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From Lab to Clinic

Transient Receptor Potential A1 and Cannabinoid Receptor Activity in Human Normal and Hyperplastic Prostate: Relation to Nerves and Interstitial Cells

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Abstract

Background: Ion channel transient receptor potential A1 (TRPA1) and cannabinoid (CB) receptors are involved in mechanoafferent signaling from the bladder and the urethra.
Objective: To characterize TRPA1-, CB1-, and CB2-receptor activities in the human prostate.
Design, setting, and participants: Prostate specimens were obtained from 12 patients undergoing radical prostatectomy. We studied expressions ($n = 6$) of TRPA1, CB1, and CB2 receptors and effects of the TRPA1 agonists allyl isothiocyanate (AI), cinnamaldehyde (CA), sodium hydrogen sulfide (NaHS), and CP 55940 (a CB1/CB2 agonist) on prostatic preparations.
Measurements: Western blot, immunohistochemistry, and functional experiments were performed.
Results and limitations: Western blot detected expected bands for CB1, CB2, and TRPA1. TRPA1 immunoreactivity was located on nerves that were positive for CB1, CB2, calcitonin gene-related peptide (CGRP), nitric oxide synthase (NOS), or vesicular acetylcholine transporter (VAChT). CB1 and CB2 immunoreactivity was found on nerves that were positive for NOS, VAChT, or CGRP. Adrenergic nerves were not immunoreactive for TRPA1, CB1, or CB2. In nodular hyperplasia, nerves containing the above markers were scarce or absent. TRPA1 immunoreactivity was detected in cyclic guanosine monophosphate–positive basal cells of the glandular epithelium. Basal or subepithelial TRPA1-immunoreactive cells contained vimentin and c-kit immunoreactivity. CA and NaHS relaxed precontracted preparations by $55 \pm 7\%$ and $35 \pm 3\%$ ($n = 6$ for each). CP 55940, NaHS, AI, capsaicin, and CA decreased nerve contractions up to 27%, 80%, 47%, and 87%, respectively ($n = 6$ for each).
Conclusions: The distribution and function of TRPA1 and CB receptors in prostatic tissue suggest a role for these receptors in mechanoafferent signals, epithelial homeostasis, emission, or inflammation of the human prostate.

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1. Introduction

Transient receptor potential (TRP) ion channels regulate transmembrane cation conductance, which is of importance for muscle contraction, transmitter release, cell proliferation, gene transcription, and cell death [1]. TRP channels are also targets for irritants, toxins, and inflammation products. Correlations between TRP expression, hereditary disorders, and symptoms have been reported for various diseases [1]. The TRP channels are subdivided into six families: TRPC, TRPV, TRPM, TRPP, TRPML, and TRPA [1]. Members of the TRPV and TRPM families have been located in rat and human prostates, and their involvement has been suggested in prostate cell homeostasis and in pain in prostatitis [2,3].

The TRP ion channel A1 (TRPA1) is expressed on sensory neurons, which, when activated, elicit pain, protective reflexes, and local release of neurotransmitters [1]. TRPA1 can be activated by cold and by compounds such as allyl isothiocyanate (AI), cinnamaldehyde (CA), hydrogen sulfide (H_2S), and menthol [1,4,5]. Cross-talk between TRPA1 and TRPV1 signals has been reported, and it has been suggested that TRPA1 might regulate cannabinoid (CB) signals in inflammatory hypersensitivity [1]. We recently located TRPA1 on sensory neurons and urothelium in rat and human lower urinary tract (LUT) and showed that TRPA1 activation causes detrusor overactivity in rats and relaxes the human urethra [4,6]. A role for TRPA1 in sensory signaling of the human outflow region is proposed. Although TRPA1 activity has been reported in the prostate epithelium [7], the distribution of TRPA1 and the types of nerves or cells expressing TRPA1 in the prostate have not been determined. To the best of our knowledge, a functional role for TRPA1 in the prostate has not been investigated.

Numerous studies have explored the endocannabinoid system in chronic diseases, including LUT dysfunction [8]. Endocannabinoids may also be involved in regulation of sexual function [9]. Reports that CB2 receptors can affect afferent signals from the LUT and cholinergic nerve functions in the urinary bladder suggest CB-receptor-mediated modulation of the micturition reflex [10]. CB1 receptors were reported to be expressed in the rat and human prostate, and axes between CB-mediated signals and prostatic endocrine functions or smooth muscle homeostasis have been proposed [11,12].

The present study examined the TRPA1 activity in normal and hyperplastic human prostatic tissue in relation to markers for subsets of neurons or interstitial cells. The effects of TRPA1 activators on isolated human prostatic smooth muscle specimens also were evaluated.

2. Material and methods

2.1. Tissue source and handling

Human prostate tissue was obtained from 12 patients (mean age: 62.1 yr) undergoing prostatectomy for prostate cancer. Tissues did not exhibit histologic signs of neoplasia, carcinoma, or inflammation. All procedures were approved by the ethics committees of Munich and Lund Universities.

2.2. Western blot analysis

Prostatic tissue was processed for western blot, as previously described [6]. Samples were probed for TRPA1 (Alomone Labs, Jerusalem, Israel) and CB1 and CB2 receptors (1:2000; Sigma, St. Louis, MO, USA). Secondary horseradish peroxidase immunoglobulin G (1:5000; Novus Biologicals, Littleton, CO, USA) was used. β-actin (Pierce Biotechnology, Inc., Rockford, IL, USA) was used as standard. Negative controls without primary antibodies were performed.

