Functional roles of TRPV1 channels in lower urinary tract irritated by acetic acid: in vivo evaluations of the sex difference in decerebrate unanesthetized mice

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Yoshiyama M, Araki I, Kobayashi H, Zakoji H, Takeda M. Functional roles of TRPV1 channels in lower urinary tract irritated by acetic acid: in vivo evaluations of the sex difference in decerebrate unanesthetized mice. Am J Physiol Renal Physiol 298: F1351–F1359, 2010. First published March 17, 2010; doi:10.1152/ajprenal.00695.2009.—Sex-specific differences in activity of the lower urinary tract (LUT) responding to acid irritation in mice have been revealed. This study, using continuous infusion cystometry with acetic acid (AA; pH 3.0), was conducted to examine whether the transient receptor potential vanilloid type 1 (TRPV1) channels expressed in the mouse LUT are involved in the sex difference in functional responses of the bladder and urethra responding to acid irritation. No differences were found between effects of capsazepine (a TRPV1 blocker; 100 μM) and those of its vehicle on any of the cystometric changes by intravesical AA in either female or male mice. However, capsazepine eliminated the acid-induced sex differences in parameters associated with bladder contraction phase (i.e., maximal voiding pressure, closing peak pressure, 2nd-phase contraction, bladder contraction duration), whereas capsazepine did not affect those in parameters associated with bladder-filling period (i.e., intercontraction interval, actual collecting time). In males, capsazepine reduced the number of bladder contractions accompanying fluid dribbling at 2nd-phase contraction, which is indicative of the urethral response to irritation, whereas in females it increased the number. Together, these results suggest the possibilities that TRPV1 channels in the bladder and urethra are involved in the sex difference in the LUT response to acid irritation and that these participate, e.g., via “cross talk” between the bladder and urethra, in the fine-tuning of intravesical pressure (or bladder emptying) at the bladder contraction phase under irritated LUT conditions but not in sensing for bladder filling during the storage period, although the contribution of the mechanism may be small.

afferent; micturition reflex; detrusor contractility; efferent; urethral resistance

INTERSTITIAL CYSTITIS or painful bladder syndrome is a chronic pain syndrome consisting of severe refractory bladder symptoms, such as suprapubic pain, urinary frequency, and urgency, without a specific identifiable cause (48). The etiology of the disease can be multifactorial and needs to be further explored. Epidemiologic studies have shown that the prevalence and incidence of interstitial cystitis for women are significantly higher than for men, with female-to-male ratios of 5:1 to 10:1 (11, 18, 22). However, pathophysiological factors involved in the sex difference are not yet known, and elucidation of the mechanism underlying the difference is thought to be helpful to develop treatments for the disease.

A recent study (54) revealed sex-specific differences in activity of the lower urinary tract (LUT) responding to intravesical acetic acid (AA) infusion in decerebrate unanesthetized mice. The study demonstrated that female bladders are more sensitive to the noxious stimulation than male bladders, implying an association between sex and bladder sensitivity to irritation. Sites responsible for the differences in bladder sensitivity for noxious stimuli between females and males can be at the local organs, the peripheral nervous system, and/or the central nervous system (i.e., urothelium, smooth muscle, peripheral nerve fiber, dorsal root ganglia, spinal cord, brain stem, or any of these in combination), and the underlying mechanisms can be via various channels, receptors, and mediators. Potential candidate molecules that may be involved in the sex difference include proton-sensing molecules, such as transient receptor potential vanilloid type 1 (TRPV1) channel (2, 3, 5, 6), and acid-sensing ion channels (ASICs) (26, 32); sex steroid receptors, such as estrogen receptors (36, 39), progestrone receptors (39), and androgen receptors (36, 37); chemical mediators, such as adenosine triphosphate, nitric oxide, and prostanooids (15); and neurotrophic factors, such as nerve growth factor, brain-derived neurotrophic factor, and glia-derived neurotrophic factor (15, 52) in bladder afferent mechanisms. Of these proteins, a possible involvement of TRPV1 in modulation of the irritated LUT activity has been suggested, since TRPV1, which is known to be an ion channel activated by capsaicin, heat (>43°C), protons (pH < 6), and endogenous ligands such as anandamide, is widely expressed in the urinary tract of mammals (2, 3, 8, 43).

