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Functional roles of TRPV1 and TRPV4 in control of lower urinary tract activity: dual analysis of behavior and reflex during the micturition cycle

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Yoshiyama M, Mochizuki T, Nakagomi H, Miyamoto T, Kira S, Mizumachi R, Sokabe T, Takayama Y, Tominaga M, Takeda M. Functional roles of TRPV1 and TRPV4 in control of lower urinary tract activity: dual analysis of behavior and reflex during the micturition cycle. *Am J Physiol Renal Physiol* 308: F1128–F1134, 2015. First published March 11, 2015; doi:10.1152/ajprenal.00016.2015.—The present study used a dual analysis of voiding behavior and reflex micturition to examine lower urinary tract function in transient receptor potential vanilloid (TRPV)1 knockout (KO) mice and TRPV4 KO mice. In metabolic cage experiments conducted under conscious conditions (i.e., voluntary voiding behavior), TRPV4 KO mice showed a markedly higher voiding frequency (VF; 19.3 ± 1.2 times/day) and a smaller urine volume/voiding (UVV; 114 ± 9 μl) compared with wild-type (WT) littermates (VF: 5.2 ± 0.5 times/day and UVV: 380 ± 34 μl). Meanwhile, TRPV1 KO mice showed a similar VF to WT littermates (6.8 ± 0.5 times/day) with a significantly smaller UVV (276 ± 20 μl). Water intake among these genotypes was the same, but TRPV4 KO mice had a larger urine output than the other two groups. In cystometrogram experiments conducted in decerebrate unanesthetized mice (i.e., reflex micturition response), no differences between the three groups were found in any cystometrogram variables, including voided volume, volume threshold for inducing micturition contraction, maximal voiding pressure, and bladder compliance. However, both TRPV1 KO and TRPV4 KO mice showed a significant number of nonvoiding bladder contractions (NVCs; 3.5 ± 0.9 and 2.8 ± 0.7 contractions, respectively) before each voiding, whereas WT mice showed virtually no NVCs. These results suggest that in the reflex micturition circuit, a lack of either channel is involved in NVCs during bladder filling, whereas in the forebrain, it is involved in the early timing of urine release, possibly in the conscious response to the bladder instability.

bladder instability; forebrain; mouse; central nervous system; transient receptor potential vanilloid

THE FREQUENCY-VOLUME CHART (FVC) is a useful and reliable tool for the objective measurement of voiding behavior in animals (1) as well as humans (7). Evaluation of FVCs in rats using metabolic cages is an often-used method of examining physiological and pathological functions of the lower urinary tract (1). Furthermore, patented metabolic cages that can reliably record FVCs in mice for durations exceeding 24 h are currently available (24), allowing for the precise evaluation of voluntary voiding behavior under conscious conditions. The availability of genetically manipulated mice has facilitated studies of the roles of various molecules in the regulation of diverse vital functions, including micturition. The combination of the novel device and mutants is expected to further enhance the progress of pathophysiological research. Nevertheless, information obtained from FVCs is limited to the timing and volume of urination and water intake and gives no indications regarding important variables such as postvoid residual volume and intravesical pressure change. In addition, it is difficult to conduct acute pharmacological studies with a metabolic cage because of limited drug administration routes. Thus, cystometry remains essential to the detailed examination of lower urinary tract activity.

Urodynamic monitoring of voiding function in conscious animals is often used to examine bladder activity and allows for the undertaking of physiopharmacological experiments (42, 46). However, greater attention should be paid to the handling of conscious animals since voluntary voiding behavior can be affected by stress from ambient circumstances (29, 35) or drugs that alter moods and emotions (23, 37, 39). Moreover, a recent study by Sorge et al. (33) revealed that the pain response in conscious rodents is remarkably influenced by olfactory stimuli from male experimenters, strongly suggesting that data analysis would be confounded when in vivo experiments are performed in awake animals. On the other hand, anesthetics are known to interfere with both reflex micturition (23, 49) and the pharmacological effects of drugs such as glutamatergic antagonists (47, 48, 50). As an alternative, animals under decerebrate unanesthetized conditions whose forebrains were previously removed under inhalation anesthesia can be used to examine reflex responses during cystometry (43, 45).

