Intravesical TRPV4 blockade reduces repeated variate stress-induced bladder dysfunction by increasing bladder capacity and decreasing voiding frequency in male rats

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Merrill L, Vizzard MA. Intravesical TRPV4 blockade reduces repeated variate stress-induced bladder dysfunction by increasing bladder capacity and decreasing voiding frequency in male rats. Am J Physiol Regul Integr Comp Physiol 307: R471–R480, 2014. First published June 25, 2014; doi:10.1152/ajpregu.00008.2014.—Individuals with functional lower urinary tract disorders including interstitial cystitis (IC)/bladder pain syndrome (BPS) and overactive bladder (OAB) often report symptom (e.g., urinary frequency) worsening due to stress. One member of the transient receptor potential ion channel vanilloid family, TRPV4, has recently been implicated in urinary bladder dysfunction disorders including OAB and IC/BPS. These studies address the role of TRPV4 in stress-induced bladder dysfunction using an animal model of stress in male rats. To induce stress, rats were exposed to 7 days of repeated variate stress (RVS). Quantitative PCR data demonstrated significant ($P \leq 0.01$) increases in TRPV4 transcript levels in urothelium but not detrusor smooth muscle. Western blot analyses of split urinary bladders (i.e., urothelium and detrusor) showed significant ($P \leq 0.01$) increases in TRPV4 protein expression levels in urothelial tissues but not detrusor smooth muscle. We previously showed that RVS produces bladder dysfunction characterized by decreased bladder capacity and increased voiding frequency. The functional role of TRPV4 in RVS-induced bladder dysfunction was evaluated using continuous, open outlet intravesical infusion of saline in conjunction with administration of a TRPV4 antagonist, GSK1016790A (3 μM), a TRPV4 antagonist, HC067047 (1 μM), or vehicle (0.1% DMSO in saline) in control and RVS-treated rats. Bladder capacity, void volume, and intercontraction interval significantly decreased following intravesical instillation of GSK1016790A in control rats and significantly ($P \leq 0.01$) increased following administration of HC067047 in RVS-treated rats. These results demonstrate increased TRPV4 expression in the urothelium following RVS and that TRPV4 blockade ameliorates RVS-induced bladder dysfunction consistent with the role of TRPV4 as a promising target for bladder function disorders.

micturition; TRPV4; stress; bladder; Q-PCR; Western blotting

UNDER HOSTILE CONDITIONS, there is a coordinated reaction known as the stress response that is activated to enhance survival. Symptom exacerbation due to stress is prevalent in many disease states, including functional disorders of the urinary bladder such as overactive bladder (OAB) and interstitial cystitis (IC)/bladder pain syndrome (BPS) (26, 44, 52). The prevalence of micturition disorders is high among people with anxiety disorders, and various stressors often increase levels of anxiety (8). Symptom exacerbation during times of stress may be partly due to disruption of the hypothalamic-pituitary-adrenal (HPA) axis. However, the pathophysiology underlying the effects of stress on micturition reflex function remains unknown.

Previous studies in our laboratory have examined stress-induced effects on micturition reflex function (37). A repeated variate stress (RVS) paradigm (17) that lacks habituation was used, in which a different stressor was presented every day for 7 days (d). RVS altered micturition reflex function by causing a decrease in bladder capacity and void volume and an increase in urinary frequency in rats exposed to the RVS paradigm (37). We also observed an increase in nerve growth factor (NGF) protein expression in the urinary bladders of rats exposed to RVS. The role of the neurotrophin NGF has been well established in urinary bladder function (7, 9), urinary bladder inflammation (45, 50), increased voiding frequency (21, 54), and functional urinary tract disorders including IC/BPS (34, 41), OAB (25, 33), and bladder outlet obstruction (32). Prior work in the laboratory has demonstrated the pleiotropic effects of NGF overexpression to modulate other proteins, including other neurotrophins/receptors, neuropeptides/receptors, and transient receptor potential (TRP) channels (36).

