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— REVIEW ARTICLE —

On the Origin of Bladder Sensing: Tr(i)ps in Urology

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The mammalian TRP family consists of 28 channels that can be subdivided into 6 different classes: TRPV (vanilloid), TRPC (canonical), TRPM (Melastatin), TRPP (Polycystin), TRPML (Mucolipin), and TRPA (Ankyrin). TRP channels are activated by a diversity of physical (voltage, heat, cold, mechanical stress) or chemical (pH, osmolality) stimuli and by binding of specific ligands, enabling them to act as multifunctional sensors at the cellular level. Currently, a lot of scientific research is devoted to these channels and their role in sensing mechanisms throughout the body. In urology, there's a growing conviction that disturbances in afferent (sensory) mechanisms are highly important in the pathogenesis of functional problems. Therefore, the TRP family forms an interesting new target to focus on. In this review we attempt to summarize the existing knowledge about TRP channels in the urogenital tract. So far, TRPV1, TRPV2, TRPV4, TRPM8, and TRPA1 have been described in different parts of the urogenital tract. Although only TRPV1 (the vanilloid receptor) has been extensively studied so far, more evidence is slowly accumulating about the role of other TRP channels in the (patho)physiology of the urogenital tract. *Neurourol. Urodynam. 27:264–273, 2008.*
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INTRODUCTION

What Are TRPs?

The transient receptor potential (TRP) channel superfamily is named after its founding member, first described in the Drosophila fly. Thirty years ago, a Drosophila mutant was discovered which exhibited a transient instead of a sustained response to light.[1] In the rhabdomeres of the mutant fly, a TRP was measured during light exposure, in contrast to a sustained, plateau-like potential in the wild-type. Therefore, the mutant was called TRP. The gene product for TRP was cloned in 1989 and it was shown that the TRP gene codes for a Ca^{2+} permeable cation channel, the founding member of the TRP family.[2]

TRP channels share some common features. They are all cation selective channels, although the permeability for different mono- and bivalent cations differs between isoforms. In general TRP channels are thought to consist of four subunits, forming functional channels as homo- or heterotetramers. The topology of a TRP subunit consists of intracellular N- and C-termini, six transmembrane spanning segments (S1—S6) and a pore-forming loop between S5 and S6.[3]

TRP channels function as multifunctional sensors at the cellular level. They can be activated by physical (voltage, heat, cold, mechanical stress) or chemical (pH, osmolality) stimuli and binding of specific ligands.[4] Activation by store depletion is recently under controversial discussion.[5−7] TRPs are the privileged candidates to function as primary sensing molecules in the cell. The channels contribute to changes in intracellular free Ca^{2+} by acting directly as Ca^{2+}-entry channels in the plasma membrane or by changing membrane potentials, modulating the driving forces for Ca^{2+}-entry mediated by other pathways. In addition TRP channels on the membranes of organelles provide an intracellular pathway for Ca^{2+}-release.[8]

More than 50 TRP channels are described in different species, ranging from yeast to man. At the moment, 28 mammalian TRP channels are known. Based on amino acid homology, mammalian TRP channels can be grouped into six different subfamilies.[9] The mammalian TRP channels most related to the Drosophila TRP are members of the TRPC (canonical) subfamily. Seven mammalian TRPCs have been described so far (TRPC1-7). The TRPM (Melastatin) and TRPV (Vanilloid) families consist of eight (TRPM1-8) and six (TRPV1-6) mammalian members respectively, the TRPP (Polycystin) family comprises three channel like and five non-channel members. There are three mammalian TRPML (Mucolipin) channels (TRPML1-3), whereas just one mammalian TRPA (Ankyrin) channel, TRPA1 is known. A seventh subfamily of TRP channels, TRPN (No mechanoreceptor potential C or NOMPC) has only been detected in non-mammalian species.[9]

Lessons Learned From Other Organs

In general, TRP channels are widely spread throughout the body. Some TRPs are ubiquitously present (e.g., TRPC1)

whereas others are more confined to specific organs (e.g., TRPV5 in the human kidney). A better understanding of the diverse functions of TRP channels can improve our insights in the pathogenesis of many systemic diseases. For a detailed review about the current knowledge on TRP channels in disease, see Nilius et al.[6] Our knowledge about the mechanisms by which TRP channels function is still very limited. Due to this lack of understanding, not many medical treatments have yet focused on TRP channels as a possible target. To date, only one member of the TRP family, TRPV1, has been targeted in the treatment of pain, gastrointestinal, and bladder disease. TRPV1 channel blockers are used to ameliorate chronic pain, whereas TRPV1 agonists that induce desensitization are used to treat diseases in which channel overexpression occurs.[6]

In urology, the vanilloid receptor (VR1) has been extensively studied for several decades. Intravesical instillations with vanilloids such as capsaicin or RTX have been used to treat overactive bladder diseases of different aetiology. Since VR1 was recognized as a member of the TRP family, there has been a rising curiosity about the function of TRP channels in the urogenital tract.[10] In this review article we will give an overview of the present data on the morphological, physiological and pathophysiological role of TRP channels in the urinary tract and discuss the role of TRP channels as possible targets for pharmaceutical interventions (Fig. 1).

TRP IN NORMAL PHYSIOLOGY

TRPV1. TRPV1 is the first discovered mammalian member of the Vanilloid TRP receptor subfamily. TRPV1 is a polymodal signal detector that is activated by vanilloids, heat and protons. At neutral pH[7,4] the channel is activated by noxious heat (>43°C), but when pH decreases (e.g., in case of inflammation or ischemia), the receptor can be activated at room temperature.[9,11]

TRPV1 is expressed on capsaicin-sensitive primary afferent neurons, mainly small unmyelinated C fiber nociceptors. TRPV1 is expressed on peripheral terminals of C fibers (innervating the skin, mucous membranes, joints, muscles, and internal organs), on cell bodies of dorsal root ganglia and trigeminal ganglia neurons and on central projections of nociceptive neurons towards the dorsal horn of the spinal cord.[11] Co-localization studies showed that TRPV1 is expressed in both neuropeptide (SP and CGRP) as well as IB4 expressing nociceptors.[11] TRPV1 is expressed in the nodose ganglion and different non-neuronal cell types as well.[9,11,12]

By creating a TRPV1 knock out mouse model it became clear that TRPV1 functions as a chemical and thermal sensor in vivo and has an essential role in inflammation, nociception and heat perception.[13]

Morphological data

TRPV1 in the bladder. Before TRPV1 specific antibodies were available, [3H] Resiniferatoxin autoradiography was used to study the distribution of vanilloid receptors.[14] The first experiments using TRPV1 specific antibodies were conducted by Tominaga et al. In urinary bladders of the rat, they showed a suburothelial and muscular plexus of tortuous TRPV1 immunoreactive (IR) nerve terminals. TRPV1 labeling was limited to unmyelinated C fibers.[11] These data
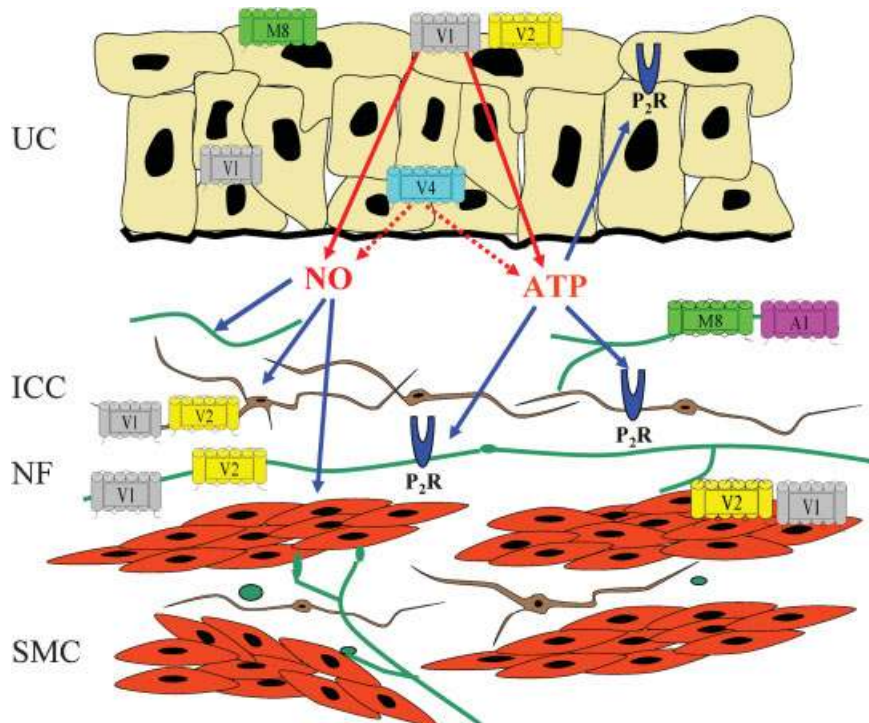

