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The role of C-fibers in the development of chronic psychological stress induced enhanced bladder sensations and nociceptive responses: a multidisciplinary approach to the study of urologic chronic pelvic pain syndrome (MAPP) research network study

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Abstract

Aims—To evaluate C fiber-mediated changes in bladder sensation and nociception in an animal model of stress induced bladder hyperalgesia and urinary frequency.

Methods—Female Wistar-Kyoto (WKY) rats were exposed to a chronic (10 days) water avoidance stress (WAS) and compared to controls. Rats were evaluated by cystometrogram (CMG) and visceromotor reflex (VMR) to bladder infusion with room temperature (RT) or cold saline. Cold saline activates afferent C-fibers via cold bladder receptors. To further evaluate bladder hyperalgesia, CMG and VMR were also obtained during RT isometric bladder distention (RT-iBD) at variable pressures.

Results—During RT infusion, WAS rats had significant decreases in pressure threshold (PT) and in the ratio of VMR threshold/maximum intravesical pressure (IVPmax), and a significant increase in VMR duration. Cold infusion also induced significant decreases in PT and in the ratio of VMR threshold/IVPmax in WAS rats. During RT-iBD, rats exposed to WAS showed a significant decrease in VMR latency and a significant increase in VMR area under the curve (AUC) compared to controls.

Conclusion—Chronic WAS induced bladder hypersensitivity manifested by earlier voiding with earlier VMR appearance. Chronic stress also enhanced bladder nociceptive responses. WAS leads

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H Chang: project design, experiments, data analysis, manuscript preparation

L Rodriguez: project design, data analysis and manuscript preparation

to increase responses to ice cold water infusion, implying a role of sensitized C-fibers and mechanoreceptors in WAS-induced bladder dysfunction and hypersensitivity.

Keywords

bladder hypersensitivity; cystometrogram; hyperalgesia; interstitial cystitis; visceromeotor reflex; water avoidance stress

INTRODUCTION

Stress is a coordinated reaction normally activated to enhance survival under hostile conditions. However, continuous exposure to stressful stimuli or abnormal enhanced sustained responses to stress has been directly linked to many pathological processes. Chronic psychological stress has been shown to aggravate lower urinary tract symptoms (LUTS), most profoundly in interstitial cystitis/bladder pain syndrome (IC/BPS) and overactive bladder.[1–4] Patients with IC/BPS experience high prevalence of comorbid stress-related mental disorders, including depression and anxiety.[1, 5] Among various contributing factors in IC/BPS, one potential mechanism is increased bladder afferent sensation and excitability. Indeed, clinical studies have showed that patients with IC/BPS experience generalized hypersensitivity to deep tissue stimulation with lower threshold and tolerance to bladder stimulation.[6] To examine stress-induced effects on bladder function, we use an animal model of chronic water avoidance stress (WAS). WAS, a well-studied stress model, induces functional and histological bladder changes quite similar to those found in human IC/BPS.[7] We have previously shown that WAS induces sustained bladder hyperalgesia as measured by suprapubic referred pain responses.[8] The purpose of the current study was two-fold: 1) to provide a detailed description of the effects of WAS on voiding function as assessed by cystometrogram (CMG) and 2) to evaluate the role of WAS on bladder sensations and pain directly elicited by bladder distention.

Iced water test (IWT) can evoke a lower motor neuron reflex, triggered by cold receptors in the bladder wall. This cold reflex is potentially mediated by C-fibers.[9] Although normally bladder C-fibers are thought to be inactive; under pathological conditions they may be activated and lead to pain and voiding frequency.[9, 10] We therefore aimed to examine, the role of C-fiber activation in WAS-induced bladder hyperalgesia.

MATERIALS AND METHODS

Animals

22 female Wistar Kyoto (WKY) rats (180–200 g) were used (Charles River Laboratories International, Inc. Wilmington, MA). WKYs are a high-anxiety strain previously proposed as a model of genetic stress vulnerability.[8] Animals were housed in pairs on a 12:12-hour light-dark cycle with standard chow and water *ad libitum*. Prior to the stress protocol, animals had 1–2 weeks to adjust to their environment. All animal procedures were carried out in strict accordance with the standards in the NIH Guide for the Care and Use of Laboratory Animals and the experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Southern California.