The TRP protein family is a group of nonspecific cation channels that are involved in sensory transduction in a variety of cellular processes. Members of the vanilloid (V) family, specifically TRPV4, are expressed in urothelial cells and lumbosacral dorsal root ganglia (5, 12, 16) and may act as sensors of stretch and/or chemical irritation in the lower urinary tract (31). The TRPV4 channel has been implicated in bladder diseases such as OAB and BPS (40). The role of TRPV4 in bladder function has been heavily studied in animal models. TRPV4 knockout (KO) mice exhibit an abnormal urine voiding pattern including a decreased frequency of voiding contractions and an increased frequency of nonvoiding contractions, intermicturition interval, bladder capacity, and total urine volume per micturition (13, 14). In addition to the TRPV4 KO mouse model, pharmacological manipulation of the channel has been used to study its role in bladder function. TRPV4 activation in rodents results in decreases in bladder capacity and increases in voiding frequency (2, 47), whereas TRPV4 blockade in animal models of cystitis decreases voiding frequency (13, 51), making the channel a promising target for bladder function disorders (2, 23).

The aim of the current study is to investigate the role of one member of the TRPV family, TRPV4, as a sensory transducer in stress-induced urinary bladder dysfunction. Bladder function was assessed following RVS using continuous, intravesical infusion of saline in conscious, unrestrained male rats with an open outlet both before and after TRPV4 blockade or activation. We also evaluated urinary bladder TRPV4 transcript...
levels and protein content in both the urothelium and detrusor smooth muscle following RVS.

MATERIALS AND METHODS

Animals

Adult, male Wistar rats (300–350 g) were purchased from Charles River Laboratories (Wilmington, MA) and housed one or two per cage and maintained in standard laboratory conditions with free access to food and water. The University of Vermont Institutional Animal Care and Use Committee (IACUC) approved all animal use procedures (Protocol 08-085). Animal experimentation was carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Repeated Variate Stress

Rats were assigned to control or RVS groups based on body weight. Rats assigned to the RVS group were exposed to 7 d of stress where rats were administered a single stressor on each day as described previously (17). Control rats were left in home cages in the animal facility following weight measurement.

Oscillation stress. Rats were placed inside a plastic chamber 28 × 17 × 13 cm [length (L) × width (W) × height (H)] that was secured to a clinical rotator (Fisher Scientific, Morris Plains, NJ) and oscillated at low to medium speed for 30 min.

Forced swim. Rats were placed in a cylindrical container 29 × 37 cm [depth (D) × H] that was filled with room temperature water to a depth that prevented the tail from touching the bottom of the container. After 5 min of monitored swimming, rats were placed in a holding chamber for 30 min before being returned to their home cage.

Electrical footshock. Rats were placed inside a Plexiglas conditioning chamber (Med Associates, St. Albans, VT) 30 × 25 × 35 cm (L × W × H). After a 5-min acclimation period, two 1.0-mA 5 s (s) scrambled footshocks were delivered through the grid floor with a 1-min intertrial interval.

Restraint. Rats were placed in a cylindrical restraining device 9 × 15 cm (D × H) for 60 min.

Pedestal. Rats were placed on an elevated platform 20 × 20 cm (L × W) that was 60 cm from the floor for 30 min.

