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ACTIVATION OF THE SUBUROTHELIAL INTERSTITIAL CELLS BY VANILLOIDS: PRELIMINARY CALCIUM IMAGING DATA FROM RAT BLADDERS

Hypothesis / aims of study

The effect of vanilloid application in detrusor overactivity is mediated by the vanilloid receptor TRPV-1, 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 [2], another in between the detrusor smooth muscle cells [3]. ICB's are immunoreactive for TRPV-1 [4] and they could be involved in ATP signalling from the urothelium to the afferent nerve endings [5].
We hypothesized that the response to vanilloids is also mediated through the interstitial cells of the bladder. Using fluorescent Calcium indicators, we examined the response of suburothelial ICB to the application of vanilloid agonists and antagonists.

Study design, materials and methods

Freshly isolated bladder tissue from female Whistar rats was used in accordance with local ethical regulations. The suburothelial network of ICB was exposed by microdissection of the urothelium. Calcium imaging was performed using the calcium indicator Fluo-4 AM. A BioRad MRC 1024 confocal laser scanning microscope with a 488nm excitation line was used to study fluorescence emission from the calcium-sensitive dye after application of the vanilloid receptor agonist resiniferatoxin (1 µM RTX in 1% ethanol) and of the vehicle. Application of RTX was repeated after incubation with the vanilloid receptor antagonist capsazepin.

Results

Calcium imaging allowed us to morphologically identify interstitial cells in the bladder suburothelium.
In the absence of stimulation, there were no spontaneous fluctuations in fluorescence. Application of RTX elicits a highly significant mean increase in fluorescence of 92% in the interstitial cells in bladder preparations from 5 different rats. After stimulation, the mean fluorescence returned to baseline. Application of the vehicle did not show significant changes in fluorescence compared to baseline.
Repetitive stimulation of the ICB did not alter their responsiveness to application of RTX.
After incubating the bladder preparations with capsazepin for 15', RTX in concentrations up to 100 µM could no longer elicit an increase in fluorescence in the ICB.

Interpretation of results

These preliminary experiments show that application of RTX elicits a rise in intracellular calcium in the ICB, thus activating the suburothelial interstitial cells. This response could be blocked by preincubation of the tissue with the vanilloid receptor antagonist capsazepin.
This suggests that the acute response of the bladder to vanilloid application is not only mediated by afferent nerve endings but at least partially by the suburothelial interstitial cells of the bladder.

Concluding message

Suburothelial interstitial cells are involved in the acute response to application of resiniferatoxin in the rat bladder.
Further experiments are ongoing to analyse the response of the ICB to different vanilloid receptor agonists and antagonists and to elucidate the role of the interstitial cells of the bladder in bladder physiology and pathophysiology.

References

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