Review article

Pathology and physiology of acid-sensitive ion channels in the bladder

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ABSTRACT

Acid-sensitive ion channels (ASICs) are sodium-permeable channels activated by extracellular acidification. They can be activated and trigger the inward flow of Na^+ when the extracellular environment is acidic, leading to membrane depolarization and thus inducing action potentials in neurons. There are four ASIC genes in mammals (ASIC1–4). ASIC is widely expressed in humans. It is closely associated with pain, neurological disorders, multiple sclerosis, epilepsy, migraines, and many other disorders. Bladder pain syndrome/interstitial cystitis (BPS/IC) is a specific syndrome characterized by bladder pain. Recent studies have shown that ASICs are closely associated with the development of BPS/IC. A study revealed that ASIC levels are significantly elevated in a BPS/IC model. Additionally, researchers have reported differential changes in ASICs in the bladders of patients with neurogenic lower urinary tract dysfunction (NLUTD) caused by spinal cord injury (SCI). In this review, we summarize the structure and physiological functions of ASICs and focus on the mechanisms by which ASICs mediate bladder disease.

1. Introduction

In 1997, Waldmann and colleagues successfully replicated the initial demonstrations of H^+-gated sensory neurons, designating the molecules responsible as acid-sensitive ion channels (ASICs) [1]. These channels are categorized as members of the epithelial sodium channel (ENaC)/degenerin (DEG) channel family [2–4]. ASICs are trimeric protein complexes that undergo activation in response to a reduction in pH within the extracellular milieu [3,5]. These complexes are mostly found in the central nervous system, and extensive research has been conducted on the physiological and pathophysiological significance of ASICs within the nervous system. These studies have explored their involvement in various processes, including synaptic plasticity, memory formation, and fear conditioning, and their associations with diseases such as ischemic brain injury and Alzheimer's disease [6–10]. However, limited attention has been given to investigating the roles of ASICs in other bodily systems.

The bladder, as part of the human urinary system, is not only a fluid reservoir but also has complex sensory and control functions. The regulation of this function involves multiple cell types, signaling pathways and molecular mechanisms [11]. The function of ASICs

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in the bladder system and their relevance to bladder diseases have become popular research topics in recent years. For example, researchers have found that ASIC3 knockout (KO) mice have reduced urinary output [12]. Bladder pain syndrome/interstitial cystitis (BPS/IC) is a persistent clinical condition distinguished by chronic discomfort localized in the bladder region [13]. A number of potential pathophysiologic pathways, including epithelial failure, mast cell activation, neurogenic inflammation, and autoimmune and occult infections, have been suggested by researchers [14]. Recent research has revealed a range of elevations in certain ASIC subunits within the bladders of individuals diagnosed with interstitial cystitis [15]. Neurogenic lower urinary tract dysfunction (NLUTD) resulting from spinal cord injury (SCI) is a pathological state that impacts both urine storage and voiding functions. The subunits of ASICs are also subject to variable degrees of modification in the bladders of individuals affected by this condition [16]. These changes in ASIC subunits may be closely related to the pathological processes of these diseases.

In this work, we first comprehensively summarized the structure and function of ASICs, emphasizing the functions of ASIC constituents and the pathophysiology involving different subunits. In addition, we explored the physiology associated with ASICs in the bladder, which provides a basis for understanding the mechanisms linking ASICs to bladder diseases. Finally, existing studies on the relationships between ASICs and BPS/IC and NLUTD have revealed the pathological mechanisms involved, emphasized that ASICs play a key role in bladder diseases, and are expected to provide new ideas for understanding the mechanisms of bladder diseases, which in turn will provide a more targeted approach for therapeutic strategies for these and other related diseases.

2. Structure and function of ASICs

2.1. Structure of ASICs

When the extracellular pH is below 7.0, ASICs can be activated and trigger the inward flow of Na^+, leading to membrane depolarization and thus inducing action potentials in neurons [1–3]. Mammals have four types of ASIC genes (ASIC1–4), which are responsible for encoding a minimum of six distinct ASIC subunits: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4 [1,17–19]. With the exception of ASIC4, all ASIC subunits are observed in sensory neurons of the peripheral nervous system. ASIC1a, ASIC2a, ASIC2b, and ASIC4 are extensively expressed in the central nervous system (CNS) [2]. Active channels are formed by the combination of three independent ASIC subunits, resulting in the formation of either homogeneous or heterogeneous channels. Different methods of combining ASIC subunits result in different pH sensitivities, desensitization kinetics, and ion selectivities; thus, these channel properties can be used to determine the subunit composition of ASICs [20].