Chronic WAS protocol

Rats were randomly divided into 2 groups: WAS (n=11) and control (n=11). Rats in the WAS group were subjected to WAS as previously described.[8] Animals were placed on a platform (6 × 6 × 10.5 cm^3) centered on the floor of a plastic container (47 × 26 × 20 cm^3) filled with 25 °C water to 1 cm below the top. Rats were exposed to WAS for 1 hour/day for 10 consecutive days. Control animals were handled for 1 minute at the beginning and end of the experimental hour. All procedures and measurements were done in the morning to minimize circadian effects.

Assessment of fecal pellet output and body weight

Fecal pellet output is a reliable measurement of autonomic regulation of colonic motility and has been used in this model as a surrogate marker of stress. Excreted fecal pellets were counted at the end of each WAS session/handle exposure on days 1 to 10. All animals were weighted daily.

Surgical procedures and preparations for CMG and VMR

Animals were anesthetized via intraperitoneal (i.p.) injection of urethane (1.2 g/kg) 1 hour before surgery. Animals were placed on a 37 °C water-circulating heating pad. For recording VMR, a pair of Teflon-coated silver wire electrodes (uncoated diameter 0.05 mm, coated diameter 0.10 mm, A-M Systems) were hooked to the tip of a 27-gauge needle and inserted into the abdominal external oblique muscle through a small skin incision. After withdrawing the needle, the electrodes were left embedded in the muscle, and the incision was closed. VMR wires were connected to a data acquisition system, and a grounding cable was placed subcutaneously. A polyethylene 50 catheter was inserted into the bladder lumen through the urethra connected via a 3-way connector to a syringe infusion pump for saline infusion (KDS-100, KD Scientific, Holliston, MA) and a pressure transducer for monitoring intravesical pressure.[8, 11] CMG and VMR were obtained at a 1-kHz sampling rate during continuous bladder infusion with saline, using a biological signal acquisition system (Biopac MP 150, BIOPAC Systems, Inc., Goleta, CA).

Recordings under room temperature (RT) or Cold Saline Infusion

CMG and VMR were recorded simultaneously at both RT and cold saline infusion in all animals. One hour after the induction of urethane anesthesia, RT physiological saline (0.9 %) was infused continuously through the catheter at a rate of 0.1 ml/min. The bladder infusion rate remained constant and three stable voiding cycles were recorded. The bladder was emptied and allowed to rest for 20 minutes. The process for recording CMG and VMR was then repeated by using cold saline (0–4 °C) infusion.

The pressure threshold (PT), contraction duration (CD), inter contraction interval (ICI), bladder compliance and the ratio of VMR threshold/maximum intravesical pressure (IVPmax) were analyzed from three consecutive voiding cycles in each animal. The VMR threshold pressure was determined as the bladder pressure at the onset of VMR firing. In the same voiding cycles, the duration, amplitude and area under curve (AUC) measurements for the VMR firing activity were also calculated. All parameters were analyzed using Acqknowledge software (BIOPAC Systems, Inc. Goleta, CA).

Room temperature isometric bladder distention (RT-iBD) test

Once the cold infusion testing was completed, the bladder was emptied and the urethral catheter was secured around the distal urethral orifice using a tight ligature. The catheter was connected via a 3-way stopcock to a saline-filled upright syringe (sans piston) and a pressure transducer for monitoring bladder pressure. After a 20-minute rest, bladder pressure was maintained at a steady 10, 20, 30 and 40 cmH_2O respectively, by adjusting the height of the upright syringe. Each isometric pressure recording lasted 2 minutes followed by a 2-minute break. VMR AUC and latency determined within each 2-minute isometric pressure session was determined.

Statistical analysis

Values are presented as means ± standard error of the mean (SEM). Data was analyzed using two-tailed, unpaired Student's *t*-test for comparing WAS and controls. *P*-value < 0.05 indicated a statistically significant difference between groups. All analyses were conducted using JMP Pro 11 (SAS Institute, Cary, NC).

