

EFFECT OF SKIN TRANSIENT RECEPTOR POTENTIAL MELASTATIN 8 (TRPM8) CHANNEL STIMULATION ON C-FOS EXPRESSION IN DORSAL HORN OF L6 SPINAL CORD

Hypothesis / aims of study

Menthol is an agonist of the transient receptor potential melastatin 8 (TRPM8) channel. It is reported that applying menthol onto back skin elicits detrusor overactivity and urinary frequency in conscious rat (1). However, the precise mechanism of menthol-induced detrusor overactivity is still unknown. C-fos expression is thought to be activated transsynaptically by peripheral stimuli in the central nerve system (CNS). Noxious stimulation of the bladder elicited c-fos expression in dorsal horn of L6 spinal cord innervating the bladder (2). In the current study, we investigated the effect of skin application of menthol on CNS by examining c-fos expression in dorsal horn of L6 spinal cord in rat model.

Study design, materials and methods

Female Sprague-Dawley rats, weighing 250-270g, were used in this study. Three days prior to the experiment, hip area was shaved by an electric hair clipper. At 3 days after shaving, these rats were randomly separated into two groups; menthol- (n=4) and saline-treatment (n=4) group. Following, 90 % of menthol solution or saline was applied onto the shaved area by spraying every 30 min for 2 hrs at room temperature (27±2°C). Just after the treatment, L6 spinal cord were removed, and then expression levels of c-fos mRNA and protein were evaluated by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry.

Results

Expression level of c-fos mRNA in L6 spinal cord in menthol-treated rats was significantly elevated compared to saline-treated rats (P=0.01) (Fig.1). In immunohistochemistry, the number of c-fos positive cell in dorsal horn of L6 spinal cord was significantly greater in menthol-treated rats than in saline-treated rats (P<0.01) (Fig.2) .

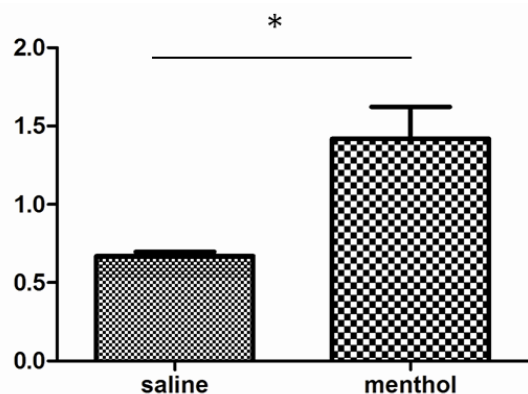
Interpretation of results

Previously, our group reported that menthol application to the back and/or leg skin induced detrusor overactivity and urinary frequency. We confirmed that TRPM8 channels were expressed in the areas by immunohistochemical analysis. In addition, those effects were antagonized by BCTC, the TRPM8 channel antagonist (1). The results of the current study suggest that skin TRPM8 channels stimulated by menthol related with menthol-induced detrusor overactivity, which results from activations of the neuron in dorsal horn of L6 spinal cord.

Concluding message

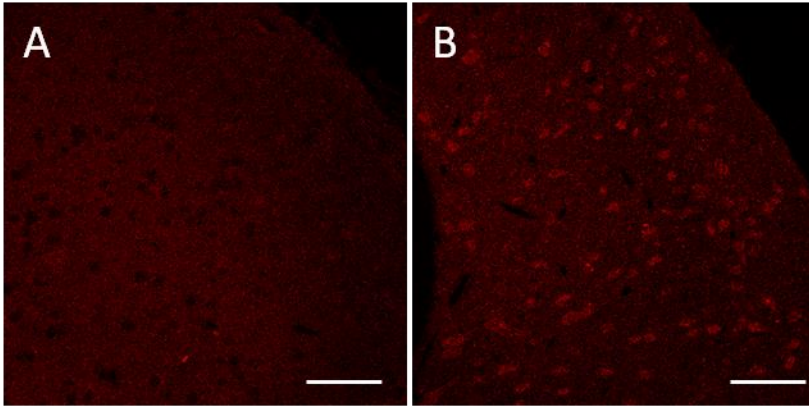
This study showed that skin TRPM 8 channels stimulated with menthol spray elevated c-fos expression levels in the dorsal horn of L6 spinal cord. Therefore, menthol-induced detrusor overactivity was composed of the activation of central nerve pathways through TRPM 8 channels.

Fig.1 RT-PCR



Expression of c-fos mRNA in L6 spinal cord after saline or menthol application onto hip area (*:P0.01).

Fig.2 Immunohistochemistry



Immunohistochemical staining of c-fos in dorsal horn of L6 spinal cord after saline (A) or menthol (B) application onto hip area. Scale bar: 50 micrometer.

References

1. Chen et al., Stimulation of skin menthol receptors stimulates detrusor activity in conscious rats: *NeuroUrol Urodyn.* 29,506-511,2010.
2. Yokoyama et al., Effects of herpes simplex virus vector-mediated enkephalin gene therapy on bladder overactivity and nociception: *Hum Gene Ther.* 24,170-180,2013.

Disclosures

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