Real-Time Quantitative RT-PCR for TRPV4

Rats

(n = 6/group) from both experimental groups (RVS and control) were euthanized 24 h following the last stressor by being deeply anesthetized with isoflurane (3–4%) and then a thoracotomy was performed. The urinary bladders (95–150 mg) were quickly dissected and homogenized separately in tissue protein extraction agent (T-PER; Roche, Indianapolis, IN), a mild zwitterionic dialyzable detergent in 25 mM bicine, and 150 mM sodium chloride (pH 7.6) containing a protease inhibitor mix (Sigma-Aldrich; 16 g/ml benzamidine and 2 μg/ml pepstatin A), and aliquots were removed for protein assay. In some instances, the bladder was cut open along the midline and pinned to a Sylgard-coated dish and the urothelium was removed with the aid of fine forceps and a dissecting microscope. Samples (35 μg) were suspended in sample buffer for fractionation on gels and subjected to SDS-PAGE. Proteins were transferred to nitrocellulose membranes, and efficiency of transfer was evaluated. Membranes were blocked for 1 h at room temperature in a solution of 5% donkey serum in Tris-buffered saline with 0.1% Tween. Membranes were incubated in anti-TRPV4 (1:500; Osselens, Fisher Scientific, Pittsburgh, PA; catalog no. OSR00136W) or 20% Tween overnight at 4°C. Washed membranes were incubated in species-specific secondary antibodies for 2 h at room temperature for enhanced chemiluminescence detection (Pierce, Rockford, IL). Blots were exposed to BioMax film (Kodak, Rochester, NY) and developed. Blots were analyzed using the Versa Doc 4000 MP Imaging System (Bio-Rad, Hercules, CA). The adjusted volume of each band was analyzed, and background intensities were subtracted using Quantity One software (Bio-Rad; Vermont Cancer Center DNA Analysis Facility). Images were scanned with a flatbed scanner, the contrast was corrected, and the images were imported and figures assembled with Adobe Photoshop (San Jose, CA). Western blot analysis of actin (1:1,000; Santa Cruz) was performed using the Bio-Rad Chemidoc XRS Imaging System (Bio-Rad, Hercules, CA). The adjusted volume of each band was analyzed, and background intensities were subtracted using Quantity One software (Bio-Rad; Vermont Cancer Center DNA Analysis Facility). Images were scanned with a flatbed scanner, the contrast was corrected, and the images were imported and figures assembled with Adobe Photoshop (San Jose, CA). Western blot analysis of actin (1:1,000; Santa Cruz Biotechnologies; catalog no. sc1616) in samples was used as a loading control. When TRPV4−/− mouse tissues were used in Western blotting procedures, we did not demonstrate a band (16).

Intravesical Catheter Implanted

A lower midline abdominal incision was made while the animals were under general anesthesia with 2–3% isoflurane using aseptic techniques (29, 48). One end of polyethylene tubing (PE-50; Clay Adams, Parsippany, NJ) was flared with a flame and inserted in the dome of the bladder and secured in place with a 6–0 nylon purse-string suture (6, 48). The distal end of the tubing was tunneled subcutaneously to the back of the neck where it was
buried in an incision in the back of the neck, out of the animal’s reach (29, 48). Rats received buprenorphine (0.05 mg/kg sc) starting at the time of surgery and then every 8–12 h postoperatively for a total of four doses. Animals were maintained for 72 h after surgery before conscious cystometry was initiated to ensure complete recovery.

Conscious Cystometry with Continuous Intravesical Infusion of Saline and Manipulation of TRPV4 Channels

The effects of TRPV4 on bladder function in control rats and rats exposed to 7 d of RVS were evaluated with both a TRPV4 agonist (GSK1016790A; 3 μM) and a TRPV4 antagonist (HC067047; 1 μM) using conscious cystometry and continuous infusion of intravesical saline (n = 4–6/group). Previous studies have shown no effects of either agent in TRPV4−/− mice, thus establishing specificity of their agonist and antagonist actions (11, 46; Nelson MT, personal communication). In addition, in pilot in vivo and ex vivo studies evaluating the roles of TRPV4 in bladder function in other animal models of bladder dysfunction, GSK agonist effects are blocked with HC067047 using the concentrations reported in the current experiments (unpublished observations). During cystometry, unrestrained and conscious rats were placed in a recording cage over a scale and pan to collect and measure voided urine. To elicit repetitive bladder contractions, room temperature saline was infused at a constant rate (10 ml/h). After an initial stabilization period (25–30 min), at least six reproducible micturition cycles were recorded. Intravesical pressure changes were recorded using a small Animal Cystometry System (Med Associates) (6, 29). Baseline resting pressure, pressure threshold for voiding, maximal voiding pressure, and intercontraction interval were measured. Nonvoiding bladder contractions (NVCs), defined as rhythmic intravesical pressure increases 7 cmH2O above baseline without the release of fluid from the urethra (38), were also determined per voiding cycle. Bladder capacity was measured as the amount of saline infused in the bladder at the time when micturition commenced (20).

