Published in final edited form as:

J Pain. 2008 November ; 9(11): 991–998. doi:10.1016/j.jpain.2008.05.006.

Footshock-Induced Urinary Bladder Hypersensitivity: Role of Spinal Corticotropin-Releasing Factor Receptors

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Abstract

Stress-induced hyperalgesia (SIH), a common clinical observation associated with multiple painful diseases including functional urinary disorders, presently has no mechanistic explanation. Using a Footshock treatment, a classical stressor, to magnify physiological responses in a model of urinary bladder pain, we examined one potential group of mediators of SIH, the corticotropin-releasing factor (CRF)-related neuropeptides. Exposure to a Footshock treatment produced bladder hypersensitivity in female Sprague Dawley rats, manifested as significantly more vigorous visceromotor responses (VMRs) to urinary bladder distension (UBD) compared to rats that were exposed to a Non-footshock treatment. This bladder hypersensitivity was significantly attenuated by blocking spinal CRF₂ receptors but not CRF₁ receptors. Furthermore, spinal administration of urocortin 2, a CRF₂ receptor agonist, augmented UBD-evoked VMRs in a way similar to what was observed following exposure to Footshock, an effect significantly attenuated by pretreatment with spinal aSVG30, a CRF₂ receptor antagonist. Surprisingly, neither spinal administration of CRF nor the CRF₁ receptor antagonist, antalarmin, had an effect on bladder nociceptive responses. The results of the present study not only provide further support for a role of stress in the exacerbation of bladder pain, but also implicate spinal urocortins and their endogenous receptor, the CRF₂ receptor, as potential mediators of this effect.

Perspective: This study presents evidence that spinal urocortins and CRF₂ receptors are involved in stress-induced hypersensitivity related to the urinary bladder. This provides a basis for investigating how urocortins mediate SIH, ultimately leading to more effective treatment options for patients suffering from painful bladder syndromes as well as stress-exacerbated chronic pain.

Keywords

Urinary bladder; hypersensitivity; visceral; urocortin; corticotropin-releasing factor

Introduction

A prominent role for stress in the pathophysiology and presentation of multiple clinical pain states has been well-documented^{9,11,13,54,64}. Acute exposure to numerous stressors can produce an inhibition of cutaneous pain-related responses, a phenomenon referred to as stress-induced analgesia^{1,59,63}. However, when stress is perceived as uncontrollable, chronic or unpredictable, it may induce long-term pathophysiological changes. Instead of being inhibited, responses to painful stimuli become augmented, a phenomenon known as stress-induced hyperalgesia.

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Clinical observations have shown that anxiety and stress may worsen symptoms in functional urinary disorders, such as interstitial cystitis/painful bladder syndrome (IC/PBS) ^{4,31}. This disorder primarily affects the female population and is characterized by pelvic and/or perineal pain, urinary urgency and frequency, and nocturia. A majority of patients report symptom exacerbation by clinical stress, and experimental stress increases bladder pain and urgency in these individuals ^{24,29}. As severity of the disease increases, the relationship between stress and symptom manifestation becomes even more evident ⁴⁵. Thus, there is clearly a relationship between stress and the exacerbation of bladder pain.

One way in which stress may worsen painful urinary bladder disorders is via mechanisms that involve the corticotropin-releasing factor (CRF) family of peptides. CRF was first characterized for its role in activation of the hypothalamic-pituitary-adrenocortical (HPA) axis, a hallmark of the stress response, and has been subsequently found to modulate autonomic function and behavior ³⁷. The discovery of structurally related peptides in the fish and frog led to the identification of the second CRF-related peptide in mammals, urocortin 1 ^{26,35,57}. The CRF family now includes urocortins 1, 2 and 3. CRF binds with high affinity to the CRF₁ receptor and with almost no affinity to the CRF₂ receptor, despite its name ^{10,57}. Urocortin 1 binds with high affinity to both CRF₁ and CRF₂ receptors, and urocortins 2 and 3 bind with high affinity to CRF₂ receptors with almost no affinity for the CRF₁ receptor ^{27,39,42}. The urocortins therefore act as the only identified endogenous agonists for CRF₂ receptors at physiological concentrations.

