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# KPR-2579, A NOVEL TRPM8 ANTAGONIST, INHIBITS ACETIC-ACID-INDUCED BLADDER HYPERACTIVITY VIA MECHANOSENSITIVE AFFERENT C-FIBERS IN RATS

Hypothesis / aims of study

Transient Receptor Potential Melastatin 8 (TRPM8) channels are widely expressed in urothelial cell and sensory nerve fibres of the bladder and in L6 dorsal root ganglia (DRG) of the rat, and also in the urothelial cells of the human bladder [1]. A previous study suggested that TRPM8 channels have a role in activation of bladder sensory pathways at least partly through mechanosensitive C-fibres during bladder filling in rats [2]. In addition, another study demonstrated a significantly increased number of TRPM8-immunoreactive nerve fibres in the bladder suburothelium taken from patients with overactive bladder and painful bladder syndromes, and its correlation with clinical symptoms of pain and urinary frequency [3]. These findings suggest that TRPM8 channels may play a pathophysiological role in the development of bladder hypersensitive disorders via activation of mechanosensitive bladder afferents. However, there have been no studies directly investigating pathophysiological roles of TRPM8 channels in a model of hypersensitive bladder. In this study, we examined the effect of KPR-2579 (KPR), a novel selective TRPM8 antagonist, on deep body temperature and acetic acid (AA)-induced bladder hyperactivity by cystometry (CMG) and measuring primary bladder single-unit afferent activities (SAAs) in rats.

Study design, materials and methods

Ninety-one female Sprague-Dawley rats were used and anesthetized with urethane (1.2 g/kg intraperitoneally). The effect of KPR on deep body temperature (intrarectal temperature) was evaluated with cumulative intravenous (i.v.) administrations of KPR (0.03 - 1 mg/kg) or its vehicle (20% *N,N*-dimethylacetamide in saline). In the CMG measurement, after baseline recordings with saline-instillation at a rate of 6 mL/hour, 0.1% AA was continuously instilled into the bladder, and 1 hour later, KPR (0.03 or 0.3 mg/kg) or vehicle was administered (i.v.), then further recordings were performed. CMG parameters were evaluated for 15 minutes in each time point. SAAs measurements were made in separate animals and Aδ- and C-fibres were identified by electrical stimulation of the pelvic nerve and by bladder distention. After baseline recordings with saline-instillation (6 mL/hour), recordings were continuously performed during intravesical AA (0.1%)-instillation after i.v.-administration of KPR (0.03 or 0.3 mg/kg) or vehicle.

Results

KPR even at the highest dose used (1 mg/kg) did not significantly affect deep body temperature. In the CMG measurements, AA-instillation significantly shortened intercontraction interval, and a high dose (0.3 mg/kg) of KPR-administration significantly attenuated the effect of AA-instillation compared to vehicle-administration (Table 1). In the SAAs measurements, 48 single afferent fibers (n = 24 in each fiber) were isolated from 41 rats. In the presence of vehicle, AA-instillations significantly increased the SAAs of C-fibers, but not those of Aδ-fibers. Pretreatment with a high dose (0.3 mg/kg) of KPR significantly inhibited the AA-induced activation of C-fiber SAAs (Figures 1 and 2).

Interpretation of results

CMG and SAAs measurements demonstrate that AA can induce bladder hyperactivity accompanied with facilitation of mechanosensitive C-fiber activities. In addition, AA-induced bladder hyperactivity was attenuated by KPR-administration at the doses which did not influence body temperature. These results suggested that KPR, a novel TRPM8 antagonist, can ameliorate AA-induced bladder hyperactivity without hypothermic effects.

Concluding message

The present results suggest that KPR, a novel TRPM8 antagonist, can inhibit frequent voiding and exaggerated activity of bladder mechanosensitive C-fibers induced by AA-instillation without affecting body temperature. TRPM8 channel may be a promising target for the treatment of bladder hypersensitive disorders.

Table 1. The effects of KPR on CMG parameters during 0.1% AA-instillation

| parameters | groups (N=10 in each group) | saline-instillation (baseline) | 0.1% AA-instillation | | | vehicle or KPR i.v.-administration (during 0.1% AA-instillation) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | (vs. baseline) | | | (vs. baseline) | (vs. AA-instillation) |
| Basal pressure ($cmH_2O$) | Vehicle | 5.6 ± 0.4 | 5.4 | ± 0.3 | (N.S.) | 5.5 | ± 0.4 | (N.S.) | (N.S.) |
| | KPR 0.03 | 5.9 ± 0.5 | 5.7 | ± 0.3 | (N.S.) | 6.1 | ± 0.4 | (N.S.) | (N.S.) |
| | KPR 0.3 | 5.2 ± 0.3 | 5.5 | ± 0.4 | (N.S.) | 5.3 | ± 0.3 | (N.S.) | (N.S.) |
| Threshold pressure ($cmH_2O$) | Vehicle | 14.5 ± 1.2 | 11.2 | ± 0.6 | (‡) | 10.7 | ± 0.6 | (‡) | (N.S.) |
| | KPR 0.03 | 18.5 ± 2.0 | 11.5 | ± 0.7 | (‡) | 11.5 | ± 0.8 | (‡) | (N.S.) |
| | KPR 0.3 | 13.4 ± 2.0 | 9.9 | ± 0.6 | (N.S.) | 11.2 | ± 0.9 | (N.S.) | (N.S.) |
| Peak pressure ($cmH_2O$) | Vehicle | 38.3 ± 1.7 | 39.5 | ± 2.9 | (N.S.) | 40.3 | ± 2.4 | (N.S.) | (N.S.) |
| | KPR 0.03 | 39.5 ± 1.6 | 36.6 | ± 2.3 | (N.S.) | 38.3 | ± 2.2 | (N.S.) | (N.S.) |
| | KPR 0.3 | 38.3 ± 1.7 | 36.4 | ± 2.0 | (N.S.) | 37.3 | ± 1.9 | (N.S.) | (N.S.) |
| Intercontraction interval (minutes) | Vehicle | 3.0 ± 0.3 | 1.9 | ± 0.2 | (‡) | 1.9 | ± 0.1 | (‡) | (N.S.) |
| | KPR 0.03 | 3.5 ± 0.5 | 1.9 | ± 0.2 | (‡) | 1.9 | ± 0.2 | (‡) | (N.S.) |
| | KPR 0.3 | 3.1 ± 0.5 | 1.8 | ± 0.2 | (‡) | 2.4 | ± 0.2# | (N.S.) | (N.S.) |

