

## THE ROLE OF TRPA1 IN THE BLADDER COOLING REFLEX IN A URETHANE ANAESTHETIZED AND SPINAL CORD INJURY RAT MODEL.

### Hypothesis / aims of study

Since decades, the bladder contraction upon instillation of ice-cold saline in the bladder has been used in the clinic as a diagnostic test for neurogenic detrusor overactivity (NDO). Similar bladder contractions have been shown in urethane anaesthetized cats, guinea pigs and rats with intact neuraxis (1). This bladder cooling reflex is also present in a significant percentage of patients with other urological conditions, such as overactive bladder (OAB) (2). It is hypothesized that this bladder cooling reflex is mediated via unmyelinated afferent C-fibers, which is different than the normal micturition reflex, which is controlled via the myelinated afferent A $\delta$ -fibers. The presence of the reflex could indicate emerging C-fiber function and could reflect modifications that occur in pathological states. The exact neuronal mechanism and the cellular origin and molecular nature of the cold sensors underlying this reflex are unknown.

The TRP superfamily represents a diverse group of ion channels which are regulated by a wide range of physical and chemical stimuli. A subset of the TRP superfamily exhibits particularly high sensitivity to changes in temperature. Expression of the cold-activated TRPA1 and TRPM8 has been reported in afferent neurons of the lower urinary tract (3).

The infliction of a spinal cord injury (SCI) in rats is well described and leads to NDO. We decided to test the bladder cooling reflex in urethane anaesthetized and in SCI rats. We hypothesized that TRPA1 plays a crucial role in the bladder cooling reflex. The long term purpose of this study will be to ameliorate the therapeutic arsenal to relieve patients with NDO and OAB.

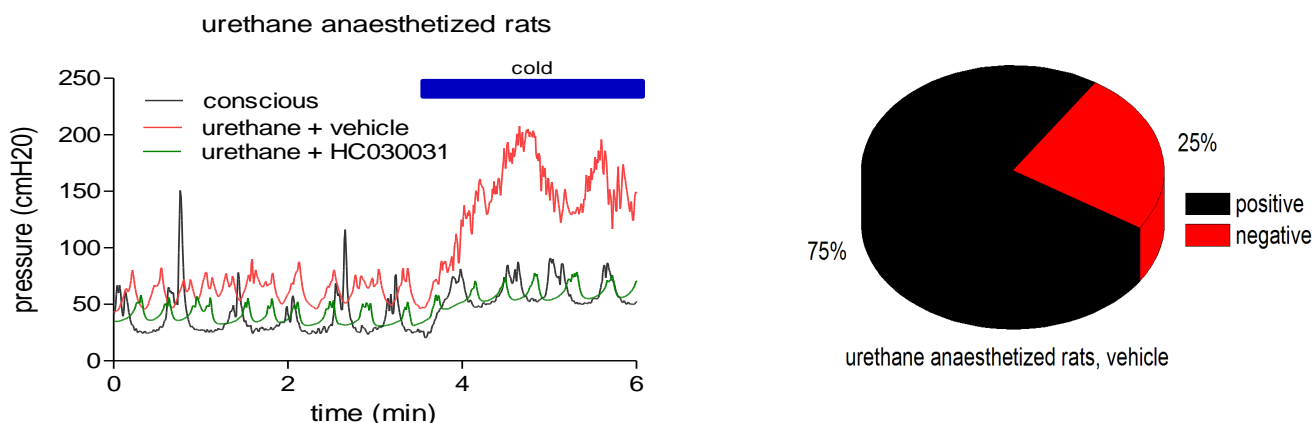
### Study design, materials and methods

All experiments were conducted in urethane anaesthetized rats (1g/kg, i.p.) and in conscious rats 3-4 weeks after SCI and sham SCI surgery.

