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Corticosterone Mediates Reciprocal Changes in CB1 and TRPV1 Receptors in Primary Sensory Neurons in the Chronically Stressed Rat

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Abstract

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 (VMR) to colorectal distension (CRD) was measured. Receptor protein levels were measured and whole-cell patch-clamp recordings employed to assess TRPV1 currents in L6-S2 DRG neurons. Mass spectrometry was used to measure endocannabinoid anandamide content.

Results—Chronic WA stress was associated with visceral hyperalgesia in response to CRD, increased stool output and reciprocal changes in 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* demonstrated 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.

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Author contribution:

Dr. Shuangsong Hong – study design and supervision, acquisition and analysis of data, drafting of the manuscript;

Dr. Gen Zheng - acquisition and analysis of data;

Dr. Natasha Snider – acquisition of endocannabinoid data

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Dr. John W Wiley - study concept and supervision, obtained funding.

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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 IBS patients demonstrated 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 demonstrated efficacy in the treatment of major depression or in the improvement of colonic transit and bowel function in female IBS patients.^{7,8}

Although the release of brain CRF induces the secretion of adrenocorticotrophic hormone (ACTH) and glucocorticoids including corticosterone, few studies have focused on a potential role for glucocorticoids in modulating visceral sensation. Recently, elevated ACTH and corticosterone levels were observed in stressed rats that demonstrated 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.

Accumulating evidence suggests that the endocannabinoid (CB) system is involved in the regulation of HPA axis and gastrointestinal function. Treatment with 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 that 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 demonstrated visceral hyperalgesia associated with elevation in endocannabinoid (anandamide) content, and reciprocal changes in TRPV1 (increased levels) and CB1 (decreased levels) receptor levels in L6-S2 DRG neurons.¹⁵ 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 the animal facility that was maintained at 22°C with an automatic 12 h 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.

Water avoidance stress paradigm

Repeated water avoidance stress was performed in adult rats as described previously.¹⁵ Briefly, the rats were subjected to water avoidance stress for 1 hour daily at 9AM – 11AM in the morning for 10 consecutive days corresponding to the chronic stress protocol. In a separate study, several groups of rats were subcutaneously injected 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 review of the literature that indicated it was associated with optimal effect to preferentially antagonize the corticoid receptor *in situ* in the rat and minimize other known non-specific 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 mg/kg to 10 mg/kg in sesame oil with 200 µl volume, was injected subcutaneously at 9AM – 11AM in the morning daily for 10 days in a group of healthy control rats. Blood was collected 60 min 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 were then subsequently injected 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 the 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 µM) in the presence or absence of RU-486 (500 nM) or WIN (1 µM) for 16 hours. The DRGs were then collected for Western blot analysis.

Measurement of Serum Corticosterone

Corticosterone measurement was conducted as described previously.¹⁵ Briefly, blood (200 µl) was collected as described¹⁸ in EDTA-containing tubes from the tail vein in the rat before the daily water avoidance (WA) exposure or corticosterone injection, and right after 1 hour WA stress or 1 hour after corticosterone injection between 9 to 11AM in the morning on day 1 and day 10 of the WA protocol. Serum was collected by centrifugation at 3000g for 10 min and stored at –80°C prior to analysis. Total corticosterone in serum samples from stressed rats and control rats was analyzed by the Assay Designs' Correlate-EIA corticosterone enzyme immunoassay kit (Ann Arbor, MI) according to the manufacturer's instructions.

Visceromotor response (VMR) to colorectal distention (CRD)

Animals were habituated in the testing room and placed in the Plexiglas cylinders for 30 minutes per day for 3 consecutive days prior to experiments. Plexiglas cylinder was used for partial restraint during the CRD experiments. Measurement of VMR to visceral stimulus was conducted in rats on day 0 and 11 of the chronic WA stress procedure or repeated

injection procedure as described.^{15,19} A series of CRD was conducted to constant pressures of 20, 40, 60, 80 mm Hg. Each distention consisted three segments: a 20-second predistention baseline period, a 20-second distention period, and a 20-second period after termination of CRD with a 4-minute inter-stimulus interval. The electromyographic (EMG) activity was amplified and digitized using a SPIKE2/CED 1401 data acquisition interface. The responses were considered stable if there was less than 20% variability between 2 consecutive trials of each CRD. The EMG activity was rectified, and the increase in the area under the curve (AUC) of EMG amplitude during CRD over the baseline period before CRD was recorded as the response.