Each individual ASIC subunit is composed of 500–560 amino acids and possesses a simple structure that is characterized by two transmembrane structural domains, a cellular N-terminal, a cellular C-terminal, and a large extracellular structural domain rich in cysteine [21]. The transmembrane structural domains are integrated into the lipid bilayer and establish ion channel pores alongside other subunits. These pores aid in the identification of extracellular ligands and regulate proton-gated currents. Additionally, they serve crucial functions in the gating and selectivity of ASICs [22,23]. The intracellular N-terminus and C-terminus are involved in
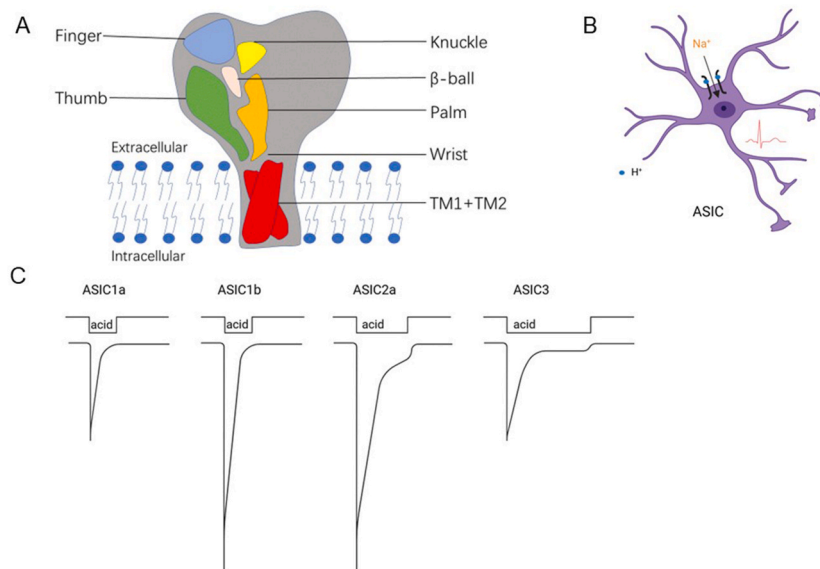
Fig. 1. Structural and biophysical properties of ASICs. **(A)** Different subdomains span the membrane segment (red), palm (orange), finger (blue), thumb (turquoise), β-ball (pink) and knuckle (yellow). **(B)** Illustration of proton activation of ASICs in neurons leading to Na^+ entry, resulting in membrane depolarization and action potential induction. **(C)** Current traces of the pH-sensitive ASIC and characteristic traces of the current recorded in the full-cell configuration of ASIC1a, ASIC2a, ASIC2b and ASIC3. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

regulating the expression and activation of ASIC subunits [24]. The large extracellular structural domain is the most likely site for the binding of some pharmacological ligands and is easily solvent accessible [25]. The large extracellular structural domain also plays a significant role in regulating ENaC and ASIC activity, and it contains H^+-sensing residues that are involved in ASIC activation. The extracellular region of each subunit resembles a clenched hand and includes the wrist, palm, finger, knuckle, thumb, and β-ball [26] (Fig. 1 A). There are many intersubunit lumens in the trimeric channel's extracellular region. One of the most significant parts is the acidic pocket, which comprises the thumb, fingers, and β-ball of one subunit, together with the palm of the adjacent subunit. Structural investigations have proposed that the activation of ASIC is influenced by the protonation of residues located in the acidic pocket. Additionally, the pH dependence of ASIC is affected by the ability of the acidic pocket to sense protons [20]. The structural domains mentioned above are crucial in various physiological processes, such as channel gating, ion permeation, intracellular protein–protein interactions, and intracellular molecular control.