A number of studies indicate that the CRF-related peptides might modulate visceral sensitivity related to stress ^{12,47,48,49}. Systemic administration of CRF₁ receptor antagonists has been demonstrated to attenuate visceral perception and anxiety after acute colorectal distension in rats and abolishes restraint stress-induced colonic hypersensitivity ^{48,49}. In humans, peripheral administration of a non-selective CRF receptor antagonist improves gastrointestinal motility, visceral perception and negative mood in response to gut stimulation ⁴⁷. Intestinal motility, abdominal symptoms and adrenocorticotrophic hormone levels are enhanced following systemic administration of CRF; these effects are more exaggerated in individuals with irritable bowel syndrome ¹². With regard to the urinary bladder, administration of CRF receptor agonists and antagonists alters cystometric parameters ^{21,22,38,40}, and CRF-related neuropeptides are abundantly expressed in areas involved in the control of micturition (Barrington's nucleus, lumbosacral areas of the spinal cord) ^{17,25}. Following stress or bladder inflammation, increases in CRF receptor expression have been detected in these areas and in the bladder itself ^{17,25}, and nociceptive responses related to the bladder are enhanced ^{41,44}.

These converging lines of evidence implicate CRF-related neuropeptides as potential mediators of stress-induced bladder hyperalgesia. The present studies therefore sought to further investigate the role of CRF receptors in stress-related augmentation of nociceptive reflexes related to the bladder. Based on the pre-existing literature related to the gut, it was expected that CRF₁ receptor agonists would be the most likely candidates for involvement. However, this did not prove to be the case.

Materials and Methods

Animal subjects

Female Sprague Dawley rats 11-12 weeks of age (Harlan, Prattville, AL) were used in experiments. Female rats were chosen since disorders of the urinary bladder that are associated with pain are prevalent in and primarily affect the female population. Estrous cycle was not controlled for in these experiments. Food and water were available on an *ad libitum* basis. A 12:12-h light-dark cycle, where lights were off between 6:00 p.m. and 6:00 a.m., was maintained. There was one week between the time of the animals' arrival and the start of any

experimental procedures. Animals were not exposed to any handling or cage habituation prior to the start of experimental procedures. All protocols were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.

Footshock Paradigm

Electrical footshock is an established and readily controlled stressor^{18,43} that has been used to produce behavioral and neurochemical changes in a variety of experiments. Factors such as timing, predictability, frequency, intensity and duration of exposure to footshock determine the characteristics of the resultant stress response. We chose to use a chronic intermittent footshock paradigm described by Imaki et al.¹⁶, which produced activation of the HPA axis evidenced by upregulation of CRF mRNA in the brain. Rats that received Footshock Treatments were placed in operant conditioning chambers enclosed in sound-attenuating cubicles and received daily intermittent footshock (15 min/day, 1.0 mA, 1s duration, total of 30 shocks each day) administered via a parallel rod floor under a variable-interval schedule for 7 days. Rats in the Non-footshock Treatment groups were treated in an identical manner except they did not receive any footshocks while in the operant conditioning chambers.

Urinary Bladder Distension(UBD)-Evoked Visceromotor Responses (VMRs)

Under mask isoflurane anesthesia (1-3% isoflurane in oxygen), a 22-gauge polytetrafluoroethylene angiocatheter was placed into the bladder via the urethra and held in place by a tight suture around the distal urethral orifice. Electrodes (silver wire) were inserted into the external oblique musculature immediately superior to the inguinal ligament. Following surgery, isoflurane anesthesia was lowered until flexion reflexes were present in the hind limbs, but spontaneous escape behaviors were absent (1-1.25% isoflurane). Urinary bladder distensions (UBDs; 20 sec) were produced by using compressed air and a previously described distension control device², and intravesical pressure was monitored using an in-line, low volume pressure transducer. Visceromotor responses (VMRs; contraction of the abdominal and hindlimb musculature), recorded as electromyographical (EMG) activity, was measured via the electrodes using standard differential amplification and rectification and saved on a computer using Spike-2 software and associated hardware (Micro 1401; CED, Cambridge, United Kingdom). Approximately 15 min after initial anesthesia induction, EMG activity to three presentations of 60 mmHg UBD at 3-minute intervals were recorded (data not shown) to overcome a period of bladder sensitization that occurs before demonstration of vigorous and reliable VMRs⁷. Responses to graded stimuli (10-60 mmHg; 1 minute intertrial intervals) were then determined. On a given day, VMRs were obtained from the same number of animals in each treatment condition/group to minimize variability.

