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DIRECT EFFECTS OF TRPV4 CATION CHANNEL ACTIVATION ON THE PRIMARY BLADDER AFFERENT ACTIVITIES OF THE RAT

Hypothesis / aims of study
It has been suggested that transient receptor potential vanilloid 4 (TRPV4) in the urothelium affects the afferent pathways innervating the bladder (1-3). 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 (SAAs) of the primary bladder afferent nerves in rats.

Study design, materials and methods
Female Sprague-Dawley rats were used in conscious and free-moving condition for CMG measurements. In SAAs measurements, under urethane anesthesia, a single nerve fiber primarily originating from the bladder was identified by electrical stimulation of the left pelvic nerve and by bladder distention, which were classified as Aδ- and C-fibers by their conduction velocity. In CMG measurements, cystometric parameters were measured before and after intravesical drug instillation. The SAAs measurements with saline instillation were served as the baseline observation before drug instillation. Then, GSK was instilled intravesically 3 times, and finally Cap was instilled to investigate the relationship with Cap-sensitivity.

Results
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). In SAAs measurements, the response of Aδ-fibers (n=7) 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).

Interpretation of results
GSK significantly decreased BC and VV at first, and then these effects were attenuated with time and disappeared, suggesting desensitization of the response. Because these effects of GSK were counteracted by RN1734, these results possibly indicate that the transient activation by GSK was mediated through TRPV4. Furthermore, both of P2X-purinoceptor antagonists blocked the effects of GSK when instilled in combination with GSK. These results assumed that activation of TRPV4 in the bladder urothelium can facilitate afferent transduction from the bladder through urothelially released ATP and subsequent stimulation of $P2X_3$-purinoceptors. Agonist-induced activation of the TRPV4 may cause desensitization when exposed continuously.
In the SAAs measurements, we have found that GSK facilitated only Cap-insensitive C-fibers but not Aδ-fibers or Cap-sensitive C-fibers. Together with the cystometric results, it is likely that activation of TRPV4 in the bladder urothelium by GSK can facilitate selectively mechanosensitive Cap-insensitive C-fibers probably though releasing ATP from the urothelium and activating its receptors ($P2X_3$). Gradual attenuation of the facilitatory effect of GSK during the second and third instillations was consistent with the results of CMG measurements, suggesting that these observations resulted from so called desensitization.

Concluding message
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.