2.3. Immunohistochemistry

Prostate specimens were processed for immunohistochemistry, as previously described [6]. Primary antibodies for TRPA1 (goat: 1:250; rabbit: 1:500; Alomone Labs, Jerusalem, Israel), calcitonin gene-related peptide (CGRP; guinea pig: 1:1000; Euro-Diagnostica, Malmö, Sweden), tyrosine hydroxylase (TH; mouse: 1:2000, Diasorin AB, Bromma, Sweden), nitric oxide synthase (NOS; sheep: 1:2000; P.C. Emson, Babraham Institute, Cambridge, UK), goat antiserum to vesicular acetylcholine transporter (VAChT; 1:1600; Chemicon, Malmö, Sweden), vimentin (goat: 1:200; Sigma Biosciences, St Louis, MO, USA), and c-kit (rabbit: 1:800; ImmunoBiological Laboratories, Gunma, Japan) were used. Cyclic guanosine monophosphate (cGMP) immunoreactivity was induced according to Smet et al [13]. A sheep cGMP antiserum (generous gift from J. de Vente, Maastricht University, the Netherlands) was used (1:1000). Alexa secondary antibodies (1:600; Molecular Probes Inc., Leiden, the Netherlands) were used. Sections were analyzed using a fluorescence microscope (Olympus Corp. Osaka, Japan). Control staining without primary antibodies was performed.

2.4. Organ bath studies

Human prostate strips were prepared for organ bath studies, as previously described [6]. Activity was registered with a Grass polygraph model 7E (Grass Technologies, West Warwick, RI, USA). Effects of CP 55940, capsaicin, AI, CA, and sodium hydrogen sulfide (NaHS; donor of H_2S) on basal tone or after precontraction with phenylephrine (Phe; 10 μM) were investigated. Frequency–response curves were obtained before and after administration of TRPA1 or CB agonists.

2.5. Drugs and solutions

CP 55940 (Sigma, St. Louis, MO, USA), capsaicin, AI, CA, NaHS, and Phe stock solutions (10^{-1} M; ethanol) were made and kept at −20 °C until use. NaHS was prepared fresh for experiments. Dilutions of the drugs were made in saline. The Krebs solution contained sodium chloride, 119 mM; potassium chloride, 4.6 mM; calcium chloride, 1.5 mM; magnesium chloride, 1.2 mM; sodium hydrogen carbonate, 15 mM; sodium dihydrogen phosphate, 1.2 mM; glucose, 5.5 mM (K^+, 60 mM).

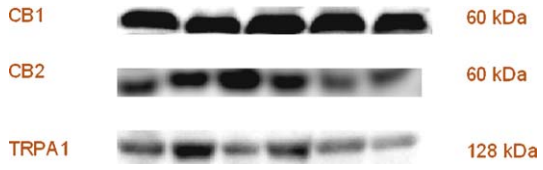

Fig. 1 – Western blot. The cannabinoid (CB) 1, CB2, and transient receptor potential A1 (TRPA1) antibody detected clear bands of the expected molecular weights (60 kDa, 60 kDa, and 128 kDa, respectively) in tissue from the human prostate ($n = 6$). Negative controls are not shown.

2.6. Calculations and statistical analysis

Values are given as mean plus or minus standard error of the mean. Two-tailed student t test was used for paired or unpaired observations. A p value of <0.05 was regarded as significant.

3. Results

3.1. Western blot analysis

Clear bands for CB1, CB2, and TRPA1 were displayed at the expected weights of 60 kDa, 60 kDa, and 128 kDa, respectively (Fig. 1). Bands for β-actin were characteristically visualized at 42 kDa (data not shown).

3.2. Immunohistochemistry

TRPA1-immunoreactive nerve fibers were detected between prostatic stromal smooth muscle bundles, around acini and ducts, and innervating arteries. Double staining revealed TRPA1 immunoreactivity on CB1- or CB2-immunoreactive nerve fibers that also expressed CGRP (Figs. 2 and 3). TRPA1-immunoreactive nerve fibers also expressed immunoreactivity for NOS or VAChT (Fig. 3). Immunoreactivity for