The activation of ASICs is contingent upon the protonation of many residues situated in distinct domains of the channel. Through functional investigations, three potential pH-sensing regions have been identified: the acidic pocket, the wrist, and the palm. In the closed state, the acidic pocket adopts an elongated shape, characterized by the displacement of the thumb away from the β-ball and finger domains. The transmembrane structural domains have a constricted shape, effectively occluding the channel and impeding the passage of ions. During the process of extracellular acidification, the reduction in pH induces contraction of the acidic pocket, resulting in enlargement of the wrist region and dilation of the lumen. This is achieved through conformational alterations in the extracellular and transmembrane structural domains, facilitating the passage of electrical current. In the event of continued acidification, the palm, wrist, and pores undergo collapse, leading to the channel entering a desensitized state [20].

2.2. Function and pathophysiology of ASICs

ASICs are triggered by a sudden increase in the extracellular proton concentration, resulting in transitory proton-gated currents that are carried mostly by Na^+ but will also allow Ca^{2+} and K^+ to pass [1,2] (Fig. 1 B). ASICs typically undergo rapid desensitization or inactivation subsequent to proton-induced activation, resulting in the generation of characteristic transient currents, such as those observed in ASIC1a and ASIC1b. During neural activity, synapses experience fast local acidification, but in conditions such as ischemia, inflammation, and tumors, there is gradual and prolonged acidification of the surrounding tissue. Owing to its Na^+ permeability, the activation of ASICs in this particular situation results in depolarization of the neuronal membrane, hence inducing neuronal excitation. In contrast, ASIC3 and certain ASIC3 complexes composed of heterodimers exhibit a notable sustained current even after partial deactivation of the first transient inward current. This sustained current is observed in the presence of persistent acidity and often has a lesser amplitude than the transient current. The observed continuous flow of current implies that ASIC3 will remain in an activated state over extended periods of tissue acidosis. This activation facilitates a persistent influx of sodium ions in sensory neurons, resulting
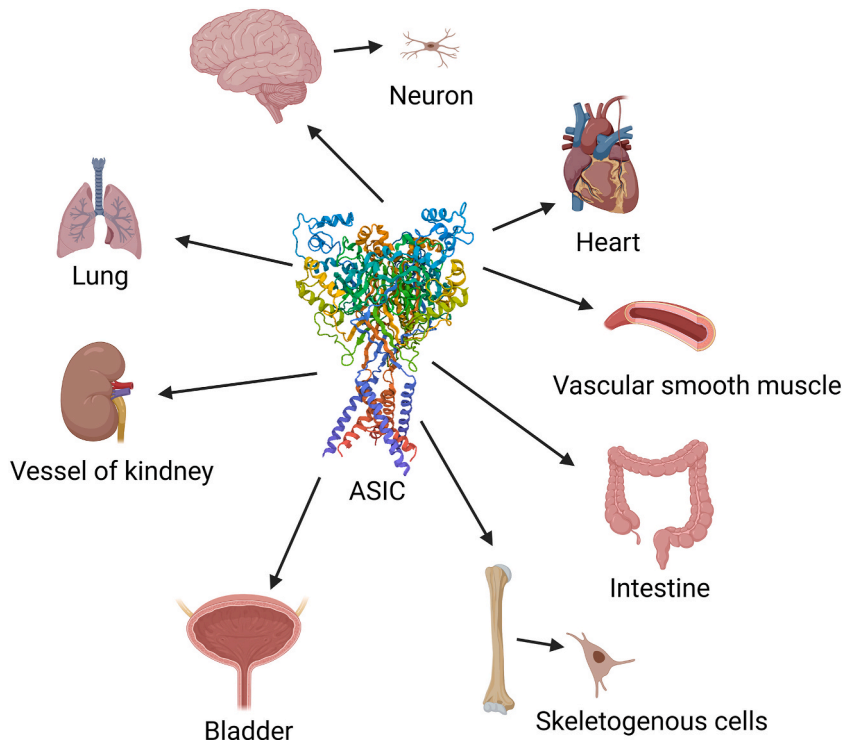

Fig. 2. ASICs are involved in physiological and pathological processes in a variety of human organs and tissues (Protein Data Bank (PDB): 6 × 9H).

in membrane depolarization and ultimately leading to prolonged discharge of action potentials (Fig. 1C). Therefore, ASIC3 has the ability to accurately convert extended tissue acidosis that arises in diseased conditions into the sensation of pain [2,27,28].

In addition, ASIC can be activated by ligands that are not protonic in nature, such as MitTx (a peptide complex from Texas coral snake venom (*Micrurus tener tener*)) and GMQ (2-guanidine-4-methylquinazoline), even at the typical physiological pH. This activation leads to the generation of prolonged ASIC currents, which have been demonstrated to be linked to the sensation of pain [29,30].