Transient receptor potential (TRP) channels are involved in sensing various exogenous and endogenous chemical stimuli as well as changes in temperature, pH, and mechanical stimuli applied to cells (10, 28, 34). Previous studies have revealed that, among the TRP channels, TRP vanilloid (TRPV)1 (2, 4, 6, 8, 9, 12, 40), TRPV4 (5, 15–17, 36), TRPA1 (13), and TRPM8 (31) have been implicated in both the pathology and physiology of lower urinary tract function. Previous studies using mice lacking TRPV1 or TRPV4 have implicated either channel in the control of bladder activity in response to an intravesical mechanical stimulus or chemical irritation. Meanwhile, the functional role of each channel remains controversial (6, 8, 16, 17, 36, 40), potentially due to differences in

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experimental design concerning whether cystometry was conducted under conscious or anesthetized conditions.

The aim of the present study was to use TRPV1 knockout (KO) mice and TRPV4 KO mice to determine the contributions of TRPV1 and TRPV4 in lower urinary tract function of wild-type (WT) phenotypes. This was achieved by applying two independent experimental approaches: one approach was to use the metabolic cage to investigate the voluntary voiding behavior of conscious mice and the other approach was to use cystometry to examine involuntary reflex micturition in decerebrate unanesthetized mice. These findings were integrated in the data interpretation. To our knowledge, this is the first study to evaluate lower urinary tract function of genetically manipulated mice using a dual analysis of behavior and reflex, both under unanesthetized conditions.

A preliminary report of this study has been previously presented in abstract form (27).

MATERIALS AND METHODS

Animals. We used 8- to 12-wk-old male TRPV1 KO mice and TRPV4 KO mice that had both been backcrossed on a C57BL/6Cr background as well as WT littermates (body weights: 23.1 ± 0.6, 22.9 ± 0.5, and 22.1 ± 0.3 g, respectively). Before each experiment, mice were housed under a 12:12-h light-dark cycle with controlled humidity and temperature and provided with food and water ad libitum. All procedures were conducted in accordance with the Institutional Animal Care and Use Committee of the University of Yamanashi. All efforts were made to minimize animal suffering and reduce the number of animals used.

Metabolic cage experiments using conscious mice. To evaluate voluntary voiding behavior, conscious mice were individually placed in metabolic cages (Fig. 1) constructed in a soundproof room at 25°C with a 12:12-h light-dark cycle. Each mouse was provided with free access to food and water. After an acclimation period of 3 days in the cage, data on voided urine (weight and timing) and water consumption (volume and timing) were continuously collected for each mouse over 2 days using a PowerLab data-acquisition system. Data were converted by a digitizer to digital signals that were entered into a computer for analysis by LabChat software (AD Instruments, Colorado Springs, CO). The specific gravity of urine was defined as 1.000 μl/μg, and urine volume was calculated using this number. The evaluated parameters were: water intake (in ml/day), urine output volume (in ml/day), voiding frequency (in times/day), urine volume per voiding (in μl), voiding duration (in s), and mean uroflow rate (in μl/s), which was calculated as the urine volume per voiding (in μl)/voiding duration (in s).

Cystometrographic experiments using decerebrate unanesthetized mice. Animals were anesthetized with sevoflurane (2–3%) in O_2 (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 catheter was exited near the xiphoid process. Precollicular decerebration was performed according to a previousy published method (32) in which both carotid arteries are ligated followed by a midline incision of the head skin with a scalpel and removal of the skull and forebrain with a fine rongeur and a blunt spatula. Sevoflurane was then discontinued. After no further intracranial hemorrhage was detected visually, the lateral flaps of the incised head skin were sutured together.

To allow for recovery from the anesthetic, cystometrograms (CMGs) were started 2 h after the surgery and conducted under

