

INVESTIGATION OF THE ROLE OF TRPM8 CHANNELS IN MODULATING CONTRACTION OF PIG URINARY BLADDER.

Hypothesis / aims of study

Transient receptor potential (TRP) channels have been shown to play an important role in the modulation of bladder sensation and afferent nerve activity. Recently a novel receptor, TRP melastatin 8 (TRPM8), activated by menthol, icilin and cool temperatures (8-28°C) has been identified in human bladders (1). The physiological and pathophysiological role of these channels remains unclear and few studies have investigated their function in mediating detrusor contraction (2). The aim of this study was to study the localisation of TRPM8 channels in pig urinary bladder and investigate the effect of TRPM8 channel agonists, menthol and icilin, on strip and whole pig bladder contraction.

Study design, materials and methods

Formalin-fixed paraffin-embedded bladder tissue was analysed using immunohistochemical staining for TRPM8. For whole organ experiments, the bladder and the associated vasculature were excised and maintained under controlled physiological conditions, perfused with Krebs buffer. The effect of intravesical (IVE) or intravascular (IVA) administration of menthol (0.1 and 0.3mM) or icilin (50µM) on carbachol-(CCH; 10µM) induced whole bladder contractions was monitored by recording the IVE pressure (cmH₂O). For isolated strip experiments, longitudinal strips of denuded detrusor or mucosa were mounted in Perspex microbaths, superfused with Krebs solution and maintained at 37°C. Strips were exposed to 10µM CCH and once contractions had stabilised, increasing concentrations of menthol (0.1-0.3mM), 50µM icilin or vehicle were applied to the strips 10min before application of CCH. All data are expressed as the mean±SEM. Statistical analysis was carried out by using repeated measure ANOVA followed by Dunett's post hoc test.

Results

Expression of TRPM8 was detected in urothelial and smooth muscle cells of pig bladders. In the whole bladder, IVA administration of 0.3mM menthol significantly ($p<0.05$) decreased the magnitude of CCH induced bladder contraction as evidenced by reduced IVE pressure rises (Fig 1A), whereas IVE administration of menthol (0.3mM) significantly increased ($p<0.05$) the response to CCH (Fig 1B). Similar results were observed with IVA and IVE administration of 50µM icilin (Fig 2). In detrusor and mucosal strips, both menthol (0.3mM) and icilin (50µM) markedly ($p<0.05-0.001$) inhibited the CCH-induced contractions. The vehicle control had no effect on bladder contractions at all concentrations

Interpretation of results

TRPM8 channels appear to have the potential to modulate pig bladder contractility. Functional activation of TRPM8 channels in the whole organ resulted in different contractile responses depending on the route of menthol and icilin administration (IVE vs. IVA). However, consistent inhibitory responses were seen when strips of tissue (muscle & mucosa) were used. The differing smooth muscle responses seen with various routes of TRPM8 agonist administration may reflect regionalisation of mechanisms in different layers of the bladder wall.

Concluding message

TRPM8 channels seem to be expressed in pig urinary bladder and appear to play an important role in modulation of pig bladder contractility.