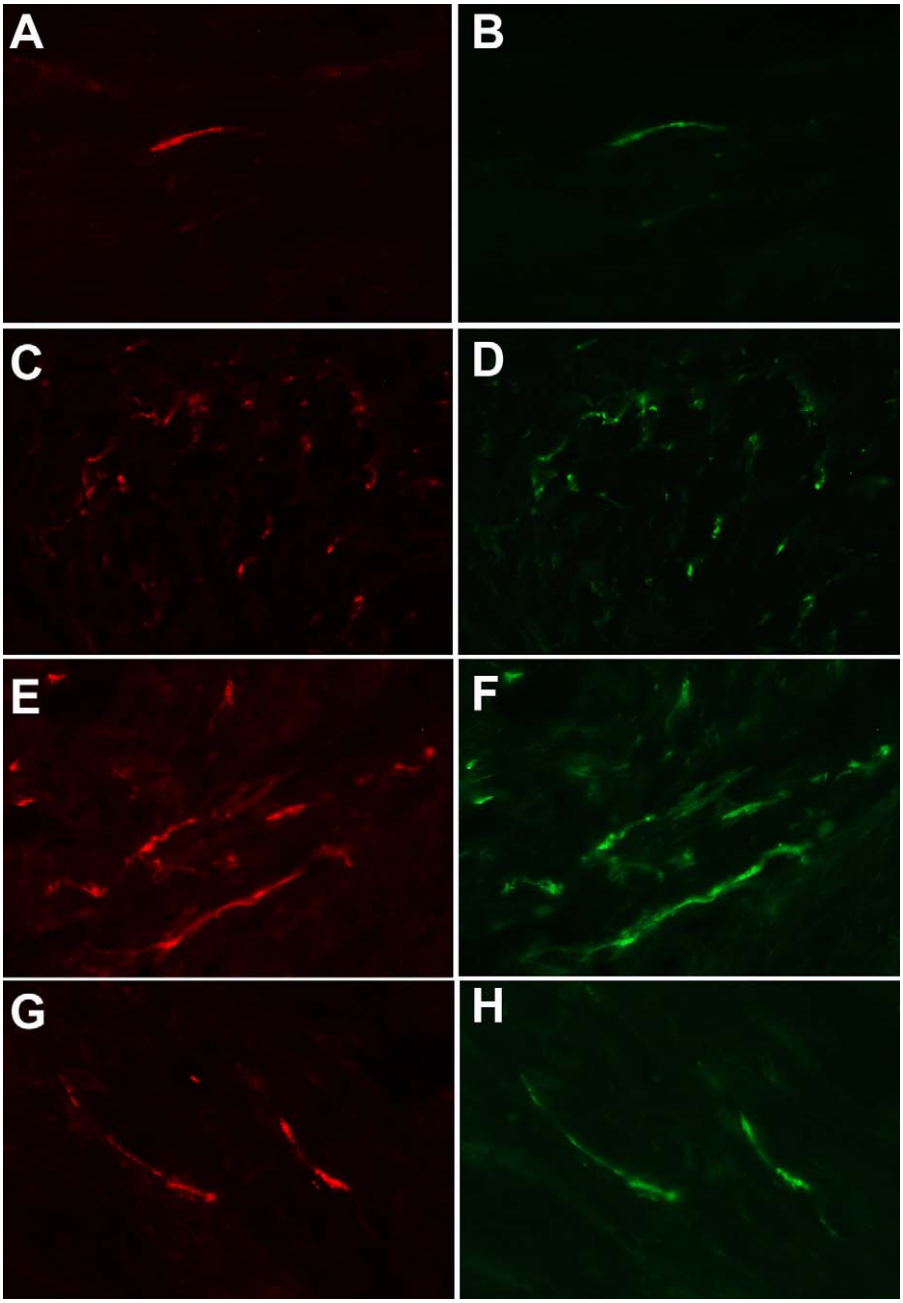

Fig. 2 – Immunohistochemistry. (A, C) transient receptor potential A1 (TRPA1)–immunoreactive (-IR) nerves in the prostatic stroma (Alexa Green). (B) Same section as in A; cannabinoid (CB) 1–IR nerve fibers with the same profiles as TRPA1-IR nerves (magnification ×400; Alexa Red). (D) Same section as in C; CB2-IR nerve fibers with the same profiles as TRPA1-IR nerves (magnification ×200; Alexa Red). (E, G) Calcitonin gene-related peptide (CGRP)–IR nerve fibers in the human prostate. (F) Same section as in E; CB1-IR nerve fibers with the same profiles as CGRP-IR nerves (magnification ×400; Alexa Red). (H) Same section as in G; CB2-IR nerve fibers with the same profiles as CGRP-IR nerves (magnification ×400; Alexa Red).

