Bladder sensory physiology: neuroactive compounds/receptors, sensory transducers and target-derived growth factors as targets to improve function.

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Running head: Bladder sensory targets to improve function

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>4α-PDD</td>
<td>4alpha-phorbol 12,13-didecanoate</td>
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<tr>
<td>AA</td>
<td>acetic acid</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<td>BOO</td>
<td>bladder outlet obstruction</td>
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<td>BPS</td>
<td>bladder pain syndrome</td>
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<td>Cap</td>
<td>Capsaicin</td>
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<td>CGRP</td>
<td>calcitonin gene-related peptide</td>
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<td>CYP</td>
<td>cyclophosphamide</td>
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<td>DO</td>
<td>detrusor overactivity</td>
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<td>DRG</td>
<td>dorsal root ganglia</td>
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<td>EFS</td>
<td>electrical field stimulation</td>
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<td>GSK</td>
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<td>IC</td>
<td>interstitial cystitis</td>
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<td>ICI</td>
<td>intercontraction interval</td>
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<tr>
<td>IR</td>
<td>immunoreactive</td>
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<td>KO</td>
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<td>LUT</td>
<td>lower urinary tract</td>
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<td>MPG</td>
<td>major pelvic ganglion</td>
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<td>NGF</td>
<td>nerve growth factor</td>
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<tr>
<td>NTF</td>
<td>neurotrophic factor</td>
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<tr>
<td>NVCs</td>
<td>non-voiding bladder contractions</td>
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<td>OAB</td>
<td>overactive bladder</td>
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<tr>
<td>OE</td>
<td>overexpression</td>
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<tr>
<td>PACAP</td>
<td>pituitary adenylate cyclase-activating polypeptide</td>
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<td>PLC</td>
<td>phospholipase C</td>
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<tr>
<td>RR</td>
<td>ruthenium red</td>
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<tr>
<td>SCI</td>
<td>spinal cord injury</td>
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<tr>
<td>SP</td>
<td>substance P</td>
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<tr>
<td>SPN</td>
<td>sacral parasympathetic nucleus</td>
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<tr>
<td>Trk</td>
<td>neurotrophic tyrosine kinase receptor</td>
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<td>TRP</td>
<td>transient receptor potential</td>
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<tr>
<td>VIP</td>
<td>vasoactive intestinal polypeptide</td>
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<tr>
<td>VV</td>
<td>void volume</td>
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Abstract

Urinary bladder dysfunction presents a major problem in the clinical management of patients suffering from pathological conditions and neurological injuries or disorders. Currently, the etiology underlying altered visceral sensations from the urinary bladder that accompany the chronic pain syndrome, bladder pain syndrome (BPS)/interstitial cystitis (IC), is not known. Bladder irritation and inflammation are histopathological features that may underlie BPS/IC that can change the properties of lower urinary tract sensory pathways (e.g., peripheral and central sensitization, neurochemical plasticity) and contribute to exaggerated responses of peripheral bladder sensory pathways. Among the potential mediators of peripheral nociceptor sensitization and urinary bladder dysfunction are neuroactive compounds (e.g., purinergic and neuropeptide/receptor pathways), sensory transducers (e.g., transient receptor potential channels) and target-derived growth factors (e.g., nerve growth factor). We review studies related to the organization of the afferent limb of the micturition reflex and discuss neuroplasticity in an animal model of urinary bladder inflammation to increase the understanding of functional bladder disorders and to identify potential novel targets for development of therapeutic interventions. Given the heterogeneity of BPS/IC and the lack of consistent treatment benefits, it is unlikely that a single treatment directed at a single target in micturition reflex pathways will have a mass benefit. Thus, identification of multiple targets is a prudent approach and use of cocktail treatments directed at multiple targets should be considered.

Keywords: urothelium, DRG, ATP, neuropeptides, TRP channels, NGF
Introduction

The storage and elimination of urine is a central and peripheral nervous system reflex involving coordinated activity between the urinary bladder and urethra. These tissues are regulated by neural circuits in the brain and spinal cord that determine appropriate smooth and striated muscle function in the lower urinary tract (LUT) (53). Disruption to the neural circuits underlying storage and elimination may often be observed with neurological injuries (e.g., spinal cord injury (SCI), stroke) or disorders (e.g., multiple sclerosis, Parkinson’s disease) and may contribute to functional disorders of the urinary bladder including overactive bladder (OAB) and bladder pain syndrome (BPS)/interstitial cystitis (IC) (5, 35, 52). We have hypothesized that BPS/IC, as well as OAB, may involve alterations to the afferent limb of the micturition reflex including bladder afferent neurons in the dorsal root ganglia (DRG) and urothelial cells in the bladder wall mucosa. The following sections will review studies related to bladder sensory neuroplasticity following injury, disease and/or inflammation in an attempt to advance functional bladder disorder understanding and to identify potential targets for therapeutic intervention.

