

THE BLADDER-COOLING REFLEX IS A LOCAL PHENOMENON, MEDIATED BY TRPA1

Hypothesis / aims of study

Ice-cold saline infusion into the bladder elicits a strong bladder contraction in patients with neurogenic detrusor overactivity (NDO). This effect has been used for decades as a diagnostic test in clinical practice. It is hypothesized that TRPA1 – a cold-activated member of the TRP ion channel superfamily - plays a crucial role in the bladder cooling reflex and is important in urological conditions, such as overactive bladder (OAB) and NDO. The effect is said to be mediated by silent C-fibers, which are activated when CNS control is removed. This way, a spinal reflex arc is induced. In this study, we tested these hypotheses by assessing the bladder cooling reflex in urethane anaesthetized and spinal cord injury (SCI) rats. Also, the expression of TRPA1 in the rat bladder was evaluated.

Study design, materials and methods

We conducted all *in vivo* experiments in urethane anaesthetized (1g/kg, i.p.) and in conscious rats 3-4 weeks after SCI and sham SCI surgery. With cystometry, we evaluated the functionality of TRPA1 *in vivo*. The bladder was filled with room temperature saline at a constant rate to get a baseline recording. The TRPA1 blockers HC 030031 (100mg/kg), the ganglionic blocker hexamethonium (20mg/kg) and the vehicles were then administered i.p. 20 minutes before a cold stimulus. This stimulus consisted of an infusion of ice-cold saline into the bladder. Meanwhile bladder pressure was monitored continuously. Expression of TRPA1 was assessed by qPCR on dorsal root ganglion neurons (L6-S1), urothelial cells and detrusor smooth muscle cells. *Ex vivo*, functional responses to cold stimulation were also evaluated using contractility experiments. In these experiments, a detrusor strip was attached to a force transducer. The organ bath was quickly cooled from 36°C to 10°C to give a cold stimulus to the detrusor muscle.

Results

From qPCR experiments, we saw that TRPA1 is highly expressed in dorsal root ganglions (L6-S1) while the expression in the urothelium and the detrusor is respectively low and very low (n=2). From the *in vivo* experiments, we saw that in urethane anaesthetized (n=8) and in SCI rats (n=7), the intravesical pressure increase upon instillation of ice-cold saline was significantly lower after administration of HC 030031, compared to administration of vehicle. Administration of hexamethonium had no effect on this bladder-cooling reflex (n=6). *Ex vivo*, in detrusor strip contractility experiments, we observed a clear cold-induced contraction (n=8), which could be largely abolished by the TRPA1 blocker HC 030031 (100µM) (n=8). This cold-induced bladder contraction was significantly diminished in a 0 Ca²⁺ BAPTA solution (n=8), in isoprenaline 1mM and in indomethacin 1µM, (n=8) but not in atropine 1µM (n=8) and suramine 300 µM (n=8).

Interpretation of results

Our *in vivo* cystometry-experiments show that the bladder-cooling reflex is mediated by TRPA1. The qPCR experiments made clear that TRPA1 is mainly expressed in dorsal root ganglions. The observation that the ganglionic blocker hexamethonium had no effect on the bladder-cooling reflex, in combination with clear cold-induced contractions in detrusor strip contractility experiments led to the conclusion that the bladder-cooling reflex is a local phenomenon, mediated by TRPA1 which is probably located at the sensory nerve terminals. Indomethacin inhibits the production of prostaglandins and could diminish the bladder contraction significantly in detrusor strip contractility experiments. Therefore, cold-induced activation of TRPA1 probably leads to the release of local mediators such as prostaglandins. Isoprenaline could abolish the bladder-cooling reflex and is a non-selective beta-adrenergic agonist. Release of mediators by the sympathetic nervous system could inhibit the bladder-cooling reflex *in vivo*. The loss of these sympathetic inhibitory pathways in pathological conditions could elicit the bladder-cooling reflex.

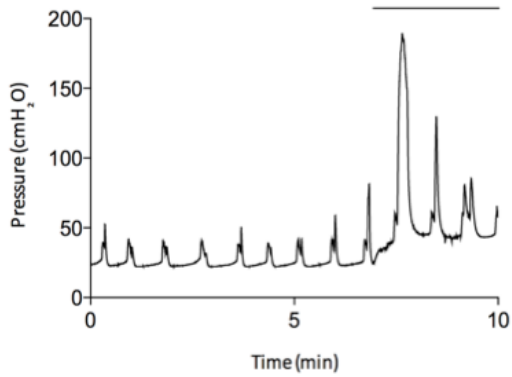
Concluding message

The bladder-cooling reflex in rats is a local phenomenon which is mediated by TRPA1. This work is a first step in assessing the “cold-activated TRP channels” in a rat model. Ultimately, TRPA1 could become an important therapeutic target to relieve symptoms in patients with NDO and OAB.

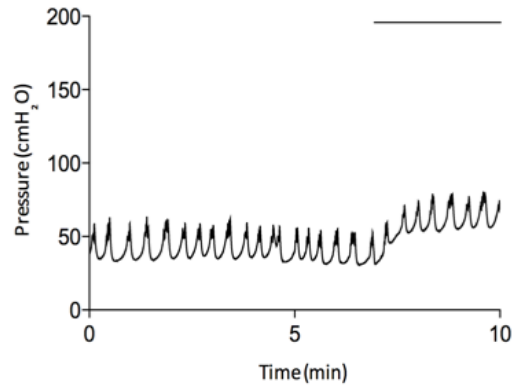


Cystometry with instillation of ice-cold saline in the bladder of urethane anaesthetized rats

20 minutes after injection of vehicle



20 minutes after injection of HC030031



Disclosures

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