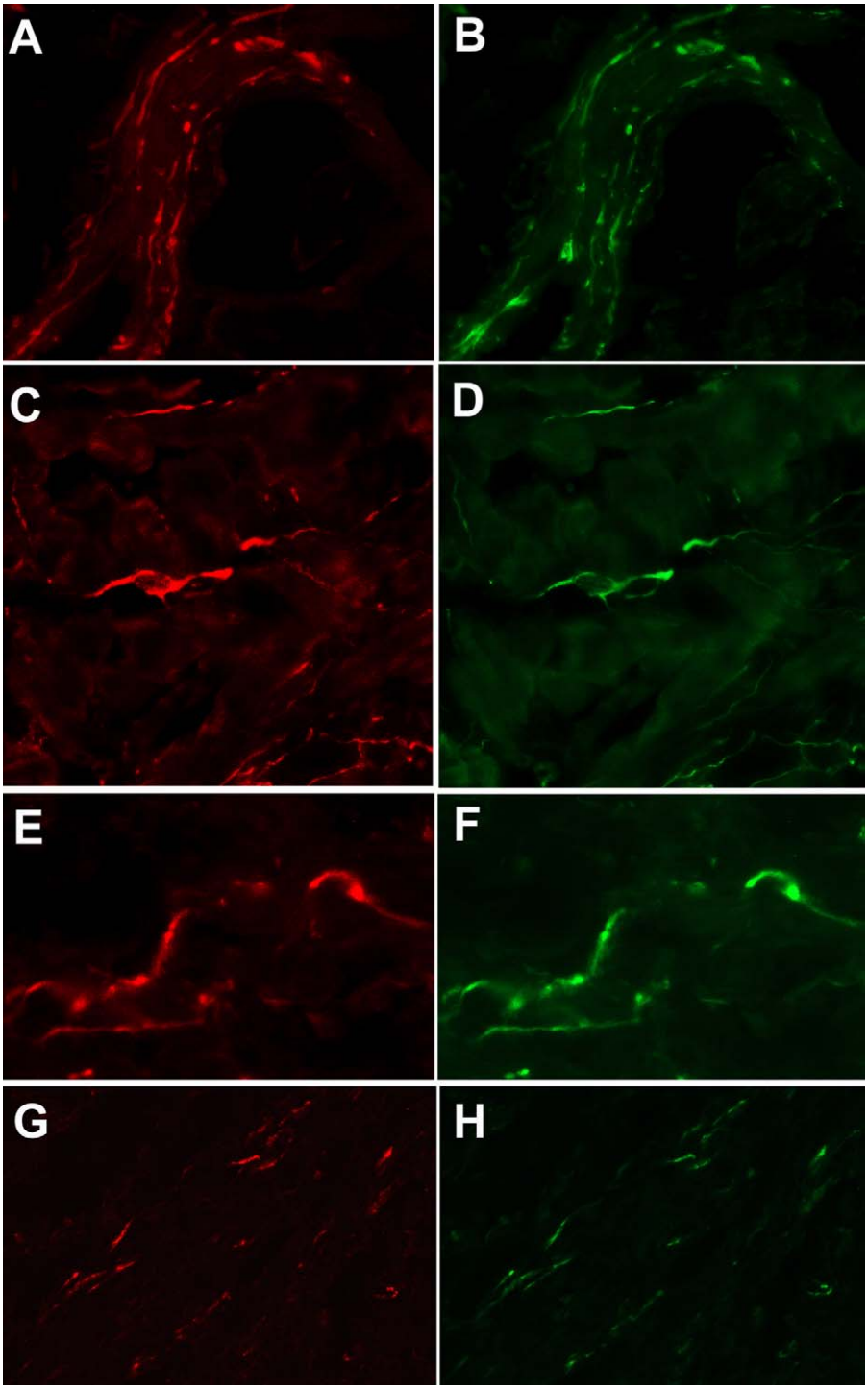

Fig. 3 – Immunohistochemistry. (A, C) transient receptor potential A1 (TRPA1)–immunoreactive (-IR) nerves in the prostatic stroma (Alexa Green). (B) Same section as in A; vesicular acetylcholine transporter (VAChT)–IR nerve fibers with the same profiles as TRPA1-IR nerves (magnification ×200; Alexa Red). (D) Same section as in C; nitric oxide synthase (NOS)–IR nerve fibers with the same profiles as TRPA1-IR nerves (magnification ×200; Alexa Red). (E, G) Cannabinoid (CB) 2–IR nerve fibers in the human prostate. (F) Same section as in E; VAChT-IR nerve fibers with the same profiles as CB2-IR nerves (magnification ×400; Alexa Red). (H) Same section as in G; NOS-IR nerve fibers with the same profiles as CB2-IR nerves (magnification ×200; Alexa Red).

CB2 (Fig. 3) or CB1 (Fig. 4) was found on VAChT- or NOS-positive nerve fibers. TH-positive nerve fibers were observed throughout the human prostate but were not immunoreactive for TRPA1, CB1, or CB2 (data not shown).

In regions with stromal nodular hyperplasia (Fig. 4), the density of TRPA1-immunoreactive nerves was significantly reduced or absent in some of these areas. Similarly, immunoreactivity for CB1 (Fig. 4) or CB2 was reduced or absent in hyperplastic prostatic tissue. Additionally, nerves

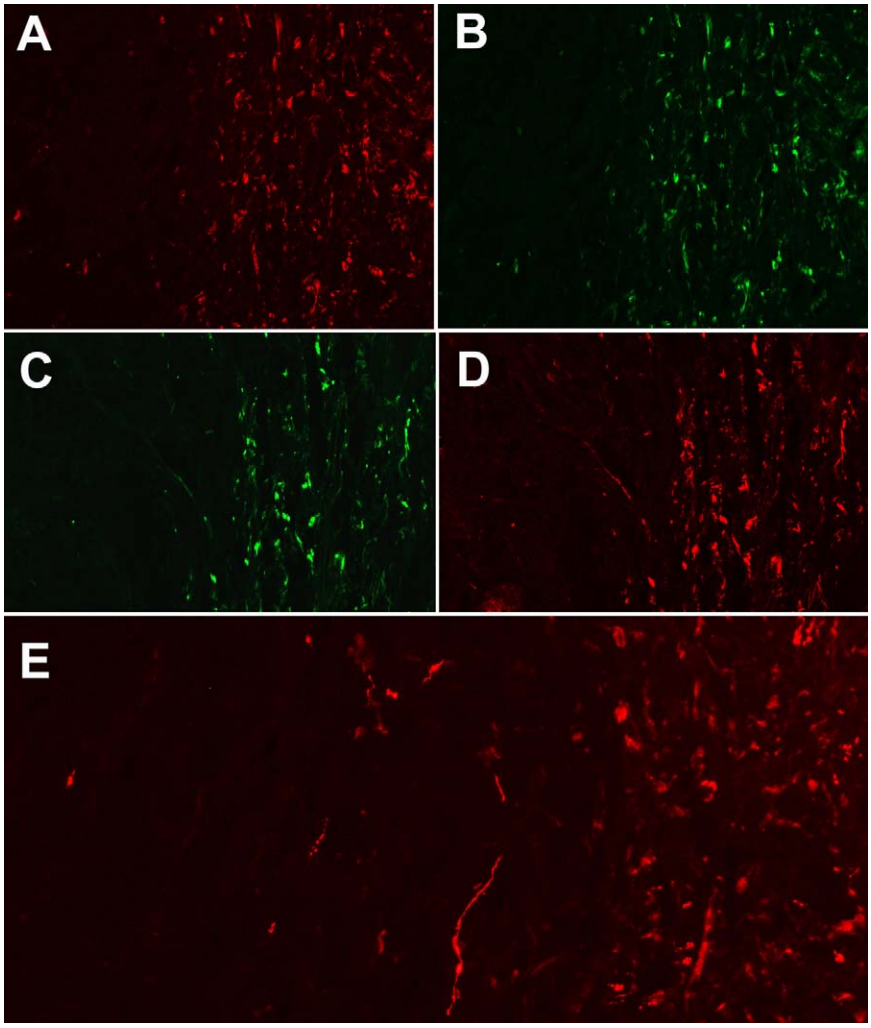

Fig. 4 – Immunohistochemistry. (A) transient receptor potential A1 (TRPA1)–immunoreactive (-IR) nerves in normal (right) and hyperplastic (left) prostatic stroma (Alexa Green). (B) Same section as in A; cannabinoid (CB) 2–IR nerve fibers with the same profiles as TRPA1-IR nerves (magnification ×100; Alexa Red). (C) CB1-IR nerves in normal (right) and hyperplastic (left) prostatic stroma (Alexa Red). (D) Same section as in C; vesicular acetylcholine transporter–IR nerve fibers with the same profiles as CB1-IR nerves (magnification ×100; Alexa Green). (E) Tyrosine hydroxylase–IR nerves in normal (right) and hyperplastic (left) prostatic stroma (Alexa Green).

containing VAChT or TH were reduced or absent in areas of prostate hyperplasia (Fig. 4).

A distinct pattern of TRPA1 immunoreactivity was detected in cGMP-positive basal cells of the prostatic glandular epithelium (Fig. 5). TRPA1 immunoreactivity also was observed in single cells that were scattered just beneath the epithelium of prostatic glandular acini. These cells also expressed immunoreactivity for vimentin and c-kit (Fig. 5).

3.3. *Organ bath experiments*

At baseline, none of the TRPA1 agonists—AI (10 nM–100 μM), CA (1 μM–1 mM), and NaHS (1 μM – 1 mM)—or capsaicin (1nM–10 μM) or CP 55940 (1 nM–10 μM) affected the level of tension of the strips of prostatic smooth muscle.