Although localizations of TRPV1 in the LUT have been well validated by previous studies (2, 3), its functional roles in the bladder response to chemical irritation as well as mechanical stimuli are still controversial (6, 16, 46). The discrepancy between results of previous in vivo studies may be attributable to various causes such as differences in animal species, sex, and consciousness (or anesthetic condition); however, these possibilities are not conclusive. To clarify the role of TRPV1 in regulation of the LUT activity, it is of interest to conduct further studies by applying alternative methods and detailed assessments.

The aim of the present study was to determine whether TRPV1 channels localized in the bladder and urethra are involved in cystometric changes and sex differences in the LUT response to acid irritation. Therefore, this study was conducted in both females and males by intravesically infusing capsazepine, a selective TRPV1 antagonist, to examine the possible involvement of TRPV1 in activity of the LUT in response to AA irritation. Decerebrate unanesthetized mice
(54) were used to avoid possible artifacts caused by behavioral responses and movements of awake animals that receive persistent intravesical irritant during cystometry (6, 34) and anesthesia-induced suppressions of the LUT activity (27, 56, 61). Moreover, to facilitate better understanding of the complex autonomic functions, we used detailed cystometric evaluations (54).

A preliminary report of this study has been presented in abstract form (55).

MATERIALS AND METHODS

Animal preparation. Twelve female and 12 male mice (C57BL/6, 12–13 wk old; Charles River Laboratories, Yokohama, Japan) weighing 19.4 ± 0.3 and 22.8 ± 0.2 g, respectively, were used in this study. The animals were housed under a 12:12-h light-dark cycle with controlled humidity and temperature. Standard pellet diet and water were available ad libitum. All animal procedures were reviewed and approved by the University of Yamanashi Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used. The animals were anesthetized with sevoflurane (2–3%) in oxygen (flow rate: 0.2 l/min) during surgery before decerebration. The trachea was cannulated with a polyethylene tube (PE-90; Clay-Adams, Parsippany, NJ) to facilitate respiration. The bladder was exposed by way of a midline abdominal incision. The bladder end of a polyethylene catheter (PE-50; Clay-Adams) was heated to create a collar and passed through a small incision at the apex of the bladder dome, and a suture was tightened around the collar of the catheter. The incised abdominal muscles as well as the incised skins were loosely sutured, and the catheter exited near the xiphoid process.

Precocious decerebration was performed according to a published method (38) that included ligating both carotid arteries, followed by midline incision of head skin with a scalpel and removal of the skull and forebrain using a fine rongeur and a blunt spatula, respectively. Sevoflurane was then discontinued. After no further intracranial hemorrhage was visually detected, both lateral flaps of the incised head skin were sutured together. Experiments were started 2 h after the decerebration and conducted under unanesthetized conditions (54).

Saline (pH 6.3) was infused into the bladders for 2 h before baseline values were measured. Bladder activity was monitored by way of the cystometry catheter connected to a pressure transducer. Cystometric recordings were performed by continuously infusing physiological saline (30 μl/min) at room temperature into the bladder to elicit repetitive voids, which allowed data collection for a large number of voiding cycles (31). Cystometric parameters measured were as follows (54): maximal voiding pressure (MVP; mmHg), which is the peak intraluminal pressure during voiding; closing pressure (CPP; mmHg), which is the peak intraluminal pressure during voiding; closing peak pressure (CPP; mmHg), which is the peak intraluminal pressure to induce a voiding contraction; resting pressure (RP; mmHg), which is the lowest intraluminal pressure during the postvoiding phase of a bladder contraction; pressure threshold (PT; mmHg), which is the intraluminal pressure to induce a voiding contraction; resting pressure (RP; mmHg), which is the lowest pressure immediately after a voiding contraction; intercontraction interval (ICI; s), which is the time lag between two voiding cycles; bladder compliance (BCP; μl/mmHg), which was calculated as the ratio of infused volume to the pressure difference between time points of RP and the following PT, i.e., (infusion rate × ICI/60) (in μl)/(PT – RP) (in mmHg). Bladder contraction duration (BCD; s) is the sum of the first-phase duration (1st PD; s) and the second-phase duration (2nd PD; s). In general, voiding occurs at a part of the first phase of bladder contraction, and the second phase of the contraction belongs to the bladder-filling period (54). Actual collecting time (ACT; s) consists of ICI and 2nd PD, which indicates the actual storage period until induction of the following voiding contraction during continuous cystometric recording. ACT was defined as being equivalent to ICI if a preceding bladder contraction exhibited persistent fluid dribbling at the second-phase contraction (54). The significance of evaluations in these parameters was stated in a previous report (54).