RESULTS

WAS-induced changes in fecal pellet output and body weight

Rats exposed to WAS showed significantly higher fecal pellet output after the first day of stress exposure than their control counterparts (4.4±0.8 vs 0.2±0.1 pellets, p=0.0002). On Day 10, WAS rats still had significantly greater fecal pellet output relative to control rats (2.9±0.8 vs 0.0±0.0 pellets, p= 0.0043). Increased fecal pelleting in the WAS model is commonly used as a surrogate measure of stress to ensure the model is effective. No significant difference in body weight between WAS and control rats on Day 1 (183.6±2.3 vs 184.7±3.7 g, p=0.8062) or Day 10 (192.4±2.1 vs 188.5.3±3.1 g, p=0.3302) was observed.

WAS-induced changes during RT infusion

During RT saline infusion, WAS rats showed significant decreases in PT (8.6±0.8 vs 12.0±1.3 cmH_2O, p=0.0404), and the VMR threshold/IVPmax ratio (53.0±3.4 vs 70.6±6.6 %, p=0.0323) (Fig 1). VMR duration significantly increased in WAS rats as compared to control rats (22.7±4.1 vs 12.2±1.2 sec, p=0.0333) (Fig 1E). WAS did not appear to alter compliance, CD, ICI, VMR amplitude or AUC when compared to controls.

WAS-induced changes during cold infusion

Cold infusion in WAS rats induced significant decreases in PT (7.4±0.8 vs 11.9±1.3 cmH_2O, p=0.0108) and in the ratio of VMR threshold/IVPmax (41.6±5.6 vs 62.1±5.9 %, p=0.0217) compared to controls (Fig 2). Differences of PT and the ratio of VMR threshold/IVPmax were increased during cold infusion as compared to RT. No significant changes appeared in other CMG parameters (compliance, CD, ICI) or VMR parameters (duration, amplitude, AUC) between WAS and control rats.

WAS-induced changes in RT-iBD

VMR was evaluated at different pressures of bladder distension (Fig 3A). Compared to controls, WAS rats showed a significant decrease of VMR latency at 20 cmH_2O (56.8±6.3 vs 113.4±3.9 sec, $p<0.0001$) and 30 cmH_2O (38.9±5.7 vs 74.7±9.9 sec, $p=0.0065$) (Fig 3B). VMR AUC was significantly increased in stressed rats compared to their control counterparts (0.96±0.2 vs 0.20±0.0 mV-sec, $p=0.0024$) at 20 cmH_2O only (Fig 3C).

DISCUSSION

In 2008, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) initiated the Multi-disciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network to rigorously study urological chronic pelvic pain syndrome (UCPPS) using an interdisciplinary approach.[12] UCPPS include IC/BPS and chronic prostatitis/ chronic pelvic pain syndrome (CP/CPPS). Part of the MAPP network has focused on the validation of rodent models that reflect multiple key characteristics of human UCPPS and provide enhanced clinical significance to mechanistic studies. The WAS model is one such model which reflects the stress variability seen in the human conditions and manifests the key features of urinary frequency and bladder hyperalgesia experienced by patients.[7]

Epidemiologic studies have showed that chronic psychological stress can exacerbate LUTS and enhance chronic pain.[1–4] By conducting this WAS model the current study provides evidence of urinary bladder hypersensitivity following WAS in female WKY rats, manifested as earlier urinary voiding and earlier VMR appearance in stressed rats. The fact that other CMG parameters such as compliance, CD, and ICI were not altered by WAS, supports altered bladder sensitivity as a driver of the observed dysfunction in animals exposed to WAS.

We have previously published the effects of WAS on voiding and water intake.[7, 8, 13] The main goal of the present study was to evaluate the urinary function of these animals by CMG. In the present study, we chose VMR as an indicator for WAS-induced bladder dysfunction and hypersensitivity. VMR is the contraction of the abdominal muscles reflexively activated by visceral stimuli. VMR is a reliable and reproducible marker and its application for visceral sensation measurement has been supported in several previous studies.[14, 15] It can be quantified by determining the vigor of the muscle contraction. The presence of VMR reflects the maintenance of the visceral pain pathway. The utility of VMR for visceral nociception has been previously validated.[11, 16] In addition, animals exposed to WAS have been shown to experience bladder hyperalgesia as demonstrated by Von Frey testing which correlates to hyperalgesic responses to bladder filling as evaluated by VMR.[8] The current study explores in more detail the VMR responses to iBD and shows that WAS leads to bladder hyperalgesia as measured by early VMR responses at low bladder pressures.