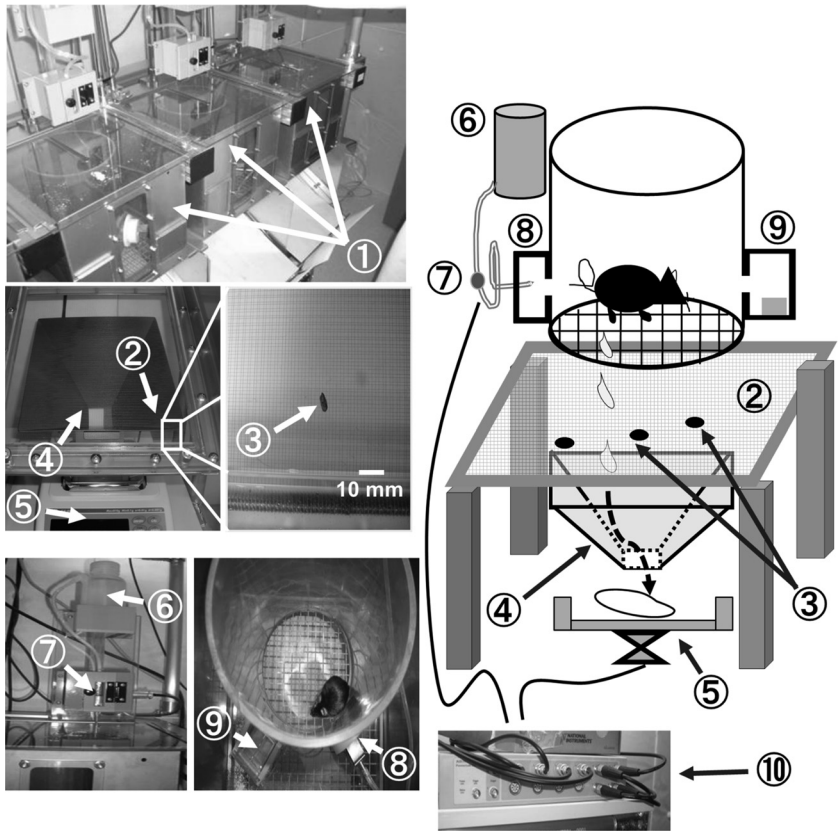

Fig. 1. Illustrated schema of the metabolic cage setup used in this study. Metabolic cages (*arrow 1*) are set up in a soundproofed room. Patented mesh made of special materials (Refs. 1 and 24) (*arrow 2*) that passes urine through but traps feces (*arrow 3*) is placed in each cage. A square-shaped water-repellent funnel (*arrow 4*) positioned below the mesh directs voided urine that passes through it into a container located on a precise balance meter (*arrow 5*). Quantities of water and food sufficient to last for a few days are prepared for administration through the supply line (*arrows 6–8*) and feeder box (*arrow 9*), respectively. Information regarding voided urine weight and consumed water volume is converted to digital signals via a digitizer (*arrow 10*), and these data are entered into a computer for analysis.

unanesthetized conditions. Saline was infused into the bladder for at least 2 h before CMG values were measured. Bladder activity was monitored with a cystometry catheter connected to a pressure transducer. CMG recordings were performed by a continuous infusion of room temperature physiological saline (10 μl/min) into the bladder to elicit repetitive voids, which allowed for data collection from a number of micturition cycles. Fluid voided from the urethral meatus was collected and measured to determine voided volume (in μl). Once constant voided volumes were collected, the infusion was stopped at the beginning of a voiding contraction, and the last voided volume was measured (44). Immediately after the final voided volume measurement, the abdomen was opened by suture removal, and the bladder was exposed. The remaining intravesical content was expelled by directly exerting pressure on the bladder with a curved forceps, and the collected fluid was estimated as residual volume (in μl). The volume threshold for inducing micturition (in μl) was calculated as the sum of the voided volume and residual volume. On the basis of these values, voiding efficiency (in %) could be estimated as follows: voided volume (in μl)/volume threshold for inducing micturition (in μl) × 100. Other evaluated parameters were: pressure at volume threshold for inducing micturition contraction (in mmHg), maximal voiding pressure (in mmHg), closing peak pressure (in mmHg), and bladder compliance (in μl/mmHg) (43, 45). Bladder compliance was calculated as the ratio of the infused volume to the pressure difference between the postvoid resting pressure and the following pressure at volume threshold for inducing micturition contraction. To examine bladder excitability, nonvoiding contractions (NVCs), rhythmic intravesical pressure waves with no fluid release from the urethra that occur before voiding, were also evaluated as amplitude changes of 7.5 mmHg or more. Variables evaluated in the analysis of NVCs included thresholds of pressure (in mmHg) and infused volume (in μl) required to elicit the first NVC, peak pressure of the first NVC (in mmHg), mean peak pressure of NVCs (in mmHg), and number of NVCs per micturition cycle (i.e., an interval between two consecutive micturition contractions).

Statistical analysis. All values are expressed as means ± SE. The Mann-Whitney *U*-test, Wilcoxon matched-pairs signed-rank test, and one-way ANOVA followed by Tukey's multiple-comparison test were used for statistical analysis, if applicable. For all analyses, P values of <0.05 were considered significant.