AA infusion into the bladder has been widely used as a model for the LUT irritation (6, 34, 54, 56, 61). Effects of diluted AA (pH 3.0) intravesically infused for 1 h on bladder activity were examined after the baseline infusion of saline. After the AA infusion, saline was again infused into the bladder to examine recovery from the irritated LUT.

Drugs. Sevoflurane (Maruishi Pharmaceutical, Osaka, Japan), AA (99.7%; Nacalai Tesque, Kyoto, Japan), ethanol (Wako Pure Chemical Industries, Osaka, Japan), and capsazepine (Sigma-Aldrich, St. Louis, MO) were used in the present study. To make 100 μM capsazepine solution with AA, we added 40 μl of the stock solution of capsazepine (50 mM in 100% ethanol) and several drops of AA to 20 ml of saline, and the pH was adjusted to 3.0 with monitoring (using a compact pH meter B-212; Horiba, Kyoto, Japan) (final concentration of diluted AA ~0.23%). Vehicle solution (saline containing 0.2% ethanol and diluted AA at pH 3.0) was also prepared.

Data analysis and statistics. Three consecutive storage periods and voiding contractions immediately before, during, and after AA infusion were evaluated as baseline control (i.e., saline infusion), effect of acidic irritation, and recovery (up to 3 h) from the irritation, respectively. All values are means ± SE. The unpaired t-test, Wilcoxon matched pairs test, and two-way analysis of variance (ANOVA) were used for statistical analyses, if applicable. For all analyses, P < 0.05 was considered significant.

RESULTS

Table 1. Comparisons between cystometric parameters of female and male mice

<table>
<thead>
<tr>
<th></th>
<th>PT, mmHg</th>
<th>MVP, mmHg</th>
<th>CPP, mmHg</th>
<th>RP, mmHg</th>
<th>BCP, μl/mmHg</th>
<th>1st PD, s</th>
<th>2nd PD, s</th>
<th>BCD, s</th>
<th>ICI, s</th>
<th>ACT, s</th>
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<tbody>
<tr>
<td>Female</td>
<td>7.1 ± 0.5†</td>
<td>26.4 ± 0.9</td>
<td>17.4 ± 1.6</td>
<td>2.7 ± 0.2</td>
<td>40.3 ± 3.9</td>
<td>10.8 ± 0.7</td>
<td>18.7 ± 1.2*</td>
<td>29.5 ± 1.2*</td>
<td>306 ± 21‡</td>
<td>324 ± 21‡</td>
</tr>
<tr>
<td>Male</td>
<td>5.2 ± 0.3</td>
<td>23.3 ± 1.3</td>
<td>16.2 ± 2.3</td>
<td>2.4 ± 0.3</td>
<td>34.9 ± 4.4</td>
<td>10.3 ± 0.9</td>
<td>26.3 ± 2.8</td>
<td>36.6 ± 2.7</td>
<td>186 ± 22</td>
<td>211 ± 21</td>
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*Values are means ± SE for 12 mice per group. PT, pressure threshold for inducing micturition contraction; MVP, maximal voiding pressure; CPP, closing peak pressure; RP, resting pressure; BCP, bladder compliance; 1st PD, 1st-phase duration of bladder contraction; 2nd PD, 2nd-phase duration of bladder contraction; BCD, bladder contraction duration; ICI, intercontraction interval; ACT, actual collecting time. *P < 0.05; †P < 0.01; ‡P < 0.001, statistical difference between female and male mice (by unpaired t-test).
infusion of the vehicle used in the present study (AA alone vs. AA with the vehicle: $P < 0.0001$, by 2-way repeated measures ANOVA).

Comparisons between effects of capsazepine and its vehicle in each sex. In female mice, significant decreases in PT, MVP, BCP, ICI, and ACT were produced by intravesical infusion of AA-containing capsazepine (100 $\mu$M) that were equivalent to decreases in these parameters, respectively, by AA with its vehicle solution; however, CPP, RP, and 1st PD were not altered by either treatment (Fig. 3). The vehicle-contained AA decreased BCD and 2nd PD, but capsazepine-contained AA did not change these parameter values ($P = 0.56$ for BCD; $P = 0.31$ for 2nd PD) (Fig. 3, G and H). No differences between effects of capsazepine and those of its vehicle on any of these cystometric changes in responses to the AA were found in female mice (Fig. 3).