Afferent and spinal pathways to the urogenital tract

The micturition reflex is organized as an on-off system, switching between two modes of action in the urinary bladder. During the storage phase, somatic and sympathetic excitatory inputs to the urethral sphincters and sympathetic inhibitory inputs to the bladder wall are tonically active whereas parasympathetic pathways are inactive (34, 79). In contrast, during reflexive or voluntary micturition, parasympathetic input to the bladder wall is excited and
somatosympathetic input to the bladder wall and urethral sphincters is inhibited (100). Unlike local spinal reflexes that underlie most of the storage phase, these LUT micturition reflex mechanisms are predominately influenced by supraspinal modulation in the pontine micturition center (59).

The switch from the storage to the elimination phase is elicited by slowly adapting mechanoreceptors in the urinary bladder wall (77). As hydrostatic pressure increases, bladder afferent ( thinly myelinated Aδ) fibers amplify their signal transduction along the hypogastric and pelvic nerves (53). Bladder afferent nerves that terminate peripherally throughout the tunica mucosa and tunica muscularis propria may also incorporate unmyelinated C-fibers, which respond to nociceptive stimulation by chemicals (e.g., capsaicin (Cap), menthol), extreme intravesical pressure and inflammation (4, 62, 97). Though typically quiescent during bladder filling, C-fiber activation may contribute to the development of LUT symptoms and pathological conditions of the urinary bladder (32, 159).

Bladder afferent fibers in the pelvic nerve travel centrally via dorsal roots and project into Lissauer’s tract where collateral branches extend ventromedially and ventrolaterally along the superficial layers of the dorsal horn (4, 34, 41). The ventromedial branches follow the medial edge of the dorsal horn into the dorsal commissure and are largely projections from the pudendal nerve and urogenital structures (4, 34, 41). The ventrolateral branches, on the other hand, project to the lateral edge of dorsal horn (laminae I) into the sacral parasympathetic nucleus (SPN) and are termed the lateral collateral pathway (4, 34, 41). Ultimately, the medial and lateral collateral pathways extend respectively to the dorsal commissure and SPN (laminae V-VII), which contain cell bodies of preganglionic parasympathetic neurons that project to the
Primary bladder afferent fibers not only synapse on preganglionic parasympathetic neurons, but also synapse on interneurons in the lumbosacral dorsal comissure, superficial dorsal horn and SPN (34, 53). These interneurons are important in the maintenance of micturition reflex function and project locally in the spinal cord or to supraspinal cortical integration centers (34, 53).

**Urothelial signaling**

The transitional epithelium lining the urinary bladder mucosa, termed the urothelium, has been demonstrated to detect mechanical, chemical and thermal stimuli (13). In response to these stimuli, urothelial cells secrete factors like urinary proteins (e.g., urokinase) and signaling molecules (e.g., adenosine triphosphate (ATP), acetylcholine, nitric oxide) through the apical and/or serosal surfaces suggesting the urothelium may have a role in urinary bladder sensory transduction (13, 15). Additionally, urothelial cells express receptors (e.g., purinergic, cholinergic, adrenergic) and mechanosensitive channels (e.g., transient receptor potential, TRP) on their surface that are responsive to signals in the extracellular environment (21, 78, 102, 150). As a result of the sensory influence the urothelium may have in micturition reflex function, any perturbation to urothelial signaling mechanisms and/or the underlying nervous network may contribute to functional disorders of the urinary bladder (13). The following sections will address the distribution, function and regulation of neuroactive compounds/associated receptors, sensory transducers and target-derived growth factors including ATP, neuropeptides, TRP channels and NGF in micturition reflex pathways under normal and pathological conditions that have been demonstrated to affect LUT function (Fig. 1).
ATP in micturition reflex function and dysfunction

