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INVOLVEMENT OF TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 (TRPA1) IN THE INFLAMMATORY BLADDER HYPERSENSITIVITY CAUSED BY INTRAVESICAL LIPOPOLYSACCHARIDE (LPS) IN MICE

Hypothesis / aims of study

TRPA1 channel is expressed in the urothelial cells and bladder sensory nerve fibers, and acts as bladder mechanosensor and nociceptor (1). A recent study revealed that mRNA expression of TRPA1 was up-regulated in human bladder tissue with Hunner type interstitial cystitis, suggesting that TRPA1 may play a role in the pathophysiology of inflammatory bladder hypersensitivity (2). In this study, to disclose roles of TRPA1 in bladder inflammatory hypersensitivity, we investigated *in vitro* and *in vivo* inflammatory responses to intravesical LPS-instillation in TRPA1-knock out (KO) mice in comparison with those in wild type (WT) mice.

Study design, materials and methods

Female WT and TRPA1-KO mice (10-12 weeks-old, N = 24 and 17, respectively) were used. Mice were received intravesical instillation of LPS (2.0 mg/ml) or saline (vehicle) for 1 hour, and all experiments were examined at 24 hours after instillation. For *in vivo* investigation, decerebrate unanaesthetized cystometry (CMG) was performed. For *in vitro* investigations, histological evaluation of the bladder, count of the infiltrating inflammatory cells in the suburothelial layer, and mRNA expression of several TRP channels known to be expressed in the bladder and L6 dorsal root ganglion (DRG) were determined.

Results

In the CMG measurements, after LPS instillation, TRPA1-KO mice showed significantly longer intercontraction interval and larger voided volume than WT mice (Figure 1). Histologically, urothelial denudation, inflammatory cell infiltration and edema in the suburothelial layer were similarly observed in the bladder of both WT and TRPA1-KO mice with LPS-instillation, whereas neither of these findings was observed in the bladder of saline-instilled WT mice (Figure 2 A-F). Compared to saline-instilled WT mice, the number of infiltrating inflammatory cells was significantly higher in both LPS-instilled WT and TRPA1-KO mice, but there was no significant difference between WT and TRPA1-KO mice with LPS-instillation (Figure 2 G). None of the other TRP channel expressions in the bladder or L6 DRG was significantly different between WT and TRPA1-KO mice. TRPM2 mRNA expression of the bladder significantly increased with LPS-instillation in both WT and TRPA1-KO mice when compared with those with saline-instillation, while LPS-instillation did not change significantly any of the other TRP channels in the bladder or L6 DRG of either group of mice (Figure 3).

Interpretation of results

The results of CMG measurements suggest that TRPA1 channel is involved in the LPS-induced bladder hyperactivity in mice. On the other hand, LPS induced similar degree of inflammatory cell infiltration in the bladders of both WT and TRPA1-KO mice, suggesting a limited role of TRPA1 in inflammatory responses themselves. Our results also revealed that no compensatory gene expression changes of other TRP channels were observed in the bladder or L6 DRG of TRPA1-KO mice. In addition, LPS-instillation did not change mRNA expression of TRPA1 in either the bladder or L6 DRG. This finding seems discrepant from the present results of CMG measurements. More detailed investigation such as evaluation of the single-cell RT-PCR of the neuronal cells in L6 DRG innervating the bladder are needed.

Interestingly, mRNA expression of TRPM2 channel, which is known to contribute to inflammatory and neuropathic pain (3), was up-regulated in the bladders of both WT and TRPA1-KO mice with LPS-instillation, suggesting that TRPM2 channel would be further explored as a target for bladder inflammatory pain disorders.

Concluding message

TRPA1-KO mice showed attenuated bladder hyperactivity induced by LPS-instillation, but similar inflammatory changes in the bladder as WT mice. These results suggest that TRPA1 is involved in bladder hyperalgesia induced by bladder inflammation, but not in inflammation itself.

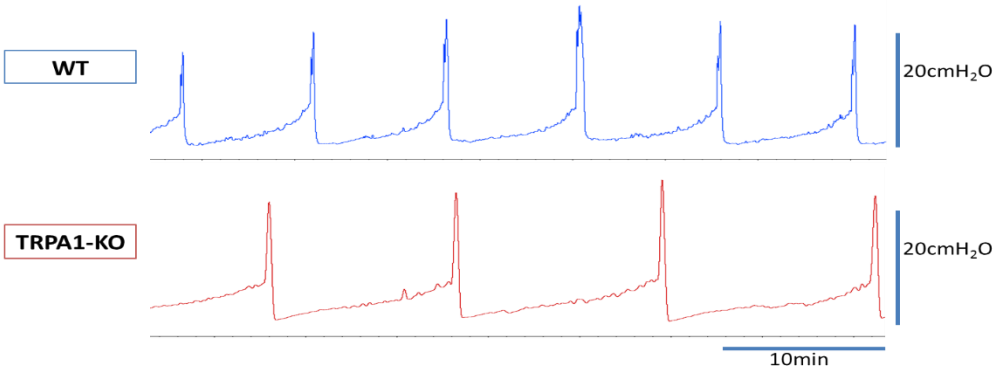

Figure 1. Representative tracings of intravisical pressure in WT and TRPA1-KO mice at 24 hours after LPS-instillation under a decerebrate unanesthetised condition