In male mice, capsazepine-contained AA significantly decreased BCP, ICI, and ACT, and these decreases were similar in degrees to changes by the vehicle-contained AA in these parameters, respectively (Fig. 3, E, I, and J). The vehicle-contained AA increased CPP, 2nd PD, and BCD, whereas capsazepine-contained AA did not change these parameter values ($P = 0.69$ for CPP; $P = 0.16$ for 2nd PD; $P = 0.16$ for BCD) (Fig. 3, C, G, and H). The AA with either capsazepine or the vehicle did not change PT, MVP, RP, or 1st PD in males. No differences between effects of capsazepine and those of its vehicle on any of these cystometric changes in responses to the AA were found in male mice (Fig. 3).

Fig. 1. Bladder activities in female mice during continuous infusion cystometry (30 $\mu$l/min) with saline (pH 6.3) and subsequent diluted acetic acid (A/A; pH 3.0)-containing vehicle (0.2% ethanol) solution (A) and saline subsequent A/A with capsazepine (CZP; 100 $\mu$M) solution (B). Notice that 1st-phase peak contraction pressures (i.e., maximal voiding pressure, MVP) and intervals between 2 consecutive bladder contractions (i.e., intercontraction interval, ICI) are markedly decreased by the acid irritation with either CZP or its vehicle. Horizontal bar with asterisk indicates fluid dribbling, which is fluid evacuation out of the urethral orifice accompanying intravesical pressure fluctuation.

Fig. 2. Bladder activities in male mice during continuous infusion cystometry (30 $\mu$l/min) with saline (pH 6.3) and subsequent diluted A/A (pH 3.0)-containing vehicle (0.2% ethanol) solution (A) and saline subsequent A/A-containing CZP (100 $\mu$M) solution (B). Notice that intravesical acid infusion, whether with CZP or its vehicle, produced only small effects on 1st-phase peak contraction pressures (i.e., MVPs) and ICIs. Horizontal bars with asterisks indicate fluid dribbling.
vehicle on any of these cystometric changes in responses to the AA were found in male mice (Fig. 3).

**Effects of capsazepine on sex differences in LUT activity in response to intravesical acid irritation.** The vehicle-contained AA produced significant differences between females and males in value changes of parameters such as PT, MVP, CPP, RP, 2nd PD, BCD, ICI, and ACT (Fig. 3). Capsazepine-contained AA produced no sex differences in value changes of parameters associated with bladder contraction phase such as MVP, CPP, RP, 2nd PD, and BCD, whereas it still elicited the sex differences in the changes of ICI and ACT (Fig. 3).

In females, five of six mice receiving intravesical infusion of the vehicle-contained AA exhibited some bladder contractions accompanying fluid dribbling at the second phase of contraction (17–33% of bladder contractions/mouse), which is considered to be a urethral response to the irritation (54) (Fig. 1A), whereas five of six mice receiving capsazepine-contained AA showed larger numbers of bladder contractions with the dribbling response (33–100% of bladder contractions/mouse) (Fig. 4A). One animal from either the capsazepine or the vehicle group did not show the dribbling response.

In males, all six mice receiving the vehicle-contained AA exhibited the dribbling response in all voiding contractions (100% of bladder contractions/mouse) (Fig. 2A). However, three of six mice receiving the capsazepine-contained AA showed some bladder contractions with no dribbling (0–50% of bladder contractions/mouse) (Fig. 4B), and the other three mice exhibited the dribbling in all voiding contractions. Fluid dribbling could be eliminated by stopping the intravesical AA infusion at the beginning of bladder contraction (i.e., before voiding started), and then smaller pressure fluctuations without fluid evacuation at the second phase of contraction were elicited, suggesting that the dribbling response is largely dependent on fluid collected during the second phase of contraction.

**Time course of recovery from acid-induced cystometric changes and nonvoiding contractions before micturition.** Figure 5 shows time courses of cystometric parameter changes during intravesical infusion of saline or AA with either capsazepine or its vehicle. In all mice (n = 24), nonvoiding contractions unrelated to trunk movements of animals were not found during intravesical infusion of either saline or AA with capsazepine or its vehicle.