The urothelium responds to changes in hydrostatic pressure by releasing factors, such as ATP, from its mucosal and serosal surfaces (50, 150). ATP released from the serosal surface may then interact with nerve terminals, interstitial cells and/or basolateral urothelial P2-purinoceptors to transduce sensory information or regulate its release, respectively (150). In functional disorders of the urinary bladder such as BPS/IC, however, urothelial cells have been demonstrated to increase stretch-evoked ATP release relative to symptom-free controls (125). It has been suggested that the increased ATP release may, in part, underlie the development of lower urinary tract symptoms in micturition reflex dysfunction due to the capacity of intravesical purinergic agonist (ATP or α,β-meATP) instillation to increase the firing rate of urinary bladder afferent nerves and induce bladder overactivity (105, 109, 161). Taken together, these studies have begun to establish the influence of purinergic signaling in urinary bladder sensory transduction and demonstrate its possible role in micturition reflex dysfunction.

P2X and P2Y receptor expression in the LUT. P2-purinoceptors are classified as ligand-gated ion channels, P2X, or G-protein coupled receptors, P2Y (54). There are currently seven P2X subunits (P2X1-7) that may arrange as heteromeric or homomeric ligand-gated ion channels and eight metabotropic P2Y subunits (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, P2Y14) that may couple to $G_s$, $G_i$ or $G_q$ (1, 106). While the kinetics and tissue distribution of each P2-purinoceptor differs, there is substantial evidence that many of these subunits are expressed throughout the urinary bladder urothelium, lamina propria and detrusor smooth muscle.
The distribution of P2X and P2Y receptors in the urothelium has been described in multiple species, including rodents, felines and humans. P2X2 and P2X4-7 receptor immunoreactivity (IR) was detected in the rodent urothelium, whereas, positive IR was detected for P2X1-7 in the feline urothelium (18, 83, 135) (Fig. 1). In the human bladder urothelium, glycosylated P2X2 and P2X3 transcript and protein expression has also been detected (128). There appears to be less diversity in the urothelial distribution of P2Y receptors where P2Y2 and P2Y4 transcript and protein expression has been demonstrated in cultured rat urothelial cells (26). Additionally, in the human urothelial cell line, UROtsa, P2Y1, P2Y2 and P2Y11 transcript expression was detected (116).

The urinary bladder lamina propria is adjacent to the mucosal basement membrane and includes loose connective tissue, vasculature, lymphatics, nerves and interstitial cells (7). A population of interstitial cells, termed myofibroblasts, in the lamina propria generates intracellular calcium and membrane transients in response to purinergic agonists and express P2X3, P2Y2, P2Y4 and P2Y6 receptors (123, 124). The presence of ATP-dependent transients suggests myofibroblasts may have a role in influencing urinary bladder sensory transduction and warrants further investigation (123, 124).

Afferent (and efferent) nerves terminating in the urothelium, lamina propria and detrusor smooth muscle have received much of the attention in characterizing P2-purinoceptor distribution. While the transcript and protein expression of all seven P2X subunits (P2X1-7) have been detected in the rodent DRG, there appears to be a differential distribution of P2X2 and P2X3 depending on spinal cord level (24, 113, 155) (Fig. 1). P2X2 mRNA has been detected in both thoracolumbar and lumbosacral urinary bladder afferent neurons, but transcripts at the
thoracolumbar level appear to be co-expressed with P2X3 (24). P2X3 transcripts, on the other hand, appear to be restricted to small- and medium-diameter afferent neurons and have a greater frequency of expression in thoracolumbar than lumbosacral neurons (20, 24, 113).

Similar to the urothelium, there appears to be less diversity in the distribution of P2Y receptors in DRG neurons. P2Y1, P2Y2 and P2Y4 transcript expression has been detected in rodent DRG neurons with P2Y1 restricted to small-diameter neurons and P2Y4 to medium- and large-diameter neurons (114). P2Y2 and P2Y4, in particular, have been detected in thoracolumbar and lumbosacral bladder afferent neurons (25).

Lastly, P2X and P2Y receptors are expressed on urinary bladder smooth muscle cells. P2X1-6 receptor IR has been demonstrated in rodent urinary bladder smooth muscle cells (44, 83). In contrast, evidence for the expression of P2Y receptors in the detrusor smooth muscle is sparse where P2Y6 has been detected and determined to augment P2X-mediated contractile force (160).