CA and NaHS concentration dependently relaxed Phe-contracted preparations (Fig. 6). Maximal relaxation amounted to 55 ± 7%, 35 ± 3%, 1.5 ± 5.1%, and 4.0 ± 5% for CA (1 mM), NaHS (1 mM), AI (100 μM), and capsaicin (10 μM), respectively (Fig. 6). In comparison, CP 55940 produced relaxation of 2.3 ± 1.2% at 10 μM.

CP 55940, H_2S, AI, capsaicin, and CA (10 μM) decreased nerve-induced contractions by 6–27%, 27–80%, 26–47%, and 67–87%, respectively ($p < 0.05$) (Fig. 7).

4. Discussion

In accordance with findings in the LUT, where TRPA1 has been found in afferents expressing CGRP, TRPV1, or substance P [4,6,14], the current study is the first to demonstrate the existence of TRPA1 in sensory nerves of the human prostate. Although an even distribution of TRPA1-immunoreactive nerves in prostatic smooth muscle bundles was noted, CB1- or CB2-immunoreactive nerves that also expressed CGRP or TRPA1 were condensed around prostatic acini and vasculature. A network of TRPV1-immunoreactive

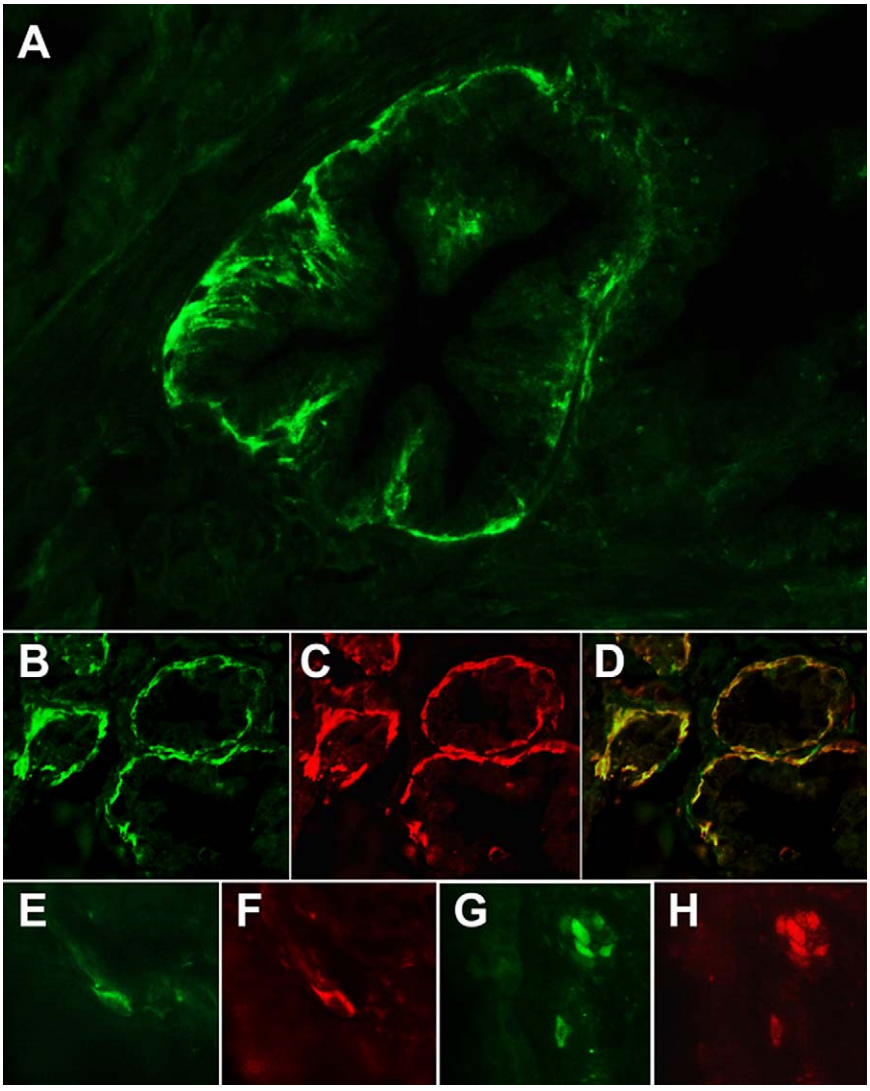

Fig. 5 – Immunohistochemistry. (A, B) transient receptor potential A1 (TRPA1)–immunoreactive (-IR) basal/subepithelial cells of a human prostatic acini (Alexa Green). (C) Same section as in B; cyclic guanosine monophosphate (cGMP)–IR cells with the coinciding profiles as TRPA1-IR cells (magnification ×400; Alexa Red). (D) Same section as in B and C with double filter settings; colocalization between TRPA1 and cGMP immunoreactivity. (E, G) TRPA1-IR basal/subepithelial cells of a human prostatic acini (Alexa Green). (F) Same section as in E; vimentin-IR cell with coinciding profile as a TRPA1-IR cell (magnification ×400; Alexa Red). (H) Same section as in G; c-kit-IR cells with coinciding profile as a TRPA1-IR cell (magnification ×200; Alexa Red).

nerve fibers has been described for the human prostate and prostatic urethra, and a role for TRPV1 signals in chronic pelvic pain syndrome (CPPS) has been suggested [2]. In a rat model of prostatitis, increased density of CGRP nerves and mast cell degranulation has been reported [15]. Because C-fibers are associated with pain transmission and supply of immunologic cells, released substances may lead to neurogenic inflammation, which in turn sensitizes afferent neurons. The TRPV1 channel may function as a heat sensor and respond to inflammatory or noxious ligands. These chemical and physical activators may have an additive effect on the gating of other TRP channels [1]. Besides the finding in this study that capsaicin and TRPA1 agonist exhibit similar effects on human prostate tissue, there is a close correlation between TRPV1 and TRPA1. This correlation is shown by the colocalization of the receptors on sensory nerve fibers in general and in the LUT and prostate,