To evaluate the effects of TRPV4 on bladder function, rats were anesthetized (1–2% isoflurane) and vehicle [0.1% DMSO (Sigma-Aldrich) in saline], GSK1016790A (3 μM), or HC067047 (1 μM) was intravesically infused for 30 min. The concentrations selected for agonist and antagonist evaluation were based on a dose-response curve (data not shown) and previous studies (13), respectively. Before intravesical drug infusion, the bladder was manually emptied using the Credé maneuver. Bladders were then infused with −1 ml (less than bladder capacity so that a bladder contraction is not elicited resulting in expulsion of instillate) of vehicle, GSK1016790A, or HC067047 as previously described (6, 29). Rats remained anesthetized during the 30-min infusion to depress the micturition reflex and prevent expulsion of the drug from the bladder. To avoid potential variation resulting from circadian rhythms, experiments were conducted at similar times of the day (10). At the conclusion of the study, rats were euthanized as described above. Micturition function before and after vehicle, GSK1016790A, or HC067047 intravesical instillation was evaluated in the same rats (control and RVS groups).

Exclusion Criteria

Rats were removed from the study when adverse events occurred that included 20% reduction in body weight postsurgery, a significant postoperative event, lethargy, pain, or distress not relieved by our IACUC-approved regimen of postoperative analgesics or hematuria in control rodents (6, 29). In the present study, no rats were excluded from the study. In addition, behavioral movements such as grooming, standing, walking, and defecation rendered bladder pressure recordings during these events unusable.

Materials

GSK1016790A (produced and distributed by GlaxoSmithKline, Dr. Kevin Thorneloe, contact) and HC067047 (Tocris Biosciences; catalog no. 4100) were prepared as stock solutions in DMSO, aliquoted, and stored at −20°C until use. Aliquots were diluted with saline to achieve final concentration at time of experimentation.

Statistical Analyses

All values represent means ± SE. Data from Q-PCR were compared using ANOVA, and Western blot studies were compared with unpaired t-tests (whole urinary bladder) and ANOVA (split urinary bladder, i.e., urothelium, detrusor). Cystometry data for vehicle groups were compared using repeated-measures ANOVA, where each animal served as its own control, and drug-treated groups were compared using two-way ANOVA. Animals, processed and analyzed on the same day, were tested as a block in the ANOVA. When F ratios exceeded the critical value (P ≤ 0.05), the Newman-Keuls or Bonferroni post hoc tests were used to compare group means. The significance level (P = 0.05) presented is for a two-tailed test.

RESULTS

TRPV4 mRNA and Protein Expression in the Urinary Bladder of Rats Following RVS

Q-PCR demonstrated a significant (P ≤ 0.01) change in TRPV4 mRNA in urothelium from rats exposed to 7 d of RVS compared with controls (Fig. 1A). No significant change was detected in TRPV4 mRNA relative expression in detrusor smooth muscle from RVS and control groups (P = 0.12; Fig. 1B). TRPV4 protein levels from whole bladder homogenates showed a significant change (P ≤ 0.01) following 7 d of RVS compared with controls as determined with Western blot analysis (Fig. 2, A and B). Western blot analyses of split urinary bladder tissues (i.e., urothelium and detrusor) demonstrated a significant change in TRPV4 protein expression in urothelial tissue, but not in detrusor smooth muscle. (Fig. 2, C and D).