During RT saline infusion, animals exposed to WAS showed significant decreases in PT and in the ratio of VMR threshold/IVPmax. In other words, a lower bladder pressure can trigger bladder voiding and VMR appearance in WAS rats, suggesting that WAS could alter the properties of sensory pathways leading to a reduction in sensory threshold to bladder stimuli. In addition, during RT infusion the longer VMR duration in WAS rats suggests a

prolonged sensory input to bladder stimuli. Furthermore, since VMR can serve as a pain or hypersensitivity marker, it was reasonable to suppose that an earlier VMR appearance induced by WAS may represent a lower pain threshold to bladder infusion. In addition, a longer VMR duration in WAS rats represents a longer sensory/pain response to bladder stimuli. A lower sensory threshold may explain the previously documented increases in urinary frequency in this animal model.[7,8,13] This is consistent with our previous publications, which demonstrates that the increased urinary frequency in rats exposed to WAS is associated with bladder hyperalgesia.[8] The WAS-induced bladder hypersensitivity is similar to other reports regarding different stress animal models. For instance, neonatal maternal separation stress induces bladder hypersensitivity in female mice, which is further exacerbated in adult individuals exposed to WAS.[14] Similarly, a 7-day exposure to footshock treatments produces bladder hypersensitivity.[17] These findings in animal models have significant translational value since they correlated to clinical findings in patients with IC/BPS, a condition long known to be exacerbated by stress. A growing number of clinical studies have demonstrated that IC/BPS patients have an altered reaction to sensory, somatic and painful stimuli.[6, 18] As reported by Ness et al, IC/BPS patients experience generalized hypersensitivity to deep tissue stimulations related to pressure, ischemia and bladder stimulation. Higher psychological reactivity has also been shown in these patients when compared to controls, including more catastrophizing, a perception of decreased health, more somatic complaints and increased vigilance to sensations.[6] Our previous study also demonstrated that IC/BPS patients show higher anxiety and depression symptoms and enhanced hypersensitivity to visceral stimuli which may be related to central hyperexcitability as indicated by increased startle responses.[19]

Sensitized C-fibers may lead to the observed lower sensory threshold to bladder stimuli in WAS rats. Particularly, C-fibers sensitization may lead to a lower pain threshold as shown by an earlier VMR appearance in WAS rats. To further explore the underlying mechanism of WAS-induced bladder hyperalgesia, IWT was utilized in this study to yield insight into C-fibers activity in WAS rats. IWT, thought to evoke the bladder cooling reflex (BCR), has been used to reveal bladder hypersensitivity and hyperalgesia in human and animal studies.[9, 20] BCR is a segmental sacral spinal reflex mediated by C-fiber afferents associated with cold receptors. Bladder C-fibers, mainly from the urothelial layer and the lamina propria, are normally quiescent during filling and not essential for normal micturition but may be activated in certain conditions.[9, 10] Sensitized C-fibers is one of the underlying mechanisms of LUTS exacerbation, including increased sensory activity (pain particularly) and enhanced bladder activity.[21–23] C-fiber bladder afferents have been proposed to mediate bladder nociceptive responses.[23] In the present study, we applied a similar IWT methodology. During cold saline infusion, WAS rats showed significant decreases in PT and in the ratio of VMR threshold/IVPmax compared to controls. These results although amplified by lower temperature, were consistent with RT saline infusion, both of which show WAS-induced reduction in sensory threshold to bladder stimuli. Thus a lower bladder pressure can trigger bladder voiding and VMR appearance in rats exposed to WAS. In addition, the observed longer VMR duration in WAS rats as compared to controls is consistent with a longer sensory/pain response to bladder stimuli. Lastly, the differences in PT and in the ratio of VMR threshold/IVPmax, respectively, are increased during cold infusion as compared to RT

saline infusion, suggesting a role of C-fibers sensitization in WAS rats. This confirms prior observations of C-fiber activation in WAS rats as indicated by more vigorous VMR during fast cold saline infusion.[8] Further studies are necessary to elicit more information about this subtype of pelvic afferents included and are a subject of ongoing investigations. Although the VMR duration in WAS rats was longer than controls, there was no statistically significant difference between these two groups during cold saline infusion. We believe this is due to the enhanced sensitivity to bladder stimuli in rats exposed to WAS. Given the earlier voiding seen in WAS rats exposed to IWS, we saw an accompanying shorter voiding duration. A shorter voiding duration may at the same time reduce the VMR duration and thus reduce the gap of VMR duration between WAS and controls. This can be directly observed by comparing the representative CMG recordings in Fig 1.A&B and Fig 2.A&B. In addition, these animals appear to experience a ceiling effect in sensory response to stimulation and thus no further increase VMR duration is seen as a response to cold temperature.

