

## STIMULATION OF COLD STRESS SKIN RECEPTOR: TRANSIENT RECEPTOR POTENTIAL MELASTATIN 8 (TRPM8) CAUSES SUDDEN URINARY FREQUENCY IN NORMAL CONSCIOUS RATS

### Hypothesis / aims of study

The mammalian transient receptor potential (TRP) channel family consists of 28 channels that can be subdivided into 5 different classes: TRPV (vanilloid), TRPC (canonical), TRPM (Melastatin), TRPML (Mucolipin), and TRPA (Ankyrin). TRPM8 could be activated by both menthol and thermal stimuli in the cool to cold temperature range (8-28 °C). In the urogenital tract, TRPM8 was first discovered in the prostate, where it is located on the cell membrane of the basal epithelial cells. Recently, it was reported that TRPM8 messenger RNA (mRNA) and protein could be detected in multiple genitourinary organs in human, such as prostate, testicle, scrotal skin, and bladder urothelium. TRPM8 immunostaining has been observed in human suburothelial nerve fibers, presumably in both A delta-fibers and C-fibers. In guinea pigs, TRPM8 has been detected in S1 dorsal root ganglia (DRG). TRPM8 expression studies in the rat showed the presence of TRPM8 not only in prostate but also in testis, penis, bladder and L6-S1 DRG tissue. However, epidermal expression of TRPM8 has yet to be demonstrated. We have previously shown that sudden drops of environmental temperature change micturition patterns in conscious rats, and that these changes are mediated, at least in part, through a resiniferatoxin-sensitive (C-fiber) nervous pathways involving stimulation of  $\alpha_1$  adrenergic receptors. The aim of the present study was to examine the expression of TRPM8 in the rat skin and to investigate if stimulation of skin TRPM8 receptors by menthol can affect detrusor activity in conscious rats.

### Study design, materials and methods

Immunofluorescence staining for TRPM8 was performed in samples of the skin from the leg and back of the rats. Continuous cystometry was performed in conscious animals. Micturition pressure (MP), voiding interval (VI), micturition volume (MV) and bladder capacity (BC) were evaluated before and after spraying menthol solution to the skin of the leg and back of the rats.

### Results

The TRPM8-antibody positive receptors could be visualized in the skin of the leg and back of the animals (Fig. 1A, B); they were strongly expressed within the cells of the epidermis. The expression of TRPM8-positive receptors did not differ between leg and back skin (leg:  $0.18 \pm 0.06$ ; back:  $0.20 \pm 0.07$  spots/ $\mu\text{m}^2$ ). Spraying the saline to the rat back and leg skin caused no significant change in these cystometric parameters ( $n=6$  in each group). In the rat leg group, there was no significant change in the cystometric parameters after spraying the 10% menthol solution to the rat leg skin ( $n=6$ ), however, the 50% and 99% menthol solution caused significantly decreased the VI, MV and BC respectively (Table 1). In the rat back group, there was no significant change in the cystometric parameters after spraying the 10% menthol solution to the rat back skin ( $n=6$ ), however, the 50% and 99% menthol solution caused significantly decreased the VI, MV and BC (Table 1). There were no significant change in VI, MV and BC between the leg and back groups before spraying the 50% menthol solution, however, there were significant changes in VI, MV and BC after spraying the 99% menthol solution (Table 1). The animals behaved normally and moved freely in the metabolic cage. No sign of skin inflammation, such as redness or erythema, was observed after the test, and we found no signs of inflammation at microscopy of the skin.

### Interpretation of results

The present study showed that spraying menthol solution to the skin of the leg and back of the rat significantly decreased the VI, MV and BC without changing MP. These results suggest that spraying menthol solution to rat skin can initiate the detrusor activity. Also, in our study, the TRPM8 receptors were visualized in the skin of the leg and back by immunohistochemistry. In the bladder, it was reported that menthol decreased the threshold for C-fiber activation by enhancing spontaneous activity. It may be speculated that TRPM8 regulates the threshold for initiating micturition reflex not only in the bladder. By a similar mechanism as exposure of the skin to cold, menthol, by stimulating TRPM8 receptors, may activate the micturition reflex. This may involve CNS regulation of the reflex, i.e. how the generated afferent impulses are handled at a central level (e.g., the pontine micturition center). However further studies are needed using "tools" such as TRPM8 KO mice, TRP antagonists, etc. in order to give further insights on how menthol applied to the skin causes stimulation of detrusor activity.

