Adrenergic Stimulation Mediates Visceral Hypersensitivity to Colorectal Distension Following Heterotypic Chronic Stress

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BACKGROUND & AIMS: Chronic stress exacerbates or causes relapse of symptoms such as abdominal pain and cramping in patients with irritable bowel syndrome. We investigated whether chronic stress increases plasma norepinephrine and sensitizes colon-specific dorsal root ganglion (DRG) neurons by increasing expression of nerve growth factor (NGF) in the colon wall. **METHODS:** Heterotypic chronic stress (HeCS) was applied to male Wistar rats and neurologic and molecular responses were analyzed. Tissues were analyzed for NGF expression. **RESULTS:** HeCS significantly increased visceromoter response to colorectal distension; expression of NGF increased in colonic muscularis externa and mucosa/submucosa. Rheobase decreased, resting membrane potential was depolarized, and electrogenesis of action potentials increased in colon-specific thoracolumbar DRG neurons. Luminal administration of resiniferatoxin in distal colon, systemic administration of anti-NGF antibody, or inhibition of the NGF receptor trkA by k252a or antisense oligonucleotides in thoracolumbar DRG blocked the chronic stress-induced visceral hypersensitivity to colorectal distension. Blockade of $\alpha 1/\alpha 2$- and $\beta 1/\beta 2$-adrenergic receptors prevented the stress-induced visceral hypersensitivity and increased expression of NGF in the colon wall. HeCS did not induce any inflammatory response in the colon wall. **CONCLUSIONS: The peripheral stress mediator norepinephrine induces visceral hypersensitivity to colorectal distension in response to HeCS by increasing the expression of NGF in the colon wall, which sensitizes primary afferents in the absence of an inflammatory response.**

Abdominal discomfort/cramping and altered bowel habits are the defining symptoms of irritable bowel syndrome (IBS).[1] The etiologies of both symptoms are not fully understood. However, clinical studies show that chronic stress exacerbates or precipitates both symptoms of IBS.[2,3] This suggests that chronic stress may impair the cellular functions of some of the same cells that cause motility dysfunction or visceral hypersensitivity in IBS patients in the first place. However, the mechanisms by which chronic stress causes cellular dysfunction in these cells may differ from those that cause the underlying dysfunction in IBS. Animal studies support this hypothesis. A recent study found that alterations in the transcription rates of genes encoding key cell-signaling proteins of the excitation-contraction coupling in colonic circular smooth muscle cells underlie motility dysfunction of faster colonic transit and increase in defecation rate in a model of postinfective IBS.[4] Another study found that chronic stress enhances the transcription rate of some of the same genes encoding key cell-signaling proteins that accelerate colonic transit and defecation rate but by different mechanisms.[5]

Clinical studies show that the primary spinal afferents mediate visceral hypersensitivity to colorectal distension (CRD) in IBS patients.[6,7] However, all psychological stress responses begin in the central nervous system. Release of corticotrophin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus is an early and essential step in the initiation of all psychological stress responses.[8] Central release of CRH and other mediators, such as arginine vasopressin, stimulate the neuroendocrine system comprised of autonomic neurons and the hypothalamus-pituitary-adrenal (HPA) axis, which modulates the adaptive and maladaptive responses of peripheral organs in a stress- and cell-type–specific manner. Both acute and chronic stressors induce visceral hypersensitivity to colorectal distension in rats by releasing CRH in the hypothalamus.[9,10] However, we do not know which pathways or which stress mediators of the neuroendocrine system transmit the central signal to colon-specific primary afferent neurons in the dorsal root ganglia (DRG) to modulate their sensitivity to CRD.

In a recent study,[5] we found that 9-day heterotypic chronic stress (HeCS) significantly increases plasma concentration of norepinephrine. In the present study, we tested the hypothesis that norepinephrine released by the

Abbreviations used in this paper: CRD, colorectal distention; CRH, corticotrophin-releasing hormone; DiI, 1,1′-dilinoleyl-3,3,3′,3′-tetramethylindocarbocynine perchlorate; DRG, dorsal root ganglia; HeCS, heterotypic chronic stress; HPA, hypothalamus-pituitary-adrenal axis; IBS, irritable bowel syndrome; MPO, myeloperoxidase; NGF, nerve growth factor; trkA, tropomyosin-related kinase A (NGF high-affinity receptor).