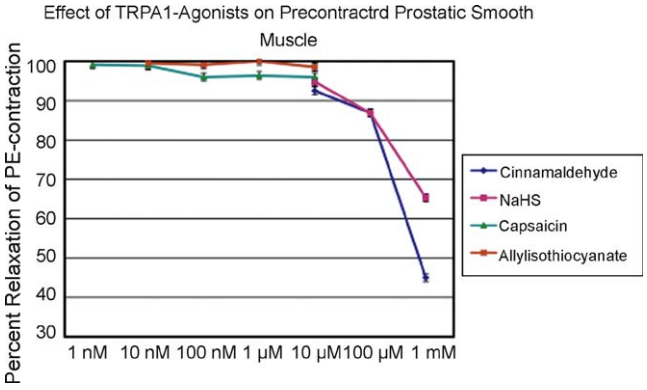

Fig. 6 – Functional in vitro experiments. Effects of different TRPA1 agonists on phenylephrine (PE; 1 mM) precontracted prostatic smooth muscle preparations ($n = 6$). Values represent mean plus or minus standard error of the mean.
NaHS = sodium hydrogen sulfide.

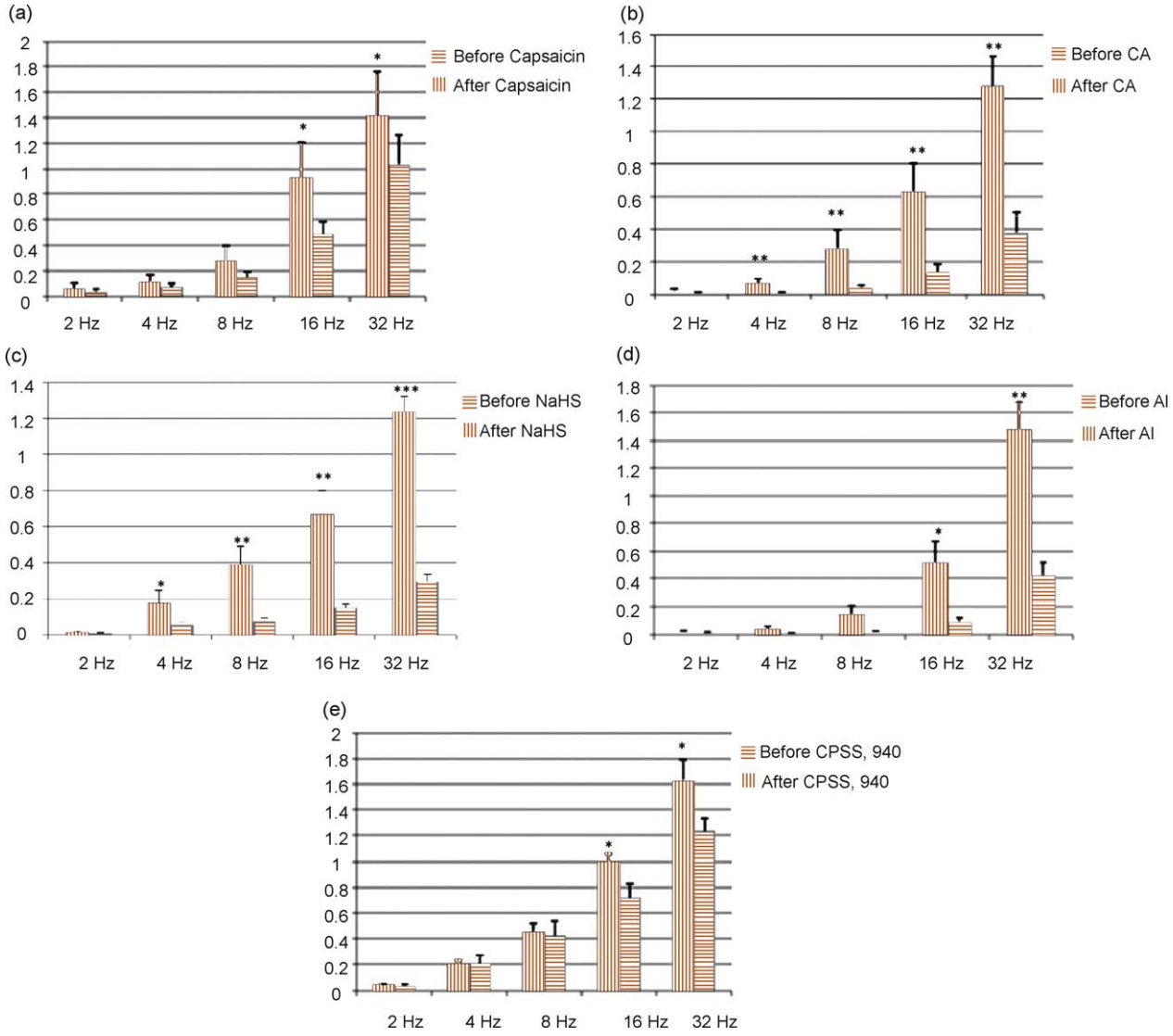

Fig. 7 – (a–e) Functional in vitro experiments. Electrical field stimulation generated frequency-dependent and tetrodotoxin-sensitive nerve-mediated contractile responses of isolated human prostatic preparations. CP 55940, hydrogen sulfide, allyl isothiocyanate (AI), capsaicin, and cinnamaldehyde (CA) at 10 μM decreased nerve-induced contractions (n = 6 for each).
NaHS = sodium hydrogen sulfide.

by cross-desensitization between TRPA1 agonists and capsaicin, by loss of effect by capsaicin or AI after pretreatment with histamine or a neurokinin-1 antagonist, by coactivation of the receptor by CBs or diallyl sulfides, and by downstream coactivation from the TRP receptors of protein kinase A and phospholipase C [1,4,6,14,16–19].

