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Gratzke C[1], Streng T[2], Waldkirch E[3], Sigl K[1], Stief C G[1], Andersson K[4], Hedlund P[5]

1. *Department of Urology, Ludwig-Maximilians University Hospital, Munich, Germany,* ***2.*** *Department of Pharmacology, Drug Development and Therapeutics, University of Turku, Turku, Finland,* ***3.*** *Department of Urology, Hannover Medical School, Hannover, Germany,* ***4.*** *Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Medical Center Boulevard, Winston Salem, NC, USA,* ***5.*** *Department of Clinical Chemistry and Pharmacology, Lund University Hospital, Lund, Sweden*

TRANSIENT RECEPTOR POTENTIAL A1 (TRPA1) ION CHANNEL ACTIVITY IN THE HUMAN URETHRA – EVIDENCE FOR A FUNCTIONAL ROLE FOR TRPA1 IN THE OUTFLOW REGION

Hypothesis / aims of study

TRPA1 is the only mammalian member of the Ankyrin TRP subfamily and generally known to be present on capsaicin-sensitive primary sensory neurons, which upon activation elicit pain, protective reflexes, and local release of neurotransmitters in the periphery 1. The TRPA1 ion channel is co-expressed with TRPV1 on unmyelinated bladder afferents and urothelium of the rat bladder 2. Intravesical TRPA1-agonists initiate detrusor overactivity in this species 3. However, it is not known if TRPA1 is expressed in the human lower urinary tract (LUT) or if activation of this receptor has functional consequences in this tissue. The aim of the current study was to characterize the distribution of TRPA1-receptors in the human urethra and to investigate the effects of the TRPA1-agonists Allyl isothiocyanate (AI), Cinnamaldehyde (CA) and NaHS (donor of H2S) on isolated preparations of the human urethra.

Study design, materials and methods

Macroscopically normal proximal urethral specimens were obtained peroperatively from 10 patients undergoing cysturethrectomy or radical prostatectomy due to cancer disease. The expression of TRPA1 was studied with western blot and fluorescence immunohistochemistry. Sections (10 µM) were incubated alone or in combination with primary antisera for TRPA1, TRPV1, CGRP, tyrosine hydroxylase (TH), nitric oxide synthase (NOS) and vimentin. The effects of AI (10nM-100µM), CA (0.1µM-100µM) and NaHS (1µM-1mM) on isometric tension of urethral strips were investigated in tissue baths.

Results

Characteristic bands for TRPA1 (128 kDa) were detected with western blot. TRPA1 immunoreactivity (-IR) was found in nerve fibers in the suburothelial space and was also located to nerve fibres of the muscle layer. Single TRPA1-IR nerves extended into the urothelium. The area occupied by TRPA1-IR nerves was 30 ± 5.0% ($p<0.04$) larger in the suburothelial region compared to the urethral muscular wall. A majority, but not all TRPA1-IR nerves also expressed immunoreactivity for CGRP or TRPV1. TRPA1-immunoreactive structures were distinct from autonomic motor nerves that contained immunoreactivity for TH or NOS. In the urothelium, TRPV1 was located to the outer layers whereas TRPA1 was observed in basal urothelial cells. Interspersed between strands of smooth muscle cells of the urethral wall, TRPA1- and vimentin-IR cells, containing central nuclei and slender cytoplasmatic extensions, were observed.

In functional experiments, AI, CA, and NaHS did not have any contractile effect per se in prestretched urethral preparations. After precontraction with phenylephrine (1µM), the TRPA1- receptor agonists produced concentration-dependent relaxations (Fig. 1). At 100 µM, a maximal relaxation of 92 ± 5% was achieved for CA. At 1mM, NaHS- and AI-induced relaxations amounted to 67 ± 2% and 25 ± 5%, respectively.

Interpretation of results

The localization of TRPA1 to sensory nerves and in urothelial cells, as well as the finding that TRPA1 agonists can modify tone of urethral preparations suggest a role for TRPA1-mediated mechanisms in afferent and efferent transduction in the human outflow region. The demonstration of TRPA1 on interstitial cells further strengthens a putative interactive role for these cells in afferent-efferent modulator activity of human urethral functions.

