



# Potential Mechanisms Underlying the Increased Excitability of the Bladder Afferent Pathways in Interstitial Cystitis/Bladder Pain Syndrome

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Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic debilitating disorder associated with lower urinary tract symptoms, including frequency, urgency, and suprapubic pain, which inconveniences the patients and seriously impairs their quality of life. Although the etiology of IC/BPS is unknown, intense research has been conducted focusing on the involvement of the bladder afferent nerve in regard to the cellular mechanisms underlying neurogenic inflammation of the urinary bladder. The involvement of neurogenic inflammation in patients with IC/BPS is supported by several animal models of bladder inflammation as well as clinical studies. Chronic bladder inflammation can result in functional and anatomical changes in the primary afferent neurons through the expression of inflammation-related proteins/receptors in the urinary bladder and bladder afferent pathways, leading to pain symptoms in patients with IC/BPS. In addition, neurogenic inflammation of the bladder mucosa can induce the central sensitization as well as the peripheral sensitization, and the neuroimmune overactivity and toll-like receptor (TLR) signaling of the immune cells involve complex mechanisms of central sensitization. This review presents the potential mechanisms underlying the afferent hyperexcitability of the bladder in IC/BPS and summarizes the neurogenic inflammation, neurotrophic factors, TLRs, and neuroimmune communication.

**Keywords:** Afferent pathways; Urinary bladder; Interstitial cystitis; Physiopathology; Neurogenic inflammation

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## INTRODUCTION

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic disease with lower urinary tract (LUT) symptoms, such as urinary frequency, urgency, and suprapubic pain with bladder filling. A community-based study of American women estimated a high prevalence of IC/BPS of 2.7% to 6.5% [1]. The estimated prevalence of IC/BPS in Korean population-based studies is 261 per 100,000 [2]. IC/BPS is a clinical diagnosis based on the chronic symptoms of

pain perceived by the patient to arise from the bladder and/or pelvis associated with the urinary urgency or frequency in the absence of infection or other identifiable causes. In 2011, the American Urological Association defined IC/BPS as “an unpleasant sensation (pain, pressure, discomfort) that was perceived to be related to the urinary bladder, associated with LUT symptoms of more than six weeks duration, and with the absence of infection or other identifiable causes” [3].

The etiology of IC/BPS is unclear, and its pathogenesis

may involve multiple pathways leading to a common clinical entity. Some of the theories suggested to explain the pathophysiology of IC/BPS include urothelial barrier alterations, plasticity of the bladder afferents and/or central nervous system (CNS) abnormalities, possible contribution of inflammatory or bacterial agents, and abnormal urothelial signaling [4].

Chancellor and Yoshimura [5] proposed the mechanism of IC, in which a bladder insult and urothelial damage enable substances in the urine to leak into the lamina propria, prompting a cascade of events, each contributing to bladder inflammation and pain. Neurogenic inflammation of the urinary bladder may involve a change in the function of the sensory nerve of some patients with IC/BPS, or it may be the result of other initiating causative events [6]. Regardless, IC/BPS can produce long-lasting functional and anatomical changes in the CNS and in the primary afferent neurons. These peripheral and central sensitizations lead to allodynia (pain from non-nociceptive stimuli) and hyperalgesia (increased intensity of pain from stimuli that usually produce pain) [7].

Toll-like receptors (TLRs) are associated with the innate immune system, which have been identified as critical factors in central sensitization. In particular, TLR4 has been shown to play a key role in the development of chronic pain in IC/BPS patients. These results may help recognize new mechanisms in IC/BPS and develop new therapeutic modalities [8]. This review presents the potential mechanisms underlying the afferent hyperexcitability of the bladder in IC/BPS, providing a concise overview of neurogenic inflammation, neurotrophic factors, TLRs, and neuroimmune communication.

## ANATOMY OF THE BLADDER

The storage and periodic elimination of urine depend on the coordinated activities of two functional units of the LUT, urinary bladder as a reservoir, and an outlet consisting of the bladder neck, urethra, and striated muscles of the external urethral sphincter (EUS). The bladder wall has three well-defined layers: the mucosa, muscularis propria, and adventitia/serosa [9]. The mucosa consists of an urothelium, basement membrane, lamina propria, and smooth muscle cells (muscularis mucosa). The muscularis propria (bladder smooth muscle) consists of a smooth

muscle bundle with collagen and elastin fibers. The adventitia and serosa, which form the outermost part of the bladder, are composed of connective tissue [9].