To better determine the thresholds of bladder distention that lead to bladder hyperalgesia, we measured VMR to RT-iBD. In healthy females with IC, experimentally induced iBD can produce discomfort and prompt immediate pain-relieving actions.[24] Animal models exposed to iBD have been developed to more easily explore the neurophysiological mechanisms underlying bladder pain.[16] iBD can serve as a mechanical stimulation and be achieved by distending the bladder with air or saline. In rats, both Aδ- and C-fibers are mechanosensitive and can respond to bladder distension.[25] Compared with other described iBD methodologies,[26, 27] our method has some unique features. Firstly, we used saline to perform an isometric distension and each pressure was maintained for 2 minutes. This allowed for determination of responses at lower pressures and to clearly differentiate responses to different pressures. More importantly, we were interested in determining bladder hypersensitivity at physiologic stimulation. Therefore, in contrast to other published studies where animals are distended to pressure of 80 mmHg, we tested the animals at physiologic bladder pressures which have the added advantage of avoiding possible bladder compliance impairment and additional pain reflex due to high pressure-induced bladder over distension. These would be consistent with clinical findings which demonstrated that IC/BPS patients with higher psychological reactivity were more sensitive to lower bladder volume.[6] In the present study, during iBD stressed rats displayed a shorter VMR latency at 20 and 30 cmH_2O. In other words, WAS induced an earlier VMR response at lower bladder pressures, which was consistent during both RT and cold saline infusion. Additionally, since iBD may serve as a mechanical stimulus, an earlier VMR appearance at low pressure may also represent decreased threshold for mechanosensitive afferents encoding changes in pressure, supporting WAS-induced afferent nerve activation and discharge. We speculate that the decreased threshold for mechanical stimuli may be derived from WAS-induced mechanoreceptor hypersensitivity in the bladder wall. Furthermore, WAS enhances nociceptive processing related to the bladder, as indicated by an increased VMR AUC at 20 cmH_2O. These results are consisted with previously published reports in which chronic WAS rats showed enhanced VMR response to compressed air bladder distension.[15]

Numerous studies have demonstrated that sensitization of bladder sensory afferents can lead to increased pain sensation.[28, 29] Compared with Aδ-fibers, hyperexcitability of C-fiber

bladder afferents has been proposed to mediate bladder nociceptive responses.[23] As evidenced by responses to IWT[8], C-fibers appear to be activated in WAS rats. Bladder responses could be trigger under a relative lower bladder pressure stimulus of 20 cmH_2O in WAS rats, which is within physiologic bladder pressures providing an additional translational value of this model to the human condition. Further studies are necessary to determine the possible mechanism underlying WAS-induced bladder dysfunction. Understanding the mechanism by which stress leads to bladder hyperalgesia in susceptible individuals will provide new treatment opportunities to IC/PBS and other such lower urinary tract conditions were stress have been shown to increased symptom duration and exacerbations. Chronic WAS-induced bladder inflammation has been validated by experimental findings from several studies, including bladder inflammatory cells infiltration, increased vascular congestion and upregulated inflammation markers and may serve as a possible mechanism.[7, 30] Bladder inflammation can modulate properties of sensory afferents and activate bladder afferent nociceptors.[28, 29] Thus it is possible that both peripheral and central effects of stress may contribute to the development and maintenance of bladder hypersensitivity and hyperalgesia.

CONCLUSION

Chronic stress can alter the properties of bladder sensory pathways, leading to reduction in sensory thresholds (pain particularly) and amplification of painful sensation. We demonstrated that stress-induced bladder dysfunction may derive from the increased sensitivity of C-fibers and mechanoreceptors. Further studies will be needed to confirm these observations. Our animal model represents a novel foundation for future studies to identify potential mechanisms underlying hypersensitive conditions of the lower urinary tract, such as IC/BPS.