Effects of TRPV4 Agonist and Antagonist on Bladder Function

Previous work in our laboratory established that RVS produces biological markers of stress similar to other studies (17, 18, 24, 43) including a robust and reproducible reduction (10%) in body weight gain during stressor exposure (37). We also demonstrated that RVS produces bladder dysfunction in rats characterized by decreased bladder capacity, intercontraction interval, and void volume (37). These results were confirmed in the present experiments (Table 1). To establish the role of TRPV4 in stress-induced bladder dysfunction, we performed intravesical instillation (30 min) of GSK1016790A (3 μM), a potent and selective TRPV4 agonist, as well as HC067047 (1 μM), a potent and selective TRPV4 antagonist, and examined urodynamics in both control rats and in rats exposed to 7 d of RVS. Vehicle (0.1% DMSO) controls were evaluated in both control and RVS-treated rats. There were no significant differences in urodynamic parameters (bladder capacity, intercontraction interval, and void volumes) or bladder pressures (baseline pressure, micturition pressure, and threshold pressure) following intravesical instillation of the vehicle in either control rats or RVS-treated rats (Table 1).
Fig. 1. Increased the transient receptor potential ion channel vanilloid 4 (TRPV4) mRNA expression in urothelium following 7 days (d) of repeated variate stress (RVS) as detected by quantitative PCR. TRPV4 mRNA transcript relative expression normalized to the relative expression of the reference gene, L32 (represented as percentage (%)) of control in the same samples. RVS significantly (P ≤ 0.01) change in bladder capacity, void volume (2.2-fold) in rats exposed to 7 d of RVS compared with the predrug state (Table 1; Fig. 3), consistent with previous findings (2). In fact, there were no significant differences in bladder capacity, void volume, or intercontraction interval between control rats with TRPV4 agonist administration and rats exposed to RVS, implying that the TRPV4 agonist produces stress-like bladder dysfunction in normal rats. In addition, there were no significant differences in these parameters in rats exposed to RVS before or after agonist exposure (Table 1; Fig. 4), indicating that the drug causes no additional changes in bladder function in RVS-treated rats. There were no significant differences in threshold or baseline pressure following intravesical instillation of the agonist (Table 1). However, a significant (P ≤ 0.05) change in micturition pressure was observed (Table 1; Fig. 4). NVCs were not observed in either control or RVS-treated rats following intravesical instillation of the agonist (data not shown). Residual volumes were not affected by agonist administration and were comparable (<10 µl) in both control and RVS groups.

**Effects of Intravesical Instillation of a TRPV4 Agonist on Urinary Bladder Function**

Intravesical instillation (30 min) of GSK1016790A (3 µM) produced a significant (P ≤ 0.0001) change in bladder capacity (3.7-fold), void volume (4.3-fold) and intercontraction interval (3.7-fold) in control rats (Table 1; Fig. 3), consistent with previous findings (2). In fact, there were no significant differences in bladder capacity, void volume, or intercontraction interval between control rats with TRPV4 agonist administration and rats exposed to RVS, implying that the TRPV4 agonist produces stress-like bladder dysfunction in normal rats. In addition, there were no significant differences in these parameters in rats exposed to RVS before or after agonist exposure (Table 1; Fig. 4), indicating that the drug causes no additional changes in bladder function in RVS-treated rats. There were no significant differences in threshold or baseline pressure following intravesical instillation of the agonist (Table 1). However, a significant (P ≤ 0.05) change in micturition pressure was observed (Table 1; Fig. 4). NVCs were not observed in either control or RVS-treated rats following intravesical instillation of the agonist (data not shown). Residual volumes were not affected by agonist administration and were comparable (<10 µl) in both control and RVS groups.

**Effects of Intravesical Instillation of a TRPV4 Antagonist on Urinary Bladder Function**

Intravesical instillation (30 min) of HC067047 (1 µM) produced a significant (P ≤ 0.001) change in bladder capacity (2.3-fold) and intercontraction interval (2.3-fold) and a significant (P ≤ 0.01) change in void volume (2.2-fold) in rats exposed to 7 d of RVS compared with the predrug state (Table 1; Fig. 5). There were no significant differences in bladder capacity, void volume, or intercontraction interval between control rats before drug instillation and rats exposed to RVS following TRPV4 antagonist administration (Table 1); administration of the antagonist rescued the phenotype to that of the control. Furthermore, administration of the TRPV4 antagonist produced significant (P ≤ 0.05) changes in bladder capacity (1.4-fold) and intercontraction interval (1.4-fold) in control rats, supporting a previous suggestion (13, 14, 47) that TRPV4 is important for normal micturition function. There were no significant differences in bladder pressure (threshold, baseline, and micturition) following intravesical instillation of the
TRPV4 antagonist (Table 1). NVCs were occasionally observed in RVS-treated rats, but no significant changes in the number of NVCs were detected following intravesical instillation of the TRPV4 antagonist (data not shown).

