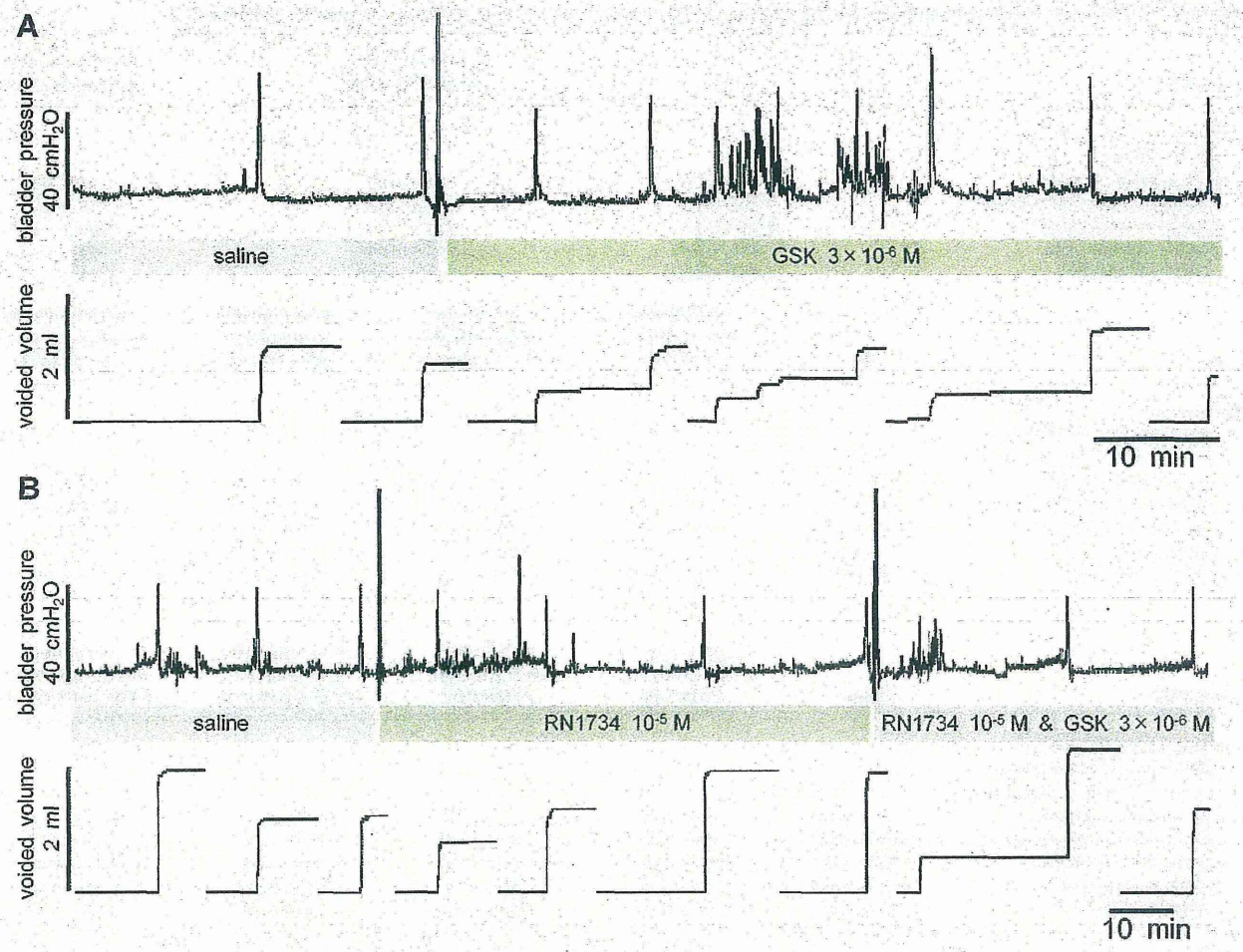

Fig. 1. Representative cystometric recordings (bladder pressure and voided volume) in a conscious free-moving rat before and during intravesical instillation of GSK (**A**) and RN1734/GSK (**B**). GSK: GSK1016790A, TRPV4 agonist; RN1734: TRPV4 antagonist.

