

INVESTIGATION OF THE ROLE OF TRPM8 CHANNELS IN MEDIATING MUCOSAL AND DETRUSOR CONTRACTION IN PIG URINARY BLADDER

Hypothesis / aims of study

Transient receptor potential (TRP) ion channels have been shown to play an important role in the mediation of bladder sensation and afferent nerve activity. Recently a novel temperature sensitive receptor, transient receptor potential melastatin (TRPM8), activated by menthol and cool temperatures (8°C to 28°C) has been identified in a number of human genitourinary tract tissues, including urinary bladder (1). The role of these channels in the physiology and pathophysiology of the bladder is unclear and only a few studies have investigated their functional role in mediating detrusor contraction (2). The aim of this study was to investigate the effect of menthol, on detrusor and mucosal contractions in pig urinary bladder.

Study design, materials and methods

Fresh female pig bladders were obtained from the local abattoir. The mucosa and the connective tissue were carefully separated from the detrusor. Longitudinal strips of detrusor (denuded) or mucosa were cut and mounted in Perspex microbaths (volume 0.2ml). The strips were superfused with carboxygenated Krebs solution at a constant rate of $1.8\text{ml} \pm 0.3\text{ml}\cdot\text{min}^{-1}$ via a peristaltic pump. The circuit passed through a heating bath, which maintained the strips at a temperature of $36 \pm 0.5^\circ\text{C}$. The strips were equilibrated under a resting tension of $1.0 \pm 0.5\text{g}$ for 60 min. Mechanical responses were recorded using an isometric force transducer. After equilibration, strips were exposed to $10\mu\text{M}$ carbachol at 20-min intervals, and washed between treatments. Once contractions induced by carbachol had stabilized, 0.1mM , 0.3mM or 1mM menthol or vehicle controls (0.05%, 0.1% and 0.5% ethanol) was administered. Strips were left to incubate for 11 min before a further carbachol response was elicited. Results are expressed as grams of tension per mg tissue. All data are expressed as mean \pm SEM. Statistical analysis was carried out using a repeated measure ANOVA followed by Dunnett's post hoc test using GraphPad Prism version 5.00.

Results

Contractile responses to carbachol of pig bladder detrusor strips ($0.25 \pm 0.07\text{ g tension/mg tissue}$) were significantly ($p < 0.01$) greater than mucosal strips ($0.023 \pm 0.007\text{ g tension/mg tissue}$). Menthol (0.3mM and 1mM) significantly inhibited the carbachol-induced contractions of both detrusor and mucosal strips (Fig 1 & 2 respectively). Ethanol which was used as a vehicle in these experiments had no effect on detrusor or mucosal contractions at all concentrations.

Interpretation of results

Both mucosal and detrusor responses to muscarinic receptor stimulation by carbachol were inhibited by menthol, demonstrating a role of TRPM8 channels in modulation of pig bladder contractility. Since TRPM8 expression has not been reported in detrusor muscle, it is speculated that menthol might inhibit the calcium influx in detrusor smooth muscle through L-type calcium channels (1 & 2). However, the possibility that urothelial TRPM8 is involved in mediating the effect of menthol in the mucosa cannot be excluded.

Concluding message

This study further confirms the contractile properties of pig bladder mucosa (3). TRPM8 channels appear to play an important role in modulation of smooth muscle and mucosal contractility in pig urinary bladder. The ability of TRPM8 channels to block contraction suggests it may be a useful target in the treatment of overactive bladder disorders.

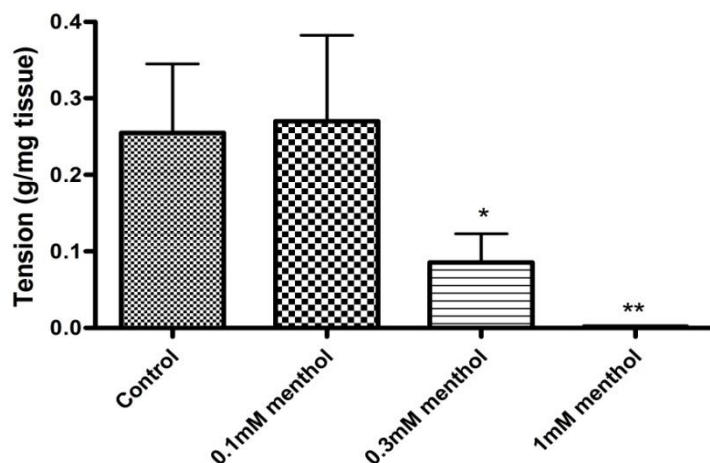


Fig 1. Effects of 0.1mM-1mM menthol on contractions induced by $10\mu\text{M}$ carbachol on pig detrusor strips. Data presented as mean \pm SEM (n=16 strips). * $p < 0.05$ & ** $p < 0.01$.

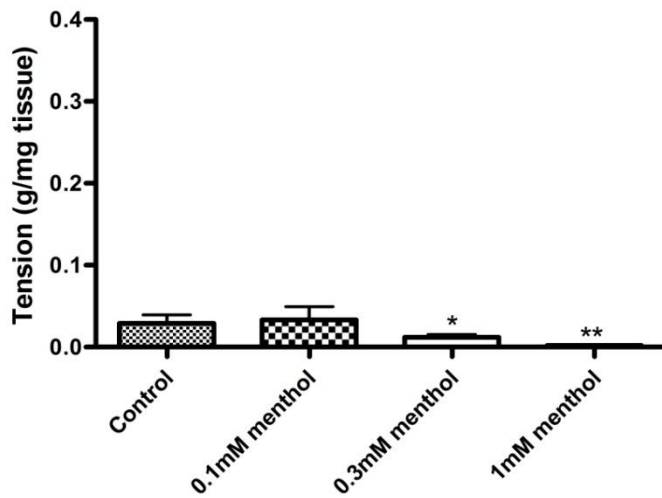


Fig 2. Effects of 0.1mM-1mM menthol on contractions induced by 10µM carbachol on pig mucosal strips. Data presented as mean±SEM (n=12 strips). *p<0.05 & **p<0.01.

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
3. Sadananda P, Chess-Williams R, Burcher E. Contractile properties of the pig bladder mucosa in response to neurokinin A: A role for myofibroblasts? Br J Pharmacol 2008; 153:1465-1473

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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?	No
Statement that no ethical approval was needed	Pig bladders were obtained from the local abattoir.