

VANILLOIDS DO NOT ACT ON THE AFFERENT NERVE FIBERS ALONE: PRELIMINARY CALCIUM IMAGING DATA FROM THE SUBUROTHELIAL INTERSTITIAL CELLS OF THE BLADDER

Hypothesis / aims of study

The effect of intravesical instillation of vanilloids in detrusor overactivity is mediated by the vanilloid receptor TRPV-1, which is located on unmyelinated afferent C-fibres and on urothelial cells.(1)

In the bladder, two networks of interstitial cells of the bladder (ICB) exist, one beneath the urothelium, another in between the detrusor smooth muscle cells. Both networks of interstitial cells express the vanilloid receptor (2) and they could be involved in ATP signalling from the urothelium to the suburothelial space.(3) We hypothesize that the bladder response to vanilloid application is mediated at least partially through the interstitial cells of the bladder. Using fluorescent Calcium indicators, we examined the role of the suburothelial network of ICB in the response to application of vanilloids.

Study design, materials and methods

We used freshly isolated bladder tissue from female Whistar rats in accordance with local ethical regulations. The bladder was opened longitudinally and the suburothelial network of ICB was exposed by careful microdissection of the urothelium. The tissue was loaded with the fluorophore Fluo-4 AM (Molecular Probes) then washed in Krebs' solution. Confocal laser scanning microscopy was used to study fluorescence emission from the calcium-sensitive dye, with a 488nm excitation line on a Bio-Rad MRC 1024 confocal microscope. Measurements were analysed with standard software for Windows (Scion Image and Microsoft Excell). Intracellular calcium oscillations were expressed at a rate of F/F_0 of the fluorescence generated by an event (F) against the baseline (F_0)

The response to application of the vanilloid agonist RTX (1 μ M in 1% ethanol) and to vehicle was calculated and compared by a t-test. The effect of the vanilloid receptor antagonist capsazepin was studied.

Results

Calcium imaging allows us to morphologically identify the suburothelial network of interstitial cells in a microdissected tissue preparation.

In the absence of stimulation, there are no spontaneous fluctuations in fluorescence. Application of the vanilloid agonist RTX elicits a significant mean increase in fluorescence of 92% in interstitial cells in 7 different preparations. After stimulation, the mean fluorescence returns to baseline.

Repetitive stimulation of the ICB did not alter their responsiveness to application of RTX.

Application of the vehicle did not show significant changes in fluorescence.

Preincubation with the vanilloid antagonist capsazepin completely blocked the response of the interstitial cells to application of RTX.

Interpretation of results

These preliminary experiments show that application of RTX elicits an activation of the suburothelial network of interstitial cells of the bladder. The effects are mediated through an activation of the vanilloid receptor TRPV-1 which can be blocked by a vanilloid antagonist. Repetitive stimulation did not seem to alter the excitability of the suburothelial ICB.

Concluding message

These data suggest that the acute effects of intravesical application of vanilloids could be mediated not only through the afferent innervation of the suburothelium, but also partially through the suburothelial interstitial cells of the bladder. Further experiments are ongoing to analyse the response of the ICB to different vanilloid receptor agonists and antagonists.

References

- (1) Intravesical treatment of overactive bladder. Urology 2000 vol 55 suppl 5A: 60-64
- (2) Topography of the vanilloid receptor in the human bladder: more than just the nerve fibers. J Urol 2002 vol 168 (1): 293-297
- (3) Electrical characteristics of suburothelial cells isolated from the human bladder. J Urol 2004 vol 171 (2 Pt 1): 938-943