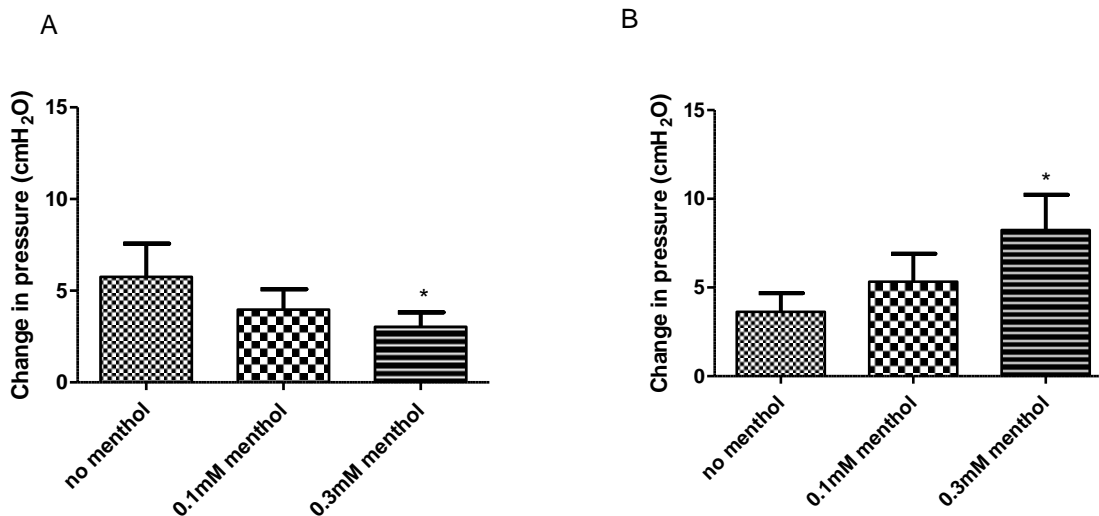


Figure 1) The effect of (A) IVA and (B) IVE menthol (0.1mM and 0.3mM) on CCH-induced (10 μ M) contractions of whole pig urinary bladder. IVA menthol (0.3mM) (N=5) significantly decreased the CCH-induced whole bladder contractions whilst IVE menthol (0.3mM) (N=5) significantly enhanced the CCH-induced contractions. *p<0.05 versus paired response with menthol omitted. Data is presented as mean \pm S.E.M.

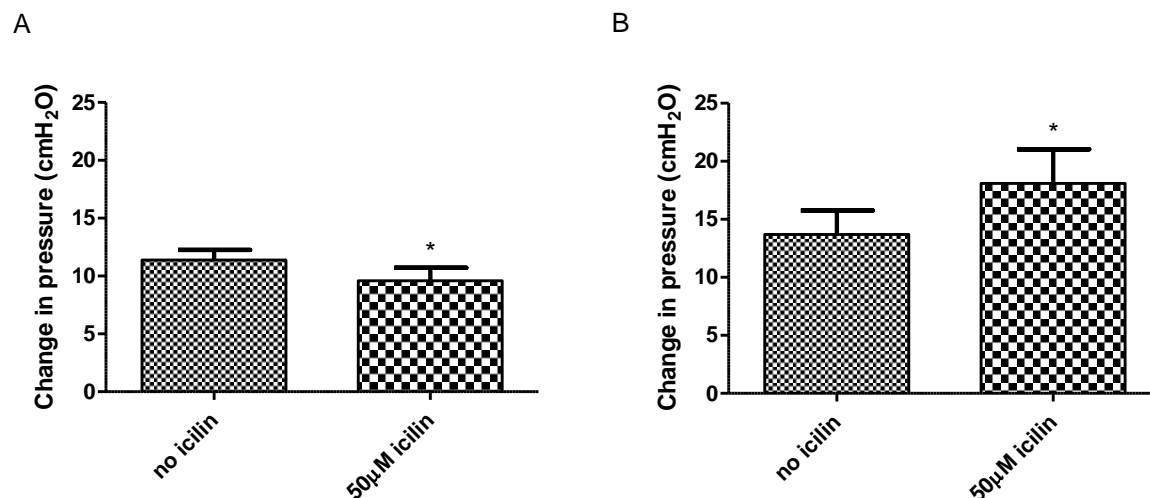


Figure 2) The effect of (A) IVA and (B) IVE icilin (50 μ M) on carbachol-induced (10 μ M) contractions of whole pig urinary bladder. IVA icilin (N=4) significantly decreased CCH-induced whole bladder contractions whilst IVE icilin (N=3) significantly enhanced CCH-induced contractions. *p<0.05 versus paired response with icilin omitted. Data is presented as mean \pm S.E.M

References

- 1) Stein RJ, Santos S, Nagatomi J, Hayashi Y, Minnery BS, Xavier M, Patel AS, Nelso JB, Futrell WJ, Yoshimura N, Chancellor MB, Miguel F. Cool (TRPM8) and hot (TRPV1) receptors in the bladder and male genital tract. J Urol 2004;172: 1175-1178
- 2) Nomoto Y, Yoshida A, Ikeda S, Kamikawa Y, Harada K, Ohwatashi A, Kawahira K. Effect of menthol on detrusor smooth muscle contraction and micturition reflex in rats. Urology 2008;72:701-705

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Is this a clinical trial?	No
What were the subjects in the study?	ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	Yes
Name of ethics committee	No ethical approval was needed as pig bladders were obtained from the local abattoir.