ASIC is widely expressed in various organs and tissues of the human body, and thus far, ASIC has been found to be involved in a variety of pathophysiological processes (Fig. 2). ASIC1a is abundantly expressed in neurons [31,32] and its expression in sensory neurons is closely related to pain perception. Under conditions of tissue injury, inflammation or an acidic environment, the activity of ASIC1a channels increases, leading to increased excitability of neurons, which is involved in pain transmission [33,34]. Second, ASIC1a is associated with the formation and maintenance of learning and memory in certain regions of the central nervous system, such as the hippocampus. It is involved in influencing spatial memory and conditioned learning [35,36]. In some cases, the activation of ASIC1a channels may be involved in neuroprotective mechanisms, such as under conditions of ischemia and brain injury. On the other hand, overactivated ASIC1a channels may lead to apoptosis and participate in the development of several neurological diseases. In the cardiovascular system, ASIC1a expression may be associated with physiological processes in the heart and blood vessels. Some studies suggest that ASIC1a may play a role in conditions such as myocardial ischemia and myocardial infarction [37,38]. Overall, the widespread expression of ASIC1a in the nervous system and other tissues makes it an important participant in a variety of physiological and pathological processes. Its role in pain perception, learning memory, neuroprotection, and cardiovascular regulation makes it a potential therapeutic target, especially in pathological processes related to pain and neurological disorders [39–41].

ASIC1b is predominantly localized in peripheral sensory neurons. Research findings indicate that channels that include ASIC1b exhibit heterogeneity and it often combines with other ASIC subtypes, such as ASIC3 or ASIC1a, to produce heterotrimeric channels [42]. The functional ASIC1b-containing channels are involved in the perception of peripheral injury-related sensations and pain [43, 44].

The distribution of ASIC2 in the brain is rather extensive, encompassing several regions, such as the hippocampus, amygdala, basal ganglia, and cerebellum [45,46]. In the peripheral nervous system, ASIC2 is expressed predominantly in sensory neurons, especially in sensory nerve endings in the skin, skeletal muscles, and visceral organs. ASIC2 has been implicated in the pathogenesis of several disorders, including ischemia, multiple sclerosis, epilepsy, migraine, arthritis, and aminoglycoside-induced hearing loss. ASIC2a/3 isoforms have also been found in cardiac dorsal root ganglion (DRG) neurons and are capable of inducing an electric current that plays a role in cardiac afferent signaling. These cardiac afferent neurons predominantly function as chemoreceptors and play a role in cardiac reflexes and the perception of pain during myocardial ischemia. The findings obtained from experiments involving blockage of the middle cerebral artery in rodents and analysis of cardiac dorsal root ganglia indicate that ASIC2 channels could serve as a viable target for therapeutic interventions aimed at mitigating neuronal damage and angina associated with ischemia injury [47–50].

ASIC3 is predominantly located within peripheral DRG neurons. ASIC3 is associated with P2X3 (a family of trimeric cation channels that are activated by extracellular ATP) ion channels [51], in addition to its ability to build functional channels with other ASIC subunits [52]. ASIC3 can convert prolonged acidosis into an injury signal. In addition, certain mediators are capable of sensitizing ASIC3, resulting in lower proton concentrations being able to activate it. There is a considerable body of information derived from transgenic mouse models and pharmacological studies that support the notion that ASIC3 holds promise as a viable target for the advancement of analgesic medications. While ASIC3 is known to have a significant effect on pain modulation [53–55], it is also involved in bladder function. Problems such as voiding and oliguria have been found in ASIC3 KO mice [12]. ASIC4 is predominantly localized within the pituitary gland, and it should be noted that ASIC4 does not exhibit functional channel formation and is not responsive to proton activation [56].