**DISCUSSION**

The present studies confirmed our recent description (37) that RVS in male rats produces urinary bladder dysfunction characterized by decreased bladder capacity and void volume and increased voiding frequency. Furthermore, these studies demonstrated several novel findings with respect to TRPV4 channel expression in the urinary bladder and the effects of pharmacological manipulation following RVS on urinary bladder function. First, we demonstrated that RVS produces increases in TRPV4 mRNA transcript levels and protein expression in urothelium using Q-PCR and Western blotting techniques, respectively. Second, we established that 1) TRPV4 activation at the level of the urinary bladder decreases bladder capacity and increases voiding frequency in control rats, consistent with a previous study (2); and 2) TRPV4 blockade at the level of the urinary bladder increases bladder capacity and decreases voiding frequency in rats exposed to 7 d of RVS.

Previous studies using the RVS model have focused on its role in anxiety-like behavior and on brain regions involved in mediating fear- and anxiety-like behavior (e.g., the bed nucleus of the stria terminalis) (17, 18). Previously, we extended the use of the RVS model to the study of micturition reflex function and demonstrated RVS-induced changes in urinary bladder function and somatic sensitivity and in the inflammatory milieu of the urinary bladder (37). In the present studies, we focused on RVS-induced urinary bladder dysfunction and pharmacological blockade of the TRPV4 channel as a potential therapeutic target. Patients with functional bladder disorders including IC/BPS often report symptom exacerbation during times of stress (52). Few approved therapies for symptoms associated with IC/BPS exist, and those available may not be effective for all patients given the heterogeneity of this chronic pain syndrome. Therefore, it is important to utilize animal models to identify potential disease mechanisms, although their value has been questioned (52). Because of the fact that these are symptom-based disorders, where chronic pelvic/bladder pain is the cardinal symptom of IC/BPS and OAB is characterized by urinary urgency (1), both of which are impossible to study in a rat, interpretation of findings in animal models of these syndromes deserves careful attention. Therefore, the focus has been on animal models that display multiple, key characteristics of symptoms of human lower urinary tract disorders such as urinary frequency and pelvic/bladder pain (42). The TRPV4 channel has been implicated as a promising target for the IC/BPS and OAB symptom of increased voiding frequency. The current studies extend the potential utility of TRPV4 blockade at the level of the urinary bladder as a therapeutic target by demonstrating improvements in stress-induced urinary bladder dysfunction.

These studies demonstrated increased expression of TRPV4 following RVS at both the transcript and the protein level in the urothelium but not detrusor smooth muscle. The expression of TRPV4 in the lower urinary tract has been widely established; TRPV4 expression was first shown in the urinary bladder urothelium of mice and rats in 2007, where it was mainly found in basal and intermediate urothelial cells (14), and has since been confirmed (4, 30, 39). In the present study, we confirmed the presence of TRPV4 specifically in the urothelium using Q-PCR and Western blotting techniques, and we also demonstrated increased TRPV4 expression in urothelial tissue but not in detrusor smooth muscle following RVS. In rats exposed to RVS, we found that TRPV4 activation by administration of the potent and highly selective agonist GSK1016790A increased voiding frequency with associated decreases in bladder capacity and void volumes. Our results are similar to a previous study (2), which used female rats of a different strain. Additionally, the previous study by Aizawa et al. (2) found that the drug effects were transient. Urodynamic parameters were averaged for 20 min (10–30 and 40–60 min after drug adminis-
The effects were attenuated 40–60 min after drug instillation. We did not observe transient pharmacologic effects in the present study, and this may be attributed to differences in the route of drug administration. Our results indicate that instillation of GSK1016790A in male Wistar rats significantly reduces bladder capacity and void volume and increases voiding frequency for 1 h following drug administration for 30 min under anesthesia. The lack of additional changes in bladder function parameters (Table 1; Fig. 4) in rats exposed to RVS and treated with TRPV4 agonist may be due to a saturation effect; increased voiding frequency cannot be further increased. Although we did not observe changes in bladder pressures (baseline, peak, and threshold) in all other groups, we did observe an increase in peak micturition pressure in rats exposed to RVS and treated with the TRPV4 agonist. This may be indicative of an effect on efferent mechanisms involved in micturition reflex function in the context of RVS. Future studies are needed to further evaluate this mechanism.