Fig. 1. Distribution of TRP channels throughout the bladder wall. Activation of urothelial TRPV1 and TRPV4 induces ATP and NO release by the urothelial cells. ATP can activate afferent nerves by acting on P_2X_3 receptors. The presence of P_2X and /or P_2Y receptors on urothelial cells, smooth muscle cells and interstitial cells also suggest a paracrien role for released ATP. NO is shown to act on smooth muscle cells, nerve fibres and interstitial cells. Most TRP channels are present on nerve fibres in both the suburothelium and the muscularis, making them sensitive to different stimuli. Direct or indirect (via second messengers like ATP) stimulation of afferent nerves fibres, modulates afferent information to the CNS and thereby influences bladder filling/voiding. UC urothelial cell, NF nerve fibre, IC interstitial cell, SMC smooth muscle cell, V1 TRPV1, V2 TRPV2, V4 TRPV4, A1 TRPA1, M8 TRPM8.

were confirmed by Avelino et al.,[15] who determined the expression of TRPV1 throughout the entire urinary tract in the rat. TRPV1 was present in a mucosal and muscular TRPV1 IR plexus, not only in the bladder, but also in the renal pelvis, the ureters and the proximal urethra. Also in the rat, Birder et al.[12] suggested that besides expression on afferent nerve fibers, TRPV1 is expressed on detrusor smooth muscle cells and urothelial cells, which respond to capsaicin by releasing NO.

In human bladder tissue comparable suburothelial and muscular networks of TRPV1 IR nerve fiber were found. However, myelinated, larger caliber fibers, possibly A delta fibers were also stained.[16,17] Our group found a non-neuronal localization of TRPV1 on detrusor smooth muscle cells and interstitial cells but not on urothelial cells.[17,18] Lazzeri et al.[19] on the other hand showed an intracytoplasmatic labeling of the urothelium, smooth muscle cells, mast cells, and endothelium.

The morphological studies cited above, show a number of discrepancies. These variations can be explained by differences in specimen collection and conservation. Human as well as rat tissue was used. Human specimens were obtained by cold cup biopsies during endoscopy or by taking full thickness biopsies during open surgery or even autopsy. In some protocols, tissues were immersion fixed while in others, tissue was fresh frozen. These different conservation techniques may contribute significantly to observed differences in staining patterns.

TRPV1 in the prostate. In the human prostate, TRPV1 is present on interstitial cells and nerve fibers spreading throughout the prostatic urethral mucosa, verumontanum, ejaculatory ducts, and periurethral prostatic acini.[18,20] No TRPV1 immunoreactive fibers can be detected in the transitional and peripheral zones of the gland. In prostate cancer cell lines PC-3 and LNCaP TRPV1 is expressed by epithelial prostate cells, while this is not the case in normal epithelial cells.[21]

TRPV1 in other parts of the urogenital tract. TRPV1 expression studies in human and rat have shown that TRPV1 is widely expressed throughout the urogenital tract. In the rat's urinary tract, TRPV1 is present on peripheral nerve fibers forming a suburothelial and a muscular network in the renal pelvis, the ureters, the bladder and the proximal urethra. In the distal urethra, TRPV1 IR fibers are only detectable in the suburothelium.[15] In the rat's genital tract TRPV1 mRNA was detected in prostate, testis and penis.[22] TRPV1 is expressed in the human prostate, testicles and seminiferous tubules. Also human specimens from the corpus cavernosum, the glans, the preputium, and the scrotal skin express TRPV1.[22]

Functional data

TRPV1 in the bladder. Bladder instillations with vanilloids like capsaicin and resiniferatoxin have been used for over a decade in the treatment of neurogenic bladder overactivity.[23]

Nevertheless, little is known about the role of TRPV1 in normal bladder physiology. With the development of TRPV1 knock out mice, a model became available to study the function of TRPV1 in the bladder in vivo.

The vanilloid pathway. Electrophysiological studies in cats and rats have revealed the importance of afferent activity in control of the bladder function. In normal circumstances, afferent bladder activity is mediated primarily by small myelinated afferent fibers (Aδ fibers). These afferents pass through the spinal cord to the periaqueductal gray matter in the brainstem, where they give information about bladder fullness to the pons. These nerves form the afferent loop of the spinobulbospinal micturition reflex pathway. A second group of bladder afferents, small unmyelinated C fibers

form an addition pathway. In normal circumstances, most of these C fibers are inactive (silent C fibers) but many of them can be activated by chemical irritants, resulting in bladder contraction and expulsion of the irritant.[24] These C fibers are capsaicin sensitive and mediate the burning sensation after intravesical capsaicin instillations.

TRPV1 as pressure sensor in the bladder. In the bladder, TRPV1 seems to play a crucial role in stretch detection by the urothelial cells. Urothelial tissue produces ATP in response to pressure application and similarly cultured urothelial cells respond to hypotonic stretch by releasing NO and ATP. In TRPV1 knock out mice this stretch evoked urothelial release of NO and ATP was significantly smaller compared to wild type littermates, suggesting that TRPV1 is directly involved in mechanosensing by the urothelium.[25] In our lab, we found that extravesically applied capsazepine (a selective TRPV1 antagonist) decreased the contractile response of isolated bladders to intravesical volume load. These findings support the role of TRPV1 in stretch detection.[26]

Finally there is evidence that bladder filling detected by TRP not only evokes local responses, but that this information is sent to the CNS. In TRPV1 knock out mice there is a significant decrease in c-fos expression in the lumbo-sacral cord in response to bladder distension.[25] Whether TRPV1 channels in the urothelium are solely responsible for this function remains unclear. The presence of TRPV1 in other cell types like interstitial cells, smooth muscle cells, and nerve fibers, suggests these cells might also have a contribution to mechanosensing in the bladder.

Effect of TRPV1 on bladder contractions. TRPV1 knock out mice show enhanced intermictional spotting, whereas normal micturitions seem to be unaffected. This was reflected in the cystometrograms by an increase in the frequency of non-voiding contractions and a regular pattern of voluntary voiding contractions. Whether these non-voiding contractions are responsible for the increased spotting is still uncertain and needs further investigation.[25]

All urethane anesthetized TRPV1 knock out mice display increases in mean bladder capacity and reductions in spinal cord c-fos induction in response to bladder distension. In conscious mice, the micturition frequency is unaffected in these knock outs, suggesting that TRPV1-mediated mechanisms are responsible for setting the micturition threshold under anesthesia, whereas non-TRPV1-mediated mechanisms set the threshold in voluntary conditions.[25]

Because TRPV1 is expressed by various cell types (urothelial cells, interstitial cells, neurons, and smooth muscle cells), it is not easy to understand how TRPV1 contributes to intermictional spotting, non-micturition contractions and abnormal reflex voiding. The use of different bladder preparations (bladders of live animals, bladder strips with or without the urothelium, in vitro isolated whole bladders, urothelial cell cultures) can help us to gain insight in the specific function of TRPV1 in each part of the bladder wall.

Our group studied autonomous bladder activity after pretreatment with piperine and RTX (30 min before pressure measurements). RTX installations induced shorter contractions (macro-transients) with increased amplitudes and a significant decrease in the number of spikes.[27] Similarly piperine instillations caused shorter contractions and more periods of inactivity.[26] In both studies the frequencies of macro-transients were unaffected.

