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Chronic psychological stress in high-anxiety rats induces sustained bladder hyperalgesia

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HIGHLIGHTS

- Anxiety-prone rats exposed to chronic water avoidance stress display increased voiding frequency.
- The urinary frequency appears to be a response to abnormal sensory processing and resultant bladder hyperalgesia.
- Chronic water avoidance stress induces sustained bladder hyperalgesia of greater than one month.
- We report a potential model for bladder hypersensitivity syndromes, i.e., interstitial cystitis/painful bladder syndrome.

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ABSTRACT

Objective: To evaluate whether anxiety-prone rats exposed to chronic water avoidance stress (WAS) develop visceral bladder hyperalgesia in addition to increased voiding frequency and anxiety-related behaviors.
Materials and Methods: Female Wistar-Kyoto (WKY) rats were exposed to chronic (10-day) WAS or sham paradigms. Referred hyperalgesia and tactile allodynia were tested using von Frey filaments applied to the suprapubic region and plantar region of the hindpaw, respectively. To confirm that suprapubic nociception represented referred visceral bladder hyperalgesia, we recorded abdominal visceromotor responses (VMR) to slow (100 μl/min) and fast (1 cc/sec) bladder filling with room temperature or ice-cold saline. We assessed the development of hyperalgesia over the 10-day WAS protocol and the durability of increased pain sensations over time.
Results: Animals exposed to chronic WAS had significantly lower hindpaw withdrawal thresholds post-stress and significant differences in referred hyperalgesia. Rats exposed to chronic WAS demonstrated an increased pain response to suprapubic stimulation and decreased response threshold to mechanical hindpaw stimulation by day 8 of the stress protocol, which persisted for more than one month. Animals exposed to chronic WAS showed increased VMR to fast filling and ice water testing in comparison to sham animals. Cystometry under anesthesia did not show increases in the frequency of non-voiding contractions.
Conclusion: Chronic WAS induces sustained bladder hyperalgesia, lasting over a month after exposure to stress. The urinary frequency demonstrated previously in anxiety-prone rats exposed to chronic WAS seems to be associated with bladder hyperalgesia, suggesting that this is a potential model for future studies of bladder hypersensitivity syndromes such as interstitial cystitis/painful bladder syndrome (IC/PBS).

1. Introduction

Stress has been shown to play a role in the exacerbation and possibly the development of lower urinary tract symptoms (LUTS), including urinary frequency, overactive bladder (OAB), and interstitial cystitis/painful bladder syndrome (IC/PBS) [1,2]. Both OAB and IC/PBS conditions exhibit urinary frequency, but differ in the absence or presence of bladder pain, respectively. Although stress can have adaptive purposes, as in the acute stress-induced analgesia seen in the "fight or

Abbreviations: WAS, water avoidance stress; VMR, visceromotor responses; IC/PBS, interstitial cystitis/painful bladder syndrome; LUTS, lower urinary tract symptoms; OAB, overactive bladder; IBS, irritable bowel syndrome; WKY, Wistar-Kyoto; EMG, electromyographic.

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flight" response, chronic or uncontrollable stress can lead to maladaptive responses, such as stress-induced hyperalgesia characterized by augmented nocioception [3]. Several reports, including the recent epidemiology of lower urinary tract symptoms (Epi-LUTS) study [4], have linked stress, anxiety, and depression to LUTS and specifically urinary frequency [5]. Men and women who reported LUTS in this study also displayed high levels of clinical anxiety (35.9% and 53.3%, respectively) and depression (29.8% and 37.6%, respectively) [4]. The majority (60%) of IC/PBS patients report that stress exacerbates their symptoms [6,7]. Symptom severity is strongly correlated with stress [1]; many patients report increases in bladder pain and urgency when exposed to an acute stressor [7]. The high prevalence of psychological comorbidity and stress in patients with LUTS, most profoundly in IC/PBS, suggests that central neuronal alterations may influence lower urinary tract function in these patients [1,8–10]. While it is unlikely that stress is the sole etiological factor in IC/PBS, it appears that stress acts as a vulnerability factor in individuals predisposed to the disease for other reasons.

Chronic water avoidance stress (WAS) in rats predisposed to anxiety induces some of the same histopathologic and clinical features of human IC/PBS, including disruption of the epithelial layer of the bladder, increased numbers of mast cells, and urinary frequency [11,12]. WAS is well studied in the gastrointestinal literature as a model for irritable bowel syndrome (IBS) [13], which is a comorbid condition frequently associated with LUTS and IC/PBS. While Robbins *et al.* demonstrated that chronic WAS enhances nociceptive responses to urinary bladder distension in high-anxiety rats [14], it remains unknown whether the urinary frequency induced by chronic stress in anxiety-prone rodents is due to sustained visceral nociception in the bladder or bladder overactivity. Therefore, we characterized the changes in somatic and visceral bladder nociception and analyzed bladder overactivity in rats exposed to chronic WAS.