DISCUSSION

In the present study, we investigated the effects of a TRPV4 agonist, GSK, on CMG and mechanosensitive primary bladder afferent activities by directly instilling this and other compounds into the bladder, hereby yielding direct exposure of the bladder urothelium. Intravesical instillation of GSK significantly decreased BC and VV at first, and then these effects were attenuated with time and disappeared, suggesting desensitization of the receptor. Such desensitization of TRPV4 was reported in a previous study with HeLa cells transiently transfected with TRPV4.[16] The effects of GSK on BC and VV were counteracted by RN1734, a TRPV4 antagonist, although instillation of RN1734 alone caused no significant changes in these cystometric parameters. This implies that the effects of GSK were indeed TRPV4-mediated and that in the absence of exogenous agonist there was little endogenous tone on the TRPV4 receptors under our experimental conditions. In previous reports,[8,21] TRPV4$^{-/-}$ mice had increased BC, suggesting a physiological role of TRPV4 for MT volume. This discrepancy between the previous findings in TRPV4$^{-/-}$ mice and the present findings with RN1734 may occur by differences in experimental condition and species of animal, or occur by the influence of systemic or local TRPV4 channel reaction. Nevertheless, Thorneloe et al.[21] demonstrated that intravesical instillation of 10^{-5} M GSK induced bladder overactivity in TRPV4$^{+/+}$ mice with no effect in TRPV4$^{-/-}$ mice, further confirming that this compound indeed selectively acts via TRPV4. The dose used in that study was higher than that (3×10^{-6} M) used in rats in the present study. These results suggest that the transient activation of the micturition reflex by GSK was mediated through TRPV4, and also suggest that under these specific conditions TRPV4 does not play a role physiologically in control of the MT.

Recently, it has been reported that activation of TRPV4 in rat and mouse bladder urothelial cells induces Ca^{2+} influx-evoked ATP release, and the released ATP modulates bladder sensory transduction.[7,8,18] To test involvement of an ATP-mediated mechanism, we further conducted cystometric investigation with P2X-purinoceptor antagonists. Although neither TNP-ATP, a $P2X_3$-purinoceptor antagonist,[27–29] nor PPADS, a nonselective P2X-purinoceptor antagonist,[30] significantly affected any of cystometric parameters, both antagonists blocked the effects of GSK when instilled in combination