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central component of the stress response acts as a messenger to induce expression of neurotrophin, nerve growth factor (NGF), in the distal colon, which sensitizes the colon-specific neurons in the thoracolumbar DRG to induce visceral hypersensitivity to CRD. We chose chronic stress because clinical findings indicate that chronic stress, rather than short-term acute stress,[2,3] exacerbates symptoms of IBS. In addition, variable stressors are less likely to show adaptation compared to repeated applications of the same stressor.

Methods

Animals

We used 6- to 10-week-old male Wistar rats housed at 22°C with a 12-hour light/dark cycle. The Institutional Animal Care and Use Committee at University of Texas Medical Branch approved all procedures performed on animals.

Heterotypic Chronic Stress Protocol

Rats were subjected to 9 consecutive days of a heterotypic stress protocol comprised of 3 randomly arranged stressors, 60 minutes of water avoidance stress, 45 minutes of cold restraint stress at 4°C, or 20 minutes of forced swimming stress, as described previously.[5] Control rats were brought to the laboratory and handled identically without subjecting them to the stress protocol.

Measurement of Visceromoter Response to Graded CRD

Electromyographic activity from the external oblique muscle was recorded in response to colorectal distention (CRD). CRD was performed by rapidly inflating the balloon to constant pressures: 20, 30, 40, 50, 60, and 80 mmHg for 20 seconds followed by 2-minute rest. Net value for each distension period was calculated by subtracting the baseline value derived from the average area under the curve for 20 seconds before and 20 seconds after the distention period. Additional details are provided in the Supplementary Materials.

Tissue Isolation

About a 6-cm length of the distal colon was opened along the mesentery. Mucosa/submucosa was separated from the muscularis externa. Tissues were either snap-frozen in liquid nitrogen or divided into 8 strips and washed in Hanks' balanced salt solution and incubated in Dulbecco's modified eagle medium + 10% fetal calf serum + penicillin/streptomycin containing norepinephrine for 24 hours.

Electrophysiology

Under general 2% isoflurane anesthesia, the lipid soluble fluorescent dye, 1,1′dioleyl-3,3,3′,3′-tetramethylindocarbocyanine methanesulfonate (DiI; Invitrogen, Carlsbad, CA), 25 mg in 0.5 mL methanol was injected in 2 μL volumes at 8 to 10 sites in the distal colon wall starting at the pelvic girdle and moving toward the cecum. These sites were within the colon segment used for colorectal distension. Thoracolumbar DRG neurons were isolated from DRG T13, L1, and L2, 16 days later. The methods used for isolation of DRG and current clamp recordings have been described previously[11] (see Supplementary Materials for additional details).

Intrathecal Catheter/Osmotic Pumps

K252a, a nonspecific antagonist of tyrosine kinase receptors including the high-affinity tropomyosin-related kinase A (trkA) receptors,[12] or previously validated antisense and mismatched oligonucleotides[13] were administered by osmotic pump attached to an intrathecal catheter. Please see Supplementary Materials for additional details.

NGF neutralizing antibody (16 μg/kg intraperitoneally in 0.5 mL in phosphate buffer saline) was purchased from R&D Systems (Minneapolis, MN). Control rats received phosphate-buffered saline containing an equal concentration of goat nonimmune serum.

Please see Supplementary Materials for additional details on mast cell counts, myeloperoxidase (MPO), and tryptase measurements, and pharmacological reagents.

Statistics

Data are expressed as mean ± standard error of mean. One-way analysis of variance followed by Fisher post-hoc analysis and *t* test were used for comparison of means. The effect of HeCS and treatments was analyzed using two-way repeated measures analysis of variance.

Results

Chronic Stress-Induced Visceral Hypersensitivity Is Associated With Increase in Excitability of Colon-Specific Thoracolumbar DRG Neurons

We found that 9-day HeCS significantly increases the visceromoter response to graded CRD at pressures of 40, 50, 60, and 80 mmHg, compared to prestressed baseline response (Figure 1*A*) (n = 14 rats). The increase in visceromoter response persists for at least 8 hours after the last stressor, but it returns to basal levels 24-hours post-HeCS. By contrast, 9-day sham stress in age-matched control rats had no significant effect on the visceromoter response to CRD (Figure 1*B*) at 8 and 24 hours (n = 10 rats). However, there was a significant decline in the visceromotor response to CRD in sham stressed rats at 7 days compared to baseline at 40, 50, 60, and 80 mmHg.

Acutely dissociated thoracolumbar colon-specific afferent neurons, identified by the presence of retrograde label DiI