Electrophysiological recordings

Capsaicin-evoked TRPV1 current was recorded in acutely dissociated DRG neurons as described previously.²⁰ Patch clamp recordings were performed under the whole-cell mode at room temperature. Standard bath solution for whole-cell recordings contained (in mM) 140 NaCl, 5 KCl, 2 MgCl₂, 2 CaCl₂, 10 HEPES, 10 glucose, pH 7.4 (adjusted with NaOH). The internal solution consisted of (in mM) 140 CsCl, 5 EGTA, 1 MgCl₂, 10 HEPES, pH 7.4. Small-sized DRG neurons with cell capacitance less than 40 pF were voltage-clamped at -80 mV, and pipette resistance was compensated as necessary. Data were acquired using an Axopatch 200B amplifier (Axon Instruments Inc., Sunnyvale, CA), low pass filtered at 5 kHz, digitized at 10 kHz through a Digidata 1322A 16-bit data acquisition system, and recorded with pClamp version 8.2 software (Axon Instruments).

Statistical analysis

The electromyographic amplitudes, represented by calculating AUC, were normalized as percentage of baseline response for the highest pressure (80 mmHg) for each rat. The effects of stress and/or pharmacologic treatment on the VMR to CRD was analyzed by comparing the post-stress or post-treatment measurements with the baseline values at each distention pressure using a repeated-measures, two-way ANOVA followed by Bonferroni post-test comparisons. Unpaired Student's *t* test was used to examine the data for other studies. Results were expressed as means $\pm$ SE. *P*<0.05 was considered statistically significant.

RESULTS

Administration of the corticoid receptor antagonist RU-486 reduced hypersensitivity in chronic WA stress rats

Previously we demonstrated that the VMR to CRD and fecal output were significantly increased in the chronic WA stressed rats compared with sham-stressed controls, supporting a role for the HPA axis in chronic stress-induced visceral hyperalgesia.¹⁵ To further establish the involvement of the HPA axis in stress-induced visceral hyperalgesia, we assessed whether the corticoid receptor antagonist RU-486 had a preventative effect on the development of visceral hyperalgesia in WA stress rats. At the time of study, the weight gain of WA stress rats was 62.9 ± 2.6 g, significantly less than the value of 79.3 ± 8.3 g in sham stress rats (*P*<0.05; *n*=8), whereas animals in each group were initially of similar average weight. As shown in Figure 1A–B, chronic WA stress resulted in significant increases in VMR to CRD for the pressures from 20 to 80 mmHg compared with the baseline, consistent with our previous results (*P*<0.05). Repeated treatment with the antagonist RU-486 during the stress procedure significantly decreased the stress-induced increases in the VMR at CRD pressures from 40 to 80 mmHg (*P*<0.05). Treatment with RU-486 had no significant effect on VMR in control rats. Moreover, fecal pellet outputs were significantly increased on day 5 and day 10 in WA stressed rats compared with the controls. The increased fecal outputs in WA stress rats were significantly reduced after RU-486 treatment (Figure 1C, *P*<0.05).