Drake et al. suggest that the bladder is arranged into modules, controlled by the central nervous system as well as the peripheral myovesical plexus. When modules contract separately, they can only cause minor intravesical pressure

changes. When all modules are activated at the same time however, they contract synchronously, resulting in an increase of the intravesical pressure, causing a micturition contraction. TRPV1 seems to play a role in the way these modules interact. More efficient macro-transients (higher amplitude and shorter duration) and a decrease in the number of spikes after treatment with specific TRPV1 agonists PIP and RTX, suggests a better electrical coupling between the different modules, leading to coordinated contractions instead of isolated module contractions.[27]

The increased number of non-micturition contractions in TRPV1 knock-out mice could be the result of a reduced coupling between the bladder modules. On the other hand, these non-micturition contractions can also be a compensatory mechanism by which the bladder tries to increase the afferent information to the central nervous system, trying to detect the amount of bladder filling in absence of the stretch detector TRPV1.

TRPV1 in diuresis and natriuresis. TRPV1 is present in the renal pelvis on afferent nerve fibers, which form a suburothelial and a muscular plexus.[15] In the rat, capsaicin instilled in the renal pelvis at one site causes ipsilateral as well as contralateral afferent renal nerve activity, resulting in an increase of diuresis and natriuresis at both kidneys, without influencing blood pressure. It is suggested that this phenomenon is caused by an ipsilateral and contralateral renorenal reflex mediated by capsaicin sensitive nerves. Renal denervation at the site of instillation and admission of capsazepine inhibits these renorenal reflexes.[28,29]

In human patients, intravesical administration of capsaicin can also increase natriuresis and diuresis. This suggests the presence of a vesico-renal reflex, mediated by TRPV1 positive nerve fibers.[30] It appears that intravesical irritation promotes expulsion of the irritant, not only by increasing bladder contractility, but also by increasing diuresis.

TRPV1 in the urethra. TRPV1 positive nerve fibers form two plexuses in the proximal urethra, but only a single suburothelial plexus in the distal part of the urethra.[15] In the dog urethra, capsaicin causes relaxation of the smooth muscles precontracted by noradrenalin. This effect is mediated by the release of NO, after activation of capsaicin sensitive afferents. Neuropeptides, like substance P and CRGP released by the afferent nerves, do not seem to be involved in this process.[31] In urethral striated muscle of the rat, capsaicin also induces relaxation, but in a CGRP mediated way.[32] In urethane anesthetized rats, intravesical infusion of saline induces reflex bladder contraction as well as activation of the external urethral sphincter, leading to intraluminal pressure high frequency oscillations (IPHFO). Intraurethral capsaicin instillation enhances these IPHFO's and when the bladder is empty, intraurethral capsaicin is able to evoke these IPHFO's itself. These capsaicin evoked contractions seem to be mediated by a urethra-urethral reflex, via the pundendal nerves. Intraurethral capsaicin activates TRPV1 positive nerve fibers and leads to the suprapinally mediated activation of the external urethral sphincter.[33] This effect seems to be opposite to the direct effect of capsaicin on isolated urethra striated muscle tissue. Depression of this TRPV1 mediated external sphincter activation however, could be a possible explanation for the increased spotting seen in TRPV1 knock out mice.[25] The exact function of TRPV1 in the urethra is still unclear, but it appears to be involved in the complex coordination between bladder and urethra.

TRPV2. TRPV2, also known as VRL1, shares a 50% homology with TRPV1. TRPV2 expressed in a subset of DRG neurons and in the hypothalamus, but is also highly expressed outside the nervous system in the heath, the gastro-intestinal tract and smooth muscle cells.[8,34] TRPV2 is activated by highly noxious temperatures ($>52°C$) and IGF-1.[35] TRPV2 can also be activated by hypo-osmotically induced cell swelling and functions as a mechanosensor in vascular myocytes.[36] TRPV2 is associated with dystrophic cardiomyopathy[37] and inflammation-induced hyperalgesia.[38] Due to lack of a TRPV2 knock out model and TRPV2 specific activators and inhibitors, the knowledge about TRPV2's physiological role is still very limited.[8]

TRPV2 in the urogenital tract. Before TRPV2 was described as a separate channel, it was thought that TRPV2, or VRL1 was a variant of VR1 (now TRPV1). In our group we used aVR1 and aVRL1 antibodies to stain for VR1 in the human bladder. Both types of antibodies gave a similar staining pattern in small nerve fibers, as well as suburothelial interstitial cells and smooth muscle cells.[17] Post factum it is tempting to relate the VRL1 immunoreactivity in the human bladder to the presence of TRPV2, but results might be influenced by the lack of specificity of the antibodies. By using RT-PCR, Birder et al. showed a similar expression pattern of TRPV1 and TRPV2. Both VR1 and VRL1 mRNA was found is cultured urothelial cells, bladder urothelium tissue, dissociated bladder smooth muscle cells and de-epithelialized bladder tissue.[12]

From these data we can conclude that TRPV2 is probably expressed in the urogenital tract, but further research is needed to assess its exact localization. Being a stretch activated channel, TRPV2 might play a role in the detection of bladder filling. If TRPV1 and TRPV2 would indeed have a similar distribution pattern in the bladder, they might form functional heterotetramers like in other organs.[39]

TRPV4. TRPV4 was discovered in 2000 by Liedtke et al.[40] as an osmotically activated channel, showing structural similarities with OSM-9 as well as VR1. TRPV4 is a non-selective cation channel activated by various physical and chemical stimuli such as hypotonicity, (cell swelling), moderate heat, acid and agonists like phorbol derivates (4αPDD).[9,40−43] TRPV4 is expressed in the neurons of circumventricular organs in the mammalian CVS, in the kidneys, the liver, the airway and the hearth.[40,44,45] TRPV4 is expressed in afferent as well as autonomous nerves.[43,45]

Using a TRPV4 knock out model, it became clear that TRPV4 plays an essential role for regulating the osmotic balance in vivo, functioning as an osmotic sensor in the CNS.[46] Knock out mice show an impaired detection of acid and pressure, but hot and touch sensation are still intact.[43] TRPV4 is therefore suggested to be an important mechanosensor in the body.

Recent articles suggest that TRPV4 is also shear stress sensitive and is able to act as a flow sensor in endothelial cells as well as in cortical collecting ducts.[47,48]

TRPV4 in the urological tract. TRPV4 is expressed in the prostate. Northern blot analysis revealed the presence of TRPV4 (TRPVL2) mRNA transcripts in the prostate.[45] Using monoclonal TRPV4 antibodies our lab showed the presence of TRPV4 on the basal urothelial cells in rat bladders. There was no TRPV4 IR in the suburothelium or in the superficial urothelial cells.[49]

TRPV4 knock out mice showed an altered micturition pattern, having significantly more intermictional spotting, whereas micturitions itself seem to be normal. Performing continuous cystometrograms we found an increase of the intermictional interval and an increased number of non micturition contractions.[49]

Nakamura et al.[50] also checked the micturition pattern in TRPV4 knock out mice. They presented an increase of both mean micturition volume per void and intermictional

interval. These results are similar to ours, suggesting that TRPV4 indeed plays an important role in bladder physiology.

Another possible role for TRPV4 might be the detection of urine flow in the urethra, hereby activating the urethra to bladder reflex, promoting bladder emptying. Once micturition is started, bladder emptying is facilitated by contraction of the detrusor. The mechanisms responsible for these detrusor contractions are still poorly understood, but bladder to bladder and urethra to bladder reflexes seem to play an important role. In the awake ewe urethra to bladder reflexes are stimulated by urethral infusion saline at body temperature, but not at temperatures below the physiological rate.[51] As TRPV4 in activated by shear stress at body temperature, it is good candidate for mediating the urethra to bladder reflex. Using the TRPV4 knock out model is would be interesting to asses this hypothesis and evaluate the possible role of TRPV4 for flow detection in the urethra.

TRPM8. TRPM8 was first discovered in the prostate. By looking for new markers of prostate cancer, Tsavaler et al.[52] found a new member of the TRP family that was expressed in normal prostate tissue and was up-regulated in prostate cancer. TRPM8 is member of the temperature sensitive TRP channels, but in contradiction to TRPV1, TRPV2, TRPV3, and TRPV4 it is activated by cold temperatures ($<23°C$).[9,53] Pharmacological agents that evoke a "cool sensation", like menthol and ilicin can activate TRPM8.[53] TRPM8 is expressed in DRG and TG neurons that do not express TRPV1, IB4, or CGRP. This suggests that TRPM8 is expressed in a subpopulation of thermoceptive and nociceptive nerves different from the TRPV1 expressing subpopulation.[53]

TRPM8 in the urogenital tract. As mentioned above, TRPM8 is expressed in the prostate, where it is located on the cell membrane of the basal epithelial cells.[52] TRPM8 expression studies in the rat showed the presence of TRPM8 not only in prostate but also in testis, penis, bladder and dorsal root ganglion tissue. In the human urogenital tract TRPM8 is expressed in the prostate, the testicles, seminiferous tubules, scrotal skin, and bladder.[54]

While in the study by Stein et al.[54] TRPM8 expression in the human bladder was limited to the urothelium, Mukerji et al.[55] showed TRPM8 immunoreactivity in the bladder urothelium as well as in fine nerve fibers scattered in the suburothelium.