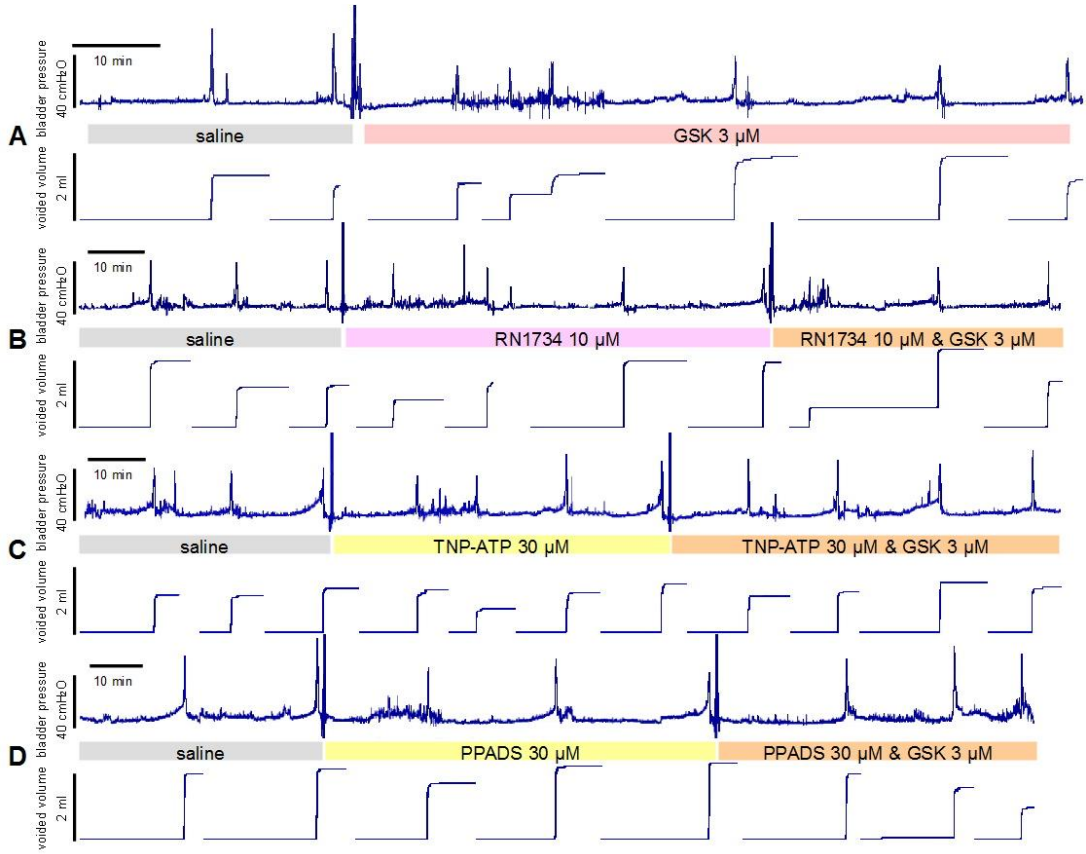

Fig. 1. Representative CMG recordings in a conscious free-moving rat before and during intravesical instillation of GSK(A), RN1734/GSK(B), TNP-ATP/GSK(C), and PPADS/GSK(D).

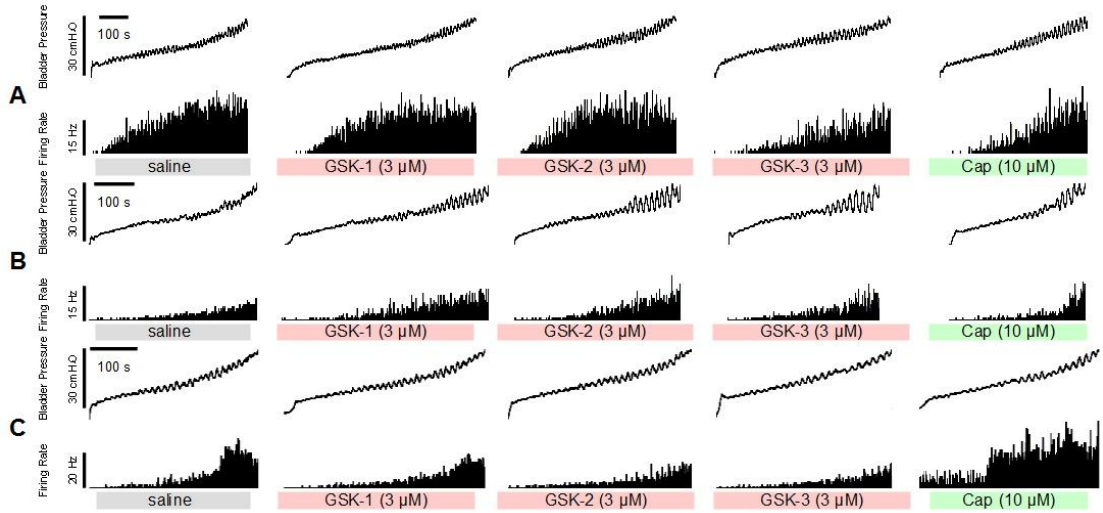

Fig. 2. Representative recordings of bladder pressure and firing rate of the Aδ-(A), Cap-insensitive C-(B), and Cap-sensitive C-fiber(C) activities during bladder filling with GSK/Cap.

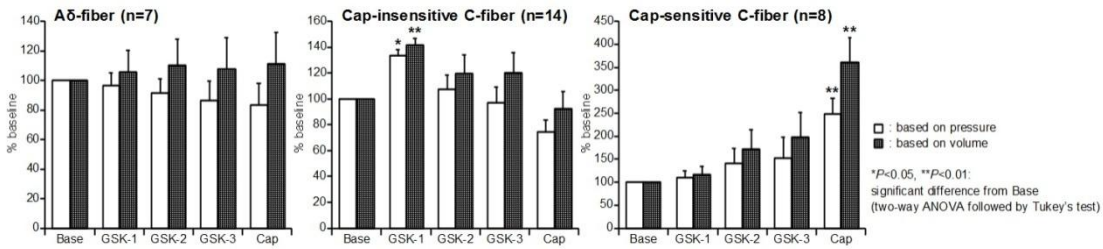

Fig. 3. Responses of the Aδ-fibers and Cap-insensitive and Cap-sensitive C-fibers integrated during the whole filling phase based on pressure and volume with intravesical instillation of GSK and Cap.

References

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2. J Clin Invest 2007; 117: 3453-62
3. J Biol Chem 2009; 284: 21257-64

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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	**Animal Ethics Committee, The University of Tokyo Graduate School of Medicine**