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Research Report

Urinary bladder hypersensitivity and dysfunction in female mice following early life and adult stress

*Angela N. Pierce, Elizabeth R. Di Silvestro, Olivia C. Eller, Ruipeng Wang, Janelle M. Ryals, Julie A. Christianson**

Department of Anatomy and Cell Biology, School of Medicine, University of Kansas Medical Center, 3901 Rainbow Blvd, MS 3038, Kansas City, KS 66160, USA

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ABSTRACT

Early adverse events have been shown to increase the incidence of interstitial cystitis/painful bladder syndrome in adulthood. Despite high clinical relevance and reports of stress-related symptom exacerbation, animal models investigating the contribution of early life stress to female urological pain are lacking. We examined the impact of neonatal maternal separation (NMS) on bladder sensitivity and visceral neuroimmune status both prior-to, and following, water avoidance stress (WAS) in adult female mice. The viscero-omotor response to urinary bladder distension was increased at baseline and 8 d post-WAS in NMS mice, while colorectal sensitivity was transiently increased 1 d post-WAS only in naïve mice. Bladder micturition rate and output, but not fecal output, were also significantly increased following WAS in NMS mice. Changes in gene expression involved in regulating the stress response system were observed at baseline and following WAS in NMS mice, and WAS reduced serum corticosterone levels. Cytokine and growth factor mRNA levels in the bladder, and to a lesser extent in the colon, were significantly impacted by NMS and WAS. Peripheral mRNA levels of stress-responsive receptors were differentially influenced by early life and adult stress in bladder, but not colon, of naïve and NMS mice. Histological evidence of mast cell degranulation was increased in NMS bladder, while protein levels of protease activated receptor 2 (PAR2) and transient receptor potential ankyrin 1 (TRPA1) were increased by WAS. Together, this study provides new insight into mechanisms contributing to stress associated symptom onset or exacerbation in patients exposed to early life stress.

Abbreviations: NMS, neonatal maternal separation; WAS, water avoidance stress; PAR2, protease activated receptor 2; TRPA1, transient receptor potential ankyrin 1; IC/PBS, interstitial cystitis/painful bladder syndrome; CRF, corticotropin-releasing factor; HPA, hypothalamic-pituitary-adrenal; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; NGF, nerve growth factor; SP, substance P; TRPV1, transient receptor potential vanilloid 1; VMR, visceromotor response; UBD, urinary bladder distension; CRD, colorectal distension; CORT, corticosterone; BDNF, brain-derived neurotrophic factor; IL6, interleukin-6; IL10, interleukin-10; SCF, stem cell factor; LTP, long term potentiation; EMG, electromyographic; TRPA1, transient receptor potential ankyrin 1

**Corresponding author*. Fax: +1 913 588 5677.

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1. Introduction

Interstitial cystitis (IC), which is associated with painful bladder syndrome (PBS), is characterized by recurrent pain in the bladder or surrounding region that is often associated with increased voiding and urgency (Offiah et al., 2013). Population prevalence of IC/PBS is nine times more common in women than in men (Berry et al., 2011; Link et al., 2008), affecting 3.3 million women in the U.S. alone. Mood disorders, such as depression, anxiety, and panic disorder, are common among chronic pelvic pain patients due to altered functioning of corticotropin releasing factor (CRF)-responsive regions of the brain, including the hypothalamic-pituitary-adrenal (HPA) axis, which regulates stress response and influences the perception of pain (Heim et al., 1998; Hubbard et al., 2011; Mayson and Teichman, 2009). Exposure to early life stress or trauma is a significant risk factor for developing HPA abnormalities and associated chronic pain syndromes (Maniam et al., 2014). As such, a significant proportion of IC/PBS patients report a history of adverse childhood events (Carrico et al., 2009; Jones et al., 2009; Mayson and Teichman, 2009; Peters et al., 2009; Seth and Teichman, 2008; Tietjen et al., 2010).

Regulation of the stress response occurs through CRF and glucocorticoid receptors located at each site along the HPA axis and on higher, regulatory limbic structures, such as the hippocampus, amygdala, and frontal cortex (Ulrich-Lai and Herman, 2009). The two CRF receptors (CRF_1 and CRF_2) serve opposing roles, as pharmacological antagonism or genetic deletion of CRF_1 has been shown to be anxiolytic and pharmacological blockade or genetic deletion of CRF_2 is anxiogenic (Bale et al., 2002). Binding at the glucocorticoid (GR) and mineralocorticoid (MR) receptors is largely anxiogenic; however, exposure to chronic stress decreases hippocampal GR expression, reducing the extent of descending inhibition onto the hypothalamus and thereby increasing CRF release and glucocorticoid production (Herman et al., 2005; Ulrich-Lai and Herman, 2009). Downstream propagation of neurogenic inflammation, as a result of improper HPA axis output, has been implicated in other chronic pain disorders (Black, 2002) and may underlie exacerbation of dormant IC/PBS symptoms during periods of high stress (Lutgendorf et al., 2000).

