Direct effects of TRPV4 cation channel activation on the primary bladder afferent activities of the rat

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Hypothesis / aims

It has been suggested that transient receptor potential vanilloid 4 (TRPV4) in the urothelium affects the afferent pathways innervating the bladder (J Pharmacol Exp Ther 2007; 323: 227-35. J Clin Invest 2007; 117: 3453-62. J Biol Chem 2009; 284: 21257-64). We investigated the effects of GSK1016790A (GSK) and RN1734, a TRPV4 agonist and antagonist, respectively, and P2X-purinoceptor antagonists (TNP-ATP and PPADS) on cystometry (CMG), and the direct effect of GSK and its relationship with capsaicin (Cap)-sensitivity on single unit afferent activities of the primary bladder afferent nerves in rats.

Materials & Methods

- Female Sprague-Dawley rats were used
- CMG measurement
  - 4 days before measurement, catheterization were performed.
  - Conscious and free-moving condition for CMG measurement.
- Cystometric parameters were measured before and after intravesical drug instillation.

Afferent measurement

- Urethane anesthesia (1.5g/kg i.p.).
- Left pelvic nerve was put on an electrode for electrical stimulation.
- A catheter (PE-50) was inserted into the bladder.
- Laminectomy: L6 dorsal roots cut and left split until < 3 fibers.
- A5-fiber: conduction velocity (CV) ≥ 2.5 m/sec, C-fiber: CV< 2.5 m/sec.
- The afferent activity originating from the bladder were identified by electrical stimulation of the pelvic nerve and by bladder filling (0.08 ml/min).
- Then, GSK was instilled intravesically 3 times, and finally Cap was instilled to investigate the relationship with Cap-sensitivity. (*Cap-sensitive” or “Cap-insensitive” afferent activities were classified based on both pressure and volume increases of more or less than 150 % from baseline, respectively, when the bladder was instilled with Cap.)

Results

CMG measurement

- Intravesical instillation of GSK transiently decreased bladder capacity (BC) and voided volume (VV), which were counteracted by pretreatment with RN1734, TNP-ATP, and PPADS (Fig. 1).

Afferent measurement

- The response of A5-fibers (n=8) to bladder filling was not affected by either GSK or Cap. Based on the Cap-sensitivity, C-fibers could be divided into two subtypes: Cap-insensitive (n=14) and Cap-sensitive (n=8). In the Cap-insensitive C-fibers, the response to bladder filling significantly increased with GSK at the first instillation, but the increase attenuated with time. On the other hand, in the Cap-sensitive C-fibers, the response was not significantly affected by GSK (Figs. 2 and 3).

Conclusion

The present results suggest that activation of TRPV4 in the bladder, probably urothelium, facilitates the micturition reflex by P2X-purinoceptor-mediated activation of the mechanosensitive Cap-insensitive C-fibers of the primary bladder afferents in rats.

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Fig. 1. Representative CMG recordings in a conscious free-moving rat before and during intravesical instillation of GSK(A), RN1734(GSK(B), TNP-ATP(GSK(C), and PPADS(GSK(D). BP: bladder pressure. VV: voided volume.

Fig. 2. Representative recordings of bladder pressure and firing rate of the A5(A), Cap-insensitive C(B), and Cap-sensitive C-fiber(C) activities during bladder filling with GSK/Cap. BP: bladder pressure. FR: firing rate.

Fig. 3. Responses of the A5-fibers and Cap-insensitive and Cap-sensitive C-fibers integrated during the whole filling phase based on pressure and volume with intravesical instillation of GSK and Cap.

*p<0.05, **p<0.01: significant difference from Base (two-way ANOVA followed by Tukey’s test)