**P2X and P2Y receptor expression and function with cystitis.** Of the seven P2X subunits, P2X2 and P2X3 have been suggested to be involved in the sensitization of urinary bladder sensory transduction with cystitis. Specifically, P2X2 and P2X3 protein expression in the human bladder urothelium has been demonstrated to increase in IC (128). Likewise, P2X2 transcript expression was increased in mouse thoracolumbar DRG neurons with cyclophosphamide (CYP)-induced cystitis (24). Inhibition of P2X3 or P2X2/3 receptors with A-317491 following CYP-induced cystitis reduced non-voiding contractions and residual urine volume and increased intermicturition intervals suggesting a role for purinergic signaling in bladder hyperreflexia with cystitis (67).
CYP-induced cystitis has also been demonstrated to increase peak urinary bladder afferent nerve activity that was significantly decreased following P2X receptor antagonist (TNP-ATP or PPADS) instillation (161). The significance of P2X3 specifically in bladder afferent nerve sensitization was demonstrated with P2X3-null mice and its attenuation of P2X agonist (ATP or α,β-meATP) induced afferent nerve excitation (148). Additionally, following CYP-induced cystitis, thoracolumbar rat DRG neurons increased homomeric P2X3 current, whereas, lumbosacral rat DRG neurons increased heteromeric P2X2/3 current (30). While CYP-treated urinary bladder neurons exhibit increased responsiveness to purinergic agonist application, the changes in P2X subtypes do not appear to be conserved across species (24, 30). For instance, homomeric P2X2 currents not seen in rat DRG neurons increased in mouse lumbosacral DRG neurons (24).

Although the role of P2Y receptors in urinary bladder sensory transduction is still maturing, P2Y2 has been implicated in bladder afferent hyperexcitability. UTP, a P2Y2 and P2Y4 agonist, application depolarized the resting membrane potential and increased action potential frequency in bladder afferent neurons (25). UTP application also potentiated homomeric P2X2 current in wildtype (WT) but not P2Y2-null mice suggesting P2Y2 may contribute to P2X-mediated afferent nerve sensitization that was observed with cystitis (25).

**Neurochemical plasticity in the LUT with bladder inflammation**

*Neurochemistry of micturition pathways.* Bladder afferent fibers contain a variety of neuropeptides, including: calcitonin-gene related peptide (CGRP), substance P (SP), neurokinin A, neurokinin B, vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase-activating
polypeptide (PACAP), cholecystokinin and enkephalins (9, 33, 37, 41, 73, 136, 141). With the exception of CGRP, all of these substances are predominantly expressed in small-diameter (presumably C-fiber) afferents (33, 37, 41, 45, 74, 122, 142, 144-147). The administration of Cap, which acts selectively on small-diameter afferent fibers to deplete neurotransmitter stores and induce neuronal degeneration, reduces the levels of SP, neurokinin A and CGRP within the pelvic viscera but does not affect VIP or enkephalin expression (31). These findings are consistent with SP, related tachykinins and CGRP expression in afferent pathways to the pelvic viscera (31). The following sections will focus on the expression, distribution and functional plasticity of members of the VIP/secretin/glucagon family of hormones, PACAP and VIP (Fig. 1). The contributions of other peptides to micturition reflex pathways have recently been described (9).

**PACAP, VIP and associated receptors signaling in micturition reflexes**

*PACAP mechanisms facilitate micturition dysfunction.* Dense PACAP-IR sensory fibers are present in the urinary bladder including in the suburothelial nerve plexus (19, 58). PACAP receptor expression has also been demonstrated in the detrusor smooth muscle, urothelium, lumbosacral DRG and spinal cord suggesting that PACAP signaling participates at multiple levels in the LUT (19, 58) (Fig. 1). Notably, in the detrusor smooth muscle, PACAP has been demonstrated to facilitate contractility by increasing smooth muscle tone and potentiating electrical field stimulation (EFS)-induced contractions (19).

Following CYP-induced bladder inflammation, PACAP/receptor expression in the DRG and afferent projections to the spinal cord are upregulated (141). The increase in PACAP
expression with cystitis may evoke ATP release from the urothelium to enhance bladder sensory transduction (58). Inhibiting the PAC1 receptor with PACAP6-38 following cystitis has been demonstrated to reduce voiding frequency suggesting PACAP signaling may have a functional role in the inflammation induced neuroplasticity of the afferent limb of the micturition reflex (19). Differential effects of PACAP have also been demonstrated in peripheral nociception (63, 71, 72). These differences may reflect the diversity of PACAP-mediated second messengers (63) that may result in increased neuropeptide/neurotransmitter production and secretion, and facilitation of neuronal depolarization by modulating nonselective cationic channels (e.g., TRP channels) (12).