Effect of RU-486 on CB1 and TRPV1 receptor protein levels *in situ*

In view of our previous results demonstrating reciprocal changes of CB1 (down-regulation) and TRPV1 (up-regulation) receptors in the WA stress rat, we examined whether these reciprocal changes of CB1 and TRPV1 in L6-S2 DRGs can be blocked by RU-486 treatment. As shown in Supplemental Figure 1, the double immunoreactivity (IR)-positive neurons for TRPV1 and CB1 were decreased to $10.2 \pm 2.1\%$ of total DRG neurons in WA stress rats whereas it was $18.2 \pm 3.4\%$ in controls ($P < 0.05$, $n = 6$). In WA stress rats treated with RU-486, the double IR-positive neurons for TRPV1 and CB1 was significantly increased to $17.3 \pm 3.2\%$ compared with non-treated stress rats ($P < 0.05$). Moreover, we carried out TRPV1/CB1 staining in colonic afferents labeled with CTB-FITC. In control rats, $33.0 \pm 5.6\%$ of the CTB-FITC labeled colonic neurons was also IR-positive for TRPV1 and this percentage increased to $58.3 \pm 3.3\%$ in stressed rats ($P < 0.05$; $n = 5$). Reciprocally, CB1 was observed in $65.0 \pm 5.1\%$ of colonic neurons and this percentage decreased significantly to $36.5 \pm 1.7\%$ in stressed rats ($P < 0.05$). RU-486 treatment during the stress phase largely blocked these differential changes in TRPV1 and CB1 in colonic neurons (Figure 2A). Quantitative densitometry of the immunoblot bands showed that the level of TRPV1 protein after RU-486 treatment in chronic WA stress rats was $112.3 \pm 3.4\%$ of the vehicle-treated controls while TRPV1 protein level in WA stress rats was $155.6 \pm 6.6\%$ of the controls. The difference in TRPV1 level between the WA stress rats and the RU-486 treated WA stress rats is significant ($P < 0.05$). In contrast, the level of CB1 protein decreased significantly to $62.5 \pm 12.3\%$ of the controls in L6-S2 DRGs in WA stress rats whereas its level increased to $96.4 \pm 8.9\%$ of the controls in WA stress rats with RU-486 treatment ($P < 0.05$; Figure 2B–C).

RU-486 treatment decreased capsaicin-evoked TRPV1 current in WA stress rats

Capsaicin-evoked TRPV1 current was recorded in acutely dissociated L6-S2 DRG neurons. As shown in Figure 3, the capsaicin ($1 \mu\text{M}$) evoked TRPV1 current was significantly higher in DRG neurons from WA stress rats compared with the controls. The mean current density was $208.9 \pm 20.6 \text{ pA/pF}$ in control rats and $321.8 \pm 32.8 \text{ pA/pF}$ in WA stress rats, respectively. In DRG neurons from RU-486 treated WA stress rats, the mean current density was significantly reduced to $225.7 \pm 22.3 \text{ pA/pF}$ ($P < 0.05$, $n = 8$). This result indicates the increased function of TRPV1 in L6-S2 DRG neurons from the WA stress rats was nearly reduced to the level of the sham controls after RU-486 treatment, consistent with the previous result showing the recovery of TRPV1 protein expression in DRGs in WA stress rats after RU-486 treatment.

Administration of corticosterone in control rats *in situ* induced visceral hyperalgesia

Repeated injection of corticosterone significantly elevated the serum corticosterone level in the control rat on day 1 and day 10 as shown in Figure 4A ($P < 0.05$; $n = 8$). The serum corticosterone level was $296.6 \pm 46.6 \text{ ng/ml}$ on day 1 and $391.7 \pm 41.36 \text{ ng/ml}$ on day 10, respectively, in corticosterone-injected rats 1 hour after corticosterone injection, similar to the levels ($303.6 \pm 25.6 \text{ ng/ml}$ on day 1 and $329.0 \pm 35.5 \text{ ng/ml}$ on day 10 after 1 h WA stress) observed in WA stress rats. It should be noted that vehicle-injected control rats displayed a higher level of serum corticosterone on day 10 after injection compared with its basal level ($P < 0.05$). However, the difference in corticosterone level after injection on day 10 between vehicle- and corticosterone-injected rats was also significant ($P < 0.05$). Rats exposed to repeated corticosterone showed a similar weight gain compared with the control group receiving the vehicles only during the 10-day period. Consistent with the increased VMR to CRD observed in WA stress rats, the EMG amplitude was significantly increased in corticosterone-injected rats compared with the vehicle-injected controls as shown in Figure 4B–C. At the CRD pressures of 40, 60 and 80 mmHg, the EMG amplitude was $58.7 \pm 11.6\%$, $80.8 \pm 14.7\%$ and $92.6 \pm 16.0\%$ in corticosterone-injected rats compared with the

baseline respectively, while it decreased significantly to $23.3 \pm 12.8\%$, $32.2 \pm 16.4\%$ and $38.5 \pm 15.4\%$ in rats injected with both corticosterone and RU-486 ($P < 0.05$, $n = 8$). There were no significant differences in the EMG amplitudes in response to CRD between the vehicle-injected control rats and the non-treated controls.