2. Materials and methods

2.1. Animals

Female Wistar-Kyoto (WKY) rats aged 11-12 weeks (200-300 g) were purchased from Charles River Laboratories International, Inc. (Wilmington, MA). This strain is genetically predisposed to elevated levels of anxiety [15]. As disorders of the urinary bladder associated with pain occur at greater frequency in women, we studied female rats only. We did not control for estrous cycle during these experiments. Animals were maintained on a normal light-dark cycle; food and water were available *ad libitum*. We allowed an adjustment period of one to two weeks between the animals' arrival and the start of experimental procedures. Animals were housed individually in standard cages. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Los Angeles (ARC#2011-143).

2.2. Chronic water avoidance stress protocol

Rats were placed on a pedestal (8 x 8 x 11.5 cm^3) in the center of the floor of a plexiglass cage (24 x 45 x 15 cm^3). The container was filled with 25 °C water up to 1 cm below the top of the pedestal. In the sham group, animals were placed on the platform in a waterless container. Animals were exposed to water avoidance (WA) stress or sham stress between the hours of 8:00 am and 12:00 pm to minimize circadian effects as previously described [11]. To generate chronic psychological stress, animals were exposed to the stress or sham protocol for one hour a day for 10 consecutive days, a well-characterized protocol representing a potent psychological stressor [16]. Rats were weighed prior to and after the stress protocol to assess weight change from baseline over time (n=11 for each group).

2.7. Assessment of fecal pellet output

Using a validated method to estimate autonomic regulation of colonic motility [17], fecal pellets were counted at the end of each WAS or sham session from day one to day ten of the stress protocol in a separate group of WKY rats (n=11 for each group).

2.3. Assessment of tactile allodynia

Calibrated von Frey nylon monofilaments (Stoelting Co., Wood Dale, IL) were used in tests of tactile allodynia (n=8 for each group) [18]. Each animal was tested at baseline and after WAS or sham during the day portion of the circadian cycle (06:00-18:00). Each rat was placed in a metabolic cage with a wire mesh bottom allowing full access to the hindpaws. Behavioral accommodation was allowed for a minimum of 10 minutes or until cage exploration and major grooming activity ceased.

Tactile allodynia was measured at the mid-plantar hindpaw, as previously described [19]. The paw was touched with a series of von Frey hairs with increasing order of force. The von Frey hair was presented perpendicular to the plantar surface with sufficient force to cause bending of the filament and held for 6-8 seconds. Stimuli were presented at intervals greater than 5 seconds, allowing for resolution of previous stimuli. Sharp withdrawal or licking of the stimulated hindpaw was recorded as a positive response. Ambulation was considered an ambiguous response; in such cases, the stimulus was repeated. The median 50% withdrawal threshold was determined using the up-down method [20]. In brief, after observing a positive response, the following measurements were performed with the next weakest stimulus. In the absence of a response, the next strongest stimulus was employed. Filaments were applied in this consecutive manner until six responses of 50% threshold were obtained[19].

2.4. Assessment of referred bladder hyperalgesia

Referred hyperalgesia of the bladder was tested using von Frey filaments applied to the suprapubic area (n=8 for each group) [21]. We tested the frequency of withdrawal responses to individual von Frey filaments (0.16, 0.4, 1, 2, 4, 8, and 15 g) applied to the abdomen in ascending order of force. Each filament was applied for 1-2 seconds for a total of 10 times with an inter-stimulus interval of greater than 5 seconds. Stimulation was applied to the lower abdominal area in the area overlying the bladder with small variations in the exact area stimulated to avoid desensitization. Sharp retraction of the abdomen, immediate licking or scratching of the area, vocalization, and jumping were considered positive responses to filament stimulation.

2.6. Time course of pain development

In a separate group of WKY rats, tactile allodynia and referred bladder hyperalgesia were assessed throughout the WAS protocol to determine the effect of stress on the development of pain responses. Animals were placed on pedestals in the Plexiglass cages for 1 hour each day as described above. Prior to the stress protocol and on days 1, 3, 5, 8, and 10 of the WAS protocol, animals were removed from the Plexiglass cages and immediately subjected to hyperalgesia testing as described above. To avoid the influence of pain testing as an additional psychological stressor, different animals were used for each time point.

2.5. Assessment of visceral pain and detrusor overactivity during bladder filling

After assessment of tactile allodynia and referred bladder hyperalgesia, the same WKY rats underwent transperitoneal suprapubic bladder catheter implantation and placement of abdominal wall wire electrodes as previously described (n=8 for each group) [22]. Animals