General Experimental Protocols

Experiment 1: Effect of Footshock Treatments on UBD-evoked VMRs—Rats were exposed to either the Footshock or Non-footshock treatments. Immediately after the final treatment (day 7), rats were anesthetized with isoflurane, and UBD-evoked VMRs were measured as described above.

Experiment 2: Effect of spinal CRF receptor antagonists on Footshock-induced bladder hypersensitivity—Rats received Footshock Treatments as in Experiment 1, and immediately after the final treatment (day 7) were anesthetized with isoflurane (1-3%) for intrathecal (i.t.) catheter placement and subsequent measure of UBD-evoked VMRs. In brief, while anesthetized, rats were mounted in a stereotaxic instrument, and a midline incision was made to expose the atlanto-occipital membrane. A small slit beginning at the base of the skull

was made along the midline of the atlanto-occipital membrane. The catheter, a length of polyethylene tubing (PE10), was advanced 7.8 cm into the i.t. space to the S1 level, and the skin incision was sutured to secure the catheter. An intravesical catheter and EMG electrodes were placed as described in Experiment 1 for recording EMG activity to UBD. Antalarmin (24 μ g), a selective CRF₁ receptor antagonist, anti-sauvagine-30 (aSVG30; 12 μ g), a selective CRF₂ receptor antagonist, or appropriate vehicle was administered i.t., and 15 min later VMRs to UBD were measured. Evan's Blue (Sigma) was injected into the spinal catheter at the conclusion of the experiment. Spinal cords were removed and examined for dye to verify proper placement of the catheter.

Experiment 3: Effect of CRF receptor agonists/antagonists on UBD-evoked VMRs—Rats were anesthetized with isoflurane for placement of catheters (i.t. and intravesical) and EMG electrodes, as in Experiment 2. In one group of rats, either CRF (CRF₁ receptor agonist; 24 μ g), urocortin 2 (CRF₂ receptor agonist; 12 μ g) or vehicle was administered i.t. In a second group of rats, antalarmin (24 μ g) was administered i.t. 10 minutes before i.t. delivery of CRF (24 μ g). Similarly, in a third group of rats, aSVG30 (12 μ g) was administered i.t. 10 minutes prior to i.t. administration of urocortin 2 (12 μ g) or vehicle. A fourth group of rats did not have an i.t. catheter placed and instead received intraperitoneal (i.p.) administration of either CRF (24 μ g), urocortin 2 (12 μ g) or vehicle. In all groups, VMRs to UBD (10–60 mmHg) were measured 15 minutes after i.t. or i.p. administration of the last drug.

Drugs

Isoflurane was obtained from Minrad, Inc. (Bethlehem, PA, USA). Antalarmin, CRF, and urocortin 2 were obtained from Sigma (St. Louis, MO, USA). aSVG30 was obtained from Phoenix Pharmaceuticals, Inc. (Belmont, CA, USA). Urocortin 2, CRF, and aSVG30 were dissolved in sterile water, a solution which served as the vehicle in associated experiments. Antalarmin was dissolved in 9% DMSO/sterile water, a solution which served as the vehicle in its associated experiments. Based on previously published studies of bladder function^{21, 22,36}, 6 μ g doses of CRF and urocortin 2 were initially chosen for i.t. administration. In preliminary experiments, neither of these agonists affected UBD-induced VMRs; thus doses were increased to 12 μ g for the present experiments. Doses of CRF receptor antagonists antalarmin and aSVG30 used in the present experiments are comparable to i.t. doses employed in other studies of bladder function and visceral perception^{21,36}.