In patients with spinal cord lesion, infusion of ice cold water in the bladder causes reflex detrusor contraction.[56] This phenomenon has been used as a diagnostic test, the ice water test, in patients with bladder overactivity to assess whether their bladder problems have a neurogenic origin.[57] This reflex activity is likely to be mediated by cold sensitive TRP channels like TRPM8 and TRPA1. In the guinea pig bladder, infusion of menthol indeed affects the micturition pattern (lowering of the volume threshold, increase of micturition pressure). In addition, when guinea pig bladders are pre-treated with menthol, cold saline itself is able to cause reflex micturition.[58]

In the human prostate, TRPM8 is expressed in the androgen dependent apical epithelial cells and smooth muscle cells.[59] TRPM8 expression is regulated by androgens and it is suggested that TRPM8 plays an important role in normal prostate secretion. Consequently TRPM8 could have an influence on sperm mobility.[59]

The role of TRPM8 in the testis and scrotal skin is still unknown, but TRPM8 may mediate temperature homeostasis of the testes, by positioning them closer or further to the body. If TRPM8 would indeed be responsible for both sperm mobility and temperature homeostasis in the testicles, it would be a highly important factor in male fertility.

TRPA1. TRPA1 is the only mammalian member of the Ankyrin TRP subfamily. TRPA1 is activated by covalent modification of cysteines at the N-terminus, for example by some plant derived agonists like allyl isothiocyanate (the pungent compound in mustard oil), allicin (garlic), and cinnamaldehyde (in cinnamon).[60,61] TRPA1 is also activated by intracellular Ca^{2+} and is a voltage dependent channel. It is considered as a mechanosensor based on genetic evidence and expression in heterologous systems.[62] There is still discussion to whether or not TRPA1 is activated by noxious cold ($<17°C$).[8] TRPA1 is expressed in DRG and TG neurons and in hair cells.[63,64] In sensory nerves, TRPA1 is co-expressed with TRPV1. It has been suggested that TRPA1 participated in noxious pain perception, mechanosensing and hearing, but conflicting observations were made in two independently developed knock out mice models.[65,66]

TRPA1 in the urogenital tract. Immunohistochemical studies revealed the presence of TRPA1 in sensory nerve fibers innervating the trigone of the bladder.[63] No non-neuronal expression of TRPA1 in the urogenital tract has been reported so far.

When TRPA1 agonists isothiocyanate and cinnamaldehyde were applied to isolated rat bladder strips, they caused concentration dependent bladder contractions. Application of maximal agonist concentrations induced rapid desensitization. Cross desensitization was noted between TRPA1 agonists and the TRPV1 agonist capsaicin and also between different TRPA1 agonists. In contradiction to TRPV1, TRPA1 mediated contractions were sensitive to tetrodoxine and indomethacin, but resistant to atropine and suramin. TRPA1 mediated contractions were dependant on extracellular Ca^{2+} and TRPA1 agonist application to the urinary bladder caused release of prostaglandine E_2 and substance P.[67]

We conclude that TRPA1 activation induces bladder contractions, mediated by sensory afferent stimulation and release of neuropeptides and prostanoids.

TRP IN PATHOPHYSIOLOGY

TRPV1

TRPV1 in bladder disorders

(1) *Neurogenic detrusor overactivity (NDO)*: In cats and rats the reflex pathways controlling the bladder after chronic spinal cord injury differ markedly from normal bladder control. Spinal cord injury (SCI) is associated with the emergency of an aberrant sacral spinal reflex that is mediated by capsaicin sensitive C-fiber afferents in the bladder suburothelium.[68] These observations formed the basis for performing bladder instillations with vanilloids in patients with neurogenic bladder overactivity. These vanilloids can desensitize the TRPV1 positive C fibers, thereby interrupting the afferent limb of the spinal reflex arc.

Patients with NDO have an increased immunoreactivity of PGP9.5 and TRPV1 in the suburothelium and an increased TRPV1 reactivity in the basal layers of the urothelium compared to control patients. In addition, patients with NDO clinically responding to intravesical instillations of resiniferatoxin show a significant decrease of this TRPV1 immunoreactivity in both the suburothelium and the basal urothelial layers compared to non-responders, suggesting a role for TRPV1 in the pathophysiology of NDO.[69,70] The effects of vanilloids on urothelial TRPV1 indicate that vanilloid actions are more complex than simple C fiber desensitization.

The exact effect of capsaicin or its analogues on the different TRPV1 positive structures (urothelium, afferent nerve fibers, interstitial cells) still needs further elucidation.

(2) *Overactive bladder (OAB) with and without detrusor over-activity (OAD): Patients with OAB without demonstrable OAD*,[71] who have a normal filling cystometry but an early first sensation during bladder filling due to sensory discomfort, show an increased TRPV1 mRNA expression in the trigonal mucosa. In these patients, TRPV1 expression levels in the trigone are inversely correlated to the volume at first sensation during bladder filling.

In patients with *idiopathic detrusor overactivity* on the other hand, there are no changes in TRPV1 expression levels, suggesting a distinct molecular basis between SU and IDO.[72] These data provide a basis for the use of intravesical vanilloid instillations in patients with OAB without demonstrable OAD. No data from clinical trials in this patient group are available so far.

(3) *Painful bladder syndrome (PBS) and interstitial cystitis (IC)*: Painful bladder syndrome is the complaint of suprapubic pain related to bladder filling, accompanied by other symptoms such as increased daytime and night-time frequency, in the absence of proven urinary infection or other obvious pathology.[73] PBS is used to describe this syndrome of complaints, while IC is preserved for typical cystoscopic and histological features. In patients with PBS there is a significant increase in the number suburothelial TRPV1 nerve fibers. In these patients the relative TRPV1 nerve density in the urothelium is correlated with their pain scores. Inflammatory bladder changes might be the driving force in these alternations, with nerve growth factor (NGF) being a key player. NGF is increased in inflammation and the levels of NGF are increased in the urine and urothelium of patients with PBS. As NGF stimulates TRPV1 expression, NGF contributes to the hyperproliferation of TRPV1 nerve fibers in patients with PBS.

Not only an increased expression, but also an increased sensitivity of TRPV1 nerve fibers in the bladder wall can contribute to the bladder dysfunction. In cats with feline interstitial cystitis (FIS) afferent nerves are larger, have an increased excitability and exhibit a slower desensitization to capsaicin as a consequence of an increased phosphorylation of TRPV1 by PKC.[74,75]

Theoretically, intravesical vanilloid instillations, causing TRPV1 nerve fiber desensitization could be an adequate therapy in people with PBS. The results from the first, small randomized controlled trial with RTX showed promising results, but a recently published RCT using a much larger population was unable to show any benefit of intravesical RTX instillations for patients with interstitial cystitis.[76,77]

(4) *Hemorrhagic Cystitis*: Irritating substances in the bladder activate the C fibers, leading to bladder contraction and expulsion of the irritant. In cyclophosphamide induced hemorrhagic cystitis anandamide levels in the bladder are increased, activating TRPV1 and leading to the development of hyper-reflexia and hyperalgesia.[78] These effects can be blocked by capsazepine and RTX pre-treatment.

