Corticosterone Mediates Reciprocal Changes in CB 1 and TRPV1 Receptors in Primary Sensory Neurons in the Chronically Stressed Rat

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BACKGROUND & AIMS: Chronic stress is associated with visceral hyperalgesia in functional gastrointestinal disorders. We investigated whether corticosterone plays a role in chronic psychological stress-induced visceral hyperalgesia. **METHODS:** Male rats were subjected to 1-hour water avoidance (WA) stress or subcutaneous corticosterone injection daily for 10 consecutive days in the presence or absence of corticoid-receptor antagonist RU-486 and cannabinoid-receptor agonist WIN55,212-2. The visceromotor response to colorectal distension was measured. Receptor protein levels were measured and whole-cell patch-clamp recordings were used to assess transient receptor potential vanilloid type 1 (TRPV1) currents in L6–S2 dorsal root ganglion (DRG) neurons. Mass spectrometry was used to measure endocannabinoid anandamide content. **RESULTS:** Chronic WA stress was associated with visceral hyperalgesia in response to colorectal distension, increased stool output and reciprocal changes in cannabinoid receptor 1 (CB1) (decreased) and TRPV1 (increased) receptor expression and function. Treatment of WA stressed rats with RU-486 prevented these changes. Control rats treated with serial injections of corticosterone in situ showed a significant increase in serum corticosterone associated with visceral hyperalgesia, enhanced anandamide content, increased TRPV1, and decreased CB1 receptor protein levels, which were prevented by co-treatment with RU-486. Exposure of isolated control L6-S2 DRGs in vitro to corticosterone reproduced the changes in CB1 and TRPV1 receptors observed in situ, which was prevented by co-treatment with RU-486 or WIN55,212-2. **CONCLUSIONS: These results support a novel role for corticosterone to modulate CB1 and TRPV1-receptor pathways in L6-S2 DRGs in the chronic WA stressed rat, which contributes to visceral hyperalgesia observed in this model.**

Keywords: Visceral Hyperalgesia; Visceral Motor Response; Water Avoidance Stress; Dorsal Root Ganglion.

A substantial number of studies support the association of acute and chronic stress with lowered pain sensation thresholds and visceral hypersensitivity, increased motility, altered brain circuitry, and hypothalamic–pituitary–adrenal (HPA) axis activity.[1] Several pathophysiological mechanisms have been implicated in the development of this stress-induced visceral hyperalgesia, including defective activation of descending opioidergic pathways,[2] up-regulation of the substance P/neurokinin-1 receptor system, and the corticotrophin releasing factor (CRF)/CRF1-receptor pathway.[3,4] The CRF signaling pathway has been studied extensively in stress-induced colonic motor activity using CRF and CRF-receptor agonists and antagonists. For instance, CRF administration mimicked stress-induced visceral hyperalgesia in rats, which was blocked by injection of CRF antagonists.[5] A recent clinical study involving irritable bowel syndrome patients showed that administration of a CRF antagonist improved gastrointestinal motility and visceral hypersensitivity in response to gut stimulation compared with healthy controls.[6] However, other clinical studies using CRF antagonists have not shown efficacy in the treatment of major depression or in the improvement of colonic transit and bowel function in female irritable bowel syndrome patients.[7,8]

Although the release of brain CRF induces the secretion of adrenocorticotropic hormone and glucocorticoids including corticosterone, few studies have focused on a potential role for glucocorticoids in modulating visceral sensation. Recently, increased adrenocorticotropic hormone and corticosterone levels were observed in stressed rats that showed visceral hyperalgesia.[9] Myers et al[10] observed that corticosterone administration in the rat amygdala induced anxiety-like behavior associated with decreased thresholds for visceral and somatic pain. However, the role of glucocorticoids in stress-induced visceral hyperalgesia remains largely unknown, particularly as it applies to primary afferent neurons.

Abbreviations used in this paper: AEA, endocannabinoid anandamide; CB1, cannabinoid receptor 1; COX-2, cyclooxygenase-2; CRD, colorectal distension; CRF, corticotrophin releasing factor; CTB, chlorotoxin B; DRG, dorsal root ganglion; EMG, electromyography; FAAH, fatty acid amide hydrolase; HPA, hypothalamic-pituitary-adrenal; IR, immunoreactivity; TRPV1, transient receptor potential vanilloid type 1; VMR, visceromotor response; WA, water avoidance; WIN, (R)-(+)-WIN 55,212-2 mesylate salt.

