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PELVIC ORGAN CROSS-SENSITIZATION TO ENHANCE BLADDER AND URETHRAL PAIN BEHAVIORS IN RATS WITH EXPERIMENTAL COLITIS

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Abstract—Neural cross-sensitization has been postulated as a mechanism underlying overlaps of chronic pelvic pain disorders such as bladder pain syndrome/interstitial cystitis (BPS/IC) and irritable bowel syndrome (IBS). Animals with experimental colitis have been used to study the underlying mechanisms for overlapped pelvic pain symptoms, and shown to exhibit bladder overactivity evidenced by frequent voiding; however, it has not directly been evaluated whether pain sensation derived from the lower urinary tract is enhanced in colitis models. Also, the cross-sensitization between the colon and urethra has not been studied previously. In the present study, we therefore investigated pain behaviors induced by nociceptive stimuli in the lower urinary tract and the involvement of C-fiber afferent pathways using rats with colitis induced by intracolonic application of 2,4,6-trinitrobenzenesulfonic acid (TNBS). In TNBS-induced colitis rats at 10 days, intravesical application of resiniferatoxin (RTx) induced a significantly greater number of episodes of both licking and freezing behaviors, which were reduced by capsaicin-sensitive C-fiber afferent desensitization. Histochemical studies using fluorescent dye tracers injected into the colon, bladder or urethra showed that dichotomized afferent neurons comprised 6.9–14.5% of L1, L6 and S1 dorsal root ganglion (DRG) neurons innervating the colon or the lower urinary tract. Transient receptor potential vanilloid 1 (TRPV1) mRNA expression was significantly increased in, the bladder, urethra and S1 DRG in colitis rats. An increase in myeloperoxidase (MPO) activity was found in the colon, but not in the bladder or urethra after intracolonic TNBS treatment. These results indicate that TNBS-induced colitis increased pain sensitivity in the bladder and urethra via activation of C-fiber afferent pathways due to colon-to-bladder and colon-to-urethral cross-sensitization, suggesting the contribution of pelvic organ cross-sensitization mechanisms to overlapped pain symptoms in BPS/IC and IBS.

Key words: pain behavior, cross-sensitization, lower urinary tract, bladder, urethra, dorsal root ganglion (DRG).

INTRODUCTION

Bladder pain syndrome/interstitial cystitis (BPS/IC) is a chronic urological disorder characterized as pelvic pain related to bladder filling, coupled with additional symptoms, such as increased urinary frequency and urgency, without proven urinary infection or other obvious pathology (Hanno et al., 2011). It is currently estimated that 3.3–7.9 million United States women 18 years old or older are suffering from BPS/IC (Berry et al., 2011). It has also been reported that over one-third of patients diagnosed with BPS/IC exhibit symptoms consistent with irritable bowel syndrome (IBS) (Alagiri et al., 1997; Novi et al., 2005), while 26–56% of patients diagnosed with IBS also have symptoms of BPS/IC (Maxton et al., 1989; Blanchard et al., 2004). In addition, BPS/IC patients often report pain in different and/or additional sites such as the urethra (Warren et al., 2008). However, the mechanisms underlying the pelvic organ cross-talk that contributes to overlapped symptoms in chronic pelvic pain syndromes such as BPS/IC, urethral pain and IBS have not been well clarified.

Previous animal studies demonstrated that 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced experimental colitis produced lower urinary tract dysfunction such as increased voiding frequency (Liang et al., 2007; Ustinova et al., 2007) and an increase in the firing rate of bladder afferent nerves in response to urinary bladder distension in rats (Ustinova et al., 2007). These animals showed increased expression of neuropeptides such as substance P and calcitonin gene-related peptide (Pan et al., 2010), growth factors and mast cells (Liang et al., 2007) in the bladder. Moreover, a recent report demonstrated that desensitization of the transient receptor potential vanilloid 1 (TRPV1) by intravesical application of resiniferatoxin (RTx) suppressed the

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Abbreviations: BPS/IC, bladder pain syndrome/interstitial cystitis; DRG, dorsal root ganglion; IBS, irritable bowel syndrome; MPO, myeloperoxidase; RTx, resiniferatoxin; RT-PCR, real-time polymerase chain reaction; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TRPV1, transient receptor potential vanilloid 1.