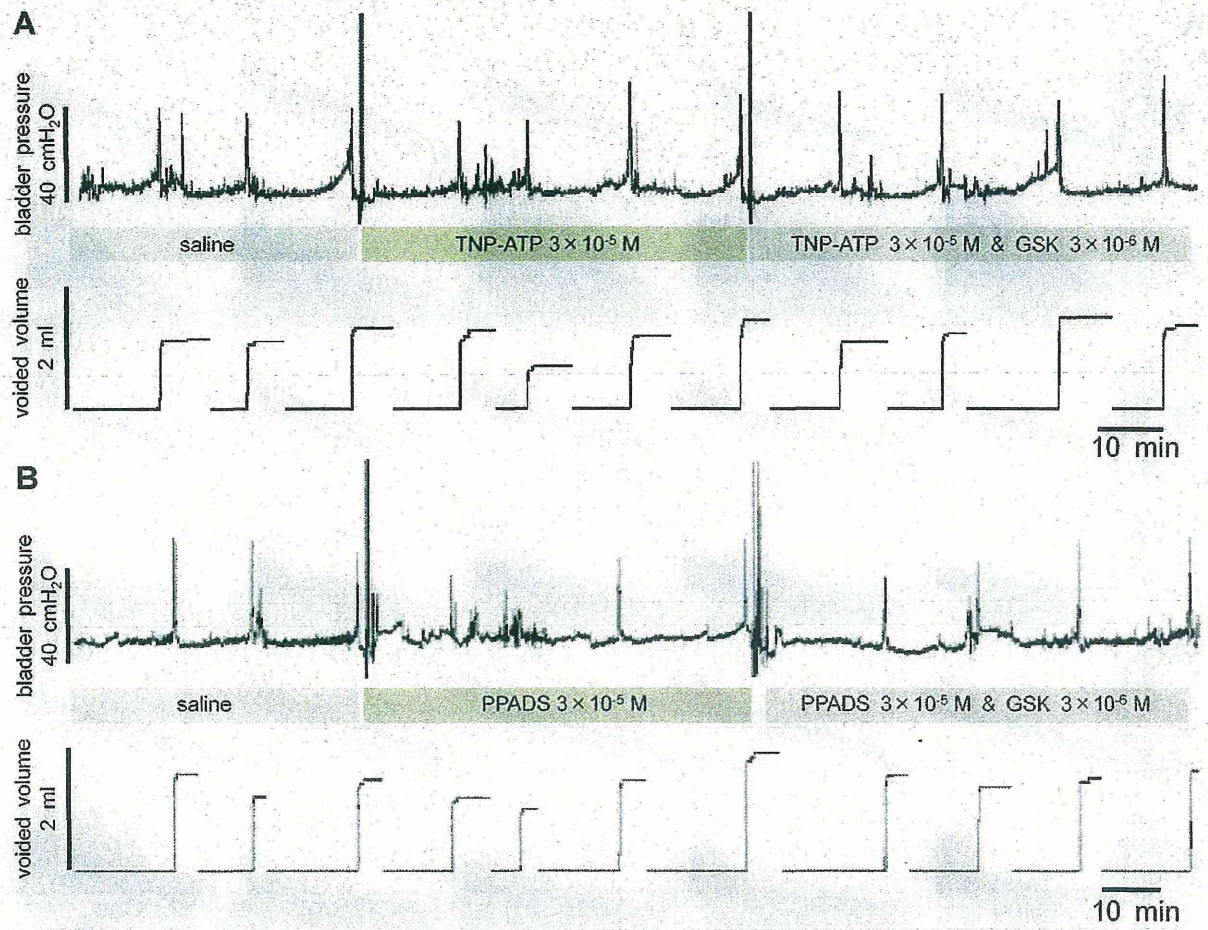

Fig. 2. Representative cystometric recordings (bladder pressure and voided volume) in a conscious free-moving rat before and during intravesical instillation of TNP-ATP/GSK (**A**) and PPADS/GSK (**B**). TNP-ATP: $P2X_3$-purinoceptor antagonist; PPADS: nonselective P2X-purinoceptor antagonist.

with GSK. Birder et al.[7] reported that continuous intravesical instillation of 4α-PDD (10^{-4} M) in conscious and restrained rats significantly increased the amplitude (referred to as PP in the present study) of reflex bladder contractions and tended to decrease the ICI, but PPADS (10^{-4} M) with or without 4α-PDD had no effect on either bladder contraction amplitude or ICI. Moreover, Thorneloe et al.[21] found that instillation of GSK into the bladders induced bladder overactivity characterized as reduction of infused volume (referred to as BC) and VV in $TRPV4^{+/+}$ mice but not in $TRPV4^{-/-}$ mice. These observations mostly consist with our results. Our experimental results were compatible with these previous observations. The desensitization effect of GSK observed in the present study was not detected in those studies, which may be due to the differences of experimental conditions (drugs/its dose, species of mouse/rat, with/without anesthesia). Taken all together, it is 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.

As the next step, we evaluated the influence of TRPV4 activation on mechanosensitive afferent fibers from the bladder. In the afferent measurements, we used pretreatment with PS to facilitate permeability of the bladder urothelium because there is a time limitation (within 1 hr) for preserving adequate condition of the afferent nerve fibers isolated for recording, and thus onset time of the drugs instilled intravesically needed to be as short as possible. It has been reported that PS exposure affects only epithelial cells while sparing the underlying layers,[31] and we have found no significant effects of PS-exposure itself on the bladder afferent activities in a pilot study. Moreover, we used urethane anesthesia in this afferent activity measurements. Although urethane has been shown to spare the micturition reflex compared with other anesthetics,[32] Birder et al. pointed out that intravesical application of 4α-PDD, a TRPV4 agonist induced an increase in micturition pressure in awake rats, but this effect was prevented by urethane anesthesia. Even though the influence of urethane–anesthesia may not be neglected under this condition, we have found that intravesical instillation of the TRPV4 agonist GSK facilitated only Cap-insensitive C-fibers but not Aδ-fibers or Cap-sensitive C-fibers among mechanosensitive afferent fibers primarily originating from the bladder. Since the bladder compliance was not significantly increased by the intravesical instillation of GSK, it is unlikely that GSK directly affected