**DISCUSSION**

In the present study we validated sex differences in the LUT response to AA irritation, as shown in a previous report (54), and revealed that intravesical infusion of capsazepine (100 μM) can produce no significant effect on any of cystometric changes in response to the irritant, compared with its vehicle (0.2% ethanol) effects, in either female or male mice. In these animals, however, capsazepine eliminated the AA-induced sex differences in cystometric parameters associated with bladder contraction phase (i.e., MVP, CPP, RP, 2nd PD, and BCD), although it did not affect the differences in parameters associated with the bladder-filling period (i.e., ICI and ACT). There

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**Fig. 3. Effects of intravesical infusion of diluted A/A (pH 3.0) with CZP or its vehicle on cystometric parameters: CZP (●) or its vehicle (○) in female mice, and CZP (▲) or its vehicle (▼) in male mice (n = 6 for each group). Statistical analysis by 2-way repeated-measures ANOVA revealed that in all of the evaluated parameters, lower urinary tract (LUT) responses to intravesical A/A infusion were not different between CZP and its vehicle treatments in either female or male mice. Comparisons between female and male mice in LUT response to A/A infusion with CZP or its vehicle were also made, and the statistical differences are indicated below each graph: #P < 0.05; ##P < 0.01; ###P < 0.001, by 2-way repeated-measures ANOVA. Effects of A/A with CZP or its vehicle were analyzed in each group using the Wilcoxon matched pairs test: *P < 0.05. All statistical analyses were performed using actual values. SAL, saline (pH 6.3) infusion; ns, not significant.**
were no sex differences in AA-induced responses of BCP and 1st PD.

Involvements of TRPV1 expressed in irritated LUT during bladder contractions. A lack of effects by capsazepine on cystometric changes in response to AA, compared with its vehicle effects, in either sex implied that TRPV1 is less likely to participate as a major contributor in control of the irritated LUT activity. However, it is of interest to note that capsazepine eliminated sex differences in changes of bladder contraction-related parameters, such as MVP, CPP, RP, 2nd PD, and BCD, produced by the noxious stimulation to the LUT. MVP and CPP are parameters indicative of intravesical peak pressures at the first and second phase, respectively, of a bladder contraction and can be influenced by complex factors such as detrusor contractility and urethral resistance (54). Thus the results suggest the possibilities that TRPV1 localized in the LUT participates in the modulation of bladder and urethral activities at the bladder contraction phase and that it is involved in the sex difference in the LUT response to the irritation. The possible involvements of TRPV1 at the bladder contraction phase are supported by Daly et al. (12). They demonstrated in a study using a technique of in vitro recording from mouse

Fig. 4. Bladder activities in a female (A) and male mouse (B) during continuous infusion cystometry (30 μl/min) with saline (pH 6.3) and subsequent diluted A/A (pH 3.0)-containing CZP (100 μM) solution. Notice that A/A-containing CZP induced bladder contractions accompanying fluid dribbling in the female mouse (see Fig. 1), whereas it produced bladder contractions without such dribbling response in the male mouse (see Fig. 2).

Fig. 5. Time courses of cystometric changes in female mice that received diluted A/A (pH 3.0) with CZP (▲) or its vehicle (○) and in male mice that received A/A with CZP (●) or its vehicle (▲) (n = 6 for each group) followed by subsequent saline infusion. Notice that CZP did not affect the time courses of any evaluated cystometric parameters during the recovery from the acid irritation, compared with the vehicle’s effects, in either females or males (by 2-way repeated-measures ANOVA). To refer to statistics on the changes by A/A with CZP or the vehicle, see Fig. 3.

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capsazepine (10 μM) on afferent discharge evoked by bladder distention can be exerted only when the intravesical pressure is at 15 mmHg or higher (12), suggesting that the signal transduction mechanism via TRPV1 in the bladder can be activated when the mechanical stimulation reaches over the pressure threshold for inducing bladder contractions. In the present study, capsazepine affected sex differences induced by the acid irritation in MVP and CPP at the bladder contraction phase (i.e., >15 mmHg), whereas it did not alter sex differences in ICI and ACT during storage period (i.e., <15 mmHg) (see Table 1 and Fig. 5), showing an intriguing consistency in concert with the study by Daly et al. (12).