Disproportionate contribution of PACAP and VIP to altered bladder function. Urinary bladder function and somatic sensitivity were recently evaluated in PACAP null (PACAP−/−), littermate heterozygote (PACAP+/-) and WT mice (96). PACAP−/− mice exhibit morphological differences in the urinary bladder including hypertrophy of the lamina propria and detrusor smooth muscle (96). Consistent with an increased bladder mass, PACAP−/− mice exhibit increased bladder capacity, void volume (VV) and intercontraction intervals (ICI) (96). Furthermore, PACAP−/− mice are less responsive to somatic stimulation and, unlike WT mice, do not exhibit altered urodynamic measurements following intravesical instillation of acetic acid (AA, 0.5%) (96). This transgenic mouse model demonstrated that PACAP gene disruption is associated with changes in bladder morphology, bladder function and reduced somatic and visceral sensitivity (96).

Unlike PACAP, the closely related neuropeptide, VIP, exhibits minimal innervation to the urinary bladder and is predominately expressed in postganglionic efferent neurons of the pelvic ganglia (22, 49, 118, 152). Minimal innervation of VIP to the urinary bladder is consistent with
the lack of functional effects on urinary bladder contractions (66). Application of VIP to detrusor
smooth muscle had no effect on spontaneous or carbachol-induced bladder contractions;
however, intrathecal or intraarterial administration of VIP facilitated micturition in the rat (66).
The diverse and conflicting roles for VIP demonstrated in the literature (46, 64, 66, 132) may be
attributable to differential VIP receptor distribution across species and target tissues. Taken
together, it appears that PACAP/receptor signaling is a more prominent regulator of rat bladder
physiology compared to VIP/receptor signaling (19, 46, 64, 66, 132).

TRP channels expression and function in the LUT

Studie...
of TRPV1 in bladder afferent fibers using TRPV1 knockout (KO) mice. Birder et al. (17)
demonstrated that TRPV1 KO mice had increased bladder capacity and decreased voiding
contractions relative to WT mice suggesting decreased afferent input to micturition reflex
pathways. In support of this interpretation, they demonstrated a decrease in c-fos expressing
cells in the sacral spinal cord in TRPV1 KO mice compared to WT mice (17).

The direct role of TRPV1 in micturition reflex sensory transduction was examined by
Daly and colleagues (29) using a bladder-pelvic nerve preparation in TRPV1 KO and WT mice.
Distension-induced afferent nerve activity was significantly diminished in TRPV1 KO mice
relative to WT controls (29). Additionally, administration of a TRPV1 agonist, Cap, increased
afferent nerve activity, whereas the TRPV1 antagonist, capsazepine, attenuated distension-
induced nerve activity in WT mice (29). Collectively, these studies confirm a role for TRPV1 in
the afferent limb of the micturition reflex.

The functional expression of TRPV1 in the urothelium is also relevant to micturition
reflex sensory transduction. Data exist in support of the hypothesis that TRPV1 at the level of
the urothelium could indirectly activate peripheral bladder afferent terminals. For example, Cap
and H+ not only activate urothelial TRPV1 channels, but also induce the secretion of signaling
mediators like nitric oxide (16). Furthermore, TRPV1 KO mice exhibit decreased stretch-evoked
ATP release from the urothelium suggesting a role for TRPV1 in purinergic signal transduction
(17).

TRPV4. The TRPV4 channel is a calcium-permeable, stretch-activated, nonselective cation
channel that is expressed throughout the renal epithelium, endothelial cells and urinary bladder
epithelium (6, 8). In addition to TRPV4 expression in urothelial cells, the channels are highly
expressed in primary sensory neurons suggesting a role in nociception and mechano- and thermosensation (14, 56, 78, 101, 156) (Fig. 1). TRPV4 was originally identified as a channel activated by hypotonic cell swelling (85, 149), but has since been shown to be activated by other physical and chemical stimuli, including mechanical or shear stress, heat (>27°C), arachidonic acid, anandamide, and the synthetic ligands 4alpha-phorbol 12,13-didecanoate (4α-PDD) and GSK1016790A (GSK) (6, 8).

