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Research Paper

Neonatal maternal separation increases susceptibility to experimental colitis and acute stress exposure in male mice

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ABSTRACT

Experiencing early life stress can result in maladjusted stress response via dysregulation of the hypothalamic-pituitary-adrenal axis and serves as a risk factor for developing chronic pelvic pain disorders. We investigated whether neonatal maternal separation (NMS) would increase susceptibility to experimental colitis or exposure to acute or chronic stress. Male mice underwent NMS from postnatal day 1–21 and as adults were assessed for open field behavior, hindpaw sensitivity, and visceromotor response (VMR) to colorectal distension (CRD). VMR was also measured before and after treatment with intracolonic trinitrobenzene sulfonic acid (TNBS) or exposure to acute or chronic water avoidance stress (WAS). Myeloperoxidase (MPO) activity, proinflammatory gene and corticotropin-releasing factor (CRF) receptor expression were measured in distal colon. Baseline VMR was not affected by NMS, but undergoing CRD increased anxiety-like behaviors and mechanical hindpaw sensitivity of NMS mice. Treatment with TNBS dose-dependently decreased body weight and survival only in NMS mice. Following TNBS treatment, IL-6 and artemin mRNA levels were decreased in the distal colon of NMS mice, despite increased MPO activity. A single WAS exposure increased VMR during CRD in NMS mice and increased IL-6 mRNA and CRF_2 protein levels in the distal colon of naïve mice, whereas CRF_2 protein levels were heightened in NMS colon both at baseline and post-WAS exposure. Taken together, these results suggest that NMS in mice disrupts inflammatory- and stress-induced gene expression in the colon, potentially contributing towards an exaggerated response to specific stressors later in life.

Patients suffering from chronic pelvic pain syndromes, including irritable bowel syndrome (IBS), commonly report symptom onset or increased severity during periods of high stress (Mayer et al., 2001; Blanchard et al., 2008). Many IBS patients also have difficulty coping with stressful situations and suffer from depression, anxiety, and/or panic disorder (Fond et al., 2014). Co-morbidity between IBS and mood disorders has been associated with altered functioning of the hypothalamic-pituitary-adrenal (HPA) axis (Chang et al., 2009), which regulates the response to stress and influences the perception of pain (Silverman and Sternberg, 2012). A history of adverse events early in life has been shown to permanently influence the functioning of the HPA axis and also increases the likelihood of developing IBS (Barreau et al., 2007; Chitkara et al., 2008; Videlock et al., 2009).

Corticotropin-releasing factor (CRF) is the primary initiator of the HPA axis and acts both centrally and peripherally in response to an acute or chronic stressor (Ulrich-Lai and Herman, 2009; Pierce and Christianson, 2015). Centrally, CRF is expressed in the paraventricular nucleus of the hypothalamus, and initiates a cascade through the HPA axis to induce a systemic release of glucocorticoids. Two CRF receptors, CRF receptor 1 (CRF_1) and CRF receptor 2 (CRF_2), largely work in opposition of one another to propagate and diminish HPA axis activation, respectively (Plotsky et al., 1993; Bale and Vale, 2004). These two CRF receptors are also found peripherally, specifically within the mucosal layer of the colon, enteric nervous system, and innate inflammatory cells (O'Malley et al.,

Abbreviations: NMS, neonatal maternal separation; VMR, visceromotor response; CRD, colorectal distension; TNBS, trinitrobenzene sulfonic acid; WAS, water avoidance stress; MPO, myeloperoxidase; CRF, corticotropin-releasing factor; IL, interleukin; IBS, irritable bowel syndrome; HPA, hypothalamic-pituitary-adrenal; EMG, electromyographic; NGF, nerve growth factor; IFN, interferon; AUC, area under the curve.

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2010a). When released peripherally, CRF effects changes in neuroimmune function, partially through mast cell activation and degranulation (Black, 2002), resulting in increased cytokine and growth factor expression, sensitization of nerve endings, and endothelial leakage of associated vasculature (Singh et al., 1999; Tomaszewski et al., 2001; Barbara et al., 2004; Barreau et al., 2008).

Neonatal maternal separation (NMS) is a well-established model of early life stress that has been shown to induce colorectal hypersensitivity (Coutinho et al., 2002; Zhang et al., 2009; O'Malley et al., 2010b; Moloney et al., 2012) and increase colonic permeability (Varghese et al., 2006; Ghia et al., 2008; Veenema et al., 2008), largely driven by dysregulation of the HPA axis (O'Mahony et al., 2011a,b). Rodents exposed to NMS are more susceptible to experimental colitis (Varghese et al., 2006) and have demonstrated increased CRF receptor, growth factor, and cytokine mRNA expression in the adult distal colon and, in particular, IL-6 and CRF have been shown to interactively alter colonic secretory activity (Barreau et al., 2004a; O'Malley et al., 2011b; O'Malley et al., 2013). Introduction of an adult stressor, generally in the form of water avoidance stress (WAS), exacerbates the colorectal hypersensitivity of NMS rodents and can be attenuated by central or peripheral administration of CRF receptor antagonists (Schwetz et al., 2005; van den Wijngaard et al., 2012).