Changes of BCD were due to those of 2nd PD in response to AA irritation in either sex. Vehicle-contained AA decreased the 2nd PD of females and increased that of males. The increased 2nd PD in males was due to fluid dribbling in which the urethral response to the acid irritation is likely to be involved (54). Fluid dribbling at the second phase induced by AA irritation (either with or without 0.2% ethanol as the vehicle) occurred in all males. The fluid dribbling was virtually not seen in females that received AA alone (54), whereas it occasionally occurred in females that received AA with the vehicle (i.e., present study). Capsazepine (100 μM) decreased the number of bladder contractions accompanying the fluid dribbling in males and conversely increased the number of such LUT responses in females, thus resulting in no sex differences in 2nd PD and BCD. These changes by capsazepine in fluid dribbling also suggested that the urethral response to the noxious stimulation during bladder contractions is sex dependent and is, at least partly, exerted via TRPV1 channels.

RP is largely affected by postvoiding residual volume, intravesical volume collected at the second phase of bladder contraction during continuous infusion cystometry, and detrusor tone at the end of bladder contraction. Thus elimination of the sex difference by capsazepine in RP was thought to be due to changes in these complex factors as well as the AA-induced characteristic response at the second phase (i.e., fluid dribbling).

Immunohistochemical study showed TRPV1-containing nerve terminals coursing the submucosa and muscular layer of the bladder wall in the rat (43). Electron microscopic study in rat bladder further revealed that TRPV1 immunoreactivity is exclusively in the cell membrane and cytoplasm of unmyelinated axons and their varicosities (3). Expression of TRPV1 in rat or mouse bladder epithelium has been reported (5) but is yet controversial because of aspecific immunoreactivity of antibodies for TRPV1 channels (10, 17, 47). Furthermore, TRPV1-immunoreactive fibers are densely found in the mucosa and muscle layer of proximal urethra but are present only beneath the epithelial cells in distal urethra (3). With these reports, together with the present study showing elimination of sex differences by capsazepine in parameters associated with the bladder contraction phase, it is suggested that TRPV1 channels in submucosal afferent fibers and smooth muscle layers of both the bladder and urethra may be involved in the putative neural communication between the bladder and urethra via the central nervous system (i.e., “cross talk” mechanism) (14).

The cystometric recording used in this study is a simple and beneficial manner to examine genuine coordinated activity of the bladder and urethra in micturition reflex under an intact serial connection between these two organs; however, information collected by the method is somewhat limited. To determine whether the cross talk between the bladder and urethra is mediated by the mechanism involved in TRPV1 signal transduction, further studies using an in vivo animal model of isolated bladder and urethra (24) are necessary.

Controversies on the role of TRPV1 in the LUT function. Results in in vivo functional studies reported by other investigators are controversial. Birder et al. (6) demonstrated that awake male TRPV1 knockout mice exhibited increased numbers of nonvoiding bladder contractions before micturition and increased bladder capacity during cystometry with normal saline, proposing that TRPV1 participates in mechanosensory signal transduction in the bladder and/or afferent signal processing from the bladder during the storage period under physiological conditions. In addition, they showed that the bladder response facilitated by intravesical AA infusion, which is exhibited as decreased ICI during the storage period of the cystometry, in the TRPV1 knockout mice is as sensitive as that of wild-type littermates, implying that TRPV1 is not involved in transmission of bladder afferent signaling generated by the irritation. Conversely, Wang et al. (46) showed that the bladder response of urethane-anesthetized female mice lacking TRPV1 is resistant, not like wild-type littermates, to intravesical infusion of acrolein, a toxic metabolite of cyclophosphamide, which is excreted into urine and causes bladder cystitis, suggesting that TRPV1 is involved in pain signal transmission in the bladder afferent pathway. A cause of this discrepancy is unknown but may be attributable to the fact that TRPV1 channels in the central nervous system as well as the peripheral nervous system are interacting with differences in diverse factors such as sex, anesthetic conditions, and irritants.