Similar to the characterization of TRPV1 in urinary bladder function, transgenic mice have been essential to study the role of TRPV4. TRPV4 KO mice have been demonstrated to have decreased voiding frequency and increased frequency of nonvoiding contractions, intermicturition intervals and total urine output suggesting diminished afferent input into micturition reflex pathways (48, 56, 129). To further determine the role of TRPV4 in bladder afferent nerve excitability, Aizawa and colleagues (2) recorded single unit afferent activity in Aδ-fibers, as well as Cap-sensitive and Cap-insensitive C-fibers. While afferent activity was not altered in Aδ-fibers or Cap-sensitive C-fibers following GSK instillation, Cap-insensitive C-fiber activity significantly increased with the first instillation of GSK (2). These studies suggest TRPV4 may facilitate sensory transduction of the micturition reflex via Cap-insensitive C-fiber afferent nerves (2).

TRPV4 at the level of the urothelium is expressed throughout the basal and intermediate cell layers and has been suggested to be involved in regulating stretch-evoked ATP release (48, 56). Functional TRPV4 channels were demonstrated in urothelial cells when the application of a TRPV4 agonist, 4α-PDD, increased [Ca²⁺], and its influx was attenuated by a nonselective TRP antagonist, ruthenium red (RR) (14). 4α-PDD mediated [Ca²⁺], was dependent
on both TRPV4 and extracellular calcium suggesting functional expression of TRPV4 on the urothelium (101). Furthermore, there is a prominent decrease in stretch-evoked ATP release in TRPV4 KO mice and following the depletion of extracellular calcium (56, 101). Taken together, these studies provide evidence for the surface expression of TRPV4 on urothelial cells and suggest a role for TRPV4 in purinergic signaling and bladder sensory transduction.

TRPV channels and nociception. The literature suggests that the TRPV channel subfamily is involved in the detection of acute noxious thermal, mechanical and chemical stimuli (134). Hypersensitivity and pain in various pathological conditions are often due to upregulated expression and/or increases in sensitivity of TRPV channels (104). For example, CYP-induced bladder inflammation resulted in mechanical hyperreactivity and hypersensitivity of the hindpaw in WT, but not TRPV1 KO, mice suggesting TRPV1 is essential for referred mechanosensitivity in peripheral tissues with visceral inflammation (151). Similarly, TRPV1 KO mice show decreased responsiveness to noxious thermal stimuli and fail to develop inflammatory thermal hyperalgesia (104). The TRPV4 channel has also been implicated in nociception and inflammatory pain because of expression on primary afferent nociceptors (23). Similar to TRPV1 KO mice, TRPV4 KO mice fail to develop thermal hyperalgesia and also have decreased sensitivity to tail pressure and deficits in mechanically evoked paw withdrawal responses (14). These studies together suggest TRPV1 and TRPV4 may have roles in the etiology of thermal allodynia and hyperalgesia and may serve as therapeutic targets for pathological pain conditions.
Role of NGF and associated receptors in LUT plasticity after inflammation

Neurotrophic factors (NTFs). A potential mechanism underlying the morphological (158), electrophysiological (69, 158) and neurochemical (136, 137, 140-142) changes in bladder afferent neurons after cystitis may involve bladder NTFs and/or neural activity (138). The concept of trophic interactions between nerve cells and their targets is clearly demonstrated during embryonic and postnatal development (81, 87, 108, 133) and more recently in adult tissues (43, 119-121, 130, 131, 138). Studies from our laboratory (138) have demonstrated that chronic CYP-induced cystitis or chronic SCI also alters the expression of NGF and NGF mRNA in the urinary bladder as well as a variety of other NTFs; however, the role of NGF and associated receptors in LUT pathways and the contribution to LUT reflex plasticity is the focus of this section.

