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Importance of epigenetic mechanisms in visceral pain induced by chronic water avoidance stress

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Summary Epigenetic molecular mechanisms, which include DNA methylation and histone deacetylation, are implicated in the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. Previously, we demonstrated that repeated water avoidance stress (WAS), a validated model of chronic psychological stress, induces heightened visceral pain behaviors in rodents that resemble irritable bowel syndrome (IBS) sequelae. However, the involvement of epigenetic molecular mechanisms in the pathophysiology of stress-induced visceral pain has not been explored. Our hypothesis is that epigenetic mechanisms within the central nervous system (CNS) are important to chronic stress-induced visceral hypersensitivity. Adult male F-344 rats with intracerebroventricular (i.c.v.) cannulae were exposed to 7 days of repeated WAS. Controls received a SHAM stress. Following the daily 1 h stressor, trichostatin A (TSA; 100 ng/ml), a potent histone deacetylase inhibitor, or vehicle (VEH; 0.1% DMSO/saline,) as control was administered via the i.c.v. cannula. Visceral sensitivity was assessed 24 h after the final WAS and quantified the visceromotor response (VMR) by recording the number of abdominal contractions in response to graded pressures (20—60 mmHg) of colorectal distensions (CRD). From a separate group of rats that were exposed to repeated WAS or SHAM stress, the amygdala was isolated to assess the methylation status of glucocorticoid receptor (GR) and corticotropin releasing-factor (CRF) genes via bisulfite sequencing and verified by pyrosequencing. GR and CRF gene expression was quantified via qRT-PCR. Stressed rats exhibited visceral hypersensitivity that was significantly attenuated by TSA. Compared to SHAM controls, methylation of the GR gene was increased following WAS while expression of the GR gene was decreased. Methylation of the CRF promoter was decreased with WAS with a concomitant increase in CRF expression. This study demonstrates the involvement of central epigenetic mechanisms in regulating stress-induced visceral hypersensitivity and provides a foundation for exploring the epigenetic mechanisms that may contribute to IBS-like symptomatology.

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Introduction

Stress and episodes of anxiety are strongly implicated in the pathophysiology of irritable bowel syndrome (IBS), a chronic functional gastrointestinal (GI) disorder characterized by abdominal pain and abnormal bowel habits (Longstreth et al., 2006; Lydiard, 2001; Mayer et al., 2009; Posserud et al., 2004). This comorbidity of stress with the exacerbation of IBS symptomatology is supported by an abnormal hypothalamic-pituitary-adrenal (HPA) axis in IBS patients (Chang et al., 2009; Dinan et al., 2006). Further supporting the link between stress and IBS, the symptoms of IBS persist long after a stressful event and are exacerbated by subsequent stress (Blanchard et al., 2008). Several preclinical experiments have revealed key factors involved in the stress-related pathology of IBS (Bradesi et al., 2005; Greenwood-Van Meerveld et al., 2001; Myers and Greenwood-Van Meerveld, 2010, 2012; Venkova et al., 2010). For example, in a rodent model, chronic psychological stress can induce visceral hyperalgesia. Specifically, rats exposed to chronic water avoidance stress (WAS), a well-characterized experimental model of psychological stress, exhibit many of the classical sequelae reported by IBS patients including visceral hyperalgesia and colonic dysmotility (Bradesi et al., 2005; Myers and Greenwood-Van Meerveld, 2012). Much like the persistent symptoms experienced by IBS patients, visceral hyperalgesia induced by chronic WAS persisted following cessation of WAS (Bradesi et al., 2005).

Our previous studies provided further insight into the mechanisms involved in the stress-induced pathophysiology of IBS. Specifically, we demonstrated that stereotaxic implantation of the stress hormone corticosterone (CORT) onto the dorsal margin of the central amygdala (CeA), a primary brain region involved in physiological responses to stress, induces chronic visceral hypersensitivity and colonic dysmotility in rats (Greenwood-Van Meerveld et al., 2001; Myers et al., 2007; Venkova et al., 2010). Molecular and pharmacological experiments revealed that glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) in the central nucleus of the amygdala (CeA) (Myers and Greenwood-Van Meerveld, 2007) and corticotropin-releasing factor (CRF) (Myers et al., 2005; Shepard et al., 2000) are critical for CORT-induced visceral hypersensitivity. Although these studies identify key central receptor-mediated mechanisms that may be involved in the pathophysiology of IBS, the complex mechanisms by which stress leads to persistent abnormalities in GI function remain unexplored.