RESULTS

Analysis of voluntary voiding behavior in the metabolic cage. Figure 2 shows representative recording charts of 48-h voluntary voiding behavior and water intake in WT, TRPV1 KO, and TRPV4 KO mice. Water intake, urine output volume, and voiding frequency were evaluated per 24 h, per 12 h in the dark period, and per 12 h in the light period (Fig. 3). As is common behavior in this species, the majority of urination and water intake took place during the dark period. No differences in water intake at any period were found among the three groups (Fig. 3*A*). Whereas there were no differences in urine output in the dark period among the three groups, urine output in the light period was greater in TRPV4 KO mice than in WT or TRPV1 KO mice (Fig. 3*B*). Regarding urine output over 24 h, there was a difference only between WT and TRPV4 KO mice, with the latter urinating a larger volume. Some WT and TRPV1 KO mice released no urine during the light period, whereas all TRPV4 KO mice did. At all periods, the voiding frequency of TRPV4 KO mice was markedly higher than that of WT or TRPV1 KO mice (Fig. 3*C*). There was no difference in voiding frequency between WT and TRPV1 KO mice at any period.

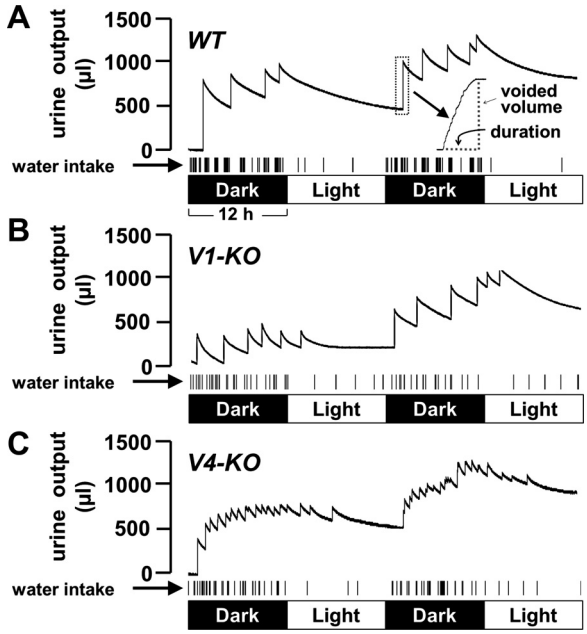

Fig. 2. Representative 48-h recording charts showing water intake and urine output of a wild-type (WT) mouse (*A*), a transient receptor potential vanilloid (TRPV)1 knockout (KO) mouse (V1-KO; *B*), and a TRPV4 KO mouse (V4-KO; *C*). The dotted rectangle in *A* indicates the voiding flow trace enlarged in the abscissa and ordinate. When analyzing each trace, each rapid increase was visually examined to determine whether it was urine flow. Each individual bar in "water intake" indicates a drop of 16.7 μl. Gradual declines after rapid increases in urine output were due to vaporization of urine collected in a container. Total urine output volume was calculated by summing the rapid increases identified as voided urine. Note that the TRPV4 KO mouse had a much higher voiding frequency and remarkably smaller urine volume per voiding compared with the WT mouse and TRPV1 KO mouse.

Comparisons among WT, TRPV1 KO, and TRPV4 KO mice in urine volume/voiding, voiding duration, and mean uroflow rate are shown in Fig. 4. Urine volume/voiding in TRPV4 KO or TRPV1 KO mice was significantly smaller than that in WT mice, and urine volume/voiding in TRPV4 KO mice was much smaller than that in TRPV1 KO mice (Fig. 4*A*). There were no differences in urine volume/voiding between the dark and light periods in WT mice (307.0 ± 35.0 and 344.3 ± 60.1 μl, respectively, $n = 11$, $P = 0.58$) and TRPV1 KO mice (258.6 ± 26.4 and 231.0 ± 18.0 μl, respectively, $n = 8$, $P = 0.15$). However, in TRPV4 KO mice, urine volume/voiding was significantly smaller in the dark period compared with the light period (98.1 ± 6.7 and 132.4 ± 13.9 μl, respectively, $n = 11$, $P = 0.002$; data not presented in the graph). Voiding duration in TRPV4 KO or TRPV1 KO mice was significantly shorter than that in WT mice, and voiding duration in TRPV4 KO mice was shorter than that in TRPV1 KO mice (Fig. 4*B*). There were no differences in mean uroflow rate among the three groups (Fig. 4*C*).

