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NEUROLOGICAL AND CELLULAR REGULATION OF VISCERAL HYPERSENSITIVITY INDUCED BY CHRONIC STRESS AND COLONIC INFLAMMATION IN RATS

J. CHEN,[a] J. H. WINSTON[a] AND S. K. SARNA[a,b]*

[a] *Enteric Neuromuscular Disorders and Visceral Pain Center, Division of Gastroenterology, Department of Internal Medicine, The University of Texas Medical Branch at Galveston, Galveston, TX 77555-1083, United States*

[b] *Enteric Neuromuscular Disorders and Visceral Pain Center, Division of Gastroenterology, Department of Neuroscience, and Cell Biology, The University of Texas Medical Branch at Galveston, Galveston, TX 77555-1083, United States*

Abstract—The role of inflammation in inducing visceral hypersensitivity (VHS) in ulcerative colitis patients remains unknown. We tested the hypothesis that acute ulcerative colitis-like inflammation does not induce VHS. However, it sets up molecular conditions such that chronic stress following inflammation exaggerates single-unit afferent discharges to colorectal distension. We used dextran sodium sulfate (DSS) to induce ulcerative colitis-like inflammation and a 9-day heterotypic chronic stress protocol in rats. DSS upregulated $Na_v1.8$ mRNA in colon-responsive dorsal root ganglion (DRG) neurons, TRPV1 in colonic muscularis externae (ME) and BDNF in spinal cord without affecting the spike frequency in spinal afferents or VMR to CRD. By contrast, chronic stress did not induce inflammation but it downregulated $K_v1.1$ and $K_v1.4$ mRNA in DRG neurons, and upregulated TRPA1 and nerve growth factor in ME, which mediated the increase of spike frequency and VMR to CRD. Chronic stress following inflammation exacerbated spike frequency in spinal afferent neurons. TRPA1 antagonist suppressed the sensitization of afferent neurons. DSS-inflammation did not affect the composition or excitation thresholds of low-threshold and high-threshold fibers. Chronic stress following inflammation increased the percent composition of high-threshold fibers and lowered the excitation threshold of both types of fibers. We conclude that not all types of inflammation induce VHS, whereas chronic stress induces VHS in the absence of inflammation.

Key words: colon inflammation, sensory neurons, inflammatory bowel disease, abdominal pain, chronic stress.

INTRODUCTION

The sensitization of primary afferent neurons by peripheral inflammation and central facilitation of peripheral nociceptive signals underlie chronic pain (Woolf, 2011). Since profound colonic inflammation occurs in ulcerative colitis patients, the tacit assumption is that colonic inflammation in these patients sensitizes primary afferent neurons to cause visceral pain. However, clinical studies in ulcerative colitis patients did not consistently find visceral hypersensitivity (VHS) in response to colorectal distension (CRD); some reported visceral hypersensitivity (Rao et al., 1987), others found normosensitivity (Bernstein et al., 1996; Mayer et al., 2005) or hyposensitivity, (Chang et al., 2000), suggesting that the afferent nervous system may not be sensitized in all patients.

Clinical findings show that chronic stress exacerbates the symptoms of IBD patients, including abdominal pain (Levenstein et al., 2000; Maunder and Levenstein, 2008). Likewise, animal studies show that the application of various chronic stress paradigms to rodents produces hypersensitivity to CRD by sensitizing colon primary afferent neurons (Bradesi et al., 2005; Winston and Sarna, 2013). Therefore, it is likely that the lack of consideration of concurrent chronic stress might be one of the reasons for the divergent findings of VHS in ulcerative colitis patients. We hypothesized that ulcerative colitis-like colonic inflammation in rats, by itself, does not sensitize the primary afferents or increase VMR to CRD. However, chronic stress following inflammation super sensitizes the primary afferents. We tested this hypothesis by applying a chronic stress protocol to rats following DSS-induced colonic inflammation that mimics the morphological, immunological, and histological features of ulcerative colitis (Elson et al., 1995). We found that acute ulcerative colitis-like inflammation alone did not induce visceral hypersensitivity, although it sensitized high threshold colonic pelvic nerve afferent fibers and up regulated the expression of pro-nociceptive genes,

*Corresponding author. Address: Division of Gastroenterology, Department of Internal Medicine, The University of Texas Medical Branch at Galveston, 8.104B Medical Research Building, Galveston, TX 77555-1083, United States. Tel: +1-409-747-0908; fax: +1 409 772 5841.

E-mail address: sksarna@utmb.edu (S. K. Sarna).

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CRD, colorectal distension; DRG, dorsal root ganglion; DSS, dextran sodium sulfate; FSS, forced swimming stress; HeICS, heterotypic intermittent chronic stress; HRP, horse radish peroxidase; HT, high-threshold; IBD, inflammatory bowel disease; LCM, laser capture microscopic; LT, low-threshold; ME, muscularis externae; MPO, myeloperoxidase; NGF, nerve growth factor; SM, submucosa; SPSS, statistical package for the social sciences; TNBS, trinitrobenzene sulfonic acid; TRAP1, transient receptor potential ankyrin repeat 1; TRP, transient receptor potential; VHS, visceral hypersensitivity; VMR, Visceromotor response; WAS, water avoidance stress.

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brain-derived neurotrophic factor (BDNF) and $Na_V1.8$ in colon afferent neurons and TRPV1 in colonic muscularis externae (ME). However, chronic stress, following colonic inflammation, induced robust sensitization of afferent neurons by up regulating the expression of nerve growth factor (NGF) and transient receptor potential ankyrin repeat 1 (TRPA1) in the ME, and down-regulating $K_v1.1$ and $K_v1.4$ in colon dorsal root ganglion (DRG) neurons. Treatment with a TRPA1 antagonist significantly reduced visceral hypersensitivity to colorectal distension and reduced sensitization of colonic pelvic nerve afferent fibers. In addition, DSS inflammation and chronic stress had differential effects on the recruitment of low-threshold (LT) and high-threshold (HT) fibers and their excitation thresholds.