Recent studies have shown that remodeling of the epigenome by the environment, or during chronic stress, may result in long-term changes in gene expression (Weaver, 2009). Gene expression patterns are controlled by the epigenome, which can include changes in the specific chromosome structure through differential histone acetylation or methylation patterns of the DNA. Here we test the hypothesis that central epigenetic changes contribute to stress-induced visceral hypersensitivity. To address this hypothesis we first examined the effect of a histone deacetylase inhibitor administered directly into the brain on stress-induced visceral hypersensitivity. We then focused our study on the amygdala to determine whether stress alters the methylation patterns of GR and CRF, two key genes previously identified to be involved in the stress-induced pathology.

Materials and methods

Animals

Experiments were performed on male Fischer-344 rats, weighing 175—200 g upon arrival (Charles Rivers Laboratory, Wilmington, MA). All animals were single-housed to prevent post-surgery complications and maintained on a 12 h light/dark cycle (lights on at 5:30 AM) at 21 °C and 70% humidity with ad libitum access to food and water. Rats were acclimated to the animal facility for one week and to the experimenter and the laboratory for an additional week before experimentation. The experiments were approved by the Oklahoma City Veterans Affairs Medical Center Animal Care and Use Committee (IACUC; protocol #0807-004) in accordance with standards established by the *Guide for Care and Use of Laboratory Animals* (1996).

Water avoidance stress (WAS) protocol

Chronic psychological stress was induced using repeated WAS, a validated model of psychological stress (Bradesi et al., 2005), for a total of 7 days. Rats in the WAS group were placed on a square platform (8 cm × 8 cm × 8 cm) mounted in the center of a white semitransparent plastic container (50 cm × 35 cm × 33 cm) filled with fresh, room temperature water to 1 cm below the surface of the platform. Animals in the SHAM stress group were placed in containers without water. Fecal-pellet output (FPO) was recorded for 60 min during the WAS or SHAM stress procedure.

Intracerebroventricular (i.c.v.) administration of compounds

To administer compounds directly into the central nervous system (CNS), a cannula was surgically implanted into the right lateral ventricle as previously described (Greenwood-Van Meerveld et al., 2005; Myers and Greenwood-Van Meerveld, 2012). Briefly, rats were anesthetized with an intraperitoneal (i.p.) injection of 100 mg/kg ketamine (Hospira, Lake Forest, IL) and 10 mg/kg xylazine (Ben Venue Laboratories, Bedford, OH). Using aseptic technique, a single cannula (PlasticsOne, Roanoke, VA) was placed at bregma −0.8 mm, medial/lateral +1.7 mm and projected anteroposterior −4 mm from skull surface. The cannula was secured in place using cerebond skull adhesive (PlasticsOne, Roanoke, VA) and anchored to 3 screws that were placed around the cannula. Patency was maintained with a dummy stylus (PlasticsOne, Roanoke, VA). The incision was closed with staples and a topical analgesic/anesthetic cream was applied to minimize pain and prevent infection. Rats were allowed to recover for 7 days before the stress treatment. Intracerebroventricular infusions were performed as previously described (Weaver et al., 2004). Each day following WAS the dummy cannula was replaced with 28-gauge infusion cannula attached to a 5.0 μl Hamilton (Reno, NV) syringe via polyurethane tubing (PE 20). Two separate syringes were used for VEH and TSA treatment. A total of 2 μl vehicle (VEH; 0.1% dimethyl sulfoxide in saline) or trichostatin A (TSA; 100 ng/μl) dissolved in VEH was infused via i.c.v. cannula at a rate of 1 μl/2.5 min. All doses and treatment protocols