Nerve growth factor (NGF). Cytokines and growth factors, including NGF, are upregulated at the site of tissue injury, inflammation and/or target organ hypertrophy (42, 84, 86, 99, 153). Following noxious peripheral stimulation, for example, levels of neuroactive compounds (e.g., enkephalin (84), dynorphin (115), CGRP (40, 55, 153), SP (55, 84, 115, 136), neuropeptide Y (84); nNOS (139, 142, 143) and PACAP (71, 141)) have been demonstrated to increase in DRG and spinal cord neurons. NGF, in particular, is also released from the target organ for tyrosine kinase receptor (Trk) type 1 (TrkA) binding and retrograde transport in DRG afferent neurons (70) (Fig. 1). The subsequent increase in NGF expression within the DRG neurons may induce increased production of neuropeptides (i.e., SP, CGRP and PACAP) and alter sensory transduction (40, 55, 153).
**NTF receptors.** A large percentage of pelvic visceral afferent neurons express NTF receptors, including Trk for NGF and related substances (98, 111, 112, 154). Following cystitis, NTF receptors exhibit neuroplastic increases in TrkA- and TrkB-IR and Trk phosphorylation in bladder afferent neurons (111, 112). Elevated levels of neurotrophins have also been detected in the urine (107) or in the urinary bladder (93) of women with BPS/IC. The activation of neurotrophin/Trk complex in the neuronal cell bodies could activate signal cascade(s) to induce long-term changes in cells, including: (1) neurotransmitter phenotype; (2) synaptic reorganization; (3) increased synaptic efficacy; and (4) target organ dysfunction (94).

**Pharmacological manipulation of NGF/receptor signaling in LUT pathways.** NTF overexpression in the urinary bladder may underlie or contribute to the diverse changes in the properties of bladder afferent neurons after CYP-induced cystitis (136, 141). As previously stated, CYP-induced bladder inflammation alters NGF and associated receptor expression in LUT tissues including the urinary bladder, DRG and major pelvic ganglion (MPG) (138). The increased LUT expression of NGF may facilitate bladder activity and afferent cell hyperexcitability (27, 157, 162). Additionally, overexpression of NGF in the bladder smooth muscle has been demonstrated to contribute to bladder hyperinnervation and bladder overactivity (28). The LUT dysfunction mediated by NGF can be reduced by instillation of anti-NGF (39) or a scavenging agent, REN1820 (65), suggesting NGF may underlie, in part, the morphological, functional and electrophysiological alterations in micturition reflex pathways with cystitis.

**Transgenic mouse model with chronic urothelial overexpression of NGF (NGF-OE).** Our laboratory has characterized a transgenic mouse model of urothelium-specific NGF-OE that represents a novel approach to exploring the role of NGF in urinary bladder inflammation and
sensory function (117). Functionally, NGF-OE mice exhibit urinary bladder hyperreflexia with frequent urination and the presence of non-voiding bladder contractions (NVCs) as well as referred somatic pelvic hypersensitivity (117). NGF-OE mice may represent a useful animal model of BPS/IC because the changes observed in the urinary bladders of these mice are consistent with certain changes observed in this syndrome. Clinically, increased NGF levels have been detected in the bladder urothelium of patients with BPS/IC (93). Elevated NGF levels were also detected in the urine of patients with BPS/IC or OAB symptoms associated with detrusor overactivity (DO), stress urinary incontinence, or bladder outlet obstruction (BOO) (75, 76, 88-92) and patients with DO who responded to treatment had reduced urinary NGF levels (90, 92).

Our studies with NGF-OE mice are consistent with numerous other studies demonstrating involvement of NGF in altered bladder sensory function (38, 39, 60, 61, 65, 68, 80, 157, 162). Pleiotropic changes, subsequent to NGF overexpression, including changes in the expression of growth factors, neuroactive compounds, and ion channels (e.g., TRP channels) (3, 110) can also directly modulate pain and bladder/visceral sensory function and could contribute to altered urinary bladder function in NGF-OE mice (51, 110, 127, 159) (Fig. 1). Recent studies (57) have demonstrated that TRPV4 transcript and protein expression are significantly increased in the urothelium, suburothelium and suburothelial nerve plexus of the urinary bladder and in small- and medium-sized lumbosacral (L1, L2, L6-S1) DRG cells from NGF-OE mice compared to littermate WT mice. These studies demonstrate NGF may, in part, regulate TRPV4 expression in LUT tissues to alter urinary bladder sensory transduction. This transgenic mouse model provides us with the opportunity to understand the contribution of NGF overexpression in the
urothelium to voiding frequency, non-voiding contractions, referred somatic sensitivity, and the mechanisms underlying these changes.