The LUT is regulated by three sets of peripheral nerves, including the sacral parasympathetic (pelvic nerves), thoracolumbar sympathetic (hypogastric nerves), and somatic nerves (pudendal nerves). These nerves include two different types of neurons: afferent and efferent axons [10]. The parasympathetic efferent nerves originate in the sacral segments of the spinal cord; they contract the bladder and relax the urethra. In contrast, the sympathetic efferent nerves originate in the thoracolumbar segments of the spinal cord; they relax the bladder and contract the urethra. Somatic efferent nerves contract the EUS.

The perception of bladder filling is transmitted to the spinal cord by the pelvic and hypogastric nerves, while sensory input from the bladder neck and urethra is carried in the pudendal and hypogastric nerves [11]. The afferent axons of these nerves are divided into two components, myelinated (A $\delta$ ) and unmyelinated (C) fibers, which terminate near and within the urothelium, suburothelium, and detrusor muscle [12,13]. The A $\delta$ -fibers transmit information on bladder filling, whereas the C-fibers are insensitive to bladder filling under normal physiological conditions ('silent' C-fibers) and essentially respond to noxious stimuli [14,15]. The afferent cell bodies are located in the dorsal root ganglia (DRG) at the S2-S4 and T11-L2 spinal segments levels, and the axons synapse with interneurons and spinal-tract neurons that are involved in spinal reflexes and bladder control, respectively [11]. The C-fibers are especially associated with a sensitivity to capsaicin, expression of the transient receptor potential vanilloid 1 (TRPV1), high threshold for activation, and long duration of action potentials that do not respond to tetrodotoxin (TTX). The A $\delta$ -fibers are insensitive to capsaicin but sensitive to TTX. Under normal conditions in conscious animals, micturition is initiated by A $\delta$ -afferents. On the other hand, C-fibers are also involved in pathological conditions and anesthetized animals [7].

The afferent nerves of the bladder have a number of receptors that detect mechanical, chemical and noxious stimuli. Some receptors have been identified in the afferent nerves of the bladder, which are involved in the detection of mechanical, chemical and noxious stimuli. The TRP channels (TRPV1, TRPA1, and TRPM8), purinergic receptors (P2X2, P2X3, and P2Y), and receptors for trophic factor

(tropomyosin receptor kinase A [TrkA], TrkB) have important roles in various pathological conditions, such as neurogenic inflammation as well as in the normal bladder function [7]. When stimuli activate these receptors, proinflammatory peptides are released from afferent neurons.

The mucosa of the bladder (urothelium and lamina propria) can influence the activity of the afferent nerves by working with other components in the bladder. Mechanical, thermal or chemical signals in the urine can activate urothelial cells to release a range of mediators and transmitters that can modulate afferent nerves and/or other cells in the bladder wall [7]. Overall, the mucosa of the bladder is likely to play a key role in the complex communication between the mucosa and nervous system.

## NEUROGENIC INFLAMMATION INDUCING AFFERENT HYPEREXCITABILITY

Pain is a hallmark of IC/BPS. One possible mechanism of IC/BPS that causes pain is the functional change in C-fiber afferents resulting from chronic tissue inflammation [16]. Neurogenic inflammation occurs when inflammatory mediators, such as substance P (SP), calcitonin gene-related peptide (CGRP), and neurokinin A (NKA), are released from the afferent neurons, leading to vasodilation and edema [17]. The experimental data suggest that neurogenic inflammation to noxious stimuli occurs in the skin, airways, and visceral organs, such as the bladder [18]. The release of proinflammatory peptides trigger a host of vascular events, including vasodilation, extravasation of plasma from the postcapillary venules, which causes edema, leukocyte adhesion to endothelial cells in the venules, and leukocyte migration into the affected tissue. As a result, these processes contribute to the activation of mast cells, disruption of the tissue architecture and function, increased afferent excitability, and smooth muscle contractility.

Many animal models and human studies have indicated the involvement of C-fibers in neurogenic inflammation. Sculptoreanu et al. [19] reported that capsaicin-responsive neurons in cats with feline interstitial cystitis (FIC) were increased in size, showed increased firing in response to depolarizing current pulses, and expressed more rapidly inactivating  $K^+$  currents. In addition, the A $\delta$  bladder afferents in cats with FIC showed a significant increase in the slope

of the pressure-firing curves compared to that in normal cats [20]. Gao et al. [21] showed that Wistar-Kyoto rats exposed to chronic water avoidance stress showed significant decreases in pain thresholds and the amplification of painful sensations, which may have resulted from the hypersensitivity of the C-fibers. Other studies using a rat model of cyclophosphamide-induced chronic cystitis reported that the expression of inflammation-related proteins/receptors, such as nitric oxide synthase, growth-associated protein, pituitary adenylate cyclase-activating polypeptide, neuropeptides, and protease activated receptors, are increased in the bladder and bladder afferent neurons [22-26].