Values are expressed as mean ± SEM. ‡P<0.01: from baseline or 0.1% AA-instillation in same group (repeated measures ANOVA followed by Tukey's test), #P<0.05: from vehicle-treated group at each time point (unpaired Student's t-test), N.S.: not significant difference

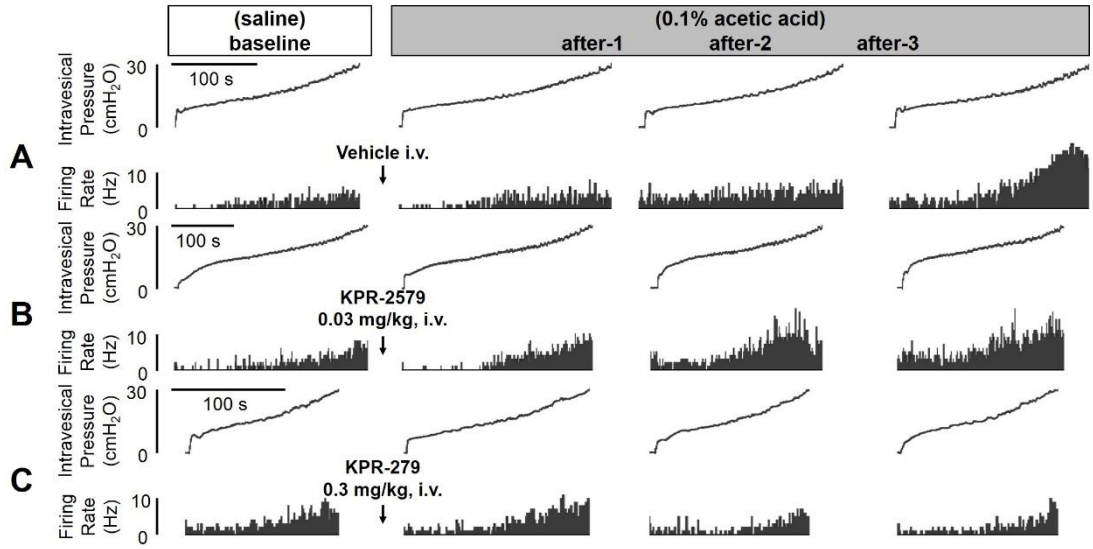


Figure 1. Representative traces of the intravesical pressure and firing rate of mechanosensitive afferent C-fiber at baseline with saline-instillation and during 0.1% acetic acid-instillation after vehicle (A) or KPR (0.03 or 0.3 mg/kg) i.v.-administration (B and C)

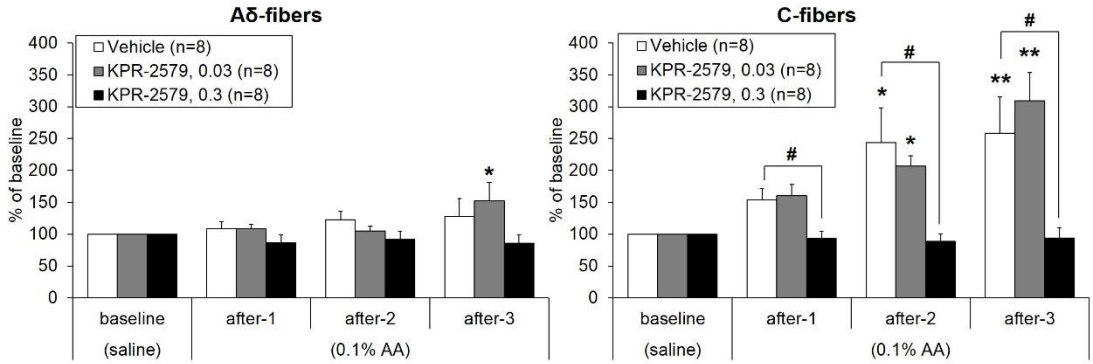


Figure 2. Effects of KPR on SAAs of Aδ-fibers (Left) and C-fibers (Right) during 0.1% AA-instillation
Values are expressed as mean ± SEM. *P<0.05, **P<0.01: from baseline in each group (repeated measures ANOVA followed by Dunnett's test), #P<0.05: from vehicle-treated group at each time point (unpaired Student's t-test)

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Disclosures

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