Evaluation of reflex activity of the lower urinary tract during CMGs. Figure 5 shows representative CMG recordings of WT, TRPV1 KO, and TRPV4 KO mice. Values of the evaluated CMG parameters are shown in Table 1. No differences among the three genotypes were found for any of the variables. Only two of eight WT mice exhibited NVCs, at an average of 1.3 contractions/micturition cycle. The remaining six WT mice showed no NVCs, suggesting that genetically normal mice

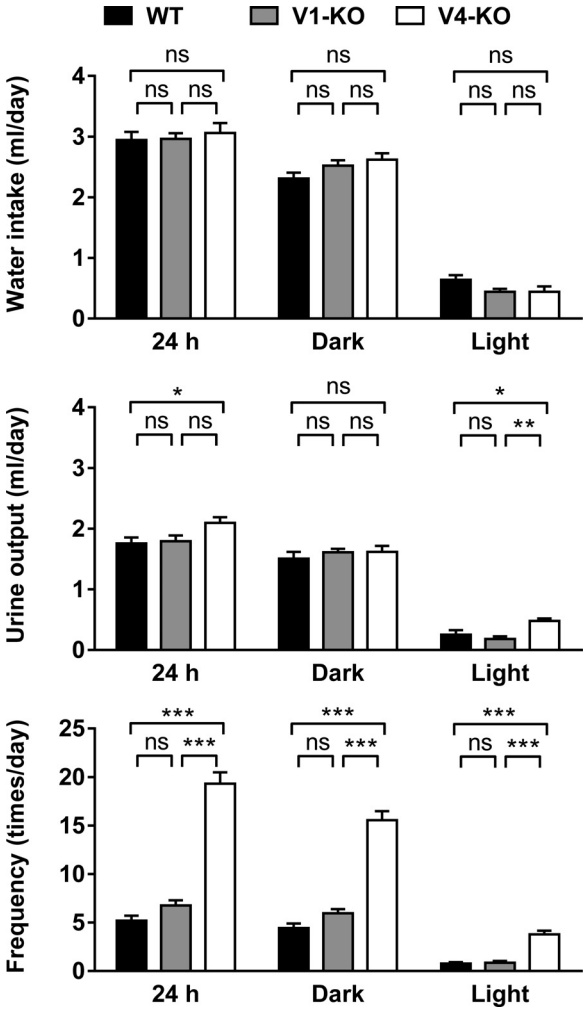

Fig. 3. Bar graphs showing quantitative analysis of metabolic cage experiments for water intake (*A*), urine output (*B*), and voiding frequency (*C*). These variables were analyzed for the 24-h period, dark period, and light period. Data are presented as means ± SE. WT ($n = 15$), TRPV1 KO ($n = 11$), and TRPV4 KO ($n = 11$) mice were compared by one-way ANOVA followed by Tukey's multiple-comparison test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ns, Not significant.