TRPV1 in vulvodynia in vulval vestibulitis. The term vulvodynia comprises all conditions of vulval pain, affecting the vulval vestibule. Vulval vestibulitis is a condition characterized by the sudden onset of painful burning sensation, mechanical allodynia and hyperalgesia at the vulval vestibule. In patients with vulvodynia of the vestibulitis type, there is an overexpression of TRPV1 positive nerve fibers in the papillary dermis and epidermis.[79] Local application of capsaicin cream in these patients successfully relieved the symptoms of vulval vestibulitis, reduced dyspareunia and resulted in more frequent sexual relations.[80]

TRPV1 in erectile dysfunction. In a study conducted in male diabetes patients with erectile dysfunctions it was suggested that dysfunction of unmyelinated C fibers plays a key role in erectile dysfunction.[81] TRPV1 positive afferents take part in a reflex arc, leading to penile erection. In impotent men, intraurethral capsaicin induces penile erections, comparable to the effect of papaverine injections. These data suggest the involvement of a capsaicin sensitive urethra-corpora cavernosa reflex arc.[82] No other clinical trials have been reported, the desensitizing effect vanilloid instillations will probably worsen erections in long term, instead of ameliorating them.

TRPV1 in transitional cell carcinoma (TCC). In normal bladder tissue TRPV1 is present on all the urothelial cells. TRPV1 staining is higher in the more differentiated superficial urothelial cells and decreases toward the basal layers.[12,19] In bladder TCC there is a progressive loss of TRPV1 expression as tumor grade increases, with TRPV1 being absent in pT4 tumors.[83] Thus, TRPV1 expression seems to be related to the degree of cell differentiation, cell growth and apoptosis.

TRPV1 activation in neurons, airway epithelial cells and even in pancreatic tumor cells can induce apoptosis.[84,85] Hence, TRPV1 on low stage TCC cells could be a possible target for intravesical vanilloid therapy. Circumin, a popular Indian food spice, derived form the rhizome of the plant *Curcuma longa Linn*, is a potent cytotoxic for in vitro bladder tumor cell lines. In a murine bladder tumor model Circumin inhibits tumor implantation and tumor growth. The action mechanism by which curcumin induces apoptosis is still poorly understood, but interestingly, curcumin has a vanilloid structure and possesses a vanilloid like activity by selectively binding TRPV1. TRPV1 activation by curcumin could be a possible mechanism by which apoptosis is induced. Future studies using other vanilloids are needed to determine the contribution of TRPV1 activation in inducing apoptosis and preventing implantation of TCC cells.

TRPM8

TRPM8 in functional bladder disorders. TRPM8 is expressed in bladder urothelium as well as myelinated (a-delta) and unmyelinated (C) nerve fibers in the suburothelium. Bladder specimens of patients with idiopathic detrusor overactivity and painful bladder syndrome show a significant increase in the number of TRPM8 positive C fibers, while the numbers of TRPM8 immunoreactive a delta fibers and urothelial cells is not significantly changed.[55] In these patients the density of TRPM8 positive nerves fibers is positively correlated to the Frequency and Pain scores. These data indicate a role for TRPM8 in the pathophysiology of IDO as well as PBS.

TRPM8 in prostate cancer. TRPM8 was initially described as a prostate specific TRP that was overexpressed in malignant tissue.[52] The expression of TRPM8 in prostate cancer seems to be regulated by androgens.[59,86,87] TRPM8 expression is reduced after anti-androgen therapy and when prostate cancer becomes androgen independent.[86] It is assumed that TRPM8 gene transcription is regulated by direct binding of

the androgen-androgen receptor complex to the androgen response elements in the TRPM8 gene.[88]

TRPM8 is a possible diagnostic marker in the differential diagnosis of prostate cancer and a prognostic marker for androgen dependency and progression of the tumor. In the future TRPM8 might also be used as a potential therapeutic target in hormone sensitive prostate cancer. Sustained calcium influx by application of menthol caused apoptosis in the androgen dependant LNCaP prostate cancer cell line.[87]

A more extensive review about TRPM8 medicated mechanisms in prostate cancer has recently been published.[88]

TRPV4 and TRPC4 in nocturnal enuresis. Hereditary factors are very important in the aetiology of nocturnal enuresis, although somatic and psychosocial environmental influences exert a major modulatory effect. Nocturnal enuresis is most commonly inherited in an autosomal dominant way. Linkage analysis in families with nocturnal enuresis associated the disorder with four different chromosomal loci, but no association with candidate genes could we proven so far.[89] Interestingly two of the associated loci refer to the location of TRP channel genes, namely TRPV4 (12q) and TRPC4 (13q13).[90] The association of these genes with nocturnal enuresis has not been checked so far. As TRPV4 has a role in bladder physiology, it would be an interesting new candidate gene.

TRPV1 as Therapeutic Target

Vanilloids. Almost two decades ago Maggi et al.[91] were the first ones to use intravesical instillations of capsaicin in patients with hypersensitive bladder disorders. Since then, a large amount of studies have been published about the use of intravesical vanilloids for different types of functional bladder disease.

The first vanilloid used was capsaicin, the pungent ingredient of hot chilli peppers (*capsicum annuum*). In patients with neurogenic detrusor overactivity capsaicin instillations are effective in improving both clinical and urodynamic parameters. In a meta-analysis by de Sèze et al. 115 patients with neurogenic detrusor hyper-reactivity, treated with capsaicin were identified. 97 (84%) of them experienced an improvement of their complaints. Only two randomized controlled trials using capsaicin in 30% ethanol in saline have been published so far. These trials by Wiart et al.[92] and de Sèze et al.[93] show a significant improvement in continence, frequency, urgency and patient satisfaction one month after capsaicin instillation. After bladder instillations, both the treatment and control (30% ethanol) group reported immediate side effects (suprapubic burning sensation, urgency, hot flushes, hot flushes, autonomic hyperreflexia) that lasted almost 2 weeks.

As previously mentioned, TRPV1 is upregulated in patients with painful bladder syndrome. A randomized controlled trial by Lazzeri et al.[94] showed that instillation of 10 μM capsaicin induces a significant improvement of frequency and nocturia but not of urgency or pain score compared to placebo.

Resiferatoxin is the active ingredient in the drug euphorbium, the air-dried latex of the cactus like plant *Euphorbia resinifera*. RTX is more than a thousand times more potent than capsaicin in desensitizing contractions in the urinary bladder, but has a similar potency in activating TRPV1.[95] It was suggested that RTX could provide better results than capsaicin with less side effects, as it can cause desensitization, without activating TRPV1.

In patients with neurogenic detrusor overactivity, Silva et al.[96] compared the effect of instillation of 50 nM RTX in ethanol 10% to instillation of vehicle alone. In the RTX treatment group, there was a significant increase of bladder capacity and a significant decrease of frequency and urinary incontinence. In patients with painful bladder syndrome however, RTX does not seem to have a beneficial effect.[97]

Several randomized controlled trials have compared the effect of RTX to capsaicin. Giannantoni et al. compared the effect of 2 mM capsaicin in 30% ethanol in saline to 100 nM RTX in saline. They showed RTX to be superior to capsaicin in improving urodynamic and clinical parameters in patients with chronic spinal cord injury. In addition, RTX did not evoke the same irritating side effects as capsaicin.[98] de Sèze et al.[99] however, did not observe any differences in efficacy and tolerance between capsaicin dissolved in glucidic solvent and RTX dissolved in 10% ethanol. They suggest that differences between the effect of capsaicin and RTX in previous studies are strongly influenced by the vanilloid solute. Our lab showed indeed that ethanol is not an inert vehicle, but strongly impairs bladder contractility.[100] Ethanol activates TRPV1 and inhibits TRPM8.[101] Therefore, the results of studies conducted with ethanol as a solvent for vanilloids need to be interpreted with care.

