

## EFFECTS OF TRPM8 CHANNELS' INHIBITION ON CONSCIOUS CYSTOMETRY AND SINGLE-UNIT MECHANOSENSITIVE BLADDER AFFERENT ACTIVITIES IN NOVEL EX VIVO MODEL OF THE RAT

### Hypothesis / aims of study

It has been reported that transient receptor potential melastatin 8 (TRPM8) ion channels are expressed in the dorsal root ganglion (DRG), urothelial cells, and sensory nerve fibers in the urothelium and suburothelial space of the rat bladder, and these channels overexpressed with bladder outlet

t obstruction (1). TRPM8 channels are expressed also in urothelial cells of the human bladder and DRG (2). Moreover, overexpression of TRPM8-immunoreactive nerve fibers in the patients with painful bladder syndrome and detrusor overactivity has been demonstrated (3). These findings suggest that TRPM8 might be involved in the bladder sensory transduction and also pathophysiology of the development of hypersensitive bladder disorders, such as overactive bladder (OAB) and bladder pain syndrome (BPS). The aim of the present study was to determine the role of TRPM8 channel in activation of bladder mechanosensory transduction by using RQ00203078 (RQ), a novel selective TRPM8 antagonist developed by RaQualia Pharma Inc., on conscious cystometry (CMG) and single-unit mechanosensitive bladder afferent activities (SAAs) measurement in a newly established *ex vivo* rat model.

### Study design, materials and methods

Female Sprague-Dawley rats were used. CMG measurements were performed with continuous saline instillation at a rate of 6 ml/hour in conscious and free-moving condition before and after intravenous (i.v.) cumulative administration of RQ00203078 (RQ, a selective TRPM8 antagonist) at three doses, 0.3, 1, and 3 mg/kg. In separate rats *ex vivo* SAAs measurements were performed. A catheter was placed in the abdominal aorta and a separate catheter in the bladder through the urethra, and the bladder neck was ligated to prevent leakage. Krebs solution was infused at a rate of 1 ml/minute through the catheter placed in the aorta to irrigate the bladder and L6 dorsal roots. Finally, the rat was sacrificed with an overdose of urethane-aesthesia and through a laminectomy, both L6 dorsal roots were cut. The pelvic organ tissues including the bladder, pelvic nerve, DRG and L6 dorsal roots nerve fibers were dissected from the rat body, then immediately moved to a chamber with warm paraffin-oil (Figure 1A). Fine filaments were dissected from the left L6 dorsal roots and placed across a bipolar electrode for monitoring SAAs. Nerve fibers primarily originating from the bladder were identified by electrical stimulation of the left pelvic nerve and by bladder distension. Nerves with conduction velocities (CV) more than 2.5 m/sec were designated as A $\delta$ -fibers and those with CV less than 2.5 m/sec as C-fibers. SAAs were recorded in response to bladder distensions induced by rapid intravesical instillation at 15 and 30 cmH<sub>2</sub>O of intravesical pressures as a base-line (Figure 1B). Then, the SAAs investigation was repeated after cumulative intra-aortal (i.a.) administration of RQ at doses of 0.3 and 3 mg/kg or vehicle. In separate experiments, after the similar baseline investigation, SAAs investigation was repeated after menthol (3 mM intravesically) with or without pretreatment with RQ-administration (3 mg/kg, i.a.).

### Results

10 rats were used for CMG measurement. Voided volume and bladder capacity significantly increased after RQ-administration at 1 and 3 mg/kg, and at 0.3 and 1 mg/kg, respectively compared with vehicle-administrations (Figure 2). Micturition pressure and basal pressure significantly increased after RQ-administration at 0.3 and 1 mg/kg and at 0.3 mg/kg, respectively (data not shown). A total of 39 SAAs of C-fibers were isolated from 35 rats, whereas A $\delta$ -fibers were detected but its SAAs were unable to measure continuously during drug-administrations in this *ex vivo* model of afferent measurement. The increased SAAs of C-fibers in response to bladder distension at 30 cmH<sub>2</sub>O of intravesical pressures were significantly inhibited by RQ-administrations at both 0.3 and 3 mg/kg (Figure 3) although RQ-administration showed similar suppressive effects on those to bladder distension at 15 cmH<sub>2</sub>O, but they were not statistically significant (data not shown). SAAs of C-fibers were significantly increased after menthol-instillation, which was suppressed by the pretreatment with RQ (Figure 4).

### Interpretation of results

In the CMG results, RQ increased the bladder capacity and voided volume, which may reflect inhibition of bladder afferent activity. In harmonize with these findings, mechanosensitive SAAs of C-fibers were attenuated with RQ-administrations at similar dose-ranges. The activation of SAAs with menthol, a TRPM8 channel agonist, was counteracted by RQ. These findings indicate that TRPM8 has a physiological role in the mechanosensation of the bladder filling in normal rats and suggest that mechanosensitive bladder afferent activities of C-fibers can be facilitated by stimulation of TRPM8 channel, and pathologically activated TRPM8 channel may enhance mechanosensation of the bladder, thereby presumably trigger OAB/BPS.

