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Evidence for the role of mast cells in colon–bladder cross organ sensitization

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ABSTRACT

This study examined the contribution of mast cells to colon–bladder cross organ sensitization induced by colon irritation with trinitrobenzene sulfonic acid (TNBS-CI). In urethane anesthetized rats 12 days after TNBS-CI, the voiding interval was reduced from 357 s to 201 s and urothelial permeability, measured indirectly by absorption of sodium fluorescein from the bladder lumen, increased six-fold. These effects were blocked by oral administration of ketotifen (10 mg/kg, for 5 days), a mast cell stabilizing agent. TNBS-CI in wild type mice produced a similar decrease in voiding interval (from 319 s to 209 s) and a 10-fold increase in urothelial permeability; however this did not occur in $Kit^{a}W^{a}/Kit^{a}W\text{-}v^{a}$ mast cell deficient mice. Contractile responses of bladder strips elicited by Compound 48/80 (50 μg/ml), a mast cell activating agent, were significantly larger in strips from rats with TNBS-CI (145% increase in baseline tension) than in control rats (55% increase). The contractions of strips from rats with TNBS-CI were reduced 80–90% by pretreatment of strips with ketotifen (20 μM), whereas contractions of strips from control animals were not significantly changed. Bladder strips were pretreated with SLIGRL-NH2 (100 μM) to desensitize PAR-2, the receptor for mast cell tryptase. SLIGRL-NH2 pretreatment reduced by 60–80% the 48/80 induced contractions in strips from rats with TNBS-CI but did not alter the contractions in strips from control rats. These data indicate that bladder mast cells contribute to the bladder dysfunction following colon–bladder cross-sensitization.

1. Introduction

Women experiencing chronic pelvic pain often exhibit overlapping symptoms of interstitial cystitis (IC) and irritable bowel syndrome (IBS) (Mathias et al., 1996). Much of this overlap is attributable to central and peripheral neural mechanisms of pelvic organ cross-sensitization (Malykhina, 2007). Specifically, the bladder and colon receive part of their afferent innervations from the same subpopulation of lumbosacral dorsal root ganglion (DRG) cells (Christianson et al., 2007), an anatomical organization that could allow afferent activity arising in one organ to be transmitted by antidromic action potentials to the other co-innervated organ. In addition, afferent activity from both organs carried by the shared DRG neurons should be received by a common population of second order neurons in the spinal cord. Thus, activation of nociceptive afferents in one organ could sensitize central sensory pathways or induce firing in peripheral afferent pathways in another organ to release afferent neurotransmitters such as substance P that trigger neurogenic inflammation (Pan et al., 2010). These mechanisms could lead to recruitment and activation of mast cells, release of inflammatory mediators, plasma extravasation and smooth muscle contraction.

The juxtaposition of substance P positive nerve fibers and mast cells (Bauer and Razin, 2000) may play an important role in pelvic organ cross-sensitization by amplifying afferent signaling. Intravenous administration of Compound 48/80, a mast cell activating and degranulating agent, which depletes mast cells of their inflammatory mediators, significantly attenuated and/or abolished ovalbumin-induced maximal bladder contractile response and bladder plasma protein extravasation in a rat model of bladder overactivity induced by sensitization with ovalbumin (Ahluwalia et al., 1998). Furthermore, mast cell deficient mice exhibit reduced pelvic pain behavior and bladder permeability changes to noxious bladder stimuli (Bauer and Razin, 2000) and do not develop pseudorabies virus induced inflammation of the bladder (Jasmin et al., 2000). Our previous studies in rats with bladder overactivity following trinitrobenzene sulfonic acid-colon irritation (TNBS-CI) revealed increased numbers of mast cells in the bladder (Ustinova et al., 2007) and that colon–bladder cross-sensitization is dependent upon capsaicin-sensitive C-fiber afferents (Ustinova et al., 2006; Christianson et al., 2007; Ustinova et al., 2007).

The goal of the present study was to further elucidate some of the major contributors to colon–bladder cross-sensitization, focusing on the role of the mast cell and its downstream targets. Specifically, we determined if mast cells are an essential link in the pathway for colon–bladder cross-sensitization by the treating the animals with ketotifen fumarate, a mast cell stabilizing agent, after TNBS-CI. We subsequently examined bladder function, including voiding interval and urothelial permeability as measures of end-organ change. In addition, we tested the mast cell activating agent, Compound 48/80,

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on in vitro smooth muscle strip preparations to determine if the responses to mast cell mediators were altered in the model. Finally, we further examined the role of mast cells in cross-sensitization by inducing TNBS-CI in the $Kit^{W}/Kit^{W\text{-}v}$ mast cell-deficient mice.

2. Materials and methods

2.1. Animal model

Virgin, female Sprague Dawley rats (200–250 g, n = 66) purchased from Hilltop Labs (Scotdale, PA) were housed in polypropylene cages with ad-libitum access to food and water in the University of Pittsburgh animal facility. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. We also studied $Kit^{W}/Kit^{W\text{-}v}$ mast cell-deficient mice (n = 42) and control wild-type (WT, $Kit^{+/+}$) mice (n = 34) purchased from Jackson Labs (Bar Harbor, Maine) as complete deletion of the c-kit gene to eliminate mast cells is a lethal mutation in rats (Waskow et al., 2004). All experimental procedures requiring anesthesia were done under isoflurane unless otherwise stated. Animals were euthanized using CO_2 asphyxiation, followed by cervical dislocation.