**Significance of non-voiding contractions (NVCs) with urinary bladder dysfunction**

Increases in micturition pressure during the filling phase without the release of urine (non-voiding bladder contractions, NVCs) are often associated with OAB and have been observed, as previously noted, in preclinical animal models with urinary bladder dysfunction including the NGF-OE mouse (117) and following CYP treatment (67) and BOO (75, 76). In addition to the increased appearance of NVCs, these animal models (NGF-OE, CYP and BOO) also demonstrate increased voiding frequency and decreased void volumes. On the other hand, it has also been reported that urinary bladder dysfunction in TRPV1−/− and TRPV4−/− mice is associated with increased NVCs, reduced voiding frequency and increased void volumes (17, 48, 56, 129). Increased NVCs and increased voiding frequency has been suggested to represent an increase in bladder afferent activity (57, 75, 76, 117) whereas decreases in bladder afferent activity have been suggested with reduced voiding frequency and increased NVCs (17, 48, 56, 129). Completely different interpretations of NVCs and their relation to bladder afferent activity most likely reflect an incomplete understanding of NVCs including their origin (neurogenic (afferent, efferent) and/or myogenic), sites of initiation in the urinary bladder, pharmacology and overall relationship to sensation. A better understanding of NVCs would be aided by additional studies including the use of an ex vivo bladder-nerve preparation where simultaneous recordings of bladder pressure and bladder afferent activity during bladder filling.
can determine the relationship between NVCs and bladder afferent nerve activity under control conditions and following urinary bladder dysfunction.

Perspectives and significance

Complex neural circuitry underlies the micturition reflex and perhaps because of this intricacy, micturition reflex function is often compromised as a result of diverse neural injuries, diseases, chronic pain and inflammatory conditions. We have emphasized the chronic pain condition, BPS/IC, and an animal model of urinary bladder inflammation induced by intraperitoneal injection of CYP in this review to illustrate the neurochemical plasticity that occurs in the sensory components (e.g., bladder afferent cells, the suburothelial plexus of the urinary bladder, urothelium) of the micturition reflex. Changes in the expression of numerous chemical mediators, receptors, sensory transducers as well as target-derived factors are associated with preclinical models of BPS/IC. Given the heterogeneity of BPS/IC, it is unlikely that identification of a single target for pharmacological intervention will have a broad impact. We draw attention to other potential targets (Fig. 1) in the sensory components of the micturition reflex to consider for therapeutic interventions, either alone or in combination, to improve bladder function including neuroactive compounds/receptor systems, the TRP family of sensory transducers as well as the target-derived growth factor, NGF.
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References


64. **Hernandez M, Barahona MV, Recio P, Benedito S, Martinez AC, Rivera L, Garcia-Sacristan A, Prieto D, and Orensanz LM.** Neuronal and smooth muscle receptors involved in the


68. **Jaggar SI, Scott HC, and Rice AS.** Inflammation of the rat urinary bladder is associated with a referred thermal hyperalgesia which is nerve growth factor dependent. *Br J Anaesth* 83: 442-448, 1999.


**Figure Legends**

**Figure 1.** Expression of neuroactive chemical/receptor systems and sensory transducers in afferent pathways of the micturition reflex emphasizing urothelium and bladder afferent nerve participation. Receptor activation and channel stimulation on urothelial cells elicits secretion of sensory mediators that may affect adjacent cells and tissues including bladder afferent nerves in the suburothelial plexus, myofibroblasts and detrusor smooth muscle. Urothelial cells are also responsive to neurotransmitters released from bladder nerves and other cell types including inflammatory cells. Abbreviations: ATP adenosine triphosphate; TrkA receptor tyrosine kinase A; TrkB receptor tyrosine kinase B; NO nitric oxide; NGF nerve growth factor; NP neuropeptides; PAC1 pituitary adenylate cyclase-activating polypeptide (PACAP) selective receptor; VPAC2 receptor with equal and high affinity for vasoactive intestinal polypeptide and PACAP; TRP transient receptor potential; V vanilloid family; M melastatin family; A ankyrin family; P2 purinergic receptor; R receptor/channel expression. See text for additional details. Figure modified from Arms and Vizzard (9).
Urothelium Receptors
P2X, P2Y, TRPV, TRPM,
TRPA, TrkA, TrkB, PAC1,
VPAC2

Bladder Afferent Receptors
P2X, P2Y, TRPV, TRPA,
TRPM, TrkA, TrkB, PAC1,
VPAC2

Chemical Mediators
ATP, NO,
NP, NGF