A relation between infectious and immunologic etiologies for prostatitis and TRPA1-mediated signals may be considered. Categories 1 and 2 prostatitis are caused by bacteria such as *Escherichia coli* and *Klebsiella*, which, together with other uropathogens involved in the development of LUT infections, produce NaHS [6,15]. NaHS, an endogenous modulator of leukocyte-mediated inflammation, has been shown to stimulate micturition in awake rats by activation of TRPA1 [4,20] and is proposed as a mediator of neurogenic inflammation that may be involved in the pathogenesis of CPPS [21].

Information is scarce on peripheral effects of TRPA1-active agents on autonomic functions, but it has been reported that AI-induced responses of the distal colon were attenuated by atropine [22]. Cholinergic NOS-containing parasympathetic nerves are believed to regulate prostatic secretory functions [23]. Considering that TRPA1 was found on VAChT-positive nerves of the human prostate, a role for TRPA1 signals in modulating prostatic emission may be suggested. This suggestion is supported by the current findings that basal cells of the prostatic glandular epithelium express cGMP, a main product of the NOS pathway. The localization of TRPA1 to cholinergic NOS-containing nerves and basal cGMP-containing cells implies that TRPA1-

mediated signals may modify secretory signals in the prostate at neuronal and epithelial levels.

In the current study, TRPA1 agonists relaxed precontracted prostatic smooth muscle and attenuated nerve-induced contractions. In line with recent findings of the human urethra, this is the first evidence that activation of TRPA1 ion channels modulates smooth muscle tone in the human prostate [6]. Taken together, administration of CA or NaHS had no effect on the basal tone of the human urethra or prostate but produced relaxant responses in Phe-activated preparations. In the isolated detrusor, effects of capsaicin and TRPA1 activators have been suggested to be dependent on the release of tachykinins and cyclooxygenase products from C-fibers [14]. Based on the present results, we cannot conclude if the relaxant responses or inhibitory effects on nerve-induced contractions by TRPA1 agonists in the human prostate are related to efferent functions of sensory nerves and/or to interaction with parasympathetic cholinergic NOS-containing nerves that previously have been described to relax the human prostate [23].

The present study provides the first evidence that CB1 and CB2 receptors are located on TRPA1- and CGRP-positive nerves of the human prostate, and it may be speculated that peripheral CB receptors can be targets in management of pain in prostatic disease. The observation that CB1 and CB2 immunoreactivity was expressed on prostatic cholinergic nerves corresponds to findings in the human corpus cavernosum and supports a role for CB-mediated modulation of autonomic neurotransmission [10]. In rat prostate, the CB agonist WIN 55212-2 inhibited electrically induced contractions [11]. We show that CP 55940 decreased nerve-induced contractions in human prostatic strips. Similarly, CB1- or CB2-induced signals have been proposed to inhibit vagal activation, cholinergic motor neurons, and endotoxin-induced motor responses of airway or gastrointestinal tissues [24–26].

Previous studies have described a decrease in autonomic nerves of the hyperplastic prostate [27]. The current results describe similar phenomena for TRPA1-, CB1-, or CB2-immunoreactive nerves and also confirm that both adrenergic and cholinergic nerves are reduced in areas of nodular hyperplasia. Based on the present and previous reports, it may be speculated whether altered innervation of the prostate is involved in the development of benign prostatic hyperplasia, whether an increased stromal cell growth exceeds the regenerative capacity of nerves, or whether changes in stromal cell homeostatis per se causes denervation.

Cells similar to the interstitial cells of Cajal that are involved in regulation of gastrointestinal motility and that may differentiate into smooth muscle cells have also been described in the LUT [13,28,29]. These cells express vimentin, c-kit, and cGMP [13,28]. Networks of interstitial cells in the LUT have been suggested to integrate signals among the urothelium, sensory nerves, and smooth muscle [6,28]. In the current study, TRPA1 immunoreactivity was detected in cGMP-, vimentin-, or c-kit-positive cells of the basal glandular epithelium or in subepithelial cells of the human prostate. Expression of c-kit, the receptor for the stem cell factor, has been found in epithelial and stromal human prostate tissue and in human hyperplastic prostate stromal cultures [30,31]. C-kit-positive cells with a basal phenotype have been reported to give rise to intermediate epithelial cells and have been implicated in epithelial renewal [32]. The present results suggest that epithelial c-kit-, vimentin-, or cGMP-positive cells that express TRPA1 can respond to exogenous or putative endogenous TRPA1-active molecules and also can form a basis that TRPA1 may be involved in prostate tissue differentiation.

5. Conclusion

The distribution of TRPA1, CB1, and CB2 on sensory nerves and the effects of TRPA1 and CB agonists on human prostatic preparations indicate that these receptors may be involved in mechanoafferent functions of the prostate. Cholinergic parasympathetic nerves that supply prostatic glandular epithelium may also be targets for TRPA1- or CB-mediated signals during epithelial homeostasis, emission, or inflammation. Altered patterns of TRPA1-, CB1-, and CB2-containing nerves in hyperplasic prostate and the coexpression of TRPA1 and markers for interstitial cells in prostatic epithelium need to be further explored in relation to prostate differentiation.

Author contributions: Petter Hedlund had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Hedlund, Stief, Andersson.
Acquisition of data: Gratzke, Weinhold, Hedlund.
Analysis and interpretation of data: Gratzke, Hedlund, Reich.
Drafting of the manuscript: Gratzke, Hedlund.
Critical revision of the manuscript for important intellectual content: Stief, Andersson, Seitz.
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References

[1] Nilius B, Owsianik G, Voets T, Peters JA. Transient receptor potential cation channels in disease. Physiol Rev 2007;87:165–217.