2.2. Trinitrobenzenesulfonic acid (TNBS) colitis model of cross-sensitization (CS)

The protocol for the subacute colon inflammation and subsequent changes in the physiologic parameters of bladder function in the TNBS-CI model have been well described in previous publications for both the rat (Ustinova et al., 2007) and mouse models (Lamb et al., 2006). The methods were the same in rats and in mast cell-deficient $Kit^{W}/Kit^{W\text{-}v}$ mice (Tsai et al., 2002) and their wild type littermates. Under isoflurane anesthesia, 50 mg/ml TNBS dissolved in 50% ethanol was administered transanally at a volume of 0.4 ml with a catheter placed 6 cm and 4 cm from the anal verge in rats and mice, respectively. The perineum, vagina, and urethra were protected with surgical lubricant while the animal remained supine to protect from TNBS leakage. Control animals in all experiments were given a saline enema vehicle of equivalent volume. Animals were then housed for 7–12 days to fully develop colitis, which has been previously defined functionally and histologically (Ustinova et al., 2007).

2.3. Ketotifen fumarate administration

On day 7 after instillation of TNBS into the colon when maximal bladder mastocytosis occurs (Ustinova et al., 2007), ketotifen fumarate salt (Sigma, St. Louis, MO), a mast cell membrane stabilizer was administered at a dose of 10 mg/kg/day via drinking water at a concentration of 0.1 mg/ml for 5 consecutive days, taking into account that, on average, a 200 g rat drinks 30 ml of water per day. Water intake in each rat was monitored to determine if variation in drinking had an influence on drug effect, though all animals drank ± 10% of the average volume. The dose of ketotifen was selected based on efficacy in comparable studies (Serna et al., 2006). Cystometric parameters and urothelial permeability were measured after 12 days and compared to measurements in intracolonic saline vehicle controls.

2.4. Mast cell histologic quantification

We also validated the development of bladder mastocytosis in the TNBS-CI model using histological methods described in our earlier studies (Ustinova et al., 2007). The urinary bladders of four groups of animals (control, ketotifen alone, 12 days of TNBS-CI, 12 days of TNBS-CI treated from day 7 to day 12 with ketotifen) 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 were obtained at a thickness of 5 μm. Serial sections were mounted and stained with hematoxylin/eosin and Giemsa stains. Mast cells were counted independently by two investigators under a 1000×-power field as previously described (Spanos et al., 1997). Ten to twenty fields per bladder section were examined from each animal (n = 4 per group), and the average number of mast cells per field was calculated.

2.5. Cystometry

On day 12 after the induction of TNBS-CI, cystometry was performed in urethane-anesthetized (1.2 g/kg sc, Sigma, St. Louis, MO) rats as previously described (Ustinova et al., 2006). Room-temperature saline was infused into the bladder constantly using PE-20 tubing (Fisher Scientific, Hanover Park, IL) at a rate of 0.05 ml/min during continuous open cystometry. In urethane anesthetized (1.2 g/kg sc) mice the bladder was constantly infused (25 μl/min) with saline using PE-10 tubing. A Transbridge transducer amplifier (World Precision Instruments, Sarasota, FL) was used to amplify the signal from the pressure transducer, which was processed using the PowerLab 8 unit data acquisition system. After an initial equilibration period multiple cystometrograms (n = 4–6) were performed to determine the average voiding interval in each animal.

2.6. Urothelial permeability

To determine if urothelial permeability was increased as a result of cross-sensitization, absorption of sodium fluorescein, a well-documented methodology for assessing biological barrier function (Natah et al., 2009) was used in rats as well as $Kit^{W}/Kit^{W\text{-}v}$ and $Kit^{+/+}$ mice . Briefly, sodium fluorescein was infused into the bladder, and then blood levels were measured to estimate absorption and in turn urothelial permeability. On day 12 after TNBS-CI, the bladder was emptied, the urethra was clamped and then the bladder was refilled for 15 min with sodium fluorescein (10 mg/ml in Sorenson buffer, pH 7.4) (0.5 ml in rats and 0.1 ml in mice). After 15 min, a blood sample (1 ml in rats and 0.1 ml in mice) was collected from the jugular vein. Blood samples (1 ml) were centrifuged (3000 rpm/5 min) and deglucuronidated by incubating 100 μl serum aliquots in 200 μl of 0.2 M acetate buffer (pH 5.1) and 50 μl of glucurase (5000 U/ml) for 30 min at 37 °C. Samples were adjusted to 2 ml with 0.2 M carbonate buffer (pH 9.15) and fluorescein levels in the blood were measured spectrofluorometrically (emission 494 nm and excitation 516 nm) using known standards.

2.7. Bladder strip experiments

In the 7–12 day time period after TNBS-CI, the bladders were removed from rats under isoflurane (4% in O_2) anesthesia, and bladder strips were prepared as described previously (Artim et al., 2009). Briefly, the bladder was placed in warm Krebs solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$1.9, $MgSO_4$ 1.2, $NaHCO_3$ 24.9, KH_2PO_4 1.2, dextrose 11.7, pH 7.4 bubbled with 95% O_2, 5% CO_2) and cut into four longitudinal strips (~1.5 mm × 8–10 mm). Strips were tied with a fine thread at each end, mounted in a vertical double-jacketed organ bath in oxygenated Krebs solution (15 ml volume) and kept at 37 °C via a circulating warm water bath. The tissue was stretched one time at the beginning of the experiment to achieve a uniform baseline tension of 1 g and allowed to equilibrate for 1–2 h prior to drug testing (Kullmann et al., 2011). Amplitude of phasic contractions was measured for a 3 min period before and for two 3 min periods at 3 and 15 min after the application of 50 μg/ml of Compound 48/80 (Sigma), a mast cell activating agent. In this paper the term phasic contraction refers to transient (less than 30 s duration) increases (greater than or equal to 0.1 g) in strip tension. Bladder activity was measured during the maximal effect of the drug; amplitude during that period was measured from the minimum to the maximum