Corticosterone administration in control rats *in situ* induced reciprocal changes in CB1 and TRPV1 receptors

As shown in Supplemental Figure 2, the TRPV1 immunoreactivity intensity was increased in whole L6-S2 DRGs from corticosterone-injected rats compared with the controls. In DRGs from control rats, the TRPV1 intensity was 73.8 ± 8.9 , whereas it was significantly increased to 105.2 ± 6.7 in DRGs from corticosterone-injected animals ($P < 0.05$). On the other hand, the CB1 immunoreactivity intensity was significantly decreased in L6-S2 DRGs from corticosterone-injected rats (88.4 ± 12.1) compared with controls (153.2 ± 10.2) ($P < 0.05$). Moreover, as shown in Figure 5A, TRPV1 was observed in $32.6 \pm 4.3\%$ of retrograde labeled colonic neurons in control rats and this percentage increased to $50.2 \pm 8.7\%$ in corticosterone-injected rats ($P < 0.05$; $n = 5$). CB1 was observed in $63.2 \pm 5.4\%$ of colonic DRG neurons and this percentage decreased significantly to $40.8 \pm 2.2\%$ in corticosterone-injected rats ($P < 0.05$). The protein level of TRPV1 increased 44% in corticosterone-injected rats compared with the controls and decreased significantly in corticosterone-injected rats treated with RU-486 (Figure 5B–C; $P < 0.05$). In addition, Western blot analysis showed that CB1 protein level decreased 38% in corticosterone-injected rats compared to the controls ($P < 0.05$) and reversed to the normal level in rats treated with the corticoid antagonist RU-486 or endocannabinoid receptor agonist WIN (Figure 5B–C).

Corticosterone administration *in situ* induced increase in anandamide content and decreased levels of endocannabinoid degradation enzymes

We examined the protein levels of endocannabinoid degradation enzymes in L6-S2 DRGs from both chronic WA stress and corticosterone-injected rats. As depicted in Figure 6A–B, the protein levels of both cyclooxygenase-2 (COX-2) and fatty acid amide hydrolase (FAAH) decreased significantly in DRGs from WA stress rats. The levels of COX-2 and FAAH decreased $48 \pm 10\%$ and $42 \pm 12\%$ in DRGs from WA stress rats compared with the controls, respectively ($P < 0.05$; $n = 6$). Similarly, repeated corticosterone injection in control rats reproduced the changes in COX-2 and FAAH in L6-S2 DRGs that were observed in the WA stress rats. The levels of COX-2 and FAAH decreased $49 \pm 16\%$ and $37 \pm 8\%$ in DRGs from corticosterone-injected rats compared with the vehicle-treated controls, respectively ($P < 0.05$; $n = 6$). In addition, the endocannabinoid anandamide content increased 42% in L6-S2 DRGs from corticosterone-injected rats compared with controls (Figure 6C). This percentage increase in anandamide content was similar to the significant increase in anandamide observed in WA stressed rats.¹⁵

Treatment of isolated control DRGs *in vitro* with corticosterone resulted in decreased CB1 and increased TRPV1 receptor levels

As shown in Figure 7A–B, corticosterone treatment induced significant down-regulation of CB1 receptor and up-regulation of TRPV1 receptor in control DRGs *in vitro*. The level of CB1 decreased 58% and the level of TRPV1 increased 39% compared with the non-treated controls, respectively ($P < 0.05$). RU-486 and WIN treatment largely blocked these effects of corticosterone on the expression of CB1 and TRPV1 proteins. These results support the interpretation that corticosterone can induce reciprocal alterations in the protein levels of CB1 and TRPV1 receptors in isolated healthy DRG neurons similar to the pattern observed in the corticosterone-injected rats *in vivo*.

DISCUSSION

The data presented in the current report provides compelling support for the interpretation that visceral hyperalgesia observed in chronic WA stress rats involves activation of the HPA axis and that corticosterone plays a potentially pivotal role in this process via modulation of the endocannabinoid (CB1) and TRP (TRPV1) receptor pathways.