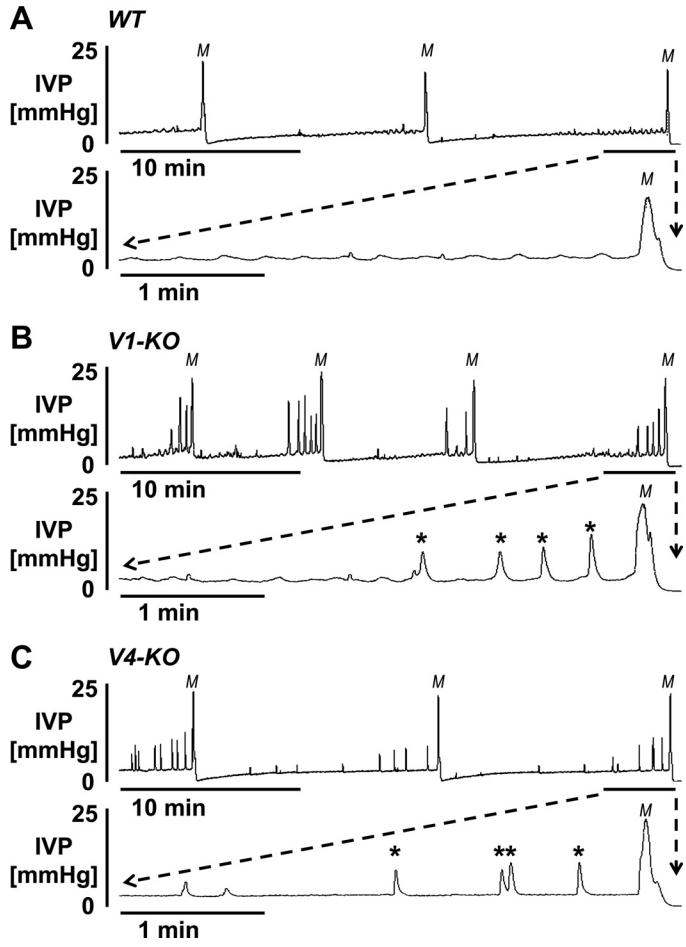

Fig. 5. Cystometrogram recording charts of decerebrate unanesthetized WT (*A*), TRPV1 KO (*B*), and TRPV4 KO (*C*) mice. Note that nonvoiding contractions (NVCs; *) appear before each micturition contraction (as indicated by *M*) in a TRPV1 KO mouse and a TRPV4 KO mouse, whereas no NVCs were detected in the WT mouse. IVP, intravesical pressure.

scarcely exhibit NVCs. NVCs of eight WT mice occurred at an average of 0.4 ± 0.1 contractions/micturition cycle. In contrast, five of six TRPV1 KO mice and six of seven TRPV4 KO mice showed a significant number of NVCs (Table 2) compared with WT mice ($P < 0.0001$ vs. TRPV1 KO mice and $P = 0.0001$ vs. TRPV4 KO mice), with only one animal of each genotype showing no NVCs. NVCs in all six TRPV1 KO mice and all seven TRPV4 KO mice were shown at averages of 2.9 ± 0.9 and 2.4 ± 0.7 contractions, respectively, showing no difference between the two genotypes ($P = 0.21$), and both were significantly larger than that of WT mice ($n = 8$, $P < 0.0001$ vs. TRPV1 KO mice and $P = 0.007$ vs. TRPV4 KO mice). No differences were found between TRPV1 KO and TRPV4 KO mice in properties of NVCs (Table 2).

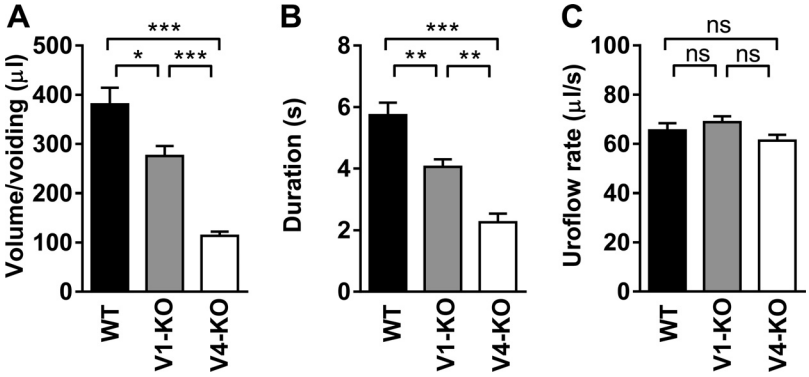

Fig. 4. Bar graphs showing quantitative analysis of metabolic cage experiments for urine volume/voiding (*A*), voiding duration (*B*), and mean uroflow rate (*C*). Data are presented as means ± SE. WT ($n = 15$), TRPV1 KO ($n = 11$), and TRPV4 KO ($n = 11$) mice were compared by one-way ANOVA followed by Tukey's multiple-comparison test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ns, Not significant.

Table 1. *Comparisons among WT, TRPV1 KO, and TRPV4 KO mice in reflex bladder activity during cystometrograms*

	PT, mmHg	MVP, mmHg	CPP, mmHg	BCP, μl/mmHg	VV, μl	RV, μl	VT, μl	VE, %
WT mice	3.8 ± 0.4	23.0 ± 2.2	10.4 ± 1.2	35.2 ± 2.3	131 ± 11	13.6 ± 3.3	144 ± 10	90 ± 2
TRPV1 KO mice	3.4 ± 0.3	21.9 ± 2.1	8.5 ± 1.7	28.2 ± 5.1	118 ± 27	17.8 ± 3.6	136 ± 27	83 ± 4
TRPV4 KO mice	3.0 ± 0.4	20.3 ± 1.2	7.7 ± 1.7	43.5 ± 9.6	135 ± 23	12.4 ± 3.6	148 ± 23	91 ± 2

Values are presented as means ± SE. PT, pressure at volume threshold for inducing micturition contraction; MVP, maximal voiding pressure; CPP, closing peak pressure; BCP, bladder compliance; VV, voided volume; RV, (postvoiding) residual volume; VT, volume threshold for inducing micturition contraction; VE, voiding efficiency. No differences were found among wild-type (WT; $n = 8$), transient receptor potential vanilloid (TRPV)1 knockout (KO) ($n = 6$), and TRPV4 KO ($n = 7$) mice in any of these variables (by one-way ANOVA with Tukey's multiple-comparison test).