3. Physiology associated with bladder ASICs

3.1. ASICs and bladder sensory neurons

Several studies have provided evidence indicating a high level of expression of ASICs in the DRGs of both rats and mice [57–60]. However, the precise manner in which ASICs are constructed in bladder sensory neurons remains poorly understood. In a recent study conducted by Montalbetti et al. [12], three distinct categories of bladder sensory neurons were identified on the basis of their response to extracellular acidification. The first group exhibited no response to extracellular acidification, whereas the second group displayed a transient increase in current, similar to ASIC-like behavior. The third group, on the other hand, presented a continuous increase in current in response to extracellular acidification. Through a comparison of the currents of bladder sensory neurons in wild-type (WT) and ASIC3 KO mice under conditions of decreased extracellular pH, it was determined that ASIC3 exists as an isomer in bladder sensory neurons. Notably, a slow desensitization of ASIC-like currents was observed in the neurons of ASIC3 KO mice, which aligns with the desensitization pattern observed for ASIC1a [61–64]. However, the potential involvement of ASIC2 in the proton-sensitization component cannot be disregarded. A previous study demonstrated that mice lacking ASIC1a, ASIC2, and ASIC3 were incapable of generating ASIC-like currents [65]. On the basis of these findings, the authors conclude that in the bladder sensory neurons of WT mice, ASIC functions in the form of ASIC1a-ASIC3 or ASIC1a-ASIC2-ASIC3 isomers.

3.2. ASICs and bladder sensory signaling

The efferent innervation of the bladder comprises superficially myelinated Aδ fibers and unmyelinated C fibers, which transmit

signals from the uroepithelium and muscular tissue to the spinal cord. The afferent fibers responsible for transmitting signals from the bladder are conveyed by the pelvic nerves situated in the DRG of the lumbosacral (L6–S2) spine, as well as the hypogastric nerves located in the DRG of the thoracolumbar spine (T13–L2) [66,67]. Nicolas Montalbetti [12] explored whether extracellular acidification might stimulate action potentials in bladder sensory neurons and whether ASIC3 was involved. They exposed bladder sensory neurons from WT and ASIC3 KO mice with ASIC-like currents to a pH of 6.0. Researchers discovered that a substantial proportion of the action potentials elicited by a decrease in extracellular pH originated from bladder sensory neurons in WT mice but not from bladder sensory neurons in mice lacking the ASIC3 gene (ASIC3 KO mice). Additionally, they reported that the ASIC-like currents were more pronounced in the neurons of ASIC3 KO mice than in those of WT mice. Researchers have hypothesized that this may be related to alterations in ASIC ion selectivity or the activation/desensitization kinetics of proton-evoked currents in ASIC3 KO sensory neurons. These findings imply that ASIC3 functions as a proton sensor in bladder sensory neurons and is an integral component of the proton sensor in bladder afferent events.

Previous research has indicated that protons might have a significant effect on the regulation of sensory signals in the bladder. Consequently, Nicolas Montalbetti proposed that ASIC3 serves as a pivotal component within the sensory system responsible for governing bladder function. Subsequent to the aforementioned study, another study yielded similar results, demonstrating that bladder sensory neurons in mice lacking the acid-sensing ion channel 3 (ASIC3) gene were incapable of generating action potentials upon exposure to reduced extracellular pH. This finding aligns with the hypothesis that ASIC functions as a genuine proton receptor during afferent events. The proper functioning of the bladder and its reactions to inflammation and damage are contingent upon the sensory input received from primary afferent fibers located inside the bladder tissue. These fibers exhibit a diverse array of ligands as well as mechanically gated ion channels and receptors, which enable the conversion of chemical and mechanical inputs into electrical impulses. The mechanisms by which these signals are processed and incorporated into afferent signals and how they transmit information on the bladder state and noxious events to the CNS, as well as the role of ASICs in these processes, remain unknown.

3.3. ASICs and bladder function

The bladder is a vital component of the human urinary system, serving as a crucial organ responsible for the storage and elimination of urine while also playing a role in urinary control and sensory perception [68]. Researchers [12] have conducted voiding studies on conscious WT and ASIC3 KO mice and reported that the major spot area and overall void area of ASIC3 KO mice are dramatically smaller than those of WT mice. This observation indicates that, compared with WT mice, ASIC3 KO mice exhibit a notable decrease in urination. This finding aligns with the notion that ASIC3 plays a crucial role in the sensory system responsible for regulating bladder function. Furthermore, the pressure necessary to initiate voiding was notably lower in ASIC3 KO mice than in WT mice.

On the basis of the observed reduced voiding volume and lowered pressure threshold for initiating voiding following ASIC3 deletion, it may be inferred that ASIC3 does not possess the ability to serve as a mechanosensor in bladder afferent neurons. The peak pressures observed in WT mice and mice without ASIC3 were similar, indicating that the absence of ASIC3 does not have an effect on detrusor function.

