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TRPV4-DEFICIENT MICE EXHIBIT INCREASED FREQUENCY OF VOIDING ~EVALUATION OF MICTURITION BEHAVIOUR USING NOVEL METABOLIC CAGE SYSTEM~

Hypothesis / aims of study

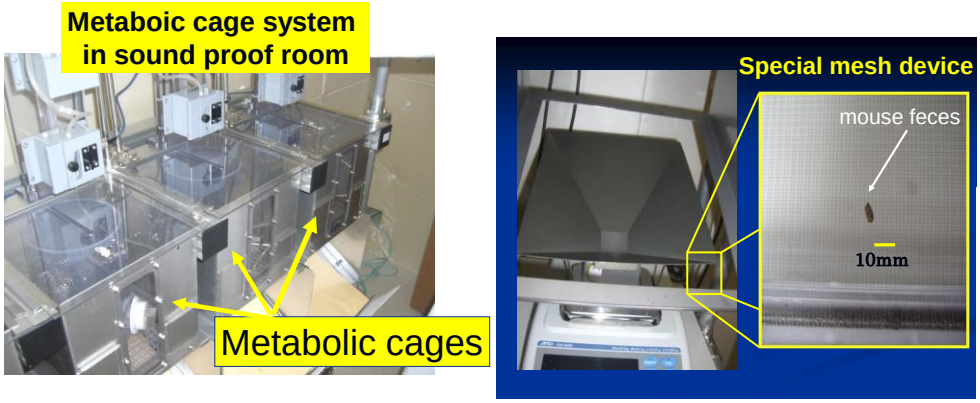
Transient receptor potential vanilloid type 4 (TRPV4) channel is known as one of the candidate molecules for the mechanosensory [1] and suggested to be involved in regulation of lower urinary tract (LUT) activity [2]. Present studies using novel metabolic cage system which allows performing detailed analysis of mouse voiding behaviour were conducted to examine a functional role of TRPV4 in regulation of LUT activity by comparing TRPV4-deficient (V4KO) mice with the wild-type (WT). In addition to this method, detailed evaluations using *in-vivo* cystometrograms (CMGs) in decerebrate unanaesthetized mice facilitated determining the possible involvement of TRPV4 in the underlying mechanism.

Study design, materials and methods

Experiments were performed using 8-10 week old male V4KO mice backcrossed on a C57BL/6Cr background weighting 22.9 ± 1.5 g (*n*=12) and age-matched wild type (C57BL/6Cr) mice weighting 23.0 ± 1.8 g (*n*=12).

(Metabolic cage study using conscious mice)

For evaluating micturition behaviour, conscious mice were individually placed in newly-designed metabolic cages in a soundproof room at 25 degrees temperature with a 12/12 hr light-dark cycle. Each mouse was provided with free access to food and water. After mice were acclimatized for 3 days in the cages, subsequent 48 hr data of voided urine and water intake were continuously recorded into a computer and analysed. Evaluated parameters are: water intake (μl/day), voided urine volume (μl/day), urine volume/void (μl), voiding time/void (s), and number of voids (/day).



(CMG study using decerebrated unanaesthetized mice)

For CMG study, mice were decerebrated under sevoflurane anesthesia and intravesical pressure was recorded *via* a PE-50 tube inserted into the bladder dome. CMG recordings were conducted under unanaesthetized conditions by continuously infusing saline (10 μl/min) at room temperature. Evaluated parameters are: maximal voiding pressure (MVP, mmHg), bladder compliance (BCP, μl/mmHg), voided volume (VV, μl), volume threshold for micturition (VT, μl), voiding efficiency (VE, %), and number of non-voiding contractions (NVCs).

All values are expressed as mean ± S.E.M. Statistical analyses were made using unpaired *t*-test: $p < 0.05$ was considered significant (*$p < 0.05$, **$p < 0.01$).

Results

Figure 1 metabolic cage analyses

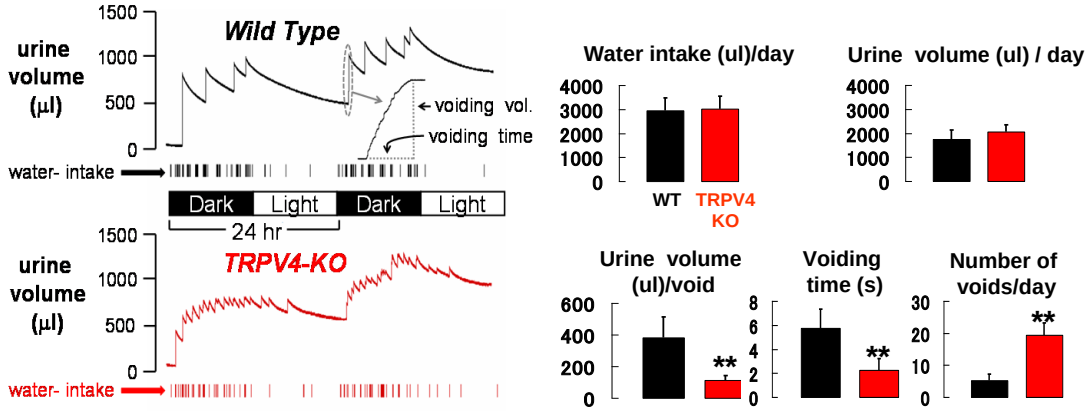
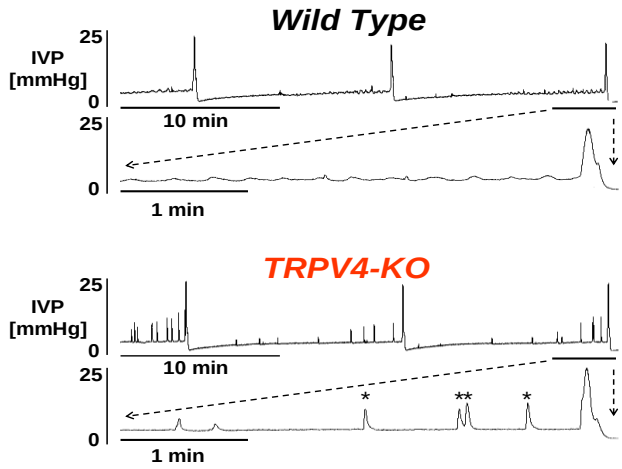


Figure 2 **CMG analyses**



	MVP (mmHg)	BCP (µl /mmHg)	VV (µl)	VT (µl)	VE (%)	**Number of NVCs**
WT	23.0 ± 2.2	35.2 ± 2.3	131 ± 11	145 ± 10	90.2 ± 2.2	**0.4 ± 0.1**
V4KO	20.3 ± 1.2	43.6 ± 9.6	136 ± 23	148 ± 23	90.7 ± 1.5	**2.4 ± 0.9****

Interpretation of results

The metabolic cage study revealed that V4KO mice exhibited higher micturition frequency, smaller urine volume per void, and shorter voiding time, compared to the parameter values of WT, respectively; however there were no differences between V4KO and WT mice in daily water intake and urine volume, showing that V4KO mice exhibit increased voiding frequency with small urine volume per void in micturition *'behaviour'*. In CMG study, no differences were found in MVP, BCP, VV, VT and VE, whereas number of NVCs in V4KO was significantly lager than that in WT, indicating that lack of TRPV4 induces bladder overactivity in storage phase although it does not disturb involuntary *'reflex'* activities of both continence and micturition. Taken together, these results suggest the possibility that in conscious V4KO mice the involuntary (unstable) bladder contractions during urine storage persistently induce the *motor urgency*, leading to an increased voiding frequency with small volume a void.

Concluding message

TRPV4 in regulation of the LUT function is likely to have an important role for stabilizing bladder activity during urine storage. In these studies, we demonstrated that evaluation of micturition behaviour using the novel metabolic cage system, in addition to *in-vivo* CMG experiments, efficiently provides with information essential to elucidate complex mechanisms controlling the LUT activity.

References

1. Nilius, B et al. Pflugers Arch 446:298-303 (2003).
2. Gevaert, T.et al. J Clin Invest 117:3453-3462 (2007).

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**University of Yamanashi Animal Care and Use Committee.**