Concluding message

In the human urethra, TRPA1 is distributed on components involved in mechanoafferent signals of the LUT. TRPA1 agonists relax human urethral preparations presumably through activation of sensory nerves, urothelial cells and / or via interaction with interstitial cells.

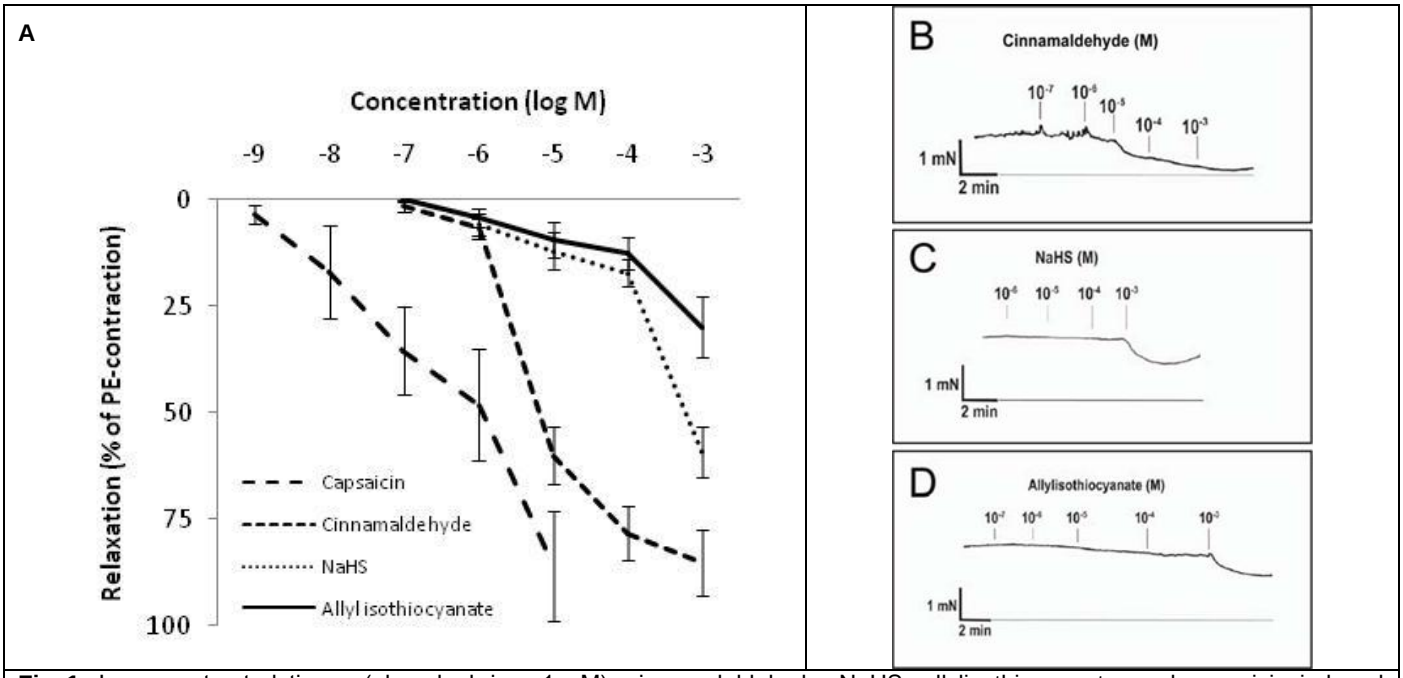

Fig 1. In precontracted tissue (phenylephrine; 1 µM), cinnamalaldehyde, NaHS, allylisothiocyanate, and capsaicin induced concentration-dependent relaxations in the order of potency: capcaisin > cinnamalaldehyde > NaHS > allylisothicyanate (n=5-8, N=5-8, A). Original tracings describing the relaxant effects by cinnamalaldehyde (B), NaHS (C) and allylisothiocyanate (D) in three separate isolated preparations of the human urethra.

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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	**Lund University Hospital Ethics Committee**