treatments. The latter finding is difficult to interpret, but there is a possibility that some of the currently undefined mechanisms such as up/downregulation of certain ion channels on bladder sensory neurons could underline synergistic effects of dual stimulation (unpublished observations). The observation that there were significant changes in the EC50 value of the CCh concentration-response curve during active colonic inflammation suggests that development of cross talk between visceral organs in pathological states may alter the properties of the muscarinic receptors within the detrusor muscle. Decreased maximal responses to CCh could be due to several reasons, including changes in the expression level or ratio between different types of muscarinic receptors, modified receptor sensitivity to agonists, and alterations of signaling pathways coupling the activation of muscarinic receptors to the contractile apparatus. Because no detectable signs of inflammation or inflammatory infiltrate were found within the bladder detrusor upon active colonic inflammation, our findings suggest that altered neuronal signaling, likely resulting fromafferent nerve sensitization and involving TRPV1 receptors, is one of the major mechanisms underlying colon-bladder cross-sensitization.

Perspectives and Significance

The results of our study provide evidence that colonic inflammation may trigger detrusor instability via activation of TRPV1-related sensory pathways as assessed by our in vitro and in vivo studies in a rat model of pelvic organ cross-sensitization. Development of cross sensitization in the pelvis is one of the suggested mechanisms underlying comorbidity of pelvic disorders that is frequently observed in the clinical setting (2, 37). Comorbid disorders are characterized by complex symptomatology and unclear underlying mechanisms, making them extremely difficult to cure. Additional animal and human studies are warranted to identify the main triggers and supporting mediators of pelvic organ cross-sensitization, as well as the time points and factors that underlie the transition of episodic painful events to persistent visceralvisceral hyper-sensitivity and CPP.

ACKNOWLEDGMENTS

We thank Dr. Shaohua Chang, Thomas Mathai, and Jocelyn McCabe for technical assistance.

Preliminary results of this work were presented in abstract form at the American Urological Association Meeting on May 28–June 3, 2010, in San Francisco, CA, and at the American Urogynecologic Society Meeting on September 30–October 2 in Long Beach, CA.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-077699 (A. P. Malykhina), DK-077699-S1 Fellowship (T. S. Asfaw and A. P. Malykhina), and DK-077699-S2 (A. P. Malykhina).

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