Statistical analysis

Similar to our previous studies⁴⁴, EMG activity was quantified as a response (change) score which represents a signal-to-noise ratio. In this case, baseline mean rectified myoelectrical activity measured prior to the presentation of UBD was treated a “noise” (in mV), and the evoked response (the rectified myoelectrical activity during UBD that exceeded the ongoing activity level immediately prior to UBD) represents the “signal” (in mV). Creating a ratio (signal divided by noise) yields a quantified measure of the vigor of the UBD-evoked physiological response that is independent of other measures. The vigor of the response is thereby represented by a signal-to-noise ratio. Low responders (signal to noise ratio <1) were treated statistically as previously described⁴⁴ and were equally distributed between groups. All data are presented as group mean $\pm$ SEM. Data were analyzed by repeated measures analysis of variance (ANOVA). Post-hoc tests were performed using Fisher's LSD.

Results

Experiment 1: Effect of Footshock Treatment on bladder nociception

The Footshock Treatment produced bladder hypersensitivity in female Sprague Dawley rats, manifested as significantly more vigorous VMRs to UBD compared to those rats in the Non-footshock Treatment group ($F=26.40$, $p<0.01$; ANOVA). Figure 1A shows typical examples of EMG responses to a 50 mmHg distension of the urinary bladder. The upper tracing is from a rat previously exposed to 7 days of Footshock Treatment, while the lower tracing is from a rat exposed to Non-footshock Treatment for 7 days. Post hoc tests revealed significant differences at UBD pressures of 20, 30, 40, 50, and 60 mmHg (Figure 1B).

Experiment 2: Effect of CRF receptor antagonists on Footshock-induced bladder hypersensitivity

Following exposure to 7 days of Footshock Treatment, VMRs of rats treated with i.t. aSVG30, a CRF₂ receptor antagonist were significantly lower than VMRs of rats that were treated with i.t. vehicle (Fig. 2A; $F=12.43$, $p=0.002$; repeated measures ANOVA). Post hoc tests indicated significant differences at 20, 30, 40, 50, and 60 mmHg. This effect was not observed after i.t. administration of antalarmin, a CRF₁ receptor antagonist (Fig. 2B; $F=0.191$, $p=0.34$). These data demonstrate that Footshock-induced bladder hypersensitivity can be significantly attenuated by blocking spinal CRF₂, but not CRF₁, receptors.

Experiment 3: Effect of CRF receptor agonists/antagonists on UBD-evoked VMRs

Naïve rats (no Footshock treatments) receiving i.t. administration of the selective CRF₂ receptor agonist, urocortin 2, exhibited greater UBD-evoked VMRs compared to those given i.t. CRF (selective CRF₁ receptor agonist) or vehicle (Figure 3A; $F=9.16$, $p=0.006$ repeated measures ANOVA), and post hoc tests indicated that UBD-evoked VMRs of urocortin 2-treated rats were significantly enhanced at distension pressures of 30, 40, 50, and 60 mmHg. I.p. administration of the same CRF receptor agonists at the same doses given spinally did not significantly affect UBD-evoked VMRs (Fig. 3B; $F=0.94$, $p=0.21$) indicating a spinal site of action. Pretreatment with i.t. administration of the CRF₂ receptor antagonist, aSVG30, significantly attenuated the augmentation of bladder nociceptive responses induced by i.t. urocortin 2 (Fig. 4A; $F=35.43$, $p<0.01$) indicating receptor specificity of the urocortin 2 action. In contrast, i.t. administration of CRF (Fig. 3A; $F=0.61$, $p=0.23$) with or without the CRF₁ receptor antagonist, antalarmin (Fig. 4B; $F=0.24$, $p=0.32$), had any effect on UBD-evoked VMRs.

Discussion

The present study demonstrates that a 7-day exposure to Footshock Treatments, a well characterized experimental stressor, is capable of producing bladder hypersensitivity as measured by augmented VMRs to UBD. Using this model system, the present study also showed that spinal administration of a selective CRF₂ receptor antagonist attenuates this Footshock-induced effect. Furthermore, spinal administration of a CRF₂ receptor agonist produces an augmentation of UBD-evoked VMRs, similar to what was observed following Footshock, an effect that was also abolished by pretreatment with a spinal CRF₂ receptor antagonist. These converging lines of evidence implicate the CRF-related neuropeptide system as a potential mediator of Footshock-induced hyperalgesia in the urologic system with a specific role for the CRF₂ receptor, and its endogenous agonists, the urocortins.