Significant increases in the number of sensory axons expressing SP, the density of messenger RNA (mRNA) encoding SP receptors (NK-1) adjacent vascular endothelial cells, and vascular-associated macrophages in the mucosa of the bladder in patients with IC have been reported [27,28]. The number of activated mast cells adjacent to SP-containing nerve endings was increased in the bladders of patients with IC [29,30]. In the submucosa tissue from patients with IC/BPS, the protein gene product 9.5 (PGP9.5) [31] and SP-containing nerve fibers [27] were significantly higher than that in healthy patients. The patients with spinal neurogenic detrusor overactivity had a greater suburothelial nerve density and vanilloid-sensitive suburothelial innervation than normal controls, which was detected by the increased PGP9.5 and TRPV1 immunoreactivity, respectively. In this cohort, intravesical resiniferatoxin resulted in a marked decrease in both PGP9.5 and TRPV1 immunoreactive nerve fibers in patients who responded to treatment, down to values similar to those found in the control tissues [32].

Chronic bladder inflammation can induce a variety of changes in the expression of inflammatory proteins/receptors in the bladder and the afferent pathway of the bladder, which can contribute to the afferent neuroplasticity, resulting in pain symptoms in IC/BPS. Therefore, the appearance of hypersensitivity and mechanical sensitivity of the C-fiber afferent nerve fibers can contribute to a painful sense to the normal innocuous distention of the bladder.

## NEUROTROPHIC FACTORS INDUCING AFFERENT HYPEREXCITABILITY

Neurotrophic factors synthesized by a range of inflammatory cells may play an essential role in linking hypersensitivity

and the development of pain with various inflammatory states, including neurogenic inflammation. Neurotrophic factors, such as nerve growth factor (NGF), brain-derived nerve factor (BDNF), neurotrophin-3 (NT-3), and NT-4 are all formed in the bladder by the urothelium, smooth muscle cell, and various cells of the mucosa; these neurotrophic factors modulate neural plasticity [33]. Neurotrophic factors have been detected in the urine of IC patients [34], and the levels of NGF are elevated in bladder biopsies from women with IC [35]. The intravesical instillation of *Escherichia coli* lipopolysaccharides (LPS) in mice increased bladder NGF in both the mucosa and detrusor [36]. The administration of exogenous NGF into the lumen of the bladders of rats produced rapid and prominent bladder hyperreflexia associated with afferent nerve sensitization. These results suggest that NGF may interact with the visceral sensory systems and play a critical role in sensory disorders associated with inflammation [37]. The exogenous infusion of NGF into the bladder wall of rodents reduced the frequency of bladder contraction, reduced the bladder capacity, increased the bladder mass, increased and altered Fos protein expression in the L6-S1 spinal cord, and increased CGRP expression in specific regions of the L6-S1 spinal cord [38].

BDNF is important in the development of bladder overactivity resulting from persistent bladder inflammation. BDNF binds to its high affinity receptor, TrkB, which is expressed abundantly in bladder afferents and the spinal cord. TrkB expression and activation is upregulated in the bladder afferents in rats with cystitis [39] and spinal cord injury [40]. The urinary concentration of BDNF was also elevated in patients with IC/BPS and was similarly suppressed following a botulinum toxin type A treatment [41]. The intravenous injection of recombinant TrkB-immunoglobulin-like domain (Ig2), which was designed to neutralize BDNF rather than NGF, reduces the frequency of reflex contractions in animals treated with cyclophosphamide [42].

NGF and BDNF are produced in the bladder by the urothelium, mast cells and smooth muscle cells upon stretching and inflammation to sensitize the underlying bladder afferent C-fibers. Activation of the TrkA receptor on urothelium by NGF binding leads to activation of TRPV1 and mechanosensory channel (MSC), which sensitizes the underlying bladder afferent fibers by urothelial mediator

release. In addition, NGF activates the TrkA receptors expressed on the suburothelial afferent C-fiber terminals, directly sensitizing neuronal TRPV1, MSCs, and voltage-gated ion channels (VGCs). The TrkA-NGF complex is transported retrogradely to the cell bodies in lumbosacral DRG, where the de novo transcription of TRPV1, VGCs, and MSCs occurs. These de novo synthesized ion channels are transported anterogradely back to the afferent terminals and consequently contribute to afferent hyperexcitability [33].