Capsaicin is a highly hydrophobic molecule that is insoluble in water. Therefore it was usually formulated in saline using 30% of ethanol. As ethanol is not considered to be an appropriate solvent any more, there is a need for better and safer vehicles to dissolve vanilloids in water. Liposomes (LP) are vesicles composed of concentric phospholipids bilayers, separated by aqueous compartments. Hydrophobic vanilloids can be entrapped in the phospholipids bilayers of liposomes and brought into an aqueous solution. The liposomes can fuse with the cell membrane and deliver capsaicin to the cell.[102] Hydrogels can also be used to dissolve capsaicin. These gels have an amphiphilic nature and can dissolve vanilloids. At room temperature these polymers are in a fluid state suitable for intravesical instillation. In the bladder they change to the gel state, covering the bladder surface.[102] In a comparable study between 30% ethanol, liposomes and hydrogels as a vehicle for capsaicin, liposomes were as effective as ethanol 30% without causing tissue damage. Hydrogels were a little less effective but equally safe as liposomes, suggesting that both liposomes and hydrogels can serve as valuable alternatives for capsaicin delivery. Another alternative to dissolve capsaicin is the use of a glucidic solute. This solute consists of a seven cycle oligosaccharide molecule dissolved in isotonic aqueous solution. The effect of capsaicin 1 mM in glucidic solute in comparison to glucidic solvent alone was compared in a randomized controlled trial in patients with neurogenic detrusor overactivity. After 1 month, patients treated with capsaicin in glucidic solute scored significantly better than controls with regard to clinical parameters, bladder capacity and quality of life. Pelvic pain during instillation was reported more frequently in the treatment group (58% compared to 12,5% in the control group).[103]

Short term evidence indicates that these new solvents are a safe and effective alternative for ethanol to dissolve vanilloids. However, long term results about efficacy and tolerance of repeated applications still need to be evaluated.

Botulinum toxin A. The rationale for the use of Botulinum neurotoxin A (BoNT/A) injections as a treatment for bladder overactivity is based on the theory that BoNT/A temporarily blocks the presynaptic release of acetylcholine from the efferent parasympatic nerves, hereby paralyzing the bladder.[104] There is accumulating evidence however that BoNT/A also has a complex effect on afferent mechanisms.[105]

Intravesical injections of BoNT/A significantly decrease the TRPV1 immunoreactivity of suburothelial nerve fibers, without influencing suburothelial PGP9.5 expression in patients with

TABLE I. Overview of TRP Channel Expression and Function in the Urogenital Tract

Channel	Proposed activation mechanisms	Localization	Proposed involvement in physiology	Proposed involvement in pathology
TRPV1	Depolarization, heat ($\geq 43°$C), low pH ($\leq 5,9$), vanilloids, endovanilloids, PKC, anandamide, 12-(S)-HPETE, 5-(S)-HETE, leukotriene B, sper mide, 2-APB, OEA, PKA, decreased PI(4,5)P$_2$, inhibited by stretch (short splice variant)	Renal pelvis, ureter, bladder, urethra (urothelial cells, interstitial cells, nerve fibers, and smooth muscle cells), prostate (interstitial cells and nerve fibers), seminiferous tubules, testicles, corpus cavernosum, glans, scrotal skin and preputium	Detection of chemical irritants, intravesical pressure sensor, diuresis and natriuresis, erections?	Neurogenic detrusor overactivity, sensory urgency, PBS/IC, cystitis
TRPV2	Noxious heat (>53°C), mechanical (stretch, swelling), growth factors, IGF-1 HA, 2-APB	Bladder (urothelium, interstitial cells, nerve fiber, smooth muscle cells)	Intravesical pressure sensor?	
TRPV4	Moderate heat (>24°C), cell welling, shear stress, PKC, epoxyeicosatrieonic acids, anan damide, 4α-PDD and other phorbols	Bladder (basal urothelial cells), prostate, testicles	Urethrovesical reflex? Intravesical pressure sensor	Nocturnal enuresis?
TRPM8	Depolarization, cold (8–28°C), menthol, ilicin, Ca^{2+}, increased intracellular pH, PI(4,5)P2	Bladder (urothelium, nerve fibers), prostate (basal epithelial cells, smooth muscle cells), seminiferous tubules, testicle, scrotal skin	Reflex micturition, normal prostate secretion, sperm mobility, testical temperature homeostasis	Prostate cancer, idiopathic detrusor over activity, PBC/IC, Ice water test
TRPA1	Δ^9-tetrahydrocannabinol (THC), prolonged noxious cold? Mechanical stress, voltage dependent, [Ca]$_i$ dependent, OAG and down stream of receptor-mediated PLC activation, covalent modification of N-terminal cystines (isothiocyanates, cinnamaldehyde, allicin)	Bladder (nerve fibers)	Bladder contractions	

NDO.(70) This means that TRPV1 expression on afferent nerves is decreased, while the nerves themselves stay intact. By inducing cleavage of SNAP-25, BoNT/A causes a destabilization of the SNARE complex and inhibits the expression of TRPV1 on peripheral nerve fibers.[106] Thus, BoNT/A affects bladder contractility by influencing both efferent and afferent innervation and its effect on afferent innervation is different than that of vanilloids.

FUTURE RESEARCH

In functional bladder disorders, most of our therapies target efferent mechanisms mediating bladder contractions. Accumulating evidence shows however that disruption of afferent mechanisms is the driving force behind pathophysiological changes in bladder overactivity.[107] This could explain why many of our conventional therapies fail to improve bladder complaints. TRP channels are able to respond to a large variety of physical and chemical stimuli, making them privileged candidates to function as primary sensing molecules in the bladder. TRPs are involved in normal bladder physiology. Overexpression or dysfunction of these channels induces pathophysiological processes, leading to functional bladder disorders. Therefore, research about TRP channels in the bladder is an unexplored and very promising domain that can help us understand the molecular mechanisms leading to urinary (in)continence (Table I).

TRP channels also seem to be involved in cell differentiation, cell growth and apoptosis. As we know, calcium overload in cells induces apoptosis. Thus, TRP channels located at the cell membrane of tumor cells, could be interesting new targets for drug therapy, immunotherapy or even gene therapy. TRPM8 and TRPV1 seem to be involved in prostate and urothelial cancer respectively. Little is known however about the changes in expression of these and other TRP channels during differentiation and carcinogenesis. Research is needed to map the expression patterns of these channels, determine their contribution to carcinogenesis and explore the application of TRP agonist in the treatment of cancer.

In different branches of urology (uro-ocology, functional urology) research on the domain of TRP channels will provide new insights and possibilities in patients who cannot be cured with our current understandings and conventional therapies.

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REFERENCES

1. Cosens DJ, Manning A. Abnormal electroretinogram from a Drosophila mutant. Nature 1969;224:285–7.
2. Montell C, Rubin GM. Molecular characterization of the Drosophila trp locus: A putative integral membrane protein required for phototransduction. Neuron 1989;2:1313–23.
3. Nilius B, Voets T. TRP channels: A TR(I)P through a world of multifunctional cation channels. Pflugers Arch 2005;451:1–10.
4. Voets T, Nilius B. TRPs make sense. J Membr Biol 2003;192:1–8.