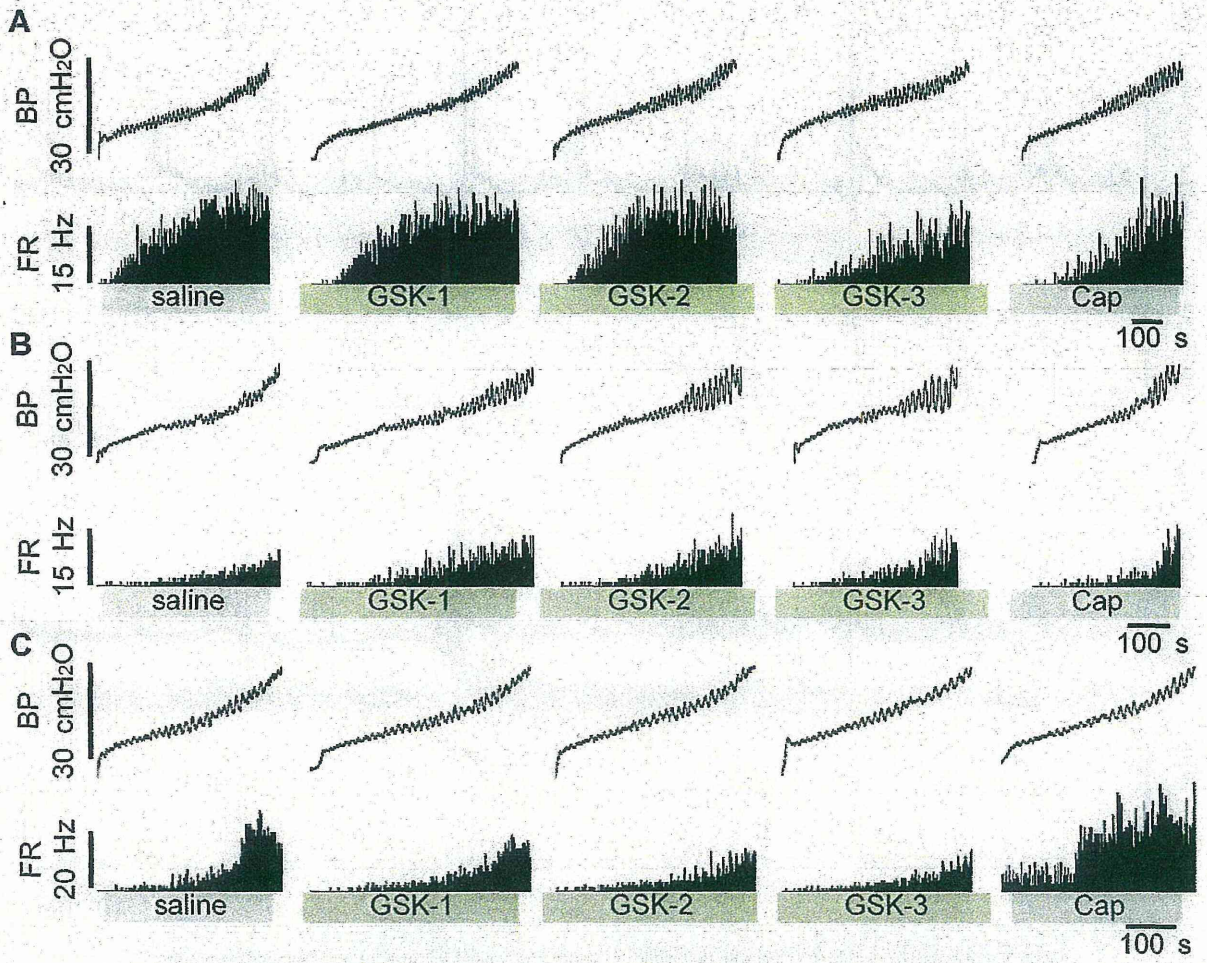

Fig. 3. Representative recordings of bladder pressure (BP) and firing rate (FR) of the Aδ- (**A**), Cap-insensitive C- (**B**), and Cap-sensitive C-fiber (**C**) activities during bladder filling with GSK (3×10^{-6} M) and Cap (10^{-5} M). GSK: GSK1016790A, TRPV4 agonist; Cap: capsaicin, TRPV1 agonist.

detrusor smooth muscle tone. These results are consistent with a previous study demonstrating a very weak expression of TRPV4 in the rat isolated smooth muscle.[7] The present findings in the afferent measurements are consistent with our previous study,[20] where we demonstrated that intravesically instilled ATP activates only Cap-insensitive C-fibers in rats. In that study, we found that approximately 2/3 of mechanosensitive C-fibers were characterized as Cap-insensitive. 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. It is conceivable that these observations resulted from desensitization, possibly Ca^{2+}-dependent desensitization of TRPV4. Strotmann et al.[13] found that Ca^{2+}-dependent potentiation of TRPV4 was often followed by inhibition during TRPV4 activation by hypotonic solutions or phorbol esters, suggesting that an excessive increase in Ca^{2+} entry via TRPV4 is prevented by a Ca^{2+}-dependent negative feedback mechanism. In the Cap-sensitive C-fibers, on the other hand, the afferent activities did not change significantly with instillation of GSK, but tended to increase gradually during repeated instillations. Although GSK is reported to be approximately 10-fold more potent activating TRPV4 than activating TRPV1 channels,[25] it is possible that GSK can also act on TRPV1 channels when instilled intravesically at a high concentration, and this might contribute to the gradual increasing tendency of the activities in Cap-sensitive C-fibers with GSK. In the afferent measurements, we did not investigate the effect of the combined drug administration (RN1734, TNP-ATP, or PPADS) with GSK because there is time limitation for keeping adequate responsiveness of SAAs, and its desensitization effect after Cap administration. However, further experiments with combined drug administrations would be helpful for our knowledge.

CONCLUSIONS

The present results suggest that activation of TRPV4 in the bladder facilitates the micturition reflex by activation of

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