

Concentration of menthol	%inhibition of CCh induced contraction	EFFECTS OF MENTHOL ON MICTURITION IN
0.1mM	11.92±1.17%* (n=5)	
0.3mM	41.22±3.18%* (n=5)	
1mM	97.32±0.28%* (n=5)	

## RATS WITH SPINAL CORD INJURY

### Hypothesis / aims of study

Menthol is known to activate cold receptor TRPM8. TRPM8 is detected in bladder and dorsal root ganglion in human and rat [1]. On the other hand, menthol has been showed to relax isolated gastrointestinal and bronchial smooth muscle [2]. The present study was conducted to investigate the effects of menthol on the micturition reflex and on the contractility of detrusor smooth muscle in spinal cord injured rats.

### Study design, materials and methods

Female Sprague-Dawley rats were used. Spinal cord was completely transected at T8 level under isoflurane anesthesia. The bladders were expressed manually three or four times daily. The experiments were performed 3 to 5 weeks after transection. *In vitro* : Detrusor strips were placed in organ baths containing Krebs-Ringer solution. The effects of menthol (0.1mM-1mM) on the contractile response to carbachol (CCh) (0.01mM) were determined. The effect of menthol (0.3mM) was also determined in the presense or absence of calcium-free Krebs solution with nifedipine 0.01µM. In another set of experiment, strips were contracted with 40mM KCl. After the contraction induced by KCl reached plateau, menthol (0.03mM-1mM) was applied cumulatively. *In vivo* : A polyethylene catheter (PE-50) was implanted into the bladder under isoflurane anesthesia. After the animals recovered from anesthesia, cystmetric investigations were performed. Saline was infused into the bladder at a rate of 12ml/h. The temperature of infused saline was maintained at 36-37°C. When three reproducible micturition cycles were recorded, saline containing 1mM or 3mM menthol was infused intravesically for 60min. The following cystmetric parameters were investigated: micturition pressure, basal pressure, threshold pressure, voided volume, residual volume and bladder capacity. The results are given as mean±S.E. Data were analyzed using student's paired *t*-test and Dunnett's *t*-test, and P<0.05 was considered statistically significant.

### Results

*In vitro* : Menthol (0.1mM-1mM) caused a concentration-dependent inhibition of the contractions induced by CCh [Table 1]. The contractile response to CCh in calcium-free Krebs solution with nifedipine in the absence or presence of menthol (0.3mM) was inhibited by 74.9± 6.0%(n=4), 94.1±1.31%(n=4) compared to control response in normal Krebs solution, respectively. Menthol (0.03mM-1mM) caused a concentration-dependent relaxation of the strips contracted with 40mM KCl. *In vivo* : Intravesical infusion of 1mM and 3mM menthol increased bladder capacity and residual volume, and decreased voided volume. Menthol did not change micturition pressure, basal pressure and threshold pressure [Table 2].

### Interpretation of results

Menthol inhibited the contraction induced by CCh and relaxed KCl precontracted preparation. The inhibitory effect of menthol on CCh induced contraction was also observed in calcium-free Krebs solution with nifedipine. These data indicate that menthol inhibits smooth muscle contraction due to inhibition of calcium influx and the intracellular calcium movement. Intravesical infusion of 1mM and 3mM menthol increased bladder capacity and residual volume, and decreased voided volume. It is likely that the effects were due to the inhibition of smooth muscle contractility by menthol. On the other hand, menthol did not decrease micturition pressure. This result suggests that there is some possibility that intravesical infusion of menthol induces detrusor-sphincter dyssynergia.

### Concluding message

Menthol inhibited rat detrusor smooth muscle contraction through the inhibition of calcium influx and the modulation of intracellular calcium movement. Intravesical infusion of menthol is likely to induce bladder relaxation and detrusor-sphincter dyssynergia.

### Referenses

1. J.Urol. 172, 1175-1178, 2004
2. Br. J. pharmacol. 121, 1645-1650, 1997

**Table 1** Effects of menthol on contractions induced by 0.01mM CCh

\*p<0.05 significance of difference from the value in the absence of menthol

**Table 2** Effects of intravesical administration of 1mM menthol (n = 6) and 3mM menthol (n=6) on cystmetric parameters in spinal cord injured rats.

M.P.	T.P.	B.P.	V.V.	R.V.	B.C.
------	------	------	------	------	------

<b>Menthol (1mM)</b>						
before	39.9±2.89	10.3±0.94	3.33±0.32	0.48±0.06	0.37±0.08	0.85±0.11
after	43.0±1.42	9.1±1.25	3.86±0.43	0.25±0.03*	0.88±0.13*	1.13±0.14*
<b>Menthol (3mM)</b>						
before	43.1±1.78	15.0±1.53	3.19±0.45	0.50±0.04	0.30±0.05	0.80±0.05
after	46.3±2.71	14.8±1.88	3.57±0.46	0.36±0.05*	0.74±0.08*	1.10±0.06*

**M.P.** : Micturition Pressure (cmH<sub>2</sub>O) **T.P.** : Threshold Pressure (cmH<sub>2</sub>O) **B.P.** : Basal Pressure (cmH<sub>2</sub>O) **V.V.** : Voided Volume (ml) **R.V.** : Residual Volume (ml) **B.C.** : Bladder Capacity (ml) \*p<0.05

**FUNDING:** Grant-in-aid for Scientific Research Japan

**DISCLOSURES:** NONE

**ANIMAL SUBJECTS:** This study followed the guidelines for care and use of laboratory animals and was approved by Animal Ethics Committee, Kagoshima University