One of the primary peripheral targets of downstream HPA axis output is the mast cell, which is a highly granulated, stem cell-derived immune cell that expresses functional CRF receptors and can respond to and release CRF and related neuropeptides (Black, 2002; Cao et al., 2005; Kempuraj et al., 2004). Multiple independent studies have confirmed that mast cell infiltration is increased in biopsies from IC/PBS patients (Christmas and Rode, 1991; Kastrup et al., 1983; Larsen et al., 2008; Peeker et al., 2000; Spanos et al., 1997; Tomaszewski et al., 2001). These observations have been correlated with increased release of granular contents (Theoharides et al., 1995), elevated nerve growth factor (NGF), histamine, and pro-inflammatory cytokine protein levels in patient serum (Jiang et al., 2013) and urine (Corcoran et al., 2013; Jacobs et al., 2010; Lotz et al., 1994; Yun et al., 1992) samples, and increased density of substance P (SP)-immunopositive nerve fibers and juxtaposition to mast cells in patient biopsies (Pang et al., 1995). Tryptase, a major component of mast cell granules, can bind to and activate protease activated receptor-2 (PAR2) located on adjacent sensory nerve endings (Cenac et al., 2002; 2007; Sipe et al., 2008). Activation of PAR2 has been shown to sensitize transient receptor potential vanilloid 1 (TRPV1) and ankyrin 1 (TRPA1) *in vivo* (Chen et al., 2011; Sipe et al., 2008), two channels that have been implicated in the development of inflammatory-induced visceral hyperalgesia (Birder et al., 2002; Brierley et al., 2009; Jones et al., 2005).

Despite its involvement in stress-related pathologies and immunomodulatory effects, dysregulation of the HPA axis has not been investigated in early life stress-induced urinary bladder hypersensitivity and dysfunction. Using a model of neonatal maternal separation (NMS), we investigated the impact of early life stress on urinary bladder sensitivity and function both prior-to, and following, exposure to water avoidance stress (WAS) in adulthood. To determine whether the effects of NMS were specific to the bladder, we also evaluated colorectal sensitivity and output prior to and following WAS exposure. Output of the HPA axis and the relevant gene expression in the hypothalamus and hippocampus were examined in NMS and naïve mice with or without exposure to WAS. Cytokine, growth factor, CRF_1, and CRF_2, mRNA levels; histological evidence of mast cell degranulation; and protein levels of TRPA1 and PAR2 were assayed in the bladder and colon. Together, these results increase our understanding of how early life stress predisposes an individual to developing stress-associated bladder pain syndromes during adulthood.

2. Results

2.1. *Neonatal and adult stress exposure differentially increase urinary bladder and colorectal sensitivity*

Previous studies in our laboratory have determined that NMS impacts adult anxiety-like behaviors and vaginal sensitivity with associated molecular changes in the pelvic viscera and HPA axis of female mice (Pierce et al., 2014). The purpose of the current study is to determine the impact of acute adult stress exposure on urinary bladder and colorectal sensitivity and associated measurements of HPA axis output and regulation in the same model of female NMS mice. Estrous cycle stage was estimated following physiological or behavioral testing or at the time of sacrifice and no significant effect of apparent cycle stage was observed for any of the reported results (data not shown).

The visceromotor response (VMR) during urinary bladder distension (UBD) or colorectal distension (CRD) was recorded in naïve and NMS mice to evaluate changes in pelvic organ sensitivity prior to additional stress exposure. In all mice, the VMR during either UBD (Fig. 1A) or CRD (Fig. 1C) significantly increased in response to greater intravesicular or balloon pressure ($p<0.0001$, two-way RM ANOVA), respectively, confirming a physiological response to organ distension. At baseline, the VMR of NMS mice during UBD was significantly higher than that of naïve mice over the entire distension series ($p<0.05$, two-way RM ANOVA) and at the highest pressure applied ($p<0.05$, Bonferroni's multiple comparisons test; Fig. 1A). In contrast, NMS mice displayed significantly decreased VMR during CRD,