EXPERIMENTAL METHODS

Animal models

We used 6–10 week-old male Sprague–Dawley rats (180–280 g). The Institutional Animal Care and Use Committee of the University of Texas Medical Branch at Galveston, TX approved the procedures. Oral administration of 5% w/v DSS in drinking water for 5 days was used to induce colonic inflammation (Shi et al., 2011). Visceromotor response (VMR) to graded (10–80 mmHg) CRD or single-fiber recordings from the sacral level S1 dorsal root molecular experiments were obtained on day 6 after the start of DSS treatment (Fig. 1A). A separate group of rats received psychological stress by a 9-day heterotypic intermittent chronic stress (HeICS) protocol comprised of randomly distributed daily application of water avoidance stress (WAS; 60 min), forced swimming stress (FSS; 20 min), or cold restraint stress (CRS; 45 min), as described previously (Winston et al., 2010) (Fig. 1A). The behavior, electrophysiological results, and tissues were obtained on day 10, one day after the end of HeICS (Fig. 1A). The third group of rats received 9-day HeICS starting on day 6 after the start of DSS treatment (Fig. 1A); experiments were performed on day 16, one day after the end of HeICS. Age-matched control rats consumed regular drinking water.

Measurement of VMR to graded CRD

Visceromotor response (VMR) or single fiber recordings from the sacral level S1 dorsal root were performed by rapidly inflating the balloon to constant pressures of 10, 20, 40, 60, and 80 mmHg for 20 s followed by 2-min rest as described previously (Winston and Sarna, 2013). The area under the curve for the electrical signal, during each 20 s of distention, was calculated using Acknowledge software (Biopac Systems, Inc., Santa Barbara, CA, USA). The net value for each distension response was calculated by subtracting the baseline

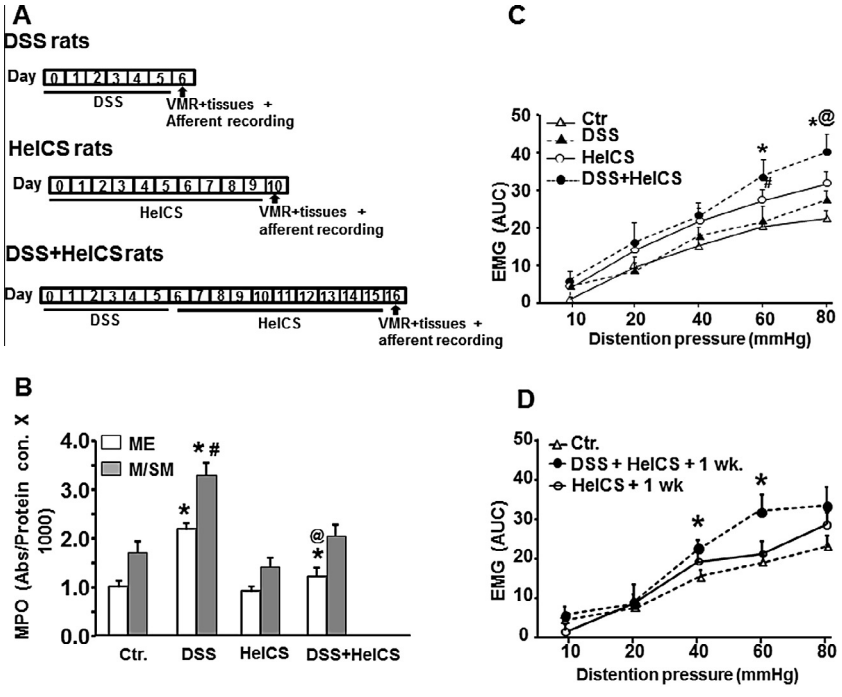

Fig. 1. Study design, inflammation and the effect of DSS, HeICS and DSS + HeICS on VMR by CRD. (A) Inflammation and chronic stress protocols in DSS-, HeICS- and DSS + HeICS-rats. (B) MPO in ME and M/SM increased significantly on day 6 in DSS-rats vs. saline-treated controls ($^{*}p < 0.05$); MPO in M/SM was significantly greater than in ME ($^{\#}p < 0.05$). HeICS did not affect MPO in ME or M/SM. In DSS + HeICS rats, MPO in ME, but not in M/SM, remained elevated on day 16 compared with controls ($n = 6$ rats, $^{*}p < 0.05$). The MPO in DSS + HeICS rats on day 16 was significantly lower than in DSS rats on day 6 ($^{@}p < 0.05$). (C) Comparison of the VMR to graded CRD in control (Ctr.), DSS, HeICS-, and DSS + HeICS-rats. Electromyographic (EMG) activity is expressed as area under the curve (AUC) in units of volts × seconds. DSS inflammation had no effect on VMR to CRD. HeICS significantly increased VMR to CRD ($n = 6$ rats, $^{\#}p < 0.05$ vs. Ctr.); HeICS + DSS further increased VMR vs. HeICS alone ($n = 6$ rats, $^{*}p < 0.05$ vs. Ctr.; $^{@}p < 0.05$ vs. HeICS). (D) The VMR to CRD in DSS + HeICS rats remained significantly elevated one week after the last stressor ($n = 6$ rats, $^{*}p < 0.05$ vs. Ctr.).