[2] Dinis P, Charrua A, Avelino A, et al. The distribution of sensory fibers immunoreactive for the TRPV1 (capsaicin) receptor in the human prostate. Eur Urol 2005;48:162–7.
[3] Wang HP, Pu XY, Wang XH. Distribution profiles of transient receptor potential melastatin-related and vanilloid-related channels in prostatic tissue in rat. Asian J Androl 2007;9:634–40.
[4] Streng T, Axelsson HE, Hedlund P, et al. Distribution and function of the hydrogen sulfide–sensitive TRPA1 ion channel in rat urinary bladder. Eur Urol 2008;53:391–400.
[5] Karashima Y, Damann N, Prenen J, et al. Bimodal action of menthol on the transient receptor potential channel TRPA1. J Neurosci 2007;27:9874–84.
[6] Gratzke C, Streng T, Waldkirch E, et al. Transient receptor potential A1 (TRPA1) activity in the human urethra—evidence for a functional role for TRPA1 in the outflow region. Eur Urol 2009;55: 696–704.
[7] Du S, Araki I, Kobayashi H, Zakoji H, Sawada N, Takeda M. Differential expression profile of cold (TRPA1) and cool (TRPM8) receptors in human urogenital organs. Urology 2008;72:450–5.
[8] Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 2006;58: 389–462.
[9] Gratzke C, Christ GJ, Stief CG, Andersson K-E, Hedlund P. Localization and function of cannabinoid receptors in the corpus cavernosum: basis for modulation of nitric oxide synthase nerve activity. Eur Urol 2010;57:342–9.
[10] Gratzke C, Streng T, Park A, et al. Distribution and function of cannabinoid receptors 1 and 2 in the rat, monkey and human bladder. J Urol 2009;181:1939–48.
[11] Tokanovic S, Malone DT, Ventura S. Stimulation of epithelial CB1 receptors inhibits contractions of the rat prostate gland. Br J Pharmacol 2007;150:227–34.
[12] Ruiz-Llorente L, Sanchez MG, Carmena MJ, et al. Expression of functionally active cannabinoid receptor CB1 in the human prostate gland. Prostate 2003;54:95–102.
[13] Smet PJ, Jonavicius J, Marshall VR, de Vente J. Distribution of nitric oxide synthase-immunoreactive nerves and identification of the cellular targets of nitric oxide in guinea-pig and human urinary bladder by cGMP immunohistochemistry. Neuroscience 1996;71: 337–48.
[14] Andrade EL, Ferreira J, Andre E, Calixto JB. Contractile mechanisms coupled to TRPA1 receptor activation in rat urinary bladder. Biochem Pharmacol 2006;72:104–14.
[15] Pontari MA, Ruggieri MR. Mechanisms in prostatitis/chronic pelvic pain syndrome. J Urol 2008;179:S61–7.
[16] Koizumi K, Iwasaki Y, Narukawa M, et al. Diallyl sulfides in garlic activate both TRPA1 and TRPV1. Biochem Biophys Res Commun 2009;382:545–8.
[17] Schnizler K, Shutov LP, Van Kanegan MJ, et al. Protein kinase A anchoring via AKAP150 is essential for TRPV1 modulation by forskolin and prostaglandin E2 in mouse sensory neurons. J Neurosci 2008;28:4904–17.
[18] Wang S, Dai Y, Fukuoka T, et al. Phospholipase C and protein kinase A mediate bradykinin sensitization of TRPA1: a molecular mechanism of inflammatory pain. Brain 2008;131:1241–51.
[19] Calixto JB, Kassuya CA, Andre E, Ferreira J. Contribution of natural products to the discovery of the transient receptor potential (TRP) channels family and their functions. Pharmacol Ther 2005;106: 179–208.
[20] Zanardo RC, Brancaleone V, Distrutti E, Fiorucci S, Cirino G, Wallace JL. Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. FASEB J 2006;20:2118–20.
[21] Nazif O, Teichman JM, Gebhart GF. Neural upregulation in interstitial cystitis. Urology 2007;69:24–33.
[22] Penuelas A, Tashima K, Tsuchiya S, et al. Contractile effect of TRPA1 receptor agonists in the isolated mouse intestine. Eur J Pharmacol 2007;576:143–50.
[23] Hedlund P, Ekstrom P, Larsson B, Alm P, Andersson KE. Heme oxygenase and NO-synthase in the human prostate—relation to adrenergic, cholinergic and peptide-containing nerves. J Auton Nerv Syst 1997;63:115–26.
[24] Duncan M, Mouihate A, Mackie K, et al. Cannabinoid CB2 receptors in the enteric nervous system modulate gastrointestinal contractility in lipopolysaccharide-treated rats. Am J Physiol Gastrointest Liver Physiol 2008;295:G78–87.
[25] Hinds NM, Ullrich K, Smid SD. Cannabinoid 1 (CB1) receptors coupled to cholinergic motorneurones inhibit neurogenic circular muscle contractility in the human colon. Br J Pharmacol 2006;148: 191–9.
[26] MacNaughton WK, Van Sickle MD, Keenan CM, Cushing K, Mackie K, Sharkey KA. Distribution and function of the cannabinoid-1 receptor in the modulation of ion transport in the guinea pig ileum: relationship to capsaicin-sensitive nerves. Am J Physiol Gastrointest Liver Physiol 2004;286:G863–71.
[27] Powell MS, Li R, Dai H, Sayeeduddin M, Wheeler TM, Ayala GE. Neuroanatomy of the normal prostate. Prostate 2005;65:52–7.
[28] McHale NG, Hollywood MA, Sergeant GP, Shafei M, Thornbury KT, Ward SM. Organization and function of ICC in the urinary tract. J Physiol 2006;576:689–94.
[29] Torihashi S, Nishi K, Tokutomi Y, Nishi T, Ward S, Sanders KM. Blockade of kit signaling induces transdifferentiation of interstitial cells of cajal to a smooth muscle phenotype. Gastroenterology 1999;117:140–8.
[30] Shafik A, Shafik I, el-Sibai O. Identification of c-kit-positive cells in the human prostate: the interstitial cells of Cajal. Arch Androl 2005;51:345–51.
[31] Lin VK, Wang SY, Vazquez DV, Xu CC, Zhang S, Tang L. Prostatic stromal cells derived from benign prostatic hyperplasia specimens possess stem cell like property. Prostate 2007;67: 1265–76.
[32] Ceder JA, Jansson L, Ehrnström RA, Rönnstrand L, Abrahamsson P-A. The characterization of epithelial and stromal subsets of candidate stem/progenitor cells in the human adult prostate. Eur Urol 2008;53:524–32.