Dinis et al. (16) demonstrated that capsazepine increases ICI and that anandamide, a TRPV1 agonist, decreases ICI during continuous saline infusion cystometry in urethane-anesthetized female rats. Since ICIs can be affected by various factors including volume threshold for inducing micturition, voided volume, residual volume (or voiding efficiency), bladder compliance (or detrusor tone), or any of these in combination, greater attention should be paid to the interpretation of changes in the parameter. For example, the increased ICI accompanying a decrease of RP and the decreased ICI accompanying an increase of RP can be indicative of the decrease and the increase, respectively, of residual volume. Changes in the postvoiding residual volume may be attributable, at least, to alterations of urethral resistance. In the study by Dinis et al. (16), each solution of drugs was extravesically and continuously applied onto the bladder serosa, and thus either drug could reach the urethra via the surface of the one serial organ. Thus it is reasonable to assume that in addition to the potential effects on detrusor, capsazepine facilitates evacuation of intravesical fluid by reducing the urethral resistance, whereas anandamide inhibits it by increasing intraurethral pressure. In our present study, capsazepine produced an increased number of bladder contractions accompanying fluid dribbling in female mice, which is likely to be linked with the urethral relaxing response. It should be noted that a reduction of urethral resistance would not produce a further decrease of RP in decerebrate unanesthetized mice because these animals have excellent voiding efficiency (i.e., higher than 95%) and thus little postvoiding residual volume (49).
In this study, we did not find any nonvoiding contractions before each micturition contraction in either female or male mice during intravesical infusion of saline or AA with either capsazepine or its vehicle solution. Charrua et al. (9) also reported that urethane-anesthetized female TRPV1−/− mice during saline infusion cystometry do not exhibit nonvoiding contractions. However, other investigators have claimed that awake or urethane-anesthetized mice with either TRPV1+/+ or TRPV1−/− present nonvoiding contractions during saline infusion cystometry with or without intravesical irritant (6, 46). What causes the difference in the incidence of nonvoiding contractions? First, we can refer to differences in animal consciousness/anesthetic conditions rather than other factors such as intravesical irritant used and sex. Our study was performed in decerebrate unanesthetized mice. Supracollicular decerebration, which removes a forebrain receiving signals from the peripheral organs via spinal cord and brain stem, and supplying both excitatory and inhibitory inputs to the brain stem, allows study of involuntary “reflex” activity of the LUT, because the brain stem (i.e., including periaqueductal gray and pons), spinal cord (i.e., including thoracic sympathetic neurons and lumbosacral parasympathetic neurons), and peripheral nerves including neural circuits responsible for voiding and storage (13) are intact. On the other hand, conscious animals presenting voluntary micturition “behavior” would be easily affected by stress from circumstances (35, 41) or chemicals that change the mood of animals (42, 50). Thus it is reasonable to speculate that nonvoiding contractions (or sudden increases of intravesical pressure) found in awake mice inflamed by intravesical irritant are caused by abdominal pressure changes, trunk movements rocking a cystometry tube connected to bladder, or both. Intriguingly, recent studies revealed that mechanisms via TRPV1 in the brain and brain stem are involved in anxiety and that blockade of TRPV1 channels in the medial prefrontal cortex of rats causes anxiolytic-like effects (1, 40), implying the possibility that micturition behavior can be affected by the lack or blockade of TRPV1 channels in the central nervous system.

Nonvoiding contractions, for example, induced by excited C-fibers transmission found in chronic neurotraumatic states such as spinal cord injury may be distinguished by systemic administration of neuromuscular blocking agents such as pancuronium bromide, vecuronium bromide, and α-h-Benzotropin with an artificial respiratory support (27, 53); however, for ethical reasons we are not allowed to conduct such studies in conscious animals. Although urethane is often used in in vivo neurophysiological and pharmacological experiments to study reflex micturition because of the convenience and suitability (29, 33), it is also known to induce nonvoiding contraction-like activity in normal rodents (30). Decerebration allows us to study reflex micturition without the use of anesthesia in the course of an in vivo experiment (51, 57–60) and with the use of a neuromuscular blocking agent (53), if necessary.

Potential influences of estrous cycle on female LUT responses to acid irritation. Johnson and Berkley (23) showed that estrous cycle influences volume threshold for inducing the first contraction (regardless of voiding or nonvoiding) of inflamed bladder cystometry in urethane-anesthetized rats, suggesting that severity in hyperreflexia and instability of the irritated bladder depends, at least in part, on the level of sex hormones in the female. Their results also suggested the possibility that estrous cycle status affects nonvoiding contractions, ICI, and ACT, which are related to the urine storage period, during continuous infusion cystometry with intravesical irritant. The present study in which estrous cycle stage in each female mouse was not determined showed no nonvoiding contraction and no differences between the capsazepine group and its vehicle group in AA-induced changes of these parameters, implying the possibilities that 1) intravesical capsaicin eliminates the estrous influence on storage function via TRPV1 in the bladder; 2) estrous variations are mingled equally between these groups; and/or 3) estrous status does not affect the storage function of irritated bladder in decerebrate unanesthetized mice. Thus it is of interest to examine whether estrous cycle status influences the amount and role of TRPV1 channels expressed in the LUT and also whether the unanesthetized condition can alter the influence of estrous variations on storage function, since an anesthetic such as urethane affects sex hormone status and ovulation (7, 28, 29, 20). In addition, it also is necessary to determine whether in in vivo cystometric study changes in intravesical pressure during irritated bladder contractions, i.e., MVP and CPP (the primary findings in the present study), are affected by the estrous status, since there are no previous reports showing influence of estrous cycle status on the bladder contraction phase of the inflamed bladder.