Altered HPA function has been reported in rodents under stressful conditions and in patients with IBS symptoms. Nevertheless, there are few studies that have specifically examined the potential role of corticosteroids in relation to visceral hyperalgesia under chronic stressful situations. We hypothesized that corticosterone plays a potentially important role in the regulation of visceral sensitivity under chronic stress conditions based, in part, on a recent report that the corticoid receptor antagonist RU-486 prevented stress-induced increase in allodynia in mice with neuropathic pain.²¹ We observed that serial injections of corticosterone in healthy rats that reproduced the serum level observed in WA stress rats induced visceral hyperalgesia. The corticoid receptor antagonist RU-486 prevented visceral hyperalgesia in the chronic WA stress rats and in the corticosterone-injected control rats, supporting a role for corticosterone in modulating visceral sensitivity. Repeated administration of exogenous corticosterone in animals has been widely used to mimic the chronic stress. However, the amount of corticosterone administered is often supraphysiological.²² In the present study, we performed serial injections of corticosterone in healthy control rats to reproduce the serum levels observed in the chronic WA stress rats. The elevated corticosterone levels in chronic WA stress rats and the corticosterone-injected control rats were similar to that reported in rats experiencing chronic mild stress, which may represent a more pathophysiologically relevant and realistic replication of levels associated with stress-induced visceral hypersensitivity.²³ Employing this methodology, repeated administration of exogenous corticosterone induced similar changes as the chronic WA stress procedure not only at the cellular level, *i.e.* the reciprocal changes in CB1 and TRPV1, decreased levels of the endocannabinoid degradation enzymes FAAH and COX-2 and increased anandamide content in primary afferent neurons, but also at the behavioral level, *i.e.* the development of visceral hyperalgesia. Moreover, we delivered the corticosterone to rats via subcutaneous injection that allows for better control of the circulating glucocorticoid levels, so that they better reproduce the levels observed in the chronic WA stress rats. Other methods of administration, *i.e.* via drinking water and through pellet implantation, may yield a more flattened variable hormone level throughout the day.²⁴ However, it is possible that the injection process itself can be a stressor and lead to release of endogenous corticosterone, which could affect the serum corticosterone level. Nevertheless, the behavioral and cellular changes we observed are quite similar in the corticosterone-injected control rats and chronic WA stress rats, supporting that repeated corticosterone administration is a valid method for studying the interaction between stress, glucocorticoids and visceral sensitivity.

Stress hormones such as glucocorticoids can enhance pain response behaviors.^{21,25} Paradoxically, corticosteroids are used in pharmacological doses to treat inflammatory conditions that activate pain pathways. Recent studies support a role for exogenous glucocorticoids in pain modulation. For example, it has been reported that administration of dexamethasone exacerbates neuropathic pain behaviors²⁶ but prevents visceral hyperalgesia induced by protease-activated receptor-2 (PAR-2) activation,²⁷ suggesting that different mechanisms are activated by glucocorticoids in different models of pain induction. Glucocorticoid receptors are progressively activated during stress and at the peaks of the diurnal rhythm for substantial receptor activation.²⁸ We observed that corticosterone administration down-regulated expression of endocannabinoid degradation enzymes FAAH and COX-2 in DRG neurons both *in vivo* and *in vitro*, most likely due to the activation of

glucocorticoid receptor by corticosterone, since glucocorticoid receptor antagonist RU-486 blocked these effects. It has been reported previously that the glucocorticoid receptor regulates FAAH and COX-2 gene expression.^{29,30} Decreased FAAH activity has also been reported in the ventral striatum in mice subjected to chronic restraint stress.³¹ Thus, the decreased expression/function of these endocannabinoid degradation enzymes might account for the increased endocannabinoid content in DRG neurons in chronic WA stress rats and the subsequent regulation of CB1 and TRPV1 receptors.¹⁵ Therefore, our data support that corticosterone can act directly on primary afferent (DRG) neurons to produce changes in the expression of CB1 and TRPV1 receptors.

The levels of endocannabinoids and their receptors are regulated in the brain-gut axis, suggesting a role for endocannabinoids in the modulation of visceral pain.³² Supporting this, we observed that the CB1 receptor decreased in DRGs innervating the colon in chronic WA stress rats and in the corticosterone-injected healthy rats which were associated with visceral hyperalgesia. The expression levels of endocannabinoid degradation enzymes FAAH and COX-2 were significantly decreased in L6-S2 DRGs in WA stress rats and in corticosterone-injected healthy rats, resulting in the increase in endocannabinoid content in DRGs, which may consequently induce the desensitization or down-regulation of the endocannabinoid receptors.^{15,33} Endocannabinoids such as anandamide are synthesized “on demand” at the cell surface membrane and act locally as paracrine/autocrine factors. The endogenous expression of synthetic/degradation enzymes and receptor targets at the cellular level are likely to play a particularly important role in the differential, tissue-specific actions of endocannabinoids in DRG neurons, supporting a peripheral action for endocannabinoids but not excluding the likely important role of endocannabinoids in the central nervous system.