5. Nilius B. Store-operated Ca2+ entry channels: Still elusive. Sci STKE 2004; 2004:e36.
6. Nilius B, Owsianik G, Voets T, et al. Transient receptor potential cation channels in disease. Physiol Rev 2007;87:165–217.
7. Lewis RS. The molecular choreography of a store-operated calcium channel. Nature 2007;446:284–7.
8. Nilius B, Voets T, Peters J. TRP channels in disease. Sci STKE 2005;295:re8.
9. Pedersen SF, Owsianik G, Nilius B. TRP channels: An overview. Cell Calcium 2005;38:233–52.
10. Caterina MJ, Schumacher MA, Tominaga M, et al. The capsaicin receptor: A heat-activated ion channel in the pain pathway. Nature 1997;389:816–24.
11. Tominaga M, Caterina MJ, Malmberg AB, et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 1998;21:531–43.
12. Birder LA, Kanai AJ, de Groat WC, et al. Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. Proc Natl Acad Sci USA 2001;98:13396–401.
13. Caterina MJ, Leffler A, Malmberg AB, et al. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 2000;288:306–13.
14. Szallasi A, Conte B, Goso C, et al. Vanilloid receptors in the urinary bladder: Regional distribution, localization on sensory nerves, and species-related differences. Naunyn Schmiedebergs Arch Pharmacol 1993;347:624–9.
15. Avelino A, Cruz C, Nagy I, et al. Vanilloid receptor 1 expression in the rat urinary tract. Neuroscience 2002;109:787–98.
16. Yiangou Y, Facer P, Ford A, et al. Capsaicin receptor VR1 and ATP-gated ion channel P2X3 in human urinary bladder. BJU Int 2001;87:774–9.
17. Ost D, Roskams T, Van der Aa F, et al. Topography of the vanilloid receptor in the human bladder: More than just the nerve fibers. J Urol 2002;168:293–7.
18. van der Aa F, Roskams T, Blyweert W, et al. Interstitial cells in the human prostate: A new therapeutic target? Prostate 2003;56:250–5.
19. Lazzeri M, Vannucchi MG, Zardo C, et al. Immunohistochemical evidence of vanilloid receptor 1 in normal human urinary bladder. Eur Urol 2004;46:792–8.
20. 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.
21. Sanchez MG, Sanchez AM, Collado B, et al. Expression of the transient receptor potential vanilloid 1 (TRPV1) in LNCaP and PC-3 prostate cancer cells and in human prostate tissue. Eur J Pharmacol 2005;515:20–7.
22. Stein RJ, Santos S, Nagatomi J, et al. Cool (TRPM8) and hot (TRPV1) receptors in the bladder and male genital tract. J Urol 2004;172:1175–8.
23. Szallasi A, Fowler CJ. After a decade of intravesical vanilloid therapy: Still more questions than answers. Lancet Neurol 2002;1:167–72.
24. Fowler CJ. Bladder afferents and their role in the overactive bladder. Urology 2002;59:37–42.
25. Birder LA, Nakamura Y, Kiss S, et al. Altered urinary bladder function in mice lacking the vanilloid receptor TR PV1. Nat Neurosci 2002;5:856–60.
26. Gevaert T, Vandepitte J, Hutchings G, et al. TRPV1 is involved in stretch-evoked contractile changes in the rat autonomous bladder model: A study with piperine: A new TRPV1 agonist. Neurourol Urodyn 2007;26:440–50.
27. Gevaert T, Vandepitte J, Ost D, et al. Autonomous contractile activity in the isolated rat bladder is modulated by a TRPV1 dependent mechanism. Neurourol Urodyn 2007;26:424–32.
28. Kopp UC, Smith LA. Inhibitory renorenal reflexes: A role for substance P or other capsaicin-sensitive neurons. Am J Physiol 1991;260:R232–R239.
29. Zhu Y, Wang Y, Wang DH. Diuresis and natriuresis caused by activation of VR1-positive sensory nerves in renal pelvis of rats. Hypertension 2005; 46:992–7.
30. Lazzeri M, Barbanti G, Beneforti P, et al. Vesical-renal reflex: Diuresis and natriuresis activated by intravesical capsaicin. Scand J Urol Nephrol 1995;29:39–43.
31. Nishizawa S, Igawa Y, Okada N, et al. Capsaicin-induced nitric-oxide-dependent relaxation in isolated dog urethra. Eur J Pharmacol 1997;335:211–9.
32. Parlani M, Conte B, Goso C, et al. Capsaicin-induced relaxation in the rat isolated external urethral sphincter: Characterization of the vanilloid receptor and mediation by CGRP. Br J Pharmacol 1993;110:989–94.
33. Conte B, Maggi CA, Giachetti A, et al. Intraurethral capsaicin produces reflex activation of the striated urethral sphincter in urethane-anesthetized male rats. J Urol 1993;150:1271–77.
34. Caterina MJ, Rosen TA, Tominaga M, et al. A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 1999;398:436–41.
35. Kanzaki M, Zhang YQ, Mashima H, et al. Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. Nat Cell Biol 1999;1:165–70.
36. Muraki K, Iwata Y, Katanosaka Y, et al. TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. Circ Res 2003;93:829–38.
37. Inoue R, Jensen LJ, Shi J, et al. Transient receptor potential channels in cardiovascular function and disease. Circ Res 2006;99:119–31.
38. Shimosato G, Amaya F, Ueda M, et al. Peripheral inflammation induces up-regulation of TRPV2 expression in rat DRG. Pain 2005;119:225–32.
39. Rutter AR, Ma QP, Leveridge M, et al. Heteromerization and colocalization of TrpV1 and TrpV2 in mammalian cell lines and rat dorsal root ganglia. Neuroreport 2005;16:1735–9.
40. Liedtke W, Choe Y, Marti-Renom MA, et al. Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. Cell 2000;103:525–35.
41. Watanabe H, Vriens J, Suh SH, et al. Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells. J Biol Chem 2002;277:47044–51.
42. Watanabe H, Davis JB, Smart D, et al. Activation of TRPV4 channels (hVRL-2/mTRP12) by phorbol derivatives. J Biol Chem 2002;277:13569–77.
43. Suzuki M, Mizuno A, Kodaira K, et al. Impaired pressure sensation in mice lacking TR PV4. J Biol Chem 2003;278:22664–8.
44. Strotmann R, Harteneck C, Nunnenmacher K, et al. OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. Nat Cell Biol 2000;2:695–702.
45. Delany NS, Hurle M, Facer P, et al. Identification and characterization of a novel human vanilloid receptor-like protein, VRL-2. Physiol Genomics 2001;4:165–74.
46. Liedtke W, Friedman JM. Abnormal osmotic regulation in trpv4-/- mice. Proc Natl Acad Sci USA 2003;100:13698–703.
47. Kohler R, Heyken WT, Heinau P, et al. Evidence for a functional role of endothelial transient receptor potential V4 in shear stress-induced vaso-dilatation. Arterioscler Thromb Vasc Biol 2006;26:1495–502.
48. Taniguchi J, Tsuruoka S, Mizuno A, et al. TRPV4 as a flow sensor in flow-dependent K+ secretion from the cortical collecting duct. Am J Physiol Renal Physiol 2007;29:F667–73.
49. Gevaert T, Vriens J, Everaerts W, et al. TRPV4 is localised on urothelium: Does it play a role in afferent bladder signalling? Eur Urol Suppl 2007;6:38.
50. Nakamura Y, Suzuki M, Mizuno A, et al. Bladder function in mice lacking TRPV4. Presented at ICS 2006 as a non-discussion poster.
51. Combrisson H, Allix S, Robain G. Influence of temperature on urethra to bladder micturition reflex in the awake ewe. Neurourol Urodyn 2007;26:290–5.
52. Tsavaler L, Shapero MH, Morkowski S, et al. Trp-p8, a novel prostate-specific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins. Cancer Res 2001;61:3760–9.
53. Peier AM, Moqrich A, Hergarden AC, et al. A TRP channel that senses cold stimuli and menthol. Cell 2002;108:705–15.
54. Stein RJ, Santos S, Nagatomi J, et al. Cool (TRPM8) and hot (TRPV1) receptors in the bladder and male genital tract. J Urol 2004;172:1175–8.
55. Mukerji G, Yiangou Y, Corcoran SL, et al. Cool and menthol receptor TRPM8 in human urinary bladder disorders and clinical correlations. BMC Urol 2006;6:6.
56. Bors EH, Blinn KA. Spinal reflex activity from the vesical mucosa in paraplegic patients. AMA Arch Neurol Psychiatry 1957;78:339–54.
57. Geirsson G, Lindstrom S, Fall M. The bladder cooling reflex and the use of cooling as stimulus to the lower urinary tract. J Urol 1999;162:1890–6.
58. Tsukimi Y, Mizuyachi K, Yamasaki T, et al. Cold response of the bladder in guinea pig: Involvement of transient receptor potential channel, TR PM8. Urology 2005;65:406–10.
59. Bidaux G, Roudbaraki M, Merle C, et al. Evidence for specific TRPM8 expression in human prostate secretory epithelial cells: Functional and androgen receptor requirement. Endocr Relat Cancer 2005;12:367–82.
60. Macpherson LJ, Dubin AE, Evans MJ, et al. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. Nature 2007;445:541–5.
61. Hinman A, Chuang HH, Bautista DM, et al. TRP channel activation by reversible covalent modification. Proc Natl Acad Sci USA 2006;103:19564–8.
62. Kindt KS, Viswanath V, Macpherson L, et al. Caenorhabditis elegans TRPA-1 functions in mechanosensation. Nat Neurosci 2007;10:568–77.
63. Nagata K, Duggan A, Kumar G, et al. Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. J Neurosci 2005;25:4052–61.
64. Story GM, Peier AM, Reeve AJ, et al. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 2003;112:819–29.
65. Bautista DM, Jordt SE, Nikai T, et al. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 2006;124:1269–82.
66. Kwan KY, Allchorne AJ, Vollrath MA, et al. TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 2006;50:277–89.
67. Andrade EL, Ferreira J, Andre E, et al. Contractile mechanisms coupled to TRPA1 receptor activation in rat urinary bladder. Biochem Pharmacol 2006;72:104–14.
68. de Groat WC. A neurologic basis for the overactive bladder. Urology 1997;50:36–52.
69. Brady CM, Apostolidis AN, Harper M, et al. Parallel changes in bladder suburothelial vanilloid receptor TRPV1 and pan-neuronal marker P GP9.5

immunoreactivity in patients with neurogenic detrusor overactivity after intravesical resiniferatoxin treatment. BJU Int 2004;93:770–6.