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Abbreviation and acronyms

AUC	area under the curve
BCR	bladder cooling reflex
BPS	Bladder pain syndrome
CD	contraction duration
CMG	cystometrogram
iBD	isometric bladder distension
IC	Interstitial cystitis

ICI	intercontraction interval
IVPmax	maximum intravesical pressure
IWT	ice water test
MAPP	a multidisciplinary approach to the study of urologic chronic pelvic pain syndrome
PT	pressure threshold
RT	room temperature
VT	VMR threshold
VMR	visceromotor reflex
WAS	water avoidance stress
WKY	Wistar Kyoto

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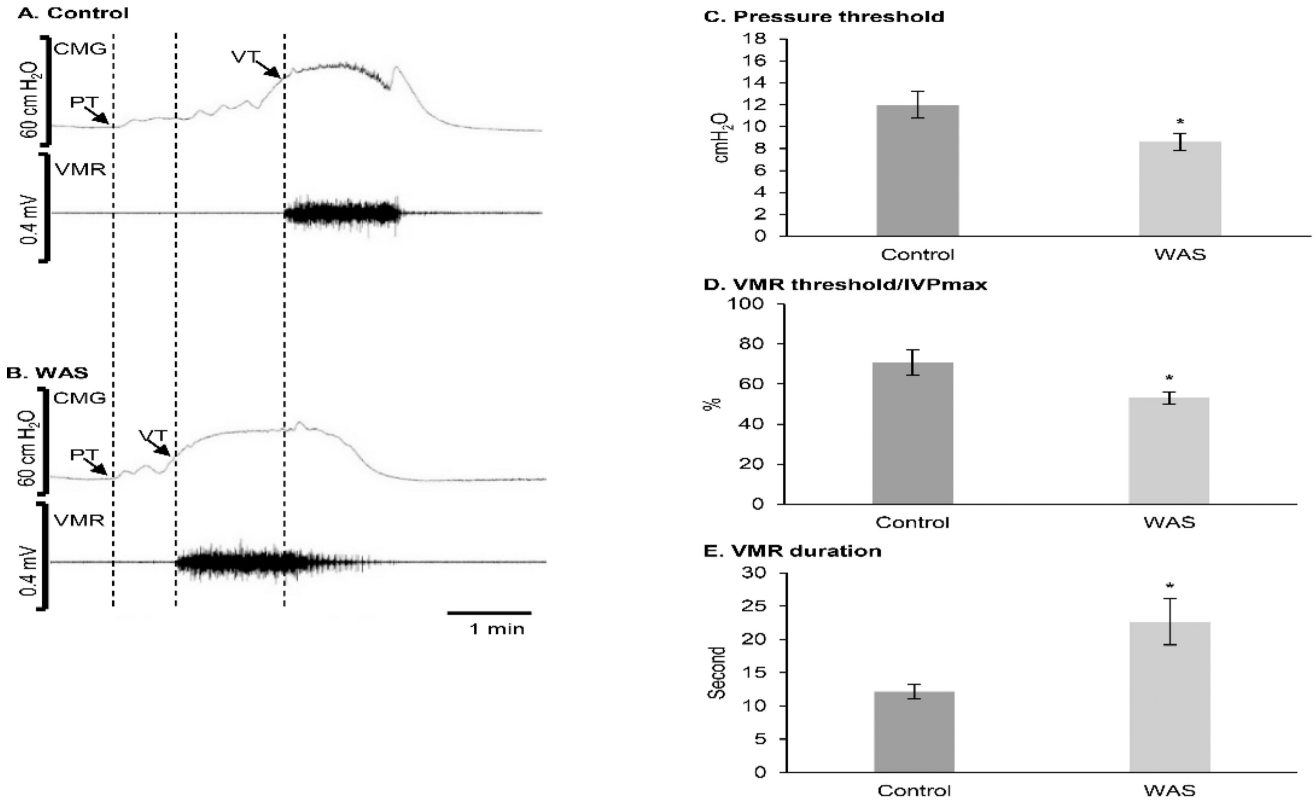

Fig 1.

CMG and VMR recordings during RT infusion. A: a representative recording example of a control rat. B: a representative recording example of a WAS rat; VMR shifted to the left compared to controls. C: WAS rats had a significant decrease in PT. D: WAS rats had a significant decrease in the ratio of VMR threshold/IVPmax. E: WAS rats had an increase in VMR duration. PT, pressure threshold; VT, VMR threshold. * represents $p<0.05$.