One of the pathways that endocannabinoids induce peripherally-mediated antinociception is *via* the regulation of peripheral nociceptor activity. It has been suggested that certain cannabinoids regulate nociceptors by inhibition of adenylyl cyclase, reducing production of the cyclic adenosine monophosphate (cAMP) and activating Ca^{2+} -permeable channels, leading to a desensitization of nociceptor activities. In colonic DRG neurons, the decreased expression of CB1 receptor may thus contrarily release the inhibitory effect in adenylyl cyclase by less endocannabinoid receptor activation, and then increase the cAMP level and promote phosphorylation of TRPV1 channel to enhance the TRPV1 function under stress conditions. Moreover, apart from activation of cannabinoid receptors, anandamide also directly activates TRPV1.¹³ Our previous results showed that treatment of anandamide was associated with increased TRPV1 expression in control DRGs *in vitro*.¹⁵ supporting that the increased anandamide content in WA stressed and corticosterone-injected rats can enhance TRPV1 function and pain response. Consistent with this interpretation, a recent paper reported that endocannabinoids produced in the spinal cord can enhance pain by dampening the synaptic activity of inhibitory interneurons that prevent the perception of innocuous stimuli as painful.³⁴ This finding may partially explain why, in human trials, cannabinoid drugs have minor effects for treatment of most types of acute and postsurgical pain but are effective for some chronic pain states.^{35,36} On the other hand, we believe that endocannabinoids such as anandamide and the CB1 receptor agonist WIN act via different signal transduction pathways because treatment of control DRGs with anandamide reproduced the reciprocal changes in CB1 and TRPV1 receptor expression observed in the stressed rat, whereas the WIN compound did not.¹⁵ It is likely that exogenous cannabinoid WIN dephosphorylates and desensitizes TRPV1, via the TRPA1 receptor and Ca^{2+} -dependent calcineurin (phosphatase 2B)-induced dephosphorylation of the channel, and contributes to the peripheral antihyperalgesic effects of cannabinoids.^{37,38} It will be interesting to investigate the detailed mechanisms underlying the receptor-receptor interactions and signal transduction pathways between CB1 and TRPV1 under chronic stress conditions.

In summary, our data suggest that visceral hyperalgesia associated with chronic WA stress is mediated, at least in part, by a corticosterone-dependent pathway in the rat. Treatment of healthy control rats with corticosterone that reproduced the serum levels observed in the chronically WA stressed rats resulted in visceral hyperalgesia in response to colorectal distension that was prevented by the corticoid receptor antagonist RU-486. Corticosterone treatment in control rats *in situ* and control DRGs explants *in vitro* reproduced the changes in CB1 (down-regulation) and TRPV1 (up-regulation) receptor levels observed in the chronic WA stress rat which were prevented by RU-486. Therefore, corticosterone likely plays an important role in regulating the reciprocal changes in CB1 and TRPV1 receptor expression and function in DRG neurons innervating the colon and concomitant visceral hyperalgesia in the chronic WA stress rat model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The abbreviations used in this paper

CB1	cannabinoid receptor 1
COX-2	cyclooxygenase-2
CRD	colorectal distension
CTB	chlorotoxin B
DRG	dorsal root ganglion
EMG	electromyography
HPA	hypothalamic-pituitary-adrenal
FAAH	fatty acid amide hydrolase
IR	immunoreactivity
TRP	transient receptor potential
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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Figure 1.

Effect of the corticoid receptor antagonist RU-486 (2 mg/kg; s.c.) on the VMR to CRD. (A) Representative EMG recordings depicting the VMR to CRD at 40 mmHg in control rats, WA stress rats, and WA stress rats treated with RU-486 during the stress procedure. (B) EMG amplitude expressed as mean change from baseline in control and WA stress rats treated with or without RU-486. (C) Effect of RU-486 on distal colonic motor function in WA stress rats. Data are expressed as mean $\pm$ SE, $n=8$ in each group. *, $P<0.05$ between control and WA stress rats; +, $P<0.05$ between WA stress rats receiving RU-486 or vehicle.

