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IMMUNOHISTOCHEMICAL EVIDENCE OF TRPV1 IN THE NORMAL HUMAN UROTHELIUM.

Hypothesis / aims of study

The vanilloid receptor type 1 (VR_1), a non-selective cation channel also known, according a new nomenclature, as transient receptor potential vanilloid 1 (TRPV1), is expressed in a peptide-containing sub-population of primary sensory nerves of the rat and human urinary bladder which are involved in the regulation of micturition reflexes. In humans, the $TRPV_1$ has been detected in the sensory nerve endings, in some of the cells present in the sub-urothelium and in the smooth muscle cells but not in the urothelium. In the rat, the presence of the TRPV1 has been showed in the urothelial cells by Birder but Avelino claimed that rat urothelium is devoid of TRPV1 [(1, 2)].

The aim of this study is to identify, by immunohistochemistry, the cell types expressing VR_1 in the human urinary bladder with special attention to the urothelium, in order to gain a better understanding of the role of this receptor in the regulation of the lower urinary tract.

Study design, materials and methods

Specimens were obtained from normal human urinary bladder by two different techniques: by multiple cold cup biopsy during cystoscopy or by cold blame cut during open surgery . Macroscopic examination of the bladder mucosa did not show any pathology at time of biopsy.

Eight series were processed for light microscope immunohistochemistry and two for fluorescence microscope immunohistochemistry. Then the sections were incubated with the primary antibodies. Three antibodies were used: the rabbit polyclonal antibody raised against capsaicin receptor (vanilloid receptor VR1, C-terminus, Chemicon International, Temecula, CA, USA), the Vanilloid Receptor, VR1 (N-15) goat polyclonal antibody (Santa Cruz Biotechnology, CA, USA) and the Vanilloid Receptor, VR1 (C-15) goat polyclonal antibody (Santa Cruz Biotechnology, CA, USA). Fluorescence microscope immunohistochemistry was also performed in some series of samples. Hematoxylin-eosin staining was also performed for all the specimens. Two non-blinded histologists reviewed the immunohistochemical preparations and two blinded independent pathologists saw the hematoxylin-eosin staining in order to confirm the diagnosis of normal urothelium.

Results

Hematoxylin-eosin staining confirmed that all the specimens were normal and no dysplasia, or tumoral cells were recorded. In one patient a moderate inflammation, characterized by the presence in the urothelium and sub-urothelium of migrating and immune cells, was detected.

Under both light and fluorescence microscopes, several cell types appeared to be labelled. Either the stained cell type or the sub-cellular distribution of the immunolabelling were similar for all of the three antibodies used and no staining was observed when the primary antibodies were omitted or the epitope pretreatment was performed. The endothelium of capillaries and arterioles was more frequently labelled with the anti-capsaicin antibody than with the anti-VR1 antibodies. The endothelium of veins and of lymphatics was always negative. In the sub-urothelium and among muscle bundles, mast cells with intensely stained granules were always detected. In the patient with a moderate inflammation, several cells, as neutrophils, were also labelled.

Interpretation of results

In the present study we found that in the human urinary bladder several cell types expressed VR_1 or TRPV1 according the new nomenclature. In particular, a VR_1-positivity was seen in the

urothelium, which is a finding not yet reported for humans, and in other structures, such as nerve fibers, mast-cells, smooth muscle cells and endothelium, that were already found to express this receptor in man and/or in the rat. The present results demonstrate that VR_1-positivity is detectable in other cells types besides the nerve tissue (i.e. smooth muscle cells, mast cells, endothelium). This finding is not surprising since recent experimental evidences and clinical hypotheses suggested that the concept of VR_1 as a "selective neuronal sensory-receptor" could no longer be maintained. Although we found differences in the staining intensity among the antibodies, no differences in the cell distribution of the labelling were seen. The negative results we obtained by omitting the primary antibody and by the pretreatment with the epitopes should give a further warrant for the specificity of the labeling and its distribution. The presence of TRPV1 at the level of urothelium might have noticeable functional implications in the regulation of the lower urinary tract and be the target of pharmacological therapies. On the basis of our results we could speculate that the urothelial TRPV1 might represent the main target of intravesical vanilloids we hypothesize that these cells might assume in humans a sensory role which allows them to mediate and/or amplify the vanilloids' effects.

Concluding message

For our knowledge, the present findings represent the first evidence of the presence of VR_1 on normal human urothelium where it could have important implications in the mechanism of action of intravesical vanilloid (capsaicin and resiniferatoxin) therapy. Furthermore, its possible localization on mitochondria might play a role in the cell growth.

References

1. Avelino A, Cruz C, Nagy I and Cruz F. Vanilloid receptor 1 expression in the rat urinary tract. Neurosci **109**: 787, 2002
2. Birder LA, Kanai AJ, de Groat WC, Kiss S, Nealen ML, Burke NE et al. Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. Proc Natl Acad Sci USA **98**: 13396, 2001