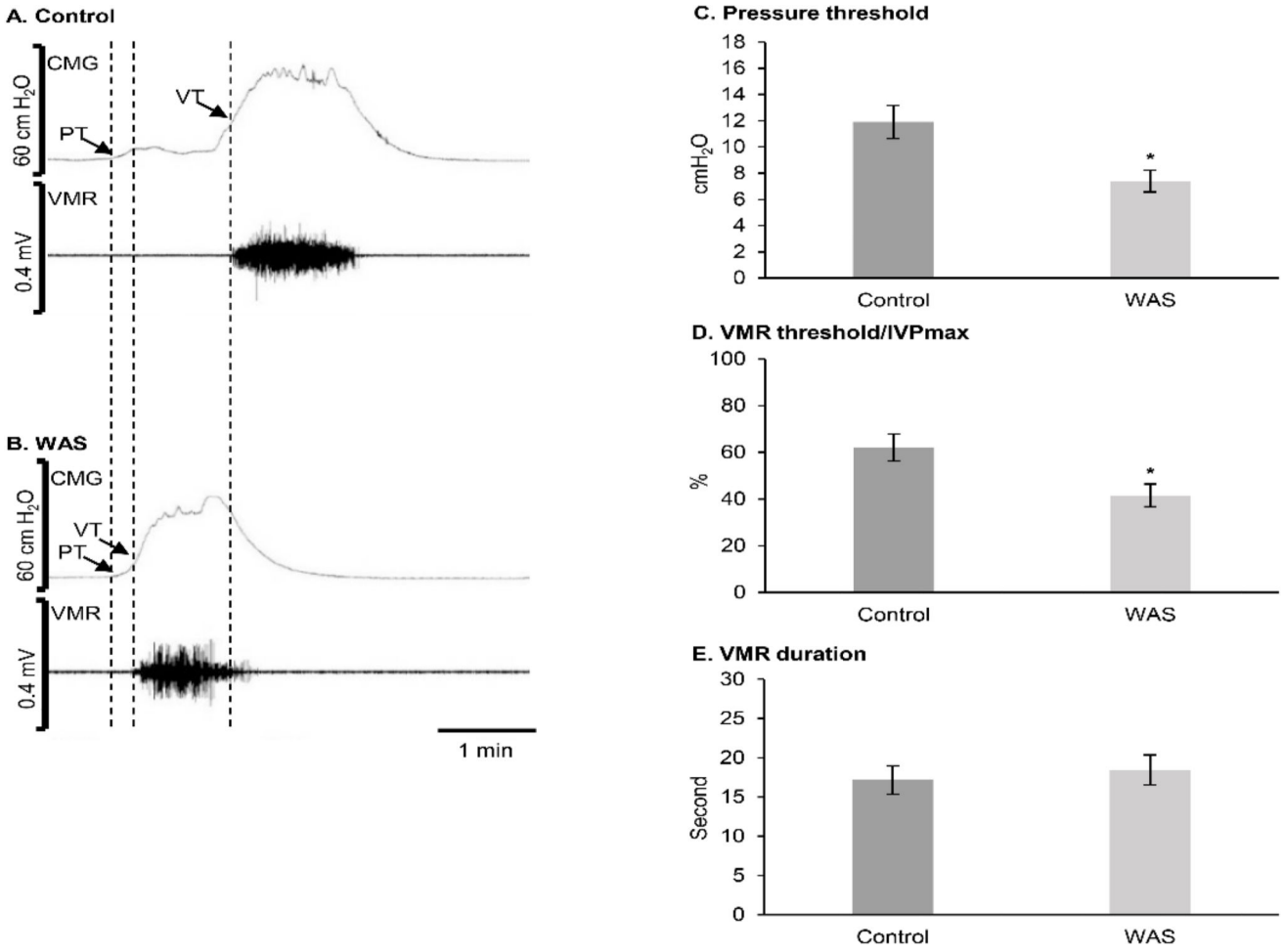

Fig 2.

CMG and VMR recordings during cold saline infusion. A: a representative recording example of a control rat. B: a representative recording example of a WAS rat; VMR shifted to the left compared to controls. C: WAS rats had a significant decrease in PT. D: WAS rats had a significant decrease in the ratio of VMR threshold/IVPmax. E: No significant difference was found in VMR duration between two groups. PT, pressure threshold; VT, VMR threshold. * represents $p<0.05$.

