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## **TRANSIENT RECEPTOR POTENTIAL MELASTATIN 2 (TRPM2) MEDIATES LIPOPOLYSACCHARIDE (LPS)-INDUCED INFLAMMATORY BLADDER PAIN AND FREQUENT VOIDING IN MICE**

### Hypothesis / aims of study

TRPM2 plays a crucial role in inflammatory and neuropathic pain (1), and its mRNA expression was up-regulated in human bladder tissue with Hunner type interstitial cystitis (2). While a recent study by using decerebrate unanesthetized cystometry (CMG) demonstrated no remarkable difference in bladder function between wild type (WT) and TRPM2-knock out (KO) mice (3), there have been no studies on pathophysiological roles of TRPM2 in the inflammatory and nociceptive responses originated from the urinary bladder. Thus in this study, we investigated inflammatory and nociceptive responses to intravesical instillation of lipopolysaccharide (LPS) in WT and TRPM2-KO mice.

### Study design, materials and methods

Female WT and TRPM2-KO mice (10-12 weeks-old, N = 31 and 20, respectively) were used. In the frequency volume (FV) measurements, voiding behaviour was monitored for 24 hours by placing the mouse without any restraints in a metabolic cage enable to measure voided urine volumes precisely. In assessment of bladder pain-like behaviour, the number of lower abdominal licking was counted for a 30-minute period. After the baseline measurements of voiding and licking behaviours by using separate animals, the mice were received intravesical instillation of LPS (2.0 mg/ml) or saline for 1 hour under isoflurane-anaesthesia, then, each behaviour was continuously monitored for further 72 hours.

In addition, at 24 hours after LPS-instillation, decerebrate unanaesthetized CMG was performed in separate mice to evaluate more detailed bladder function. After the CMG measurements, the mice were sacrificed with deep anaesthesia, and the bladders were harvested and evaluated histologically.

### Results

In the FV measurements, LPS-instillation, not saline-instillation, increased the number of voiding and decreased mean voided volume in WT mice at 24 - 48 hours after instillation, whereas such voiding behaviour changes were not observed in TRPM2-KO mice (Figure 1A).

In pain-like behaviour, both saline- and LPS-instillation in WT mice increased the number of licking behaviour compared with each baseline at 2 hours after instillation, but LPS-instillation continuously and remarkably increased this response compared with saline-instillation until further 24 hours after instillation. In contrast, the number of licking behaviour in TRPM2-KO mice at 24 hours after LPS-instillation was significantly lower than that in WT mice, which were similar level to that in WT mice with saline-instillation (Figure 1B).

In the CMG measurements, LPS-treated WT mice showed significantly shorter intercontraction interval and lower voided volume than those in LPS-treated TRPM2-KO mice (Figure 2). Histologically, inflammatory cell infiltration in the bladder submucosal layer of TRPM2-KO mice was much less than that of WT mice, whereas submucosal edema was similarly observed in both WT and TRPM2-KO mice (Figure 3).

### Interpretation of results

Intravesical instillation of LPS in WT mice induced frequent voiding at 24 - 48 hours after instillation, and an increase in the number of licking and inflammatory cell infiltration within 24 hours, suggesting that LPS caused inflammatory pain in the bladder followed by frequent voiding. In contrast, such changes induced by LPS on voiding- and licking-behaviours and on inflammatory cell infiltration were obscured in TRPM2-KO mice, which was associated with elongated intercontraction interval in TRPM2-KO mice in CMG measurements. These results suggest that TRPM2 contributes to the inflammatory bladder pain accompanied with frequent voiding induced by intravesical LPS-instillation in mice.

### Concluding message

Intravesical instillation of LPS caused inflammatory bladder pain and frequent voiding in WT mice, but such changes were obscured in TRPM2-KO mice, suggesting that TRPM2 contributes to an inflammatory nociception in the mouse bladder.

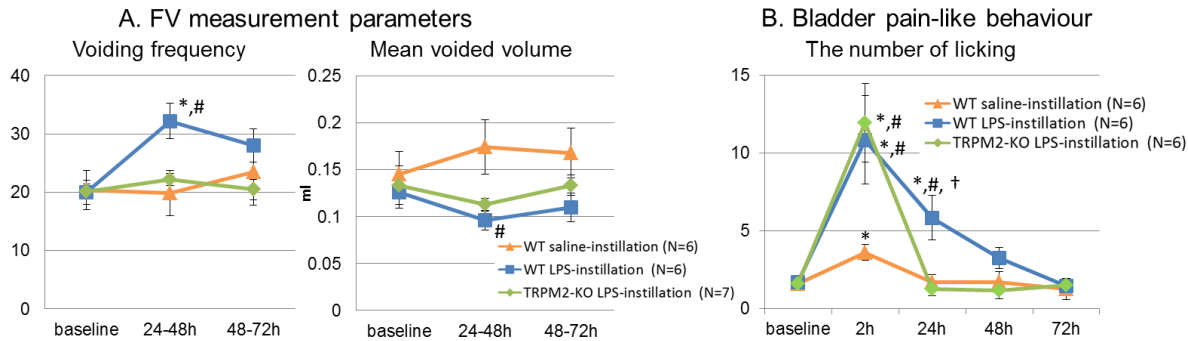


Figure 1. Changes in voiding behaviour (A) and licking behaviour (B) after intravesical instillation of LPS or saline in WT or TRPM2-KO mice

The values are expressed as mean  $\pm$  SEM.

