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TRPV4 DETECTS BLADDER STRETCH. MOLECULAR MECHANISMS OF TRPV4 ACTIVATION THROUGH A CONNECTION WITH ADHERENCE JUNCTIONS IN THE UROTHELIUM.

Hypothesis / aims of study

Transient receptor potential vanilloid receptor 4 (TRPV4) is a cation channel that is activated by physical stimuli like 'stretch'. It is found in different circumventricular organs in the body. Much of the localization of TRPV4 in the human bladder is unknown, but expression of TRPV4 in the urothelium of the human urinary bladder has already been reported. This may imply that in the urinary bladder, TRPV4 has a sensory role in detecting changes in 'stretch' in the bladder and therefore might also be involved in the sensory disregulation that is seen in diseases like OAB and PBS. Recent experiments detected a colocalization between TRPV4 and adherence junctions (AJ's) in the urothelium of the bladder and the kidney. AJ's are rigid junctions that connect urothelial cells together. The intracellular component of AJ's is connected to the actin cytoskeleton. This complex is able to equally divide mechanical forces that originate during bladder filling. When actin-freezing agents are applied, TRPV4 responses are reduced[1]. This implies that TRPV4 needs an interaction with the actin cytoskeleton and AJ's to detect stretch. AJ's have different proteins. The extracellular complex is formed by E-cadherin proteins and the intracellular complex is formed by β-, y- and α-catenins. The latter is connected to the actin cytoskeleton. The aim of this study is to investigate the connection between TRPV4 and the intracellular complex of adherence junctions.

Study design, materials and methods

We used a well-differentiated urothelial cell line (RT4). TRPV4 -/- and wildtype mice were respectively used as positive and negative controls. *Immunohistochemistry*: RT4 cells were grown on glass slides for 1 week (37°C). Mouse bladders were snap frozen, cut and mounted on glass slides. After this, cells / tissue were fixated with 3% paraformaldehyde in PBS for 10 min and prepared for immunohistochemistry. Antibodies for TRPV4 and E-cadherin were used. *Western blotting:* RT4 cells were grown in culture bottles for 1,5 weeks, following harvesting and lysation. TRPV4 antibody was used and standard electrophoresis techniques were applied. *Immunoprecipitation*: RT4 was grown in culture bottles for 1,5 week. Cell cultures (RT4) were harvested and lysated with an extraction buffer. Antibodies for α-catenin, β-catenin and tubulin (negative control) were used for precipitation. Precipitates were evaluated with standard electrophoresis techniques and considered successful when the antibody that was used for precipitation, was also detected in the precipitate. We used the antibody for TRPV4 to evaluate the existence of TRPV4 in the different precipitates.

Results

A colocalisation between TRPV4 and AJ's was seen in immunohistochemical stainings on wildtype mouse bladder urothelium (fig 1) and the RT4 cell line (fig 2). Western blotting experiments confirmed these results with a TRPV4 signal at the 95-100 kDa range (fig 3). No TRPV4 signal was seen in TRPV4 -/- mice. Immunoprecipitation experiments detected a connection between TRPV4 and α-catenin, but not with β-catenin or tubulin.

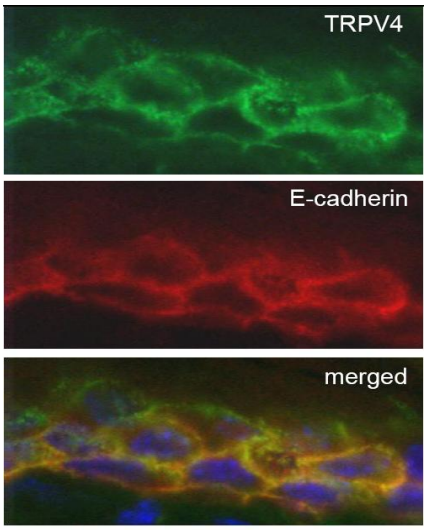

Fig 1: mouse (wildtype) bladder urothelium. Colocalisation between TRPV4 (green) & E-cadherin (red). Blue = nuclei (DAPI). Pictures taken at 60x with binocular fluorescence microscope.

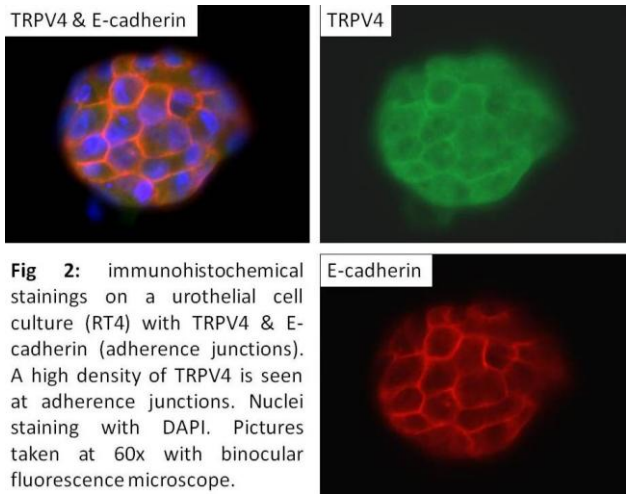

Fig 2: immunohistochemical stainings on a urothelial cell culture (RT4) with TRPV4 & E-cadherin (adherence junctions). A high density of TRPV4 is seen at adherence junctions. Nuclei staining with DAPI. Pictures taken at 60x with binocular fluorescence microscope.

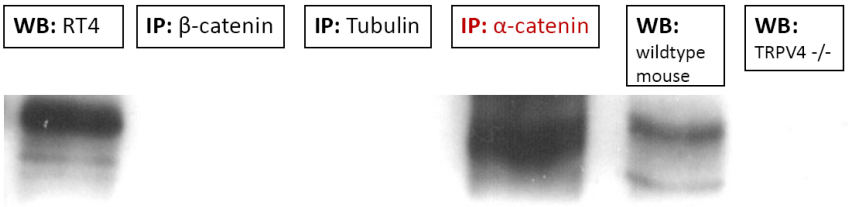

Fig 3: Western blotting (**WB**) & Immunoprecipitation (**IP**) experiments. **WB:** electrophoresis with TRPV4 antibody. TRPV4 is present in RT4 urothelial cell line and wildtype mouse, but not in TRPV4 -/- mouse. **IP:** TRPV4 was detected in the precipitate of the α-catenin precipitation (red), but not with β-catenin and tubulin (**=** negative control), implying a connection between TRPV4 and α-catenin proteins from AJ's.

Interpretation of results

Results show a connection between TRPV4 and the intracellular domain of AJ's (α-catenin). This proves that TRPV4 is connected to a rigid intercellular network that consist of AJ's and the actin cytoskeleton. This connection explains how TRPV4 can be activated by bladder stretch. To take into consideration the enlarged bladder capacities and the dysfunctional voiding seen in TRPV4-knockout mice [2], our findings provide new evidence on a molecular base, that TRPV4 is involved in measuring stretch in the bladder. TRPV4 is therefore a potential pharmacological target for the treatment of OAB.

Concluding message

TRPV4 is located on the intracellular domain of adherence junctions in bladder urothelial cells, implying a link between motoric 'stretch' and urothelial sensation. These results provide evidence that TRPV4 in the urothelium of the bladder is involved in the detection of stretch.

References

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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	**Animal Ethical Committee Radboud University (RUDEC)**