NGF is a central element in conditions associated with neurogenic inflammation and has been proposed as a biomarker for these conditions. Monoclonal antibodies against NGF have been used in preclinical and clinical studies to modulate pain. Tanezumab, which is a monoclonal antibody against NGF, prevents NGF from interacting with its receptors, TrkA and p75 [43]. The results from clinical trials using tanezumab have indicated improvements in urgency and pain in patients with IC/BPS [44,45]. On the other hand, adverse events of abnormal peripheral sensations, have been reported following tanezumab treatment.

## CENTRAL SENSITIZATION

Pain that arises in response to tissue damage is a complex and unpleasant sensory and emotional experience. Although the mechanisms associated with the development and maintenance of chronic pain have not been entirely elucidated, a primary mechanism for these is the processing of central sensitization, whereby long-term molecular alterations result in the abnormal and significant amplification of pain sensations in the CNS [46].

Repeated peripheral insults result in the activation of afferent C-fibers synapsing at the dorsal horn of the spinal cord, and give rise to alterations in the circuits in the spinal cord and brain. In the pathogenesis of central sensitization, the peripheral nerves usually maintain their normal function, but functional changes occur in central neurons. Hyperexcitable spinal neurons show reduced thresholds, increased receptor field sizes, and continuing stimulus-independent activity, as well as greater evoked responses [47]. Central sensitization is maintained through continuous input from the periphery, but it is also modulated by descending controls from the midbrain and brainstem. The projections

of sensitized spinal neurons to the brain, in turn, alter the processing of painful messages by higher centers [47]. In the environment of central sensitization, stimuli that do not usually produce pain can provoke pain (allodynia) and stimuli that normally produce pain can increase the level of pain (hyperalgesia). The process of the development of central sensitization is complex, and a range of factors are involved. Glutamate, the predominant excitatory neurotransmitter involved in nociception, binds to the N-methyl-D-aspartate (NMDA) receptor. The recruitment and activation of NMDA receptors in the spinal cord dorsal horn appear to be one of the key mediators of central sensitization [48].

The CNS contains two basic types of cells: neurons and non-neuronal cells (glial cells). Glial cells include satellite glial cells, astrocytes, oligodendrocytes, and microglia. They maintain the CNS homeostasis by providing structural and metabolic support and communicate with the neurons by bidirectional signaling (neuroimmune communication). The neuroimmune communication allows glia cells to respond rapidly to disruptions in neuronal signaling [49,50]. The strong or long-term noxious activation of astrocytes and microglia within the spinal dorsal horn can lead to aberrant synthesis and the release of proinflammatory mediators, such as interleukin (IL)-6, IL-1 $\beta$ , and TNF $\alpha$ . The overarching effect of these neurokinine signals in excitatory synapses contribute to central sensitization and facilitate the transmission of nociceptive signals to the brain [46]. In addition, inside the inhibitory synapse of the spinal cord dorsal horn, the effects of these mediators sequentially give rise to a decrease in inhibitory neurotransmission, thereby promoting central sensitization [46]. Therefore, neurogenic inflammation of the bladder mucosa can cause long-lasting alterations in the central and peripheral nervous system. Moreover, it is possible that these changes contribute to chronic pain condition in patients with IC/BPS.

## A ROLE OF TOLL-LIKE RECEPTORS IN INTERSTITIAL CYSTITIS/BLADDER PAIN SYNDROME

A urinary tract infection (UTI) is considered the most common type of bacterial infection [51]. The severity of UTI is determined directly by the microbial virulence factors and the quality of the host immune response [52]. Uropathogenic *E. coli* (UPEC) is responsible for more than

80% of UTIs. Almost all UPEC strains encompass the virulence factors of type I fimbriae and FimH [53], which enables UPEC to adhere to uroplakin 1a molecules, a major surface molecule on the superficial epithelial cells lining the bladder [54]. The mechanisms of the immune system, including the innate and adaptive immunities, are activated by pathogenic microorganisms, such as UPEC, entering the human urinary tract. The effective resistance of the bladder to colonization by active microorganisms is due to both the anatomical characteristics and antimicrobial compounds secreted from the urothelium.

As microorganisms enter the urothelial cells, macrophages simultaneously produce proinflammatory responses and pathogen recognition receptors (PRR) molecules. PRRs are comprised of five families, including TLRs, C-type lectin-like receptors, retinoic acid-inducible gene I-like receptors, nucleotide-binding oligomerization domain-like receptors, and absent-in-melanoma-like receptors [55]. To date, ten members of TLRs (TLR1 to TLR10) are recognized in the human innate immune system [56], the importance of TLRs has been recognized as a key regulator for the innate and adaptive immune responses. TLRs are transmembrane signaling proteins expressed in peripheral immune cells and glia that play a crucial role in the innate immune system against external pathogens, such as bacteria, fungi, and viruses, as well as different endogenous factors arising as a result of any type of tissue damage [57,58]. After the entrance of uropathogenic microorganisms into the urinary tract, the innate immune responses are activated via expression of the related TLRs within the urothelial cells of bladder. Consequently, the expression of related TLRs triggers different cascading responses, including the release of chemokines, interferons, interleukins, antimicrobial substances, and proinflammatory cytokines [59].