We have previously shown that NMS results in perigenital mechanical hypersensitivity in male mice (Fuentes et al., 2015) and vaginal (Pierce et al., 2014) and bladder (Pierce et al., 2016) hypersensitivity in female mice, with varying adult stress-induced changes. Here we are testing the hypothesis that NMS in male mice will increase susceptibility to colorectal hypersensitivity following adult stress exposure or experimental colitis. We have measured the visceromotor response (VMR) during colorectal distension (CRD) at baseline and following exposure to increasing doses of Trinitrobenzene sulfonic acid (TNBS) or acute or chronic exposure to WAS. We also assayed the distal colon for Interleukin (IL)-6 and artemin mRNA levels and CRF receptor protein levels.

1. Experimental procedures

1.1. Animals

Experiments were performed on male C57Bl/6 mice (Charles River, Wilmington, MA; ages listed in Table 1) born and housed in the Research Support Facility at the University of Kansas Medical Center. Mice were housed on a 12-h light cycle from 600 to 1800 h and received water and food (8604; Harlan Teklad, Madison WI) ad libitum. All research performed conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals in accordance with the guidelines specified by the University of Kansas Medical Center Animal Care and Use Protocols.

Table 1
Age of mice at experimental time points.

	Baseline	Insult	Post-insult measurements
Colorectal distension			
No insult (n = 10)	13		
Saline (n = 6)	8	9	10
TNBS 2 mg (n = 6)	10	11	12
TNBS 5 mg (n = 6)	8	9	10
1d WAS (n = 6)	10	10	11
7d WAS (n = 6)	10	10–11	12
Open Field			
CRD (n = 8)	6	13	17
Hindpaw Sensitivity			
CRD (n = 8)	7	13	19

Naïve and NMS mice underwent behavioral or physiological testing at the above noted ages (in weeks) prior to and/or following introduction of an insult, including colorectal distension (CRD), intracolonic trinitrobenzene sulfonic acid (TNBS), or acute (1d) or chronic (7d) exposure to water avoidance stress (WAS). Repeated tests were performed on the same mice for all CRD experiments involving post-insult measurements. Open field and hindpaw sensitivity tests were performed on separate groups of mice either at baseline or following CRD.

1.2. Neonatal maternal separation

Beginning on postnatal day 1 (P1, date of birth was considered P0), pups were removed daily from their home cages for 180 min (generally 1100–1400 h) and placed as a litter, with a small amount of home bedding material, into a clean glass beaker and held at 34 °C and 50% humidity as previously described (Fuentes et al., 2015). NMS mice were weighed immediately prior to and after the NMS period from P1 through P14 or P21 and were weaned at P22. Naïve mice were born in-house and remained undisturbed in their home cages, with the exception of daily weighing and routine animal husbandry, until weaning at P22. Five separate cohorts of NMS mice were used in this study and each cohort was compared to a corresponding naïve group of mice that were born, housed, and weaned during the same time frame to avoid potential complications arising from variations in prenatal shipping conditions, housing environment, and investigator handling.

1.3. Trinitrobenzene sulfonic acid (TNBS) treatment

Naïve and NMS mice were anesthetized with inhaled isoflurane (4% induction, 2% maintenance) and secured on a platform that elevated the pelvic region approximately 5 cm above the working surface. A water-based lubricant (KY Jelly, Johnson & Johnson, New Brunswick, NJ) was liberally applied to the perianal region to avoid sensitization of surrounding somatic tissues. Mice received an intracolonic instillation of TNBS (0.1 ml of 50 mg ml^{-1} or 20 mg ml^{-1} in 50% EtOH) or saline (0.1 ml) using an oral feeding needle attached to a 50 μl Hamilton syringe. Mice remained in an elevated position for 5 min to prevent leakage. Mice were then allowed to recover from anesthesia and were returned to their home cages.

1.4. Water avoidance stress

Water avoidance stress (WAS) was performed for 1 h, within the first 6 h of the light cycle, for 1 day or 7 consecutive days. Mice were placed individually on a round platform (5 cm diameter) centrally affixed to the bottom of a container (36 cm length x 31 cm width x 27 cm height) filled with room temperature tap water up to 1 cm below the top of the platform.

1.5. Electromyographic electrode implantation and colorectal distension

The visceromotor response (VMR) to colorectal distension (CRD) was evaluated in naïve and NMS mice. Electrode implantation was performed as previously described (Christianson and Gebhart, 2007). Under inhaled isoflurane (4% induction, 2.5% maintenance) and aseptic conditions, the bare ends of two Teflon-coated stainless steel wires (3 mm; Grass Technologies, West Warwick, RI) were inserted into the right lateral abdominal musculature, secured via 5-0 prolene sutures, tunneled subcutaneously to a small incision made in the nape of the neck, and externalized for access during testing. Skin incisions were closed using 5-0 silk suture. Following recovery from anesthesia, mice were housed singly and allowed to recover for a minimum of 4 days before undergoing testing.

To facilitate balloon insertion and obtain proper restraint during CRD, mice were briefly sedated with inhaled isoflurane (4%