It should be noted that the present study examined both CRF₁ and CRF₂ receptor effects *after* the presentation of an environmental stressor. These receptor-mediated effects are therefore envisioned not as anxiolytic effects but rather as representative of spinal mechanisms

related to hypersensitivity. The findings of the present study were surprising because pre-existing literature related to visceral sensation suggested a predominant role for the CRF₁ receptor. In contrast to the present findings, it appears that CRF, via the CRF₁ receptor, is involved in modulation of stress-related functional gastrointestinal disorders such as irritable bowel syndrome (IBS). Colorectal distention (CRD) enhances abdominal EMG activity, induces noradrenaline release in the rat hippocampus, and causes a significant rise in plasma adrenocorticotrophic hormone^{36,48}. Administration of CRF₁ receptor antagonists significantly suppresses all of these CRD-induced effects^{14,36}. Anxiety-like behaviors evoked by CRD and stress-induced increases in VMRs to CRD are also significantly attenuated by selective CRF₁ receptor antagonists⁴⁹. CRF₁ receptor-deficient mice have greater response thresholds to CRD⁵⁵. Results of human studies provide further support for a role of the CRF₁ receptor in IBS. Compared to normal control subjects, individuals with IBS exhibit robust colonic motility, exaggerated colonic motility indices, and enhanced secretion of adrenocorticotrophic hormone following exogenous administration of CRF¹². Thus, results of both animal and human studies support a pronociceptive role for the CRF₁ receptor, in the pathophysiology of stress-related digestive diseases. In contrast, activation of CRF₂ receptors blunts CRD-induced gastrointestinal pain^{34,36}. Together with the findings of the present study, this suggests that the function of these receptors may be organ-specific instead of applicable to stress-related pain responses in general. Obviously, great differences in methodological specifics are also present between the present study and the previously noted studies of gastrointestinal sensation which include the spinal versus systemic administration of drugs, the use of anesthetics, differing rat strains, the stressors employed and pre- versus post-stressor drug treatments. As such, a precise comparison of studies is not possible.

Several other lines of evidence, in addition to the present findings, implicate the urocortins in the regulation of stress-related physiology. Urocortins are localized to stress-related neuronal groups involved in the integration of afferent signals from the viscera (PVN, supraoptic nucleus, locus coeruleus), and the expression of urocortins in the PVN, supraoptic nucleus and Edinger-Westphal nucleus is upregulated by different stressors^{14,52,58,60}. Infusion of urocortins into the lateral septum or amygdala increases anxiogenic-like behavior^{16,46}, and uncontrollable stressors sensitize serotonergic neurons in the dorsal raphe, an effect that is mediated by activation of CRF₂ receptors³². Urocortins are also expressed in immunological tissues, and urocortin 1 has been detected in various immune cells (lymphocytes, mast cells, macrophages, fibroblasts). Stress induces mast cells to synthesize and release both CRF and urocortin 1, and activation of CRF receptors on mast cells potentiates the secretion of other pro-inflammatory mediators^{19,24,50,53}. This suggests that CRF-related neuropeptides may mediate stress-related inflammatory conditions via enhanced mast cell activation. Indeed, urocortin 1-like immunoreactivity and mRNA are elevated in the synovium of patients with rheumatoid arthritis and in rats with asthma^{56,61,62}. Inflammatory conditions of the reproductive tract, such as endometriosis, are also associated with elevated urocortin 1-like immunoreactivity, as well as increased numbers of mast cells²⁰.

With regard to the bladder, central nervous system areas involved in the control of micturition (i.e., Barrington's nucleus, lumbosacral areas of the spinal cord) express CRF-related neuropeptides and administration of nonselective CRF receptor agonists and antagonists alters cystometric parameters^{21,22,38,40}. These studies found that the state of arousal profoundly influences the effect of CRF agonists and antagonists on urodynamic function (inhibition versus facilitation) and clearly demonstrated a role for the CRF-related neuropeptides in micturition. Imaki et al. reported that both acute and chronic footshock stress enhanced CRF mRNA expression in Barrington's nucleus¹⁷. Significant increases in CRF mRNA were also observed in the hypothalamus following chronic exposure to footshock. LaBerge et al. demonstrated increases in CRF and CRF₂ receptor expression in the lumbosacral spinal cord and in the bladder itself as a result of cyclophosphamide-induced bladder

inflammation²⁵. These converging lines of evidence implicate the CRF-related neuropeptide family, including urocortins, as potential mediators of stress-induced bladder hyperalgesia, and the present studies provide further evidence for CRF₂ receptors in stress-related sensory augmentation in the bladder. Doses of CRF and urocortin 2 used in the present study are at least two-fold higher than doses previously reported to affect urodynamic function²¹ suggesting that the doses used in these studies should have been adequate to observe CRF₁ receptor-related effects.