\*  $p < 0.05$ : significant differences from baseline (Dunnett's test)

#  $p < 0.05$ : significant differences from WT saline-instillation (Tukey's test)

†  $p < 0.05$ : significant differences from TRPM2-KO LPS-instillation (Tukey's test)

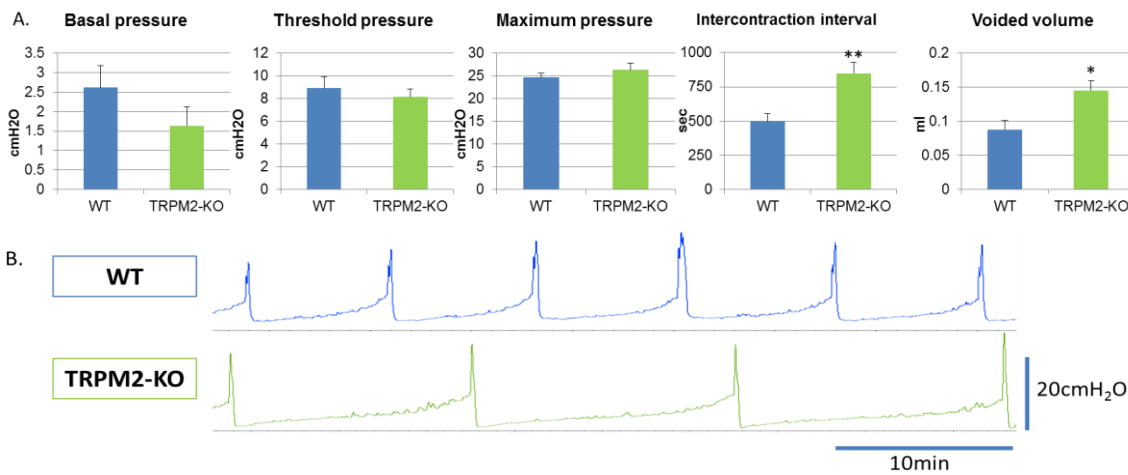


Figure 2. CMG parameters (A, N=7 in each group) and representative tracings of intravesical pressure (B) in WT and TRPM2-KO mice at 24 hours after LPS-instillation under a decerebrate unanesthetised condition

\*  $p < 0.05$ , \*\*  $p < 0.01$ : significant differences from WT mice (unpaired Student's t-test)

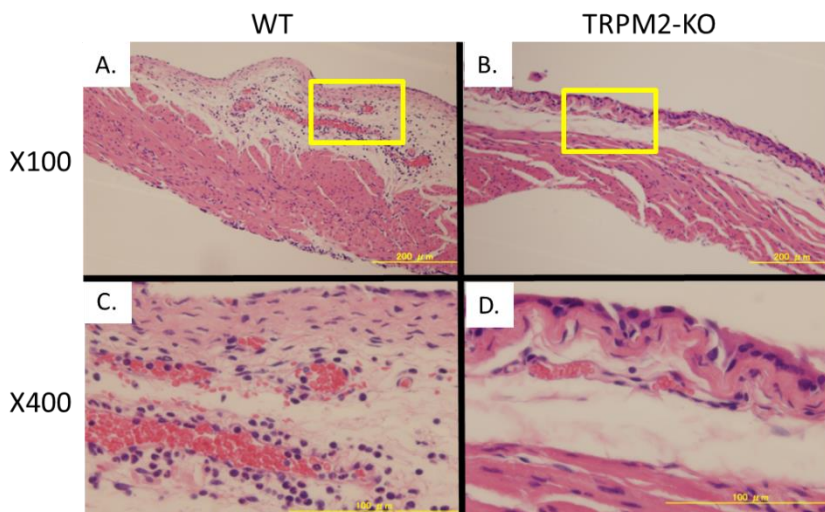


Figure 3. Representative images of the bladder in WT and TRPM2-KO mice at 24 hours after LPS-instillation

A and B: Scale bar, 200 $\mu$ m. C and D: Scale bar, 100 $\mu$ m. Square in each upper panel is corresponding to each lower panel.

## References

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Disclosures

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