### Concluding message

TRPM8 is expressed in the skin of the rat leg and back. Applying menthol solution to rat skin induced detrusor activity, probably mediated by stimulation of dermal TRPM8 receptors.

Fig. 1. The TRPM8-antibody positive receptors could be visualized in the skin of the leg (A) and back (B), Red: TRPM8. Blue: nuclei. Green: nerve fiber.

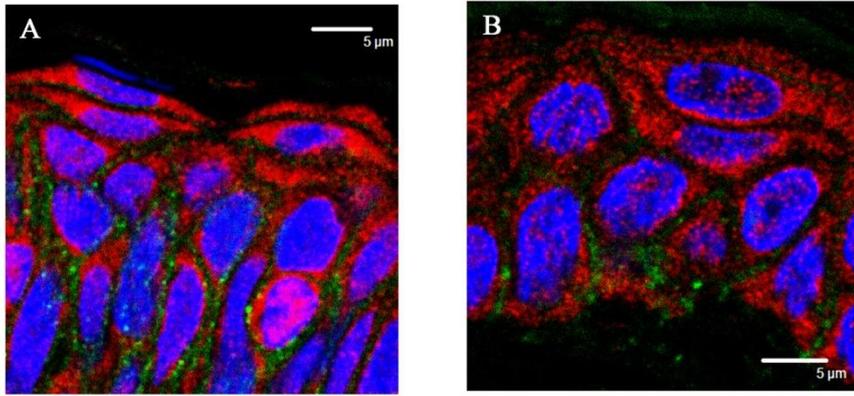


Table 1. Effects of spraying menthol solution to rat leg and back skin on cystometric parameters in conscious rats

		MP cmH20	VI min	MV ml	BC ml
leg vehicle (n=6)	before	50.58±3.54	4.93±0.45	0.83±0.08	0.82±0.07
	after	48.76±3.64	4.90±0.36	0.82±0.06	0.82±0.06
leg mentol (n=6)	before	53.56±2.03	5.39±0.30	0.89±0.10	0.90±0.09
	10%menthol	51.16±3.21	3.79±0.57	0.63±0.11	0.63±0.11
	before	60.05±1.53	5.03±0.30	0.84±0.05	0.84±0.05
	50%menthol	58.62±2.67	2.59±0.34*	0.44±0.06*	0.43±0.06*
	before	54.31±3.78	5.37±0.44	0.89±0.07	0.89±0.07
	99%menthol	52.53±5.47	2.39±0.29**	0.39±0.05**	0.40±0.05**
back vehicle (n=6)	before	54.36±3.50	4.99±0.59	0.83±0.10	0.83±0.10
	after	52.49±3.68	5.37±0.43	0.89±0.07	0.90±0.07
back mentol (n=6)	before	54.58±2.79	5.97±0.24	0.99±0.04	1.00±0.04
	10%menthol	50.54±3.14	5.53±0.26	0.92±0.04	0.92±0.04
	before	55.69±1.68	5.87±0.51	0.97±0.08	0.98±0.08
	50%menthol	54.93±1.60	3.59±0.45*	0.60±0.07*	0.60±0.08*
	before	59.78±3.43	5.25±0.52	0.87±0.09	0.88±0.09
	99%menthol	60.31±2.70	3.28±0.18*†	0.55±0.03*†	0.55±0.03*†

BP: basal pressure; MP: micturition pressure; VI: voiding interval; MV: micturition volume; BC: bladder capacity; RV = (infusion volume rate x VI)-MV; BC = infusion volume rate x VI

\* P<0.05; \*\* P<0.01 student's paired t-test

comparison the effect of before and after spraying menthol solution to rat leg and back skin

† P<0.05 one-way factorial ANOVA followed by Scheffe's F-test

comparisons between leg saline and back saline, and leg mentol and back mentol group

<b>Specify source of funding or grant</b>	<b>none</b>
<b>Is this a clinical trial?</b>	<b>No</b>
<b>What were the subjects in the study?</b>	<b>ANIMAL</b>
<b>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</b>	<b>Yes</b>
<b>Name of ethics committee</b>	<b>The Animal Ethics Committee of Shinshu University School of Medicine</b>