There remain a number of unanswered questions related to the precise mechanism by which the urocortins and their endogenous receptors mediate urinary bladder hypersensitivity. Our preliminary data demonstrate that the site of action of urocortins is spinal, however the source of these urocortins, whether they are intrinsic to the spinal cord or released from a supraspinal site, is unknown. Intrinsic urocortin 1 and 2 expression have been reported in the spinal cord^{5,42}, but given that this has been localized predominantly to lamina IX, release from this area is unlikely to activate CRF₂ receptors in the dorsal cord. There are a number of supraspinal sites which have spinal projections, including the lateral septum, hypothalamus, Edinger-Westphal nucleus, dorsal raphe, locus coeruleus, and amygdala^{23,28,33,42}, that express urocortin immunoreactivity and thus may release urocortins that activate spinal CRF₂ receptors. It remains to be determined which, if any, of these candidate sites contains urocortin-immunoreactive neurons with axonal extensions to the spinal cord.

In summary, while CRF has been extensively studied as a mediator of endocrine, autonomic and behavioral functions, particularly in terms of the stress response, these data suggest that it is not CRF, but rather urocortin 2 and the CRF₂ receptor in the spinal cord that is critical to the bladder hypersensitivity related to stress. There is very little known about urocortin 2 in mammalian systems, and a role for this neuropeptide in nociceptive processing is nonexistent, apart from the present work. Additional studies investigating the precise mechanism(s) by which urocortins mediate stress-induced bladder hypersensitivity would prove beneficial, not only as the first step in devising more effective treatment options for patients suffering specifically from painful bladder syndromes, but potentially for individuals with stress-exacerbated chronic pain, in general.

Acknowledgement

This work was supported by DK51413 and 1K99DK080981-01.

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Figure 1.

A: Typical examples of EMG activity in response to a 50 mmHg urinary bladder distension in a rat exposed to 7 days of the Footshock Treatment (upper) or the Non-Footshock Treatment (lower) for 7 consecutive days. B: Mean data demonstrated that Footshock Treatment rats exhibited significantly greater VMRs to UBD relative to their Non-Footshock Treatment counterparts. ** indicates $p < 0.01$. N=10-11/group.

Figure 2.

A: Footshock-induced bladder hypersensitivity is significantly attenuated by i.t. administration of a CRF₂ receptor antagonist, aSVG30. Following 7 day exposure to the Footshock Treatment, VMRs of rats treated with i.t. aSVG30 were significantly less than VMRs of rats that were given i.t. vehicle. B: This effect was not observed after i.t. administration of antalarmin, a CRF₁ receptor antagonist. * indicates $p < 0.05$; ** indicates $p < 0.01$. N=6-8/group.

Figure 3.

Activation of spinal CRF₂ receptors significantly enhances VMRs evoked by UBD. A: I.t. administration of the CRF₂ receptor agonist, urocortin 2, but not the CRF₁ receptor agonist, CRF, significantly increased bladder nociceptive responses relative to i.t. administration of vehicle (* and ** indicate significantly different from vehicle at $p < 0.05$ and $p < 0.01$, respectively). B: I.p. administration of the same CRF receptor agonists at the same i.t. doses did not affect EMG responses. $N = 5-8/\text{group}$

Figure 4.

A: I.t. administration of the CRF₂ receptor antagonist, aSVG30, significantly attenuated the augmentation of bladder nociceptive responses induced by urocortin 2 (** indicates significant difference from aSVG30 + urocortin 2 and from aSVG30 + vehicle at $p < 0.01$). B: In contrast, i.t. administration of the CRF₁ receptor antagonist, antalarmin, had no effect on UBD-evoked VMRs. N=5-8/group.