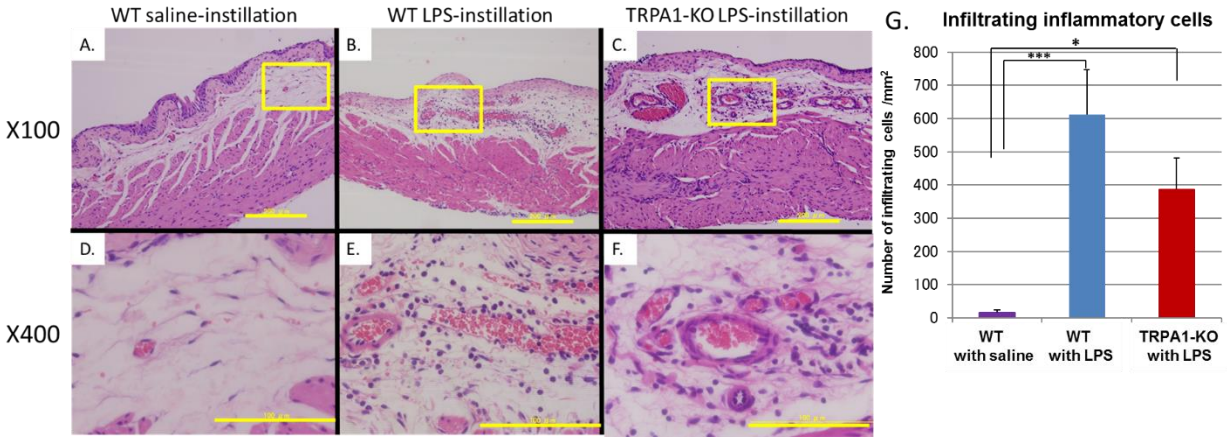

Figure 2. Representative images of the bladder in saline-instilled (A, D) and LPS-instilled (B, E) WT mice and LPS-instilled TRPA1-KO mouse (C, F) at 24 hours after instillation
Comparison between groups of the number of infiltrating inflammatory cells in the suburothelial layer of the bladder (N = 7 in each group) (G)
Scale bar, A-C: 200μm. D-F: 100μm. The square area in each upper panel (A-C) was corresponding to each lower panel (D-F).
* p<0.05, *** p<0.001: significant differences from WT mice with saline-instillation. No significant differences were found between LPS-treated WT and TRPA1-KO mice (Tukey test).

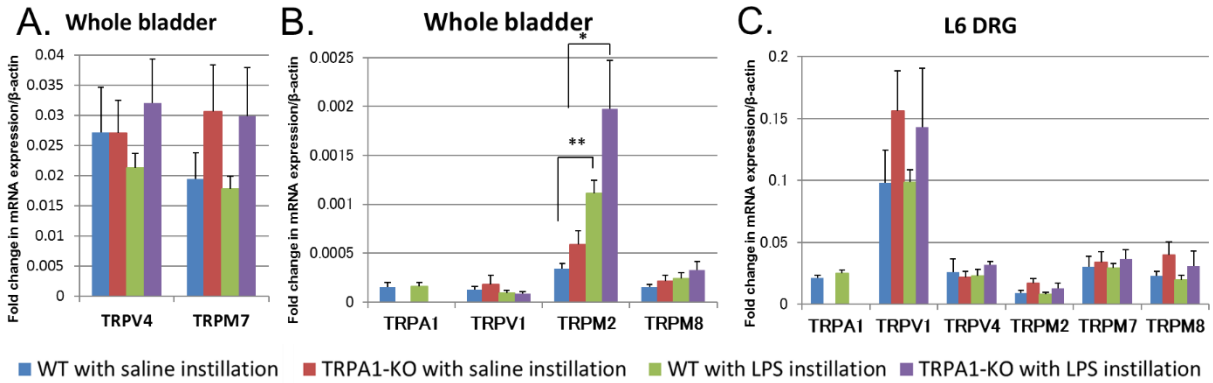

Figure 3. mRNA expressions of TRP channels in the bladder (A, B) and L6 DRG (C) of WT and TRPA1-KO mice at 24 hours after saline- or LPS-instillation (N = 5 in each group)
*p<0.05, ** p<0.01: significant differences between WT mice with saline-instillation and WT mice with LPS-instillation (unpaired t-test).

References

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Disclosures
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