We also found that TRPV4 blockade using intravesical administration of the selective antagonist HC067047 alleviated RVS-induced bladder dysfunction. We showed previously (37) and confirmed in this study (Table 1) that rats exposed to 7 d of RVS exhibit decreased bladder capacity and void volumes and increased voiding frequency. The present studies show that, following TRPV4 blockade, bladder capacities and void volumes are increased and voiding frequency is decreased in these rats. Most notably, there are no statistical differences in urodynamic parameters between these rats and control rats. This suggests that TRV4 blockade using intravesical instillation of the TRPV4 antagonist HC067047 fully recovers the phenotype found in rats that have been exposed to RVS. HC067047 has been used previously in regards to urinary bladder function following cyclophosphamide-induced cystitis (13), which is a well-known animal model of urinary bladder inflammation that induces urinary bladder dysfunction. Following intraperitoneal administration of HC067047, both mice and rats with cyclophosphamide-induced cystitis displayed a decrease in micturition frequency and an increase in bladder capacity using cystometric recordings (13). The present study, using an animal model of repeated stress that reproducibly causes bladder dysfunction in rats, provides further evidence that TRPV4 antagonists may offer a prom-
ising means of treating bladder dysfunction resulting from various causes.

The urinary bladder dysfunction induced by RVS is likely regulated by either central nervous system (CNS) or peripheral nervous system mechanisms, or both. CNS modulation of the micturition reflex in response to stress would likely occur through the secretion of stress hormones involved in the HPA axis. Corticotropin-releasing hormone (CRH) is released by the paraventricular nucleus of the hypothalamus in response to stressful stimuli and acts as both a steroid hormone and a neurotransmitter in the stress response. The role of CRH in micturition reflex function is well established. Neurons in Barrington’s nucleus, a brain region in the pons that controls the descending limb of the micturition reflex, highly express CRH (26, 49), and animal models of stress increase CRH mRNA expression in this brain region (22, 53). Conflicting evidence suggests either an excitatory (27) or inhibitory (35) role of CRH in the micturition reflex. Corticosterone is another steroid hormone involved in the HPA axis that is secreted by the adrenal gland in rodents during stressor exposure. Several animal models of stress have produced increased plasma corticosterone levels throughout stressor exposure (19). Therefore, it is important to consider and study the role of CNS modulation, particularly the role of corticosterone, in stress-induced bladder dysfunction. Ongoing studies in our laboratory are examining the potential role of circulating corticosterone as well as HPA axis function in our model of RVS-induced bladder dysfunction.

Stress is also known to potentiate the immune response, and we have shown previously that RVS causes an increase in a number of inflammatory mediators in the urinary bladder, including NGF (37). The role of NGF in the inflammatory milieu of the urinary bladder is well established, and NGF has been suggested as a potential biomarker for lower urinary tracts including IC/BPS (34, 41). We have shown previously the ability of NGF to modulate several TRP channels including TRPV4 (16, 36). Therefore, it is possible that increases in NGF in the urinary bladder following RVS at least in part contribute to the increases reported in TRPV4 expression, but future studies are necessary to further address this possibility.