Mitsuharu Yoshiyama [69] reported that the intraperitoneal injection of A-317567, an ASIC blocker, into mice increased bladder capacity and attenuated bladder hyperreflexia stimulated by intravesical acid. These results suggest that ASIC is involved in mechanosensation and injury perception in the bladder. This is contrary to Montalbetti's results. However, bladder measurements under polyurethane anesthesia conditions revealed no significant difference in void volume between ASIC3-KO and WT mice, which may be due to compensatory effects produced by gene knockouts in the central and peripheral nervous systems. For the differences between the other results under conscious and polyurethane anesthesia conditions, the authors considered the following factors: 1. ASIC3 gene deletion. ASIC3 is expressed in several organs, such as the testis, ovary, adrenal glands, and bladder [70]. Its involvement in testosterone generation and/or release in animals has been suggested. Testosterone plays a crucial role in the regulation of ion channels [71], including calcium ion channels, in smooth muscle, skeletal muscle, and cardiac muscle. Furthermore, empirical evidence has demonstrated a substantial correlation between decreased levels of blood testosterone and the occurrence of overactive bladder [72]. Hence, it is plausible that reduced serum testosterone levels in ASIC3-KO mice could exert a notable influence on the duration of urine storage, leading to increased excitability of the urethral muscle and a subsequent decrease in urination volume. Additionally, the use of polyurethane anesthetics is worth considering. One study revealed that, compared with WT mice, mice with a deficiency in genes associated with bladder afferent transmission exhibited distinct responses to polyurethane. These responses manifested as either inhibition or disruption of the micturition reflex [73]. In a comparable manner, the absence of ASIC3 within the neuronal network that regulates bladder function could interact with the effects of polyurethane, leading to modified bladder activity.

In their study, Yoshiyama [69] reported that the administration of A-317567 via systemic injection did not result in any significant changes in the maximal voiding pressure during saline infusion bladder surgery. Additionally, the researchers reported that the reduction in maximal voiding pressure caused by intravesical acid stimulation was not reversed by the systemic injection of A-317567. These findings indicate that ASICs may not play a predominant role in regulating bladder contraction. The administration of A-317567 via intravesical instillation did not elicit any alterations in bladder activity, regardless of the presence or absence of acid stimulation of the bladder. These findings suggest that ASICs located in the uroepithelium may not play a substantial role in the regulation of bladder activity.

The potential involvement of rat urinary epithelial ASICs in bladder function has been investigated through in vivo and in vitro research. A previous study [58] reported a reproducible 75 % reduction in transient Ca^{2+} responses in the presence of the nonselective ASIC blocker amiloride (10 mM). The majority of uroepithelial cells respond to changes in pH induced by acetic acid at a pH of 5.5. These cells presented biphasic-like currents sensitive to amiloride at a concentration of 10 mM. According to the researchers [58],

these characteristics resembling those of neurons, which are facilitated by ASIC activity, may play a role in the secretion of neurotransmitters that influence afferent nerves or smooth muscle, hence regulating bladder responses.

A further investigation was conducted to assess cystometry in rats, which revealed that the introduction of amiloride (1 mm) into the bladder increased the interstitial space between the bladder walls. This finding implies that epithelial sodium-selective ion channels, specifically ASICs, might play a role in sensory signaling during the process of bladder control. Nevertheless, the analysis of this outcome is intricate because the effects induced by amiloride could be ascribed to several channels, such as TRPA1 [74], ENaC, T-type calcium channels, and undetermined high concentrations. The underlying factors contributing to the variations in the reactions to ASIC blockers between rats and mice remain unclear. Consequently, additional investigations are warranted to ascertain the significance of uroepithelial ASIC in the modulation of bladder function.

Additionally, it was discovered that the DRGs are the region responsible for the effects of ASIC blockers. In a study employing reverse transcription–polymerase chain reaction (RT–PCR), the gene expression levels of ASIC2 were the highest among the ASIC subunits within the mouse DRGs. Notably, the expression levels of the ASIC2 and ASIC3 genes were considerably elevated in the DRGs of female mice compared with those of male mice. This observation implies the existence of sexual dimorphism in the