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Accumulating evidence suggests that the endocannabinoid (CB) system is involved in the regulation of HPA axis and gastrointestinal function. Treatment with cannabinoid receptor 1 (CB1)-receptor agonist suppresses stress-induced corticosterone release in rats.[11] Increased levels of adrenocorticotrophin and corticosterone were observed in CB1 knockout mice, which displayed an enhanced susceptibility to chronic variable stress.[12] It is noteworthy that endocannabinoids, such as anandamide, also act as agonists at other receptors, including the transient receptor potential vanilloid type I (TRPV1),[13] which plays an important role in pain signaling. TRPV1 expression and function has been described in dorsal root ganglion (DRG) neurons that innervate the gastrointestinal tract, supporting a role for the TRPV1 channel in visceral sensation and nociception.[14] Recently, we reported that the chronic water avoidance (WA) stress rat model showed visceral hyperalgesia associated with an increase in endocannabinoid (anandamide) content, and reciprocal changes in TRPV1 (increased levels) and CB1- (decreased levels) receptor levels in L6-S2 DRG neurons.[1] A subpopulation of these neurons innervate the colon. However, it is not known whether the reciprocal changes in TRPV1- and CB1-receptor expression and function observed in chronic stress are linked to the HPA axis. The aim of the current study was to investigate the effect of corticosterone on TRPV1 and CB1 receptor expression and function in vivo in DRG neurons innervating the colon in control and WA stressed rats and to perform parallel in vitro studies examining the effect of corticosterone on TRPV1 and CB1-receptor expression in control DRG explants.

Materials and Methods

Animals

Male Sprague–Dawley rats (200–220 g) were obtained from Charles River Laboratories (Wilmington, MA). Animals were housed in an animal facility that was maintained at 22°C with an automatic 12-hour light/dark cycle. All experiments were approved by the University of Michigan Committee on Use and Care of Animals according to National Institutes of Health guidelines. The experimenter was blinded to animal treatment during behavioral experiments.

WA Stress Paradigm

Repeated WA stress was performed in adult rats as described previously.[15] Briefly, the rats were subjected to WA stress for 1 hour daily at 9 to 11 AM in the morning for 10 consecutive days corresponding to the chronic stress protocol. In a separate study, several groups of rats were injected subcutaneously with 2 mg/kg RU-486 (Sigma-Aldrich Corp, St. Louis, MO) in sesame oil or sesame oil only consecutively for 10 days during the WA stress procedure. This dose of RU-486 was chosen based on a review of the literature that indicated it was associated with an optimal effect to preferentially antagonize the corticoid receptor in situ in the rat and minimize other known nonspecific actions.[16,17]

Administration of Corticosterone In Situ and In Vitro

To reproduce the serum corticosterone level observed in the stressed rats, corticosterone (Sigma-Aldrich), ranging from 0.5–10 mg/kg in sesame oil with 200 μL volume, was injected subcutaneously at 9 AM to 11 AM in the morning daily for 10 days in a group of healthy control rats. Blood was collected 60 minutes after injection and serum corticosterone levels were measured to determine the correct dose to inject in control rats to reproduce the level observed in the WA stressed rats. Several groups of control rats then were injected subsequently with optimal doses of corticosterone (3 mg/kg; 200 μL) or vehicle in the presence and absence of RU-486 (2 mg/kg; 200 μL) or endocannabinoid-receptor agonist WIN55,212-2 (WIN; Sigma-Aldrich; 200 μL in 10% DMSO/5% Tween 80/85% saline) at a dose of 2 mg/kg during the repeated injection period as described previously.[15] For in vitro studies, L6–S2 DRGs from control rats were incubated with corticosterone (10 μmol/L) in the presence or absence of RU-486 (500 nmol/L) or WIN (1 μmol/L) for 16 hours. The DRGs then were collected for Western blot analysis.

Measurement of Serum Corticosterone

Corticosterone measurement was conducted as described previously.[15] Briefly, blood (200 μL) was collected as described[18] in ethylenediaminetetraacetic acid–containing tubes from the tail vein in the rat before the daily WA exposure or corticosterone injection, and right after 1 hour of WA stress or 1 hour after corticosterone injection between 9 and 11 AM in the morning on days 1 and 10 of the stress or injection procedure. Serum was collected by centrifugation at 3000*g* for 10 minutes and stored at –80°C before analysis. Total corticosterone in serum samples from stressed rats and control rats was analyzed by the corticosterone EIA kit (Cayman Chemical Co, Ann Arbor, MI) according to the manufacturer's instructions.

Visceromotor Response to Colorectal Distention

Animals were habituated in the testing room and placed in the plexiglass cylinders for 30 minutes per day for 3 consecutive days before experiments. The plexiglass cylinder was used for partial restraint during the colorectal distension (CRD) experiments. Measurement of visceromotor response (VMR) to visceral stimulus was conducted in rats on days 0 and 11 of the chronic WA stress procedure or repeated-injection procedure as described.[15,19] A series of CRDs was conducted to constant