Increased TRPV4 expression in the urothelium may lead to changes in the micturition reflex and urinary bladder dysfunction seen following 7 d of RVS. TRPV4 channels are sensory cation channels found in the urothelium and are activated by...
Stretch (52). Stretch stimulation or TRPV4 activation with the selective TRPV4 agonist 4α-phorbol 12,13-didecanoate evokes intracellular Ca²⁺ increases in urothelial cells from wild-type mice, and this effect is significantly attenuated in urothelial cells from TRPV4 KO mice (14, 39). Similar results are observed with a cultured rat urothelial cell model (4). In addition, the increases in Ca²⁺ in wild-type urothelial cells were reduced in the presence of the relatively nonselective TRP channel antagonist ruthenium red (39). It has been reported that activation of TRPV4 in bladder urothelial cells induces Ca²⁺ influx-evoked ATP release and that released ATP can modulate bladder sensory transduction by subsequent stimulation of P2X3-purinoceptors (4). Therefore, it is possible that increases in TRPV4 expression following RVS cause urinary bladder dysfunction in part through a similar mechanism. Future studies involving the use of the RVS model in TRPV4 KO mice, blockade of P2X3-purinoceptors during cystometry, or the use of a novel ex vivo preparation where urodynamic parameters and afferent nerve recordings are collected simultaneously can begin to address this possibility.

Perspectives and Significance

Many disorders of the urinary bladder, including IC/BPS and OAB, exhibit symptom (i.e., urinary frequency) exacerbation due to stress. Previous studies have characterized the effects of RVS on the inflammatory milieu of the urinary bladder and bladder function, and others have identified the role of TRPV4 in bladder dysfunction disorders. These studies further identify the role of TRPV4 in the urinary bladder in regards to RVS and also provide further support of TRPV4 as a potential target for therapeutic intervention. Increased expression of TRPV4 was detected in the urinary bladder, specifically the urothelium, following exposure to RVS. Therefore, it is possible that increases in TRPV4 expression following RVS cause urinary bladder dysfunction in part through a similar mechanism. Future studies involving the use of the RVS model in TRPV4 KO mice, blockade of P2X3-purinoceptors during cystometry, or the use of a novel ex vivo preparation where urodynamic parameters and afferent nerve recordings are collected simultaneously can begin to address this possibility.

Fig. 5. Increased bladder capacity and void volume following intravesical administration of a TRPV4 antagonist to rats exposed to 7 d of RVS. Representative cystometrogram recordings of effects of a TRPV4 antagonist (HC067047) in a rat that has been exposed to 7 d of RVS using continuous intravesical infusion of saline. Pre-HC067047 (A) and post-HC067047 (B) bladder function traces in an RVS-treated rat are shown. TRPV4 receptor blockade with intravesical infusion of HC067047 (1 μM) increased both bladder capacity (measured as the amount of saline infused in the bladder at the time when micturition commenced) and void volume compared with pretreatment conditions. Infused volume (ml, top), bladder pressure (kPa, middle), and void volume (ml, bottom) are shown in A and B, which were recorded from the same rat.

**R478 TRPV4 BLOCKADE AMELIORATES STRESS-INDUCED BLADDER DYSFUNCTION**

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explore underlying mechanisms of the role of TRPV4 in RVS-induced changes in urinary bladder structure and function by examining other components of the micturition reflex pathway (e.g., bladder afferent nerves, DRG, spinal cord, etc.), referred hyperalgesia using somatic sensitivity testing of pelvic and hindpaw regions, or other possible contributions of signaling molecules (e.g., ATP), inflammatory mediators (e.g., NGF), and/or neurochemicals (e.g., pituitary adenylate cyclase-activating peptide).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: L.M. and M.A.V. conception and design of research; L.M. performed experiments; L.M. analyzed data; L.M. and M.A.V. interpreted results of experiments; L.M. and M.A.V. prepared figures; L.M. drafted manuscript; L.M. and M.A.V. edited and revised manuscript; L.M. and M.A.V. approved final version of manuscript.

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