Figure 2.

Treatment with RU-486 prevented the reciprocal changes in TRPV1 and CB1 receptors in DRG neurons innervating the colon in WA stress rats. (A) Representative immunofluorescence images (left) of double immunoreactivity (IR)-positive neurons for CB1 (red) and TRPV1 (red) in colonic DRG neurons retrograde labeled with CTB-FITC (green) from control, WA stress, and RU-486 treated WA stress rats. Scale bar: 40 μ m. Bar graph (right) depicting the changes in the percentage of double IR-positive neurons for CB1 and TRPV1 with CTB-FITC. (B) Immunoblots depicting the protein levels of CB1 and TRPV1 in L6-S2 DRGs in WA stress rats in the absence and presence of RU-486. (C) Histogram showing the immunoblot band density of CB1 and TRPV1 protein (n=5). *, $P < 0.05$.

Figure 3. DRG (L6-S2) neurons from WA stress rats demonstrated increased capsaicin-evoked TRPV1 current that was prevented by treatment with RU-486 *in situ*. (A) Representative inward current traces of neurons responding to the application of 1 μ M capsaicin (2 s) in L6-S2 DRG neurons isolated from control, WA stress and RU486-treated stress rats. (B) Bar graph depicting the mean peak current density of the capsaicin-evoked currents (n=8). *, $P < 0.05$.

Figure 4. Serial injections of control rats with corticosterone *in situ* induced visceral hyperalgesia that was prevented by co-treatment with RU-486. (A) The level of serum corticosterone was significantly increased in rats following repeated injection with corticosterone. (B) Representative EMG recordings depicting the VMR to CRD at 40 mmHg in control rats, corticosterone-injected rats, and rats treated with both corticosterone and RU-486. (C) EMG amplitude expressed as mean change from baseline in corticosterone-injected rats in the absence or presence of RU-486 (n=8). *, $P < 0.05$.

Figure 5. Serial injections of corticosterone induced reciprocal changes in CB1 and TRPV1 expression in L6-S2 DRG neurons. (A) Immunofluorescence staining of double immunoreactivity (IR)-positive neurons for TRPV1 (red) and CB1 (red) in colonic DRG neurons retrograde labeled with CTB-FITC (green) from control and serially treated corticosterone (CORT)-injected rats. Scale bar: 30 μ m. (B) Co-treatment with RU-486 or WIN prevented the changes in TRPV1 and CB1 protein expression in the corticosterone-injected control rats. (C) Statistical analysis of the immunoblot band intensity of TRPV1 and CB1 receptors (n=5). *, $P < 0.05$.

Figure 6. Changes in endocannabinoid degradation enzymes and anandamide content in L6-S2 DRGs. (A) Western blot analysis showed decreased expression levels of COX-2 and FAAH protein in L6-S2 DRGs in both chronic WA stress rats and corticosterone-injected control rats compared with untreated controls. (B) Histogram depicting the relative changes of COX-2 and FAAH protein levels in chronic WA stress rats and corticosterone-injected control rats (n=6). (C) The bar graph illustrated the increase in anandamide content in L6-S2 DRGs in rats following corticosterone-injection compared to controls receiving vehicle only. L6-S2 DRGs from 10 rats in each group were combined for sample extraction. *, $P < 0.05$.

Figure 7.

Effects of corticosterone in the absence and presence of RU-486 or WIN on the levels of CB1 and TRPV1 in L6-S2 DRGs isolated from control rats *in vitro*. (A) Representative Western blots for TRPV1 and CB1 in L6-S2 DRGs isolated from control rats treated with corticosterone in the absence and presence of RU-486 (500 nM) or WIN (1 μ M). (B) Exposure of control DRG explants to corticosterone significantly decreased the expression level of CB1 and increased the level of TRPV1 receptor protein levels ($P < 0.05$, $n = 4$). Co-treatment with RU-486 or WIN blocked corticosterone-induced changes in CB1 and TRPV1. *, $P < 0.05$.