70. Apostolidis A, Brady CM, Yiangou Y, et al. Capsaicin receptor TRPV1 in urothelium of neurogenic human bladders and effect of intravesical resiniferatoxin. Urology 2005;65:400–5.

71. Abrams P, Cardozo L, Fall M, et al. The standardisation of terminology of lower urinary tract function: Report from the Standardisation Sub-committee of the International Continence Society. Am J Obstet Gynecol 2002;187:116–26.

72. Liu L, Mansfield KJ, Kristiana I, et al. The molecular basis of urgency: Regional difference of vanilloid receptor expression in the human urinary bladder. Neurourol Urodyn 2007;26:433–8.

73. Abrams P, Cardozo L, Fall M, et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Am J Obstet Gynecol 2002;187: 116–26.

74. Sculptoreanu A, de Groat WC, Buffington CA, et al. Protein kinase C contributes to abnormal capsaicin responses in DRG neurons from cats with feline interstitial cystitis. Neurosci Lett 2005;381:42–6.

75. Sculptoreanu A, de Groat WC, Buffington CA, et al. Abnormal excitability in capsaicin-responsive DRG neurons from cats with feline interstitial cystitis. Exp Neurol 2005;193:437–43.

76. Lazzeri M, Beneforti P, Spinelli M, et al. Intravesical resiniferatoxin for the treatment of hypersensitive disorder: A randomized placebo controlled study. J Urol 2000;164:676–9.

77. Payne CK, Mosbaugh PG, Forrest JB, et al. Intravesical resiniferatoxin for the treatment of interstitial cystitis: A randomized, double-blind, placebo controlled trial. J Urol 2005;173:1590–4.

78. Dinis P, Charrua A, Avelino A, et al. Anandamide-evoked activation of vanilloid receptor 1 contributes to the development of bladder hyperreflexia and nociceptive transmission to spinal dorsal horn neurons in cystitis. J Neurosci 2004;24:11253–63.

79. Tympanidis P, Casula MA, Yiangou Y, et al. Increased vanilloid receptor VR1 innervation in vulvodynia. Eur J Pain 2004;8:129–33.

80. Steinberg AC, Oyama IA, Rejba AE, et al. Capsaicin for the treatment of vulvar vestibulitis. Am J Obstet Gynecol 2005;192:1549–53.

81. Wellmer A, Sharief MK, Knowles CH, et al. Quantitative sensory and autonomic testing in male diabetic patients with erectile dysfunction. BJU Int 1999;83:66–70.

82. Lazzeri M, Barbanti G, Beneforti P, et al. Intraurethrally infused capsaicin induces penile erection in humans. Scand J Urol Nephrol 1994;28:409–12.

83. Lazzeri M, Vannucchi MG, Spinelli M, et al. Transient receptor potential vanilloid type 1 (TRPV1) expression changes from normal urothelium to transitional cell carcinoma of human bladder. Eur Urol 2005;48:691–98.

84. Agopyan N, Head J, Yu S, et al. TRPV1 receptors mediate particulate matter-induced apoptosis. Am J Physiol Lung Cell Mol Physiol 2004;L563–L572.

85. Hartel M, di Mola FF, Selvaggi F, et al. Vanilloids in pancreatic cancer: Potential for chemotherapy and pain management. Gut 2006;55:519–28.

86. Henshall SM, Afar DE, Hiller J, et al. Survival analysis of genome-wide gene expression profiles of prostate cancers identifies new prognostic targets of disease relapse. Cancer Res 2003;63:4196–203.

87. Zhang L, Barritt GJ. Evidence that TRPM8 is an androgen-dependent Ca^{2+} channel required for the survival of prostate cancer cells. Cancer Res 2004;64:8365–73.

88. Zhang L, Barritt GJ. TRPM8 in prostate cancer cells: A potential diagnostic and prognostic marker with a secretory function? Endocr Relat Cancer 2006;13:27–38.

89. von GA, Schaumburg H, Hollmann E, et al. The genetics of enuresis: A review. J Urol 2001;166:2438–43.

90. Loeys B, Hoebeke P, Raes A, et al. Does monosymptomatic enuresis exist? A molecular genetic exploration of 32 families with enuresis/incontinence. BJU Int 2002;90:76–83.

91. Maggi CA, Barbanti G, Santicioli P, et al. Cystometric evidence that capsaicin-sensitive nerves modulate the afferent branch of micturition reflex in humans. J Urol 1989;142:150–4.

92. Wiart L, Joseph PA, Petit H, et al. The effects of capsaicin on the neurogenic hyperreflexic detrusor. A double blind placebo controlled study in patients with spinal cord disease. Preliminary results. Spinal Cord 1998;36:95–9.

93. de Sèze SM, Wiart L, Joseph PA, et al. Capsaicin and neurogenic detrusor hyperreflexia: A double-blind placebo-controlled study in 20 patients with spinal cord lesions. Neurourol Urodyn 1998;17:513–23.

94. Lazzeri M, Beneforti P, Benaim G, et al. Intravesical capsaicin for treatment of severe bladder pain: A randomized placebo controlled study. J Urol 1996; 156:947–52.

95. Maggi CA, Patacchini R, Tramontana M, et al. Similarities and differences in the action of resiniferatoxin and capsaicin on central and peripheral endings of primary sensory neurons. Neuroscience 1990;37:531–9.

96. Silva C, Silva J, Ribeiro MJ, et al. Urodynamic effect of intravesical resiniferatoxin in patients with neurogenic detrusor overactivity of spinal origin: Results of a double-blind randomized placebo-controlled trial. Eur Urol 2005;48:650–5.

97. Payne CK, Mosbaugh PG, Forrest JB, et al. Intravesical resiniferatoxin for the treatment of interstitial cystitis: A randomized, double-blind, placebo controlled trial. J Urol 2005;173:1590–4.

98. Giannantoni A, Di Stasi SM, Stephen RL, et al. Intravesical capsaicin versus resiniferatoxin in patients with detrusor hyperreflexia: A prospective randomized study. J Urol 2002;167:1710–4.

99. de Sèze SM, Wiart L, de Seze MP, et al. Intravesical capsaicin versus resiniferatoxin for the treatment of detrusor hyperreflexia in spinal cord injured patients: A double-blind, randomized, controlled study. J Urol 2004; 171:251–5.

100. Ost D, Van der Aa F, De Ridder D. Intravesical ethanol 10% in saline is not an inert vehicle. Neurourol Urodyn 2003;22:353–5.

101. Benedikt J, Teisinger J, Vyklicky L, et al. Ethanol inhibits cold-menthol receptor TRPM8 by modulating its interaction with membrane phosphati-dylinositol 4,5-bisphosphate. J Neurochem 2007;100:211–24.

102. Tyagi P, Chancellor MB, Li Z, et al. Urodynamic and immunohistochemical evaluation of intravesical capsaicin delivery using thermosensitive hydrogel and liposomes. J Urol 2004;171:483–9.

103. de Sèze SM, Gallien P, Denys P, et al. Intravesical glucidic capsaicin versus glucidic solvent in neurogenic detrusor overactivity: A double blind controlled randomized study. Neurourol Urodyn 2006;25:752–7.

104. Schurch B, Stohrer M, Kramer G, et al. Botulinum-A toxin for treating detrusor hyperreflexia in spinal cord injured patients: A new alternative to anticholinergic drugs? Preliminary results. J Urol 2000;164:692–7.

105. Apostolidis A, Popat R, Yiangou Y, et al. Decreased sensory receptors P2X3 and TRPV1 in suburothelial nerve fibers following intradetrusor injections of botulinum toxin for human detrusor overactivity. J Urol 2005; 174:977–82.

106. Morenilla-Palao C, Planells-Cases R, Garcia-Sanz N, et al. Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity. J Biol Chem 2004;279:25665–72.

107. de Groat WC. A neurologic basis for the overactive bladder. Urology 1997; 50:36–52.