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increased excitability of bladder spinal neurons in rats with acute colitis induced by intracolonic instillation with TNBS (Malykhina et al., 2013). These results suggest that intracolonic irritation can sensitize bladder afferent pathways, resulting in bladder overactivity. Also, in previous studies, pelvic pain conditions in the colitis model were assessed by the visceromotor response elicited by colorectal distension (Greenwood-Van Meerveld et al., 2005) and referred somatic hyperalgesia in the paw and/or abdominal skin regions (Cameron et al., 2008; Claudino et al., 2010). Thus, although the rat with experimental colitis has been used as an animal model of chronic pelvic pain, pain sensation derived from the bladder has not directly been evaluated in experimental colitis. In addition, the cross-talk between the colon and urethra to induce urethral pain has not been studied previously in the colitis model. Since our laboratory developed a rat model that can be used to investigate pain sensation from the bladder and urethra under the freely moving condition by monitoring pain behaviors such as freezing and licking (Saitoh et al., 2008), we investigated whether nociceptive behaviors induced by chemical stimuli in the lower urinary tract are enhanced in colitis rats. We also investigated the number of dichotomized afferent neurons that innervate both colon and bladder or both colon and urethra; as well as changes in gene expression of TRPV1 channels in dorsal root ganglia (DRG), bladder and urethral tissues.

EXPERIMENTAL PROCEDURES

Animals

Sixty-four female Sprague-Dawley rats (206–268 g) were used in this study. Rats were divided into the following groups: (1) 20 rats for behavioral testing, (2) 24 rats for mRNA measurement with real-time polymerase chain reaction (RT-PCR), (3) eight rats for a histochemical study with fluorescent dye tracers, and (4) 12 rats for MPO activity assay. We used female rats because of the higher prevalence of BPS/IC and IBS in women than in men (Clemens et al., 2007; Ito et al., 2007; Hall et al., 2008; Lovell and Ford, 2012) and the technical easiness in urethral catheterization during behavioral studies in female rats compared to male rats. All experiments were conducted in accordance with institutional guidelines and approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Induction of experimental colitis

Experimental colitis was induced as described in a previous report (Liang et al., 2007) with slight modification in the injection volume. Briefly, rats fasted for 24 h were anesthetized with isoflurane. A polyethylene catheter (PE90) was inserted from the anus and placed approximately 6 cm proximal to the anal verge, and then TNBS (50 mg/mL in 50% ethanol, 0.4 mL) was administered through the catheter and retained in the distal colon with the rats in a vertical position for several minutes. Thereafter, Surgilube® (E. Fougera & Co., Melville, NY, USA) was inserted into the anal canal to prevent the leakage of TNBS and then rats were returned to the housing facility after the recovery from anesthesia until each assay. Control animals received the vehicle treatment with 0.4 mL of 50% ethanol. In some rats used for behavioral testing, capsaicin (total 125 mg/kg) was given subcutaneously in divided doses on two consecutive days: 25 and 50 mg/kg at a 12-h interval on the first day and 50 mg/kg on the second day, to induce desensitization of capsaicin-sensitive C-fiber afferent pathways as described in previous reports (Cheng and de Groat, 2004; Kullmann et al., 2008).

Nociceptive behavior study

The measurement of nociceptive behaviors was conducted according to the previously reported method (Saitoh et al., 2008). In brief, rats were acclimated in metabolic cages (Nalgene Co., Rochester, New York) for 3 h, and then placed in a Bollman-type restraining device (KN-326; Natsume Seisakusho, Tokyo, Japan). A polyethylene tube (PE-50; Clay Adams Division of Becton Dickinson, Parsippany, NJ, USA) was inserted into the bladder through the urethra, and residual urine was withdrawn. Thereafter, RTx (0.3 μM), or the corresponding vehicle alone (10% ethanol, 10% Tween 80, and 80% physiological saline), was instilled into the bladder via the catheter at a volume of 0.3 mL and kept for 1 min. The transurethral catheter was then removed and rats were placed back into metabolic cages. Two types of behaviors, licking (lower abdominal licking) and freezing (motionless head-turning toward the lower abdomen), were scored for a 15-min interval that was divided into 5-s intervals. When licking or freezing occurred during each 5-s interval, it was scored as one positive event. The number of licking or freezing behavior events was summed for each of 5-min periods (0–5, 5–10 and 10–15 min) following the RTx treatment. The intravesical application of the solution (RTx or vehicle) was conducted in a blinded manner for assessors of animal behaviors.

Retrograde labeling of colon, bladder and urethral afferent neurons

Under isoflurane anesthesia, rats underwent a midline laparotomy to gain access to the pelvic organs. The distal colon (2.5–3.5 cm from the rectum) and the bladder or urethra were exposed, and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI, Invitrogen Inc., Paisley, UK; 1% w/v in methanol) was injected to 6–8 different sites of the colonic wall using a 30-G needle syringe. Additionally, Fast Blue (Polysciences Inc., Warrington, PA, USA; 1% w/v in water) was injected into 4–5 different sites of the bladder or urethral wall in order to examine the presence of DRG neurons innervating the colon and bladder or the colon and urethra, respectively. The total volume of dye injected into each organ was 25 μL. To prevent leakage and labeling of adjacent tissues, the needle was left in place for 30 s after injection, and then a cotton swab was applied to prevent leaking. Abdominal incisions were closed with sutures and rats were returned to housing facility until each assay. The rats were post-operatively treated with ampicillin