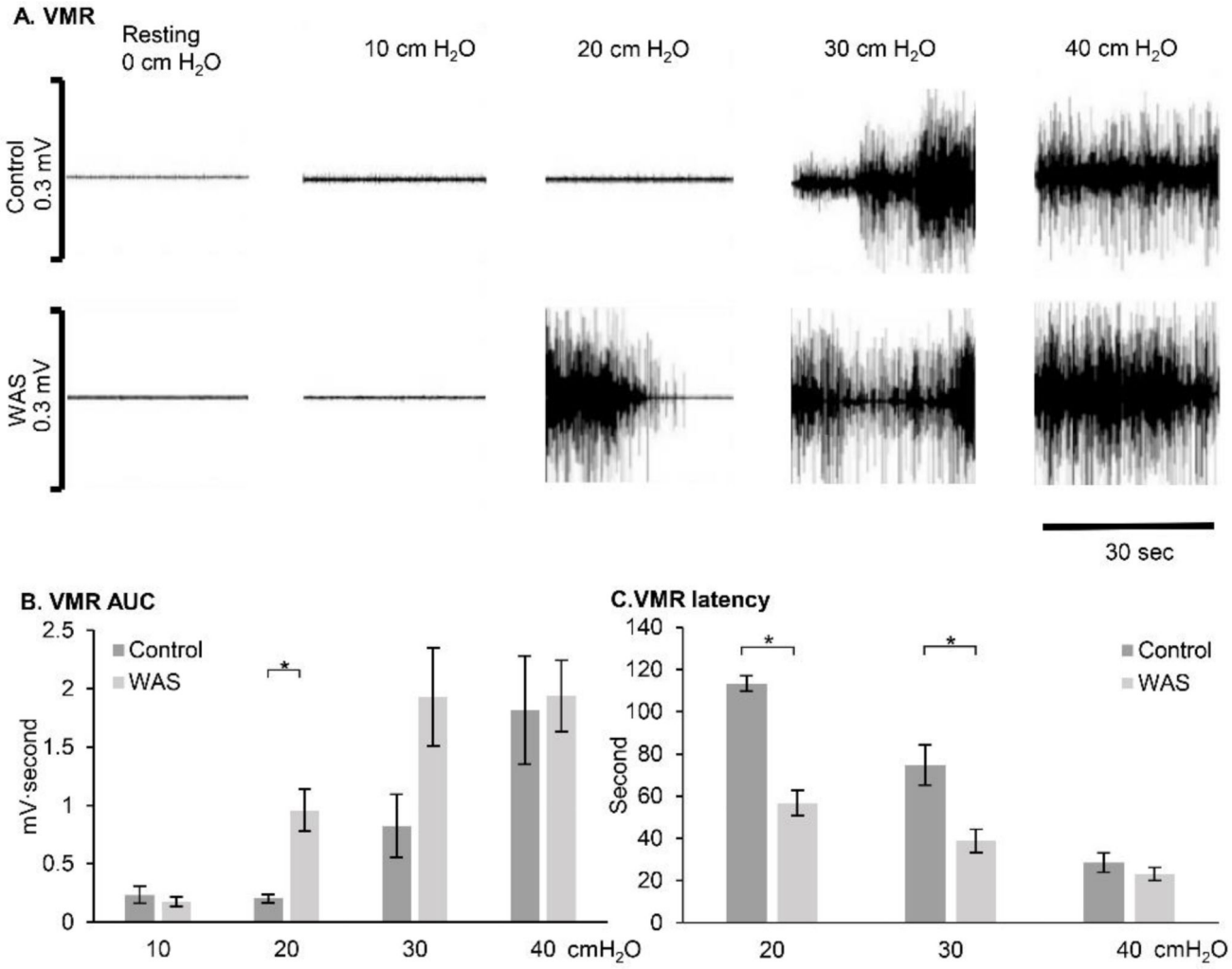

Fig 3.

VMR during RT-iBD. A: Stronger VMR with increases in bladder pressure in both control and WAS rats; at 20 cmH_2O, only WAS rats exhibited a VMR. B: Shorter VMR latency in WAS rats. C: Increased VMR AUC in WAS rats. * represents $p<0.05$.