DISCUSSION

Our dual analysis of voluntary voiding behavior (of conscious mice) and involuntary reflex micturition (of decerebrate unanesthetized mice) revealed intriguing differences among the 3 studied genotypes. In the metabolic cage study, TRPV4 KO mice showed smaller urine volume/voiding and a higher voiding frequency compared with WT mice, whereas TRPV1 KO mice exhibited smaller urine volume/voiding than that of WT mice, but with a similar voiding frequency. In the CMG experiments, no differences were found in any variables, including voided volume among the three groups; however, TRPV1 KO and TRPV4 KO mice showed a significant number of NVCs, which are associated with bladder instability during filling, even though WT mice showed virtually no NVCs. These results suggest that the forebrain plays a key role in voided volume differences among these genotypes. Furthermore, the earlier determination to void may be attributable to the bladder instability caused by the deficiency of either TRP channel.

With regard to methodology, during cystometry, but not during metabolic cage experiments, bladder expansion is restricted by the catheter fixed in the apex. In addition, the attribution of surgical manipulations such as bladder catheterization and related trauma to the bladder capacity should not be considered trivial. Moreover, voluntary voiding generally yields a larger voiding volume than reflex micturition because in the latter, tonic inhibition from the forebrain is removed (42). The cumulative influence of these were validated in WT and TRPV1 KO mice (380 vs. 131 μl in WT mice and 276 vs. 118 μl in TRPV1 KO mice). Meanwhile, voluntary voiding and reflex micturition voiding volumes were comparable in TRPV4 KO mice (114 vs. 135 μl, respectively). The reasons for this are unclear and may require further study.

TRPV4 in lower urinary tract function. A previous study by Gevaert et al. (17) revealed that conscious TRPV4 KO mice also exhibit NVCs during CMGs. Intriguingly, a study by the same group (17) examining the spontaneous voiding patterns of awake animals unrestrained in a large box revealed that WT mice show a strict voiding pattern characterized by a high frequency of evacuations in the corner of the cage, whereas TRPV4 KO mice disperse urine voiding, with a number of urine spots located away from the cage corners. These results may suggest that TRPV4 KO mice present a pathological voiding pattern or uncontrolled voiding similar to incontinence, implying an intriguing connection with our present findings. Furthermore, the clinical relevance of the TRPV4 gene was reported by Landouré et al. (19), who described a significant relationship between a TRPV4 gene mutation and Charcot-Marie-Tooth disease type 2C, an unusual peripheral neuropathy that manifests more severe axonal degeneration in the motor system than in the sensory system. Many patients severely affected with the disease present with urinary urgency and incontinence (19), thereby suggesting the possibility that loss of normal TRPV4 function is associated with lower urinary tract dysfunction.

Previous CMG studies have shown that conscious TRPV4 KO mice exhibit larger voided volumes than WT mice (17, 36), which contradicts our present findings in decerebrate unanesthetized mice. The reasons for this discrepancy are unknown, although the focus should be on the fact that the volume threshold (for inducing micturition) of conscious WT mice in the previous studies (17, 36) was markedly smaller than that of the decerebrate unanesthetized WT mice in our study. This suggests that in the former mice, the awake forebrain was the site likely responsible for the decreased volume threshold, since it has an excitatory input as well as an inhibitory input into the brain stem, which contains the switching mechanism for micturition initiation (11). In general, neurally intact conscious mice under normal circumstances are known to have a larger bladder capacity than decerebrate (unanesthetized) mice (42, 49).

TRPV1 in lower urinary tract function. Birder et al. (6) reported that compared with WT mice, conscious TRPV1 KO mice show a similar intercontraction interval (i.e., parameter associated with voided volume) and a larger number of NVCs. In addition, they revealed that urethane anesthesia increases bladder capacity (i.e., parameter associated with volume threshold for inducing micturition) to a greater extent in TRPV1 KO mice than in WT mice. Wang et al. (40) showed that under anesthetized conditions (urethane or a combination

Table 2. *Comparisons between TRPV1 KO mice and TRPV4 KO mice in properties of NVCs*