Various receptors and channels including TRPV1 expressed in the peripheral nervous system, the central nervous system including dorsal root ganglia, and the local sites of the bladder and urethra are involved in the micturition reflex (15) and may be influenced by estrous status in the female. Thus, whether or not there is estrous cycle-related differential expression of TRPV1 in the reflex pathways controlling the LUT activity, much attention should be also paid to other mechanisms via proton-sensing molecules, sex steroid receptors, chemical mediators, and neurotrophic factors in the peripheral nervous system and the central nervous system (15), which may be influenced by estrous status.

Effects of ethanol used as vehicle. It was necessary to carefully examine the effects of ethanol on LUT activity because ethanol has an agonistic property on TRPV1 channels (44), whereas ethanol is generally used as a solvent for capsaicin (Refs. 12, 21; personal communication from Sigma-Aldrich, Tokyo, Japan). In the present study, an intravesical infusion of AA contained in the vehicle solution, i.e., ethanol at low concentration (0.2%), produced changes in LUT activity that were similar to those in our previous study using AA alone (54), presenting no statistical differences between these two treatments, except for the effects on RP (i.e., postvoiding intravesical pressure) in females. Although the vehicle-contained AA produced no change in RPs in both females and males, the AA alone significantly increased the RPs of female and male mice in a similar fashion (in comparisons by Wilcoxon matched pairs test) (54). This study together with the previous results (in comparisons by 2-way repeated-measures ANOVA) showed that ethanol at low concentration (0.2%) used as the vehicle had little effect on activity of the LUT responding to the irritation, although the increase in RP values by AA in female mice was antagonized by the concomitant infusion of the vehicle. It has been reported that 4% ethanol reduces the resting pressure of in vitro male rat bladder muscle strip preparation, whereas 1% ethanol does not show such effect (25), and thus it is speculated that various factors such as...
the sex, animal species, and irritated conditions may influence the bladder sensitivity to the ethanol’s relaxing effect.

Hydrophobic compounds such as capsazeine can be dissolved in dimethyl sulfoxide (DMSO). However, intravesical infusion of DMSO is known to markedly facilitate bladder contraction frequency during continuous infusion cystometry (4). Liposomes or hydrogel is suggested to be an alternative solvent for capsaicin, a TRPV1 agonist, superior to ethanol at high concentration (e.g., 30%, concentration often used for dissolving the compound) in saline, because either solvent produces less damage of bladder tissue (45). However, the use of liposomes alone decreases bladder contraction frequency in rat cystometry (19), and hydrogel may lack efficacy in drug delivery (45). Thus ethanol at concentrations as low as 0.2% is still superior to these alternatives in dissolving capsazeine.

Other candidates as acid sensor in the bladder. Daly et al. (12) showed that the afferent response to intravesical application of capsaicin (100 μM) or HCl (50 mM) in TRPV1+/− mice is markedly increased, whereas that in TRPV1+/+ mice is small, suggesting the possible implication of TRPV1 in bladder afferent sensitivity for intravesical noxious stimulation. The results are intriguing because gene expression of TRPV1 is extremely limited in the mouse bladder (26). On the other hand, gene expressions of ASICs, other candidate molecules for the acid sensing, are rich in the bladder, being 165- to 185-fold higher than TRPV1 expression (26), and thus further studies are warranted to investigate the role of ASICs in control of the irritated LUT function.

Conclusion. The present study using decerebrate unanesthetized mice suggests that, at least under the irritated LUT conditions, TRPV1 channels in the bladder and urethra via afferent/efferent signaling mechanisms of “bladder-to-urethra,” “urethra-to-bladder,” or both are not involved in afferent signal transduction during the urine storage period. It is also suggested that the cross talk mechanism between the bladder and urethra via TRPV1 channels is associated with the sex-dependent differences in activity of LUT in response to acid irritation.

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DISCLOSURES

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

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