### Concluding message

TRPM8 channel has a role in activation of bladder afferent pathways at least partly via mechanosensitive C-fibers of the rat bladder.

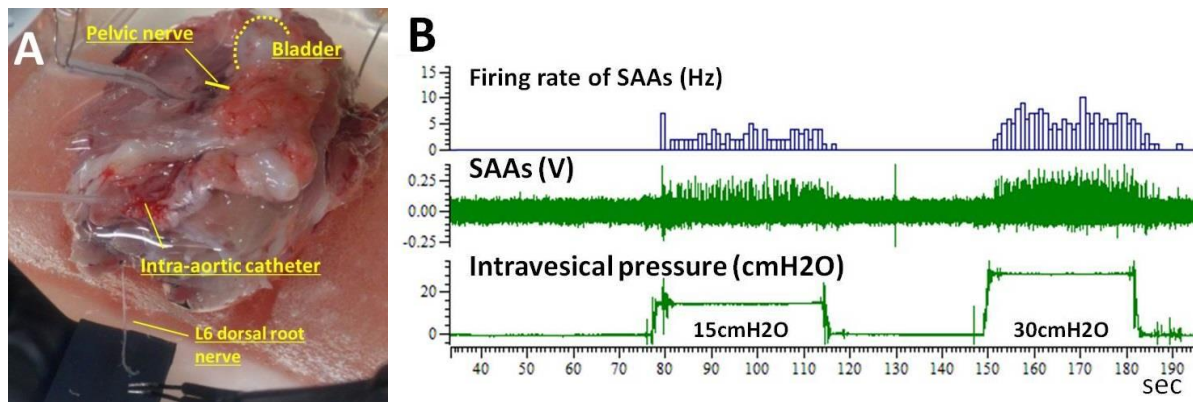


Figure 1. (A) Experimental diagram of novel established *ex vivo* model for the SAAs measurement, and (B) representative bladder pressure and SAAs of C-fibers.

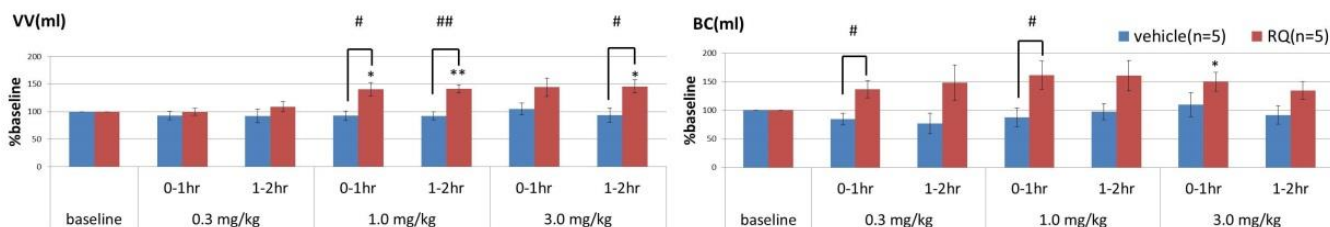


Figure 2. The effects of vehicle and RQ00203078 (RQ) on voided volume (VV) and bladder capacity (BC) on CMG in conscious rats

0-1hr: 0-1 hour after drug-administration and 1-2hr: 1-2 hour after drug-administration

\* $P < 0.05$ , \*\* $P < 0.01$ : significant differences from base-line (paired Student's t-test)

# $P < 0.05$ , ## $P < 0.01$ : significant differences between groups (unpaired Student's t-test)

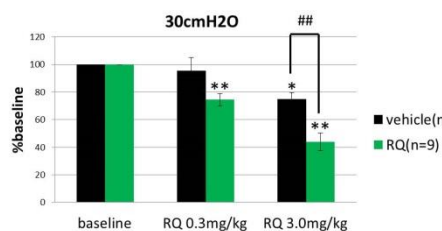


Figure 3. The effects of vehicle and RQ on SAAs of C-fibers in response to bladder distension at 30cmH2O of intravesical pressure

\* $P < 0.05$ , \*\* $P < 0.01$ : significant differences from base (Wilcoxon signed-rank test)

## $P < 0.01$ : significant difference between groups (Mann-Whitney U test)

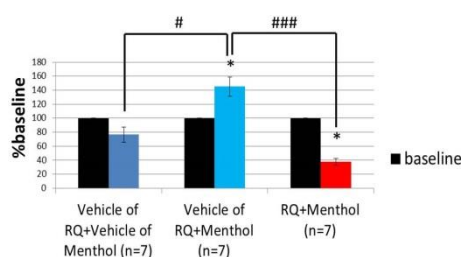


Figure 4. The effects of intravesical instillation of menthol (3 mM) with RQ (3mg/kg i.a.) and its vehicle on SAAs of C-fibers in response to bladder distension at 30cmH2O of intravesical pressure

\* $P < 0.05$ : significant differences from base (Wilcoxon signed-rank test)

# $P < 0.05$ , \*\*\* $P < 0.001$ : significant difference between groups (Mann-Whitney U test)

## References

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## Disclosures

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