In the urinary tract, TLRs 2, 4, and 5 are the most functional molecules as well as the most effective TLRs against UTIs [59]. The most important target ligands for TLR4 molecules are bacterial LPS; Type I and P fimbriae; and heat shock proteins molecules of 60, 70, and 90 [59]. At the time of the identification of bacterial LPS, TLR4/CD14 complexes on bladder epithelial cells are believed to initiate the classical signaling pathway involving NF- $\kappa$ B [56]. On the other hand, the second and parallel signaling pathways induced by TLR4 in bladder epithelial cells have recently been described, in which cyclic adenosine monophosphate (cAMP) and

cAMP response element-binding protein are important components [60]. The second pathway leads to much faster cytokine release than the classical pathway.

TLRs (particularly TLR4) have also been recognized as important factors in central sensitization, explaining chronic and persistent pain. In animal studies, inflammatory signaling secondary to TLR-4 stimulation has been reported to play an important role in the development of hyperalgesia and allodynia [50,61], but their role in IC/BPS is unknown. Schrepf et al. [8,62] reported that the peripheral blood mononuclear cells of patients with BPS increase the inflammatory response to TLR2 and TLR4 stimulation *in vitro*, and the degree of the proinflammatory response is positively correlated with the severity of pelvic pain. As the TLR response of peripheral blood and spinal cord is related to pain, the clinical outcome may act as a systemic biomarker in the case of chronic pain, including IC/BPS [63]. Therefore, the inflammatory signaling secondary to TLR-4 stimulation plays a critical role in the development of pain in patients with IC/BPS based on the evidence from animal models of chronic pain [50,61,64].

Ichihara et al. [65] studied TLR7 expression in bladder biopsy specimens from patients with Hunner-type IC (HIC) and examined the functional role of TLR7 in bladder inflammation and nociception in mice. They reported increased TLR7-gene expression and TLR7 immuno-reactive cells in the specimens of patients with HIC compared to the controls. When loxoribine, a TLR7 agonist, is instilled in the bladder of C57BL/6N female mice, it induces edema, congestion, and inflammation, and increases TLR7-mRNA expression significantly [66]. The intravesical instillation of hydroxychloroquine reversed the loxoribine-induced cystometric and voiding behavioral changes. Therefore, inhibition of the TLR7 pathway in the bladder of patients with IC/BPS can be a potential new therapeutic option.

## URINARY TRACT INFECTION-ASSOCIATED PAIN

Although most UTIs are caused by UPEC [51] and result in pelvic pain and voiding dysfunction [67], the role of a UTI in the pathogenesis of IC/BPS is unclear. Despite there being little data supporting the role of infectious causes, researchers continue to be interested in infectious theories.

LPS consists of three regions: acylated lipid A, a core

oligosaccharide, and O-antigen. Although the lipid A component is the major inflammatory mediator through interactions with TLR4 [56,68], the diversity of *E. coli* serotypes is determined largely by the O-antigen structure of more than 180 serotypes [69]. In the murine UTI model, *E. coli* strains show a spectrum of pain phenotypes, ranging from the null phenotype to a phenotype that induces chronic pelvic pain following a single, transient infection. The pain phenotype is independent of inflammation, but is mediated by LPS and TLR4 as well as by the subsequent afferent responses involving TRPV1 and chemokine (C-C motif) receptor 2. Post-UTI chronic pain modulated by O-antigen in mice can recapitulate the key features of urological and cognitive dysfunction observed in patients with IC [67].

## CONCLUSIONS

The etiology of IC/BPS is not completely understood, but there may be multiple causes with similar clinical manifestations. Various forms of long-lasting stimuli can cause neurogenic inflammation of the bladder mucosa, and patients with IC/BPS often show neurogenic inflammation of the bladder mucosa. Chronic bladder inflammation can give rise to a variety of alterations in the expression of pro-inflammatory and inflammation-related proteins/receptors in the bladder and result in central sensitization as well as hyperexcitability of the bladder afferent neurons. In addition, the inflammatory signaling response to TLR-4 stimulation plays a critical role in the development of pain in IC/BPS patients. Further intense research on the pathophysiology of IC/BPS will lead to more effective treatments for patients with IC/BPS.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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