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FUNCTIONAL ROLE OF TRANSIENT RECEPTOR POTENTIAL MELASTATIN 8 (TRPM8) CHANNEL IN THE RAT URINARY BLADDER ASSESSED BY USING A NOVEL POTENT ANTAGONIST

Hypothesis / aims of study

TRPM8 is a non-selective cation channel having multiple modes of activation such as cold, and has been widely expressed in urothelial cells 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 by measuring *ex vivo* bladder afferent activities revealed that TRPM8 channels may have a role in activation of mechanosensitive afferent pathways of the normal rat bladder, which was mediated at least partly via C-fibres [2]. RQ-00434739 (RQ) was newly developed as a potent antagonist selective for TRPM8, and it displayed analgesic efficacy in an oxaliplatin-induced cold allodynia model in the rat and cynomolgus monkey [3]. In this study, to disclose the functional role of TRPM8 channel in the bladder, we examined effects of intravesical instillation of L-menthol (a TRPM8 agonist), and counter-effects of RQ on bladder function, especially on the *in vivo* primary bladder single-unit afferent activities (SAAs) in rats.

Study design, materials and methods

Forty-two female Sprague-Dawley rats were used. In conscious cystometry (CMG), after baseline recording with saline-instillation at a rate of 6 mL/hour, further recording was performed with an intravesical instillation of L-menthol (6 mM) after intravenous (i.v.) pretreatment with RQ (1 mg/kg) or vehicle. CMG parameters were analysed before and after drug-administrations for 1 hour in each. In the SAAs measurements by using separate animals, rats were anesthetized with urethane (1.2 g/kg, intraperitoneally) and SAAs recorded from the left L6 dorsal root were classified by conduction velocity as Aδ- or C-fibers by electrical stimulation of the left pelvic nerve and by bladder distention. Thereafter, SAAs were recorded as baseline with saline-instillation (6 mL/hour), and further recordings were performed during intravesical instillation of L-menthol (6 mM) after i.v. administration of RQ (1 mg/kg) or vehicle.

Results

In the CMG measurements, intravesical L-menthol after pretreatment with vehicle showed decreases in threshold pressure and mean voided volume, whereas such decreased responses were not observed with RQ-pretreatment (Table 1). In the SAAs measurements, 32 single afferent fibers (n = 16 in each fiber) were isolated from 30 rats. In the presence of vehicle, after L-menthol-instillations SAAs of C-fibers significantly increased, whereas those of Aδ-fibers tended to increase, but not significantly. In the presence of RQ, L-menthol-instillation did not significantly affect SAAs of either Aδ- or C-fibers (Figures 1 and 2).

Interpretation of results

CMG and SAAs measurements showed that intravesical L-menthol-instillation provoked bladder hyperactivity, which was mediated mainly via facilitation of mechanosensitive C-fiber activities in rats. The L-menthol-induced bladder hyperactivity was attenuated by pretreatment with RQ-administration, suggesting that TRPM8 channel in the rat bladder has a functional role in the activation of bladder mechanosensitive C-fibers whereas its role in bladder mechanosensitive Aδ-fiber activation may be limited. These results are in line with those of a previous study by measuring *ex vivo* afferent activities [2].

Concluding message

The present results demonstrated that TRPM8 channel in the rat bladder has a role in activation of mechanosensitive C-fibers. Functional inhibition of TRPM8 channel should be further explored as a novel tool for the treatment of bladder hypersensitive disorders.

Table 1. CMG results in conscious rats at baseline with saline-instillation and during L-menthol-instillation after vehicle or RQ (1 mg/kg) i.v.-administration

parameters	groups (N=6 in each group)	saline-instillation (baseline)	L-menthol-instillation after vehicle or RQ (1mg/kg) i.v.-administration
Basal pressure (cmH_2O)	Vehicle	2.76 ± 0.49	3.46 ± 0.65
	RQ	3.31 ± 0.53	3.68 ± 0.61
Threshold pressure (cmH_2O)	Vehicle	10.32 ± 1.30	7.39 ± 0.82*
	RQ	11.28 ± 1.30	8.48 ± 1.45
Peak pressure (cmH_2O)	Vehicle	39.03 ± 1.98	40.10 ± 2.57
	RQ	37.15 ± 2.98	34.89 ± 5.22
Mean voided volume (mL)	Vehicle	1.34 ± 0.06	0.86 ± 0.05**
	RQ	1.29 ± 0.25	1.19 ± 0.17

Values are expressed as mean ± SEM.
*P<0.05, **P<0.01: from baseline in each group (paired Student's t-test)

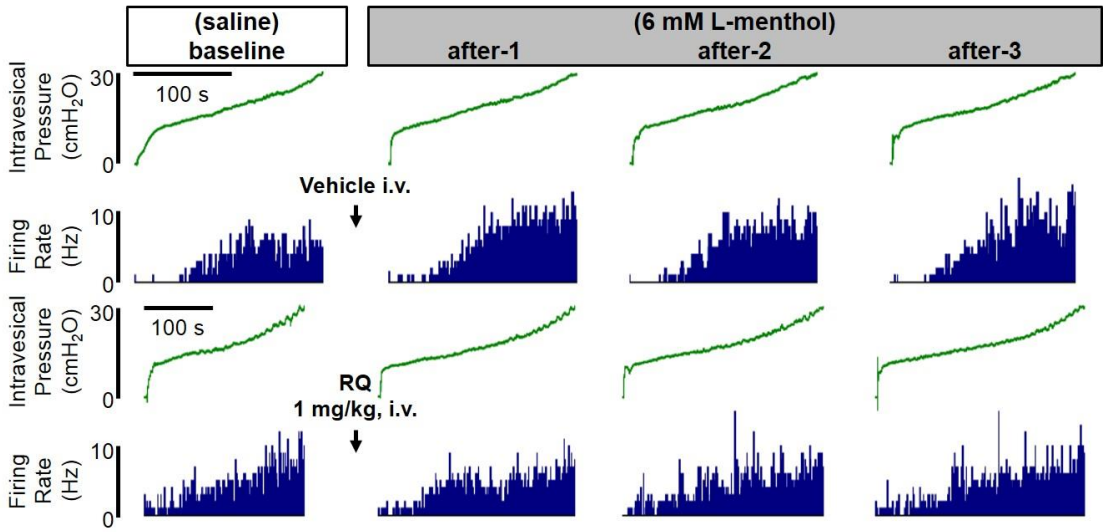

Figure 1. Representative traces of the intravesical pressure and firing rate of mechanosensitive afferent C-fiber at baseline with saline-instillation and during L-menthol-instillation after vehicle (upper trace) or RQ (1 mg/kg) i.v.-administration (lower trace)

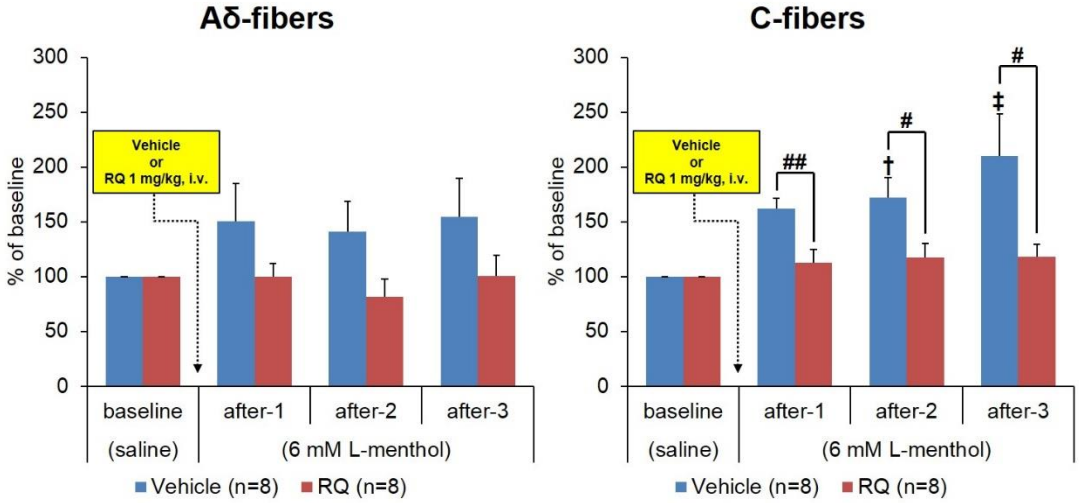

Figure 2. Effect of intravenous RQ (1 mg/kg)-administration on SAAs of Aδ-fibers (Left) and C-fibers (Right) during 6 mM L-menthol-instillation

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, ##P<0.01: between vehicle- and RQ-treated groups at each time point (unpaired Student's t-test)

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Disclosures

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