	Pressure Threshold for Inducing the First NVC, mmHg	Volume Threshold for Inducing the First NVC, μl	Peak Pressure of the First NVC, mmHg	Mean Peak Pressure of NVCs, mmHg	Number of NVCs/Micturition Cycle
TRPV1 KO mice	3.3 ± 0.5	98.1 ± 17.1	11.3 ± 0.7	14.8 ± 1.5	3.5 ± 0.9
TRPV4 KO mice	2.8 ± 0.2	106.6 ± 10.4	12.8 ± 0.9	13.4 ± 0.7	2.8 ± 0.7

Values are presented as means ± SE. No differences were found between TRPV1 KO mice ($n = 5$) and TRPV4 KO mice ($n = 6$) in any of these parameters (by Mann-Whitney *U*-test). NVC, nonvoiding contraction.

of ketamine and xylazine), TRPV1 KO mice exhibit intercontraction intervals and bladder capacities comparable with those of WT mice but a larger number of NVCs compared with WT mice. With respect to NVCs and voided volume (or the intercontraction interval), the results of both research groups are consistent with those of the present study using decerebrate unanesthetized mice. However, Charrua et al. (8) found that urethane-anesthetized TRPV1 KO mice had no NVCs, although they exhibited a bladder contraction frequency comparable with that of WT mice. The cause of this discrepancy between results is unknown.

NVCs during bladder filling. The present findings obtained using CMGs in decerebrate unanesthetized mice indicate that the site responsible for the NVCs induced by TRP channel deficiency is located somewhere in the reflex micturition circuit. This circuit includes the bladder, peripheral nerves, dorsal root ganglia, and central nervous system except for the forebrain, in which TRPV1 (3, 4, 14, 38, 41) and TRPV4 (14, 18, 20, 21, 26, 41) are widely distributed. Because NVCs were constantly exhibited in TRP-deficient mice, it is reasonable to speculate that the functional role of TRP channels is to stabilize bladder activity during the storage phase. Further investigations are warranted to elucidate the underlying mechanism of NVCs.

Comparisons of water intake, urine output, and urine volume per voiding. TRPV4 is associated with the osmotic regulation and secretion of antidiuretic hormone (22, 25). Regarding water balance, there are discrepant findings in studies using TRPV4 KO mice: one study (22) found lower drinking water intake in mutants compared with WT mice, whereas another study (25) found equal volumes among genotypes in terms of water intake and urine output. The present study supports the latter finding as far as water intake but not urine output, since TRPV4 KO mice in our study voided a larger volume. Urine output in the light period was significantly larger in TRPV4 KO mice than in WT or TRPV1 KO mice, although no differences were found among the three groups for the dark period (Fig. 3*B*). Intriguingly, there was no difference between water intake and urine output in TRPV4 KO mice during the light period, whereas in the other two genotypes, urine output was markedly smaller during the light period than during the dark period. A previous study (25) indicated that the level of serum arginine vasopressin (i.e., antidiuretic) is unaltered in TRPV4 KO mice compared with WT mice under normal conditions. Thus, the mechanism accounting for the abnormal water balance control remains to be determined.

A recent study (30) on the circadian clock of micturition rhythm suggested that urine volume/voiding is larger during the light period than during the dark period in genetically normal mice. However, our study using the metabolic cage did not detect such a change. The discrepancy may be related to a sex difference, since the previous study used female mice, whereas we used male mice. There were no differences between the two studies in age (i.e., ~10 wk old) or mouse strain (i.e., C57BL/6).

Conclusions. Using the metabolic cage and cystometry, both under unanesthetized conditions, we were able to make detailed comparisons of voluntary voiding behavior and reflex micturition, respectively, among three genotypes. The dual analysis revealed that a deficiency of either TRPV1 or TRPV4 results in a loss of bladder stability during the storage phase and that a lack of TRP channels in the forebrain contributes to the early timing of urine release. Integration of these results facilitated our understanding of the pathology of lower urinary tract function in the mutants.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: M.Y., T. Mochizuki, and R.M. conception and design of research; M.Y., T. Mochizuki, H.N., T. Miyamoto, and S.K. performed experiments; M.Y. and T. Mochizuki analyzed data; M.Y. and T. Mochizuki interpreted results of experiments; M.Y., T. Mochizuki, and H.N. prepared figures; M.Y. and T. Mochizuki drafted manuscript; M.Y., T. Mochizuki, T.S., Y.T., and M. Tominaga edited and revised manuscript; M.Y., T. Mochizuki, and M. Takeda approved final version of manuscript.

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