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## IN VIVO AND IN VITRO FUNCTIONAL CHARACTERISTICS OF THE BLADDER IN MICE LACKING TRANSIENT RECEPTOR POTENTIAL MELASTATIN 2 (TRPM2) CHANNEL

### Hypothesis / aims of study

TRPM2 channel is known as a member of the melastatin subfamily of TRP channels and expressed broadly such as in the brain, peripheral nerve, bladder, pancreas, and immune cells. It has been reported that TRPM2 stimulation produces chemokines in monocytes (1), playing an important role in the exacerbation of inflammatory process and neuropathic pain (2). A recent study revealed that mRNA expression of TRPM2 was up-regulated in human bladder tissue with Hunner type interstitial cystitis(IC) (3). These findings make us presume that TRPM2 has a potential role in pathophysiology of IC and a promising therapeutic target. However, the functional role of TRPM2 channel in the lower urinary tract has not been evaluated. In this study, to disclose the role of TRPM2 in the bladder function, we investigated the phenotypes of TRPM2 knock-out (TRPM2<sup>-/-</sup>) mice by using frequency-volume (FV), *in vitro* organ bath, and conscious cystometry (CMG) measurements.

### Study design, materials and methods

Female TRPM2<sup>+/+</sup> and TRPM2<sup>-/-</sup> mice (10-12 weeks-old) were used. In the FV measurements, voiding behaviour was monitored for 24 hours by placing the mouse without any restraints in a metabolic cage that enables to measure voided urine volumes precisely. The mice had free access to water and food during recording. After FV measurements, *in vitro* organ bath studies were carried out by using longitudinal strips of the bladder. We evaluated their contractile responses to high potassium (High K<sup>+</sup>: KCl 124 mM), carbachol (CCh: 10<sup>-8</sup> - 10<sup>-3</sup> M), ATP (10<sup>-6</sup> - 10<sup>-2</sup> M), and electrical field stimulation (EFS: 2 - 48 Hz) in the absence and presence of atropine(10<sup>-6</sup> M), alpha-beta methylene ATP (M-ATP: 10<sup>-5</sup> M x 5 times), and tetrodotoxin (TTX: 10<sup>-6</sup> M). In separate animals, CMG measurements were performed with continuous saline instillation at a rate of 0.015 ml/min in a conscious and free-moving condition at 5 days after bladder catheter implantation.

### Results

In the FV measurements during 24 hours, mean voided volume in TRPM2<sup>-/-</sup> mice was significantly greater than that in TRPM2<sup>+/+</sup> mice, although voiding frequency and total voided volume were not different between the two groups (Table 1). Similar tendencies were observed when the values were divided into light and dark cycles (data not shown).

*In vitro* organ bath studies showed that there were no significant differences in the contractile responses either to high K<sup>+</sup>, CCh, ATP or EFS between the two groups (Figure 1).

In the CMG measurements, threshold pressure, mean voided volume and bladder capacity in TRPM2<sup>-/-</sup> mice were significantly larger than those in TRPM2<sup>+/+</sup> mice (Figures 2 and 3).

### Interpretation of results

The present *in vitro* organ bath studies indicate that TRPM2 channel apparently has no action on the contractile function of detrusor smooth muscle in the female mouse. On the other hand, the larger mean voided volume in TRPM2<sup>-/-</sup> mouse was observed in the FV measurements, and similar observations were confirmed in the CMG measurements as the greater mean voided volume and bladder capacity. Moreover, threshold pressure was higher in the CMG measurement in knock-out mice. These results suggest that TRPM2 channel may play a physiological role in the modulation of bladder mechano-sensory transduction in female mice.

### Concluding message

The present study demonstrates that female TRPM2<sup>-/-</sup> mice have larger bladder capacity and larger mean voided volume, although it is unlikely that TRPM2 channel contributes to the detrusor contractile function. Basal activity of TRPM2 channel may be involved in physiological regulation of bladder sensing.

Table 1. FV measurement parameters of TRPM2<sup>+/+</sup> and TRPM2<sup>-/-</sup> mice

	TRPM2 <sup>+/+</sup> (n=10)	TRPM2 <sup>-/-</sup> (n=10)	p value
Total voided volume (ml)	2.02±0.33	2.45±0.28	0.33
Voiding frequency (times)	22.5±2.2	19.2±1.3	0.21
Mean voided volume (ml)	0.088±0.010	0.128±0.011	0.013*
Water intake (ml)	4.32±0.52	4.27±0.46	0.95

The numerical values are expressed as mean ±SEM.

\*p<0.05: significant difference from TRPM2<sup>+/+</sup> mice (unpaired Student's t-test)

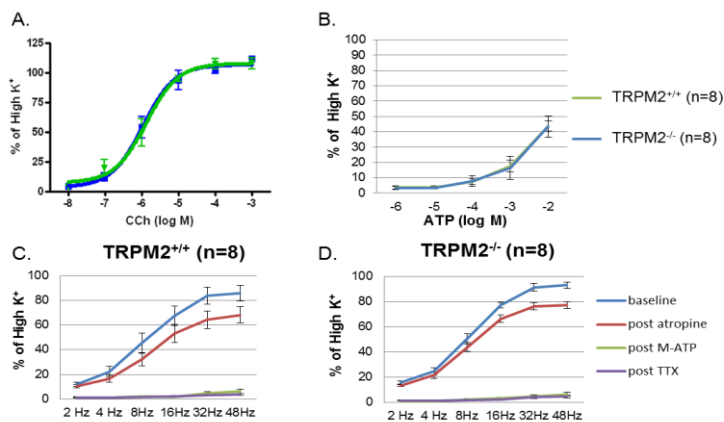


Figure 1. Concentration-response curves for CCh (A) and ATP (B), and frequency-response curves for EFS-induced bladder contractions in TRPM2<sup>+/+</sup>(C) and TRPM2<sup>-/-</sup> mice (D)

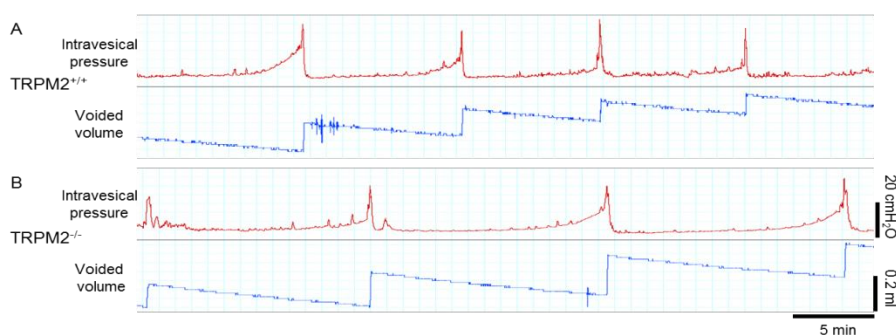


Figure 2. Representative tracings of intravesical pressure and voided volume during CMG with constant saline-filling (0.015 ml/min) in a TRPM2<sup>+/+</sup> mouse (A) and a TRPM2<sup>-/-</sup> mouse (B)

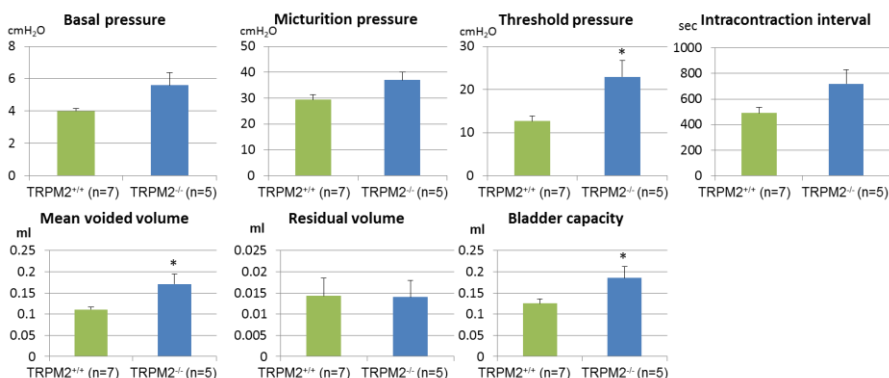


Figure 3. Comparative results of cystometric parameters between TRPM2<sup>+/+</sup> and TRPM2<sup>-/-</sup> mice. The values are expressed as mean ± SEM. \*P < 0.05: significant difference between groups (unpaired Student's t-test)

## References

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## Disclosures

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