

TRPV1 EXPRESSION IN THE BLADDER IS ESSENTIAL FOR NGF-INDUCED DETRUSOR OVERACTIVITY

Hypothesis / aims of study

The transient receptor potential vanilloid subfamily 1 (TRPV1) is an ion channel widely expressed in the urinary tract of mammals, both in neuronal and non-neuronal structures. TRPV1 participates in micturition control, mainly during pathological conditions such as cystitis or spinal cord transection. It is known for many years that NGF administration to the bladder of intact rats increases the frequency of bladder reflex contractions. On the other hand, NGF sequestration in animal models of spinal cord transection was shown to reduce detrusor overactivity. As high levels of this neurotrophin were recently detected in the urine of patients with detrusor overactivity (DO) and chronic bladder inflammatory conditions, NGF has gained a sudden importance as a modulator of micturition reflex and as a potential therapeutic target.

NGF binds to its specific TrkA receptor, present in the vast majority of bladder sensory afferents. However, the mechanisms by which NGF sensitizes bladder afferents remain essentially unclear. Available data indicates that NGF may alter the function of bladder sensory fibres by interfering with Na⁺ currents either acutely (by phosphorylating molecules that mediate Na⁺ transient currents) or chronically (by changing the expression of Na⁺ channels).

The possible interplay of TRPV1 and NGF for the latter-induced bladder afferent sensitization was never fully investigated. However, the peripheral expression of TRPV1 was shown to be under the regulation of NGF. High levels of this neurotrophic factor were shown to increase TRPV1 translation and the transport of the protein into the peripheral sensory nerve endings. In addition, NGF was shown to open the TRPV1 channel by promoting the cleavage of phosphatidylinositol-4,5-bisphosphate that exerts a tonic negative control on TRPV1. Thus, the aim of the present study was to explore the role of TRPV1 in the excitatory effects of NGF on micturition reflex.

Study design, materials and methods

TRPV1-knockout (KO) and wildtype (WT) mice (n=4 per experimental group) received a single intraperitoneal injection of cyclophosphamide (200 mg/Kg; CYP). Animals were euthanized 3 days after CYP injection and the bladder, spinal cord and dorsal root ganglia (DRG) homogenized. The protein concentration of each sample was determined using the Bradford assay. The samples were assayed in duplicate by ELISA, following the manufacturer's protocol. Plates were read at 450 nm and the absorbance of the blank value (the mean of the assay wells without sample) was subtracted from each sample's absorbance. Tissue NGF values were normalized against the protein concentrations of each sample.

In another group of TRPV1 KO- and WT mice, cystometrograms were obtained in intact conditions and 3 days after CYP-injection. A 3rd group of TRPV1 KO- and WT mice received daily intraperitoneal injections of NGF (1µg/10g) for a period of 3 days. On the 4th day, cystometrograms were obtained.

In all cases, cystometrograms were performed under urethane anaesthesia. Briefly, bladders were exposed and a catheter inserted in its dome. Body temperature was kept stable (36-37°C) and the urethra remained unobstructed throughout the recording period while saline was infused at a constant rate (1.6ml/h) through the catheter. The frequency of bladder contractions was then measured.

Results

Analysis of NGF content by ELISA showed a significant increase in the bladder, DRG and spinal cord of TRPV1-KO animals treated with cyclophosphamide. Similar results were found in wild type mice.

The frequency of reflex bladder contractions was 0.51±0.05 in non-injected WT mice and 0.46±0.14 in intact KO -mice. The frequency increased to 1.16 ± 0.14 in CYP-inflamed WT mice and was 0.71±0.10 in CYP-inflamed TRPV1 KO-mice (p<0.05).

In WT animals treated with NGF the frequency of bladder contractions increased to 0.8±0.05 (p<0.01). In TRPV1 KO-animals treated with intraperitoneal NGF, the frequency of bladder contractions was statistically similar to controls (0.6±0.05).

Interpretation of results

These results indicate that bladder inflammation results in the upregulation of NGF synthesis, irrespective of TRPV1 expression. Despite NGF increase, TRPV1 KO mice do not exhibit an increase in the frequency of bladder reflex contractions during bladder inflammation. Similarly, systemic NGF administration to TRPV1 KO mice does not change the frequency of bladder reflex contractions.

Concluding message

TRPV1 expression is essential to the NGF-induced excitation of the micturition reflex. This finding is highly relevant for the design of an effective strategy of combating the consequences of high levels of NGF in the urine of patients with detrusor overactivity.

Specify source of funding or grant	Associação Portuguesa de Urologia; InComb project.
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?	Yes
Name of ethics committee	Ethics committee of the Faculty of Medicine of Porto