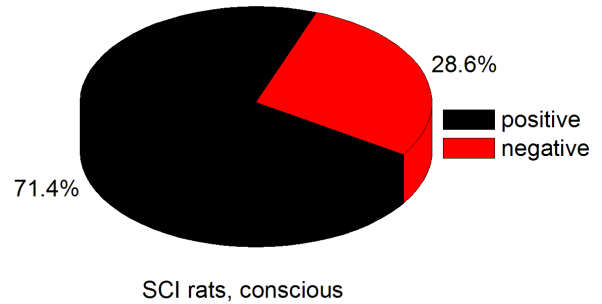
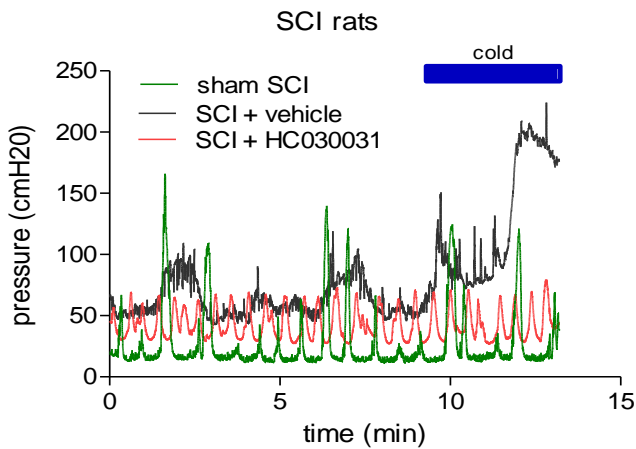
The functionality of TRPA1 was assessed *in vivo* by cystometry. Two TRPA1 blockers (HC 030031, 100mg/kg and TCS 5861528, 30 mg/kg) and the vehicle were administrated intraperitoneally 20 minutes before the cold stimulus. Calcium imaging on dorsal root ganglion neurons (L6-S1), urothelial cells and detrusor smooth muscle cells was used to assess the *in vitro* functionality and functional localization of TRPA1.

### Results

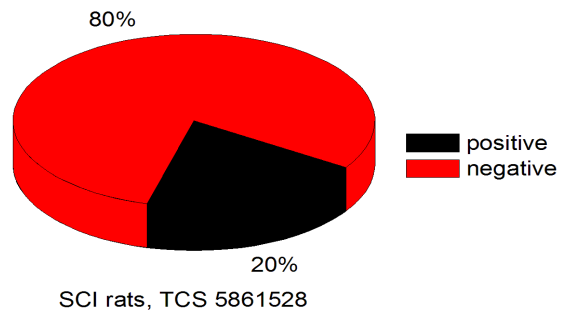
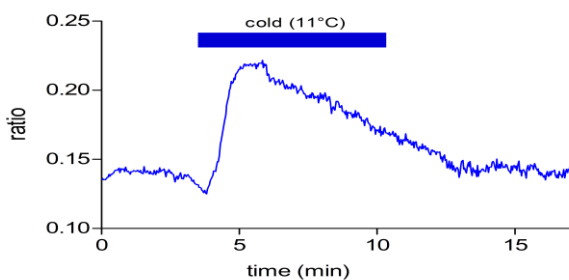
We were able to elicit the bladder cooling reflex in 75% of urethane anaesthetized rats after administration of the vehicle (n=4). The pharmacological inhibition of TRPA1 with HC 030031 (n=4) and TCS 5861528 (n=5) abolished the occurrence of the bladder cooling reflex in 100% of cases.



Then, we succeeded in provoking the bladder cooling reflex in 71,4% of spinal cord injury rats (n=7). This reflex was never present in sham operated rats (n=5). In SCI rats, we were able to inhibit the occurrence of the bladder cooling reflex in all cases with the use of HC 030031 (n=4) and in 4 out of 5 cases with the use of TCS 5861528 (n=5).



In calcium imaging experiments, we clearly saw an activation during cold stimulus of the urothelial cells of rat bladders 3-4 weeks after spinal cord injury.



#### Interpretation of results

We describe for the first time the prominent role of TRPA1 in the bladder cooling reflex in a urethane anaesthetized and a spinal cord injury rat model. The induction of the bladder cooling reflex in the rat is a powerful tool to dissect pathological OAB and NDO mechanisms. The characterization of this model will help in future work to modulate pharmacologically and by using gene therapy molecules of interest.

#### Concluding message

The bladder cooling reflex is, at least partially, mediated by TRPA1. TRPA1 could become an important therapeutic target to relieve patients with NDO and OAB. This work highlights the first step in the assessment of the “cold activated TRP channels” in a rat model.

#### References

1. Cheng, C.L., Chai, C.Y. & de Groat, W.C. Detrusor-sphincter dyssynergia induced by cold stimulation of the urinary bladder of rats. *Am J Physiol* 272, R1271-1282 (1997).
2. Mukerji, G., et al. Pain during ice water test distinguishes clinical bladder hypersensitivity from overactivity disorders. *BMC Urol* 6, 31 (2006).
3. Streng, T., et al. Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. *Eur Urol* 53, 391-399 (2008).

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<b>What were the subjects in the study?</b>	ANIMAL
<b>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</b>	Yes
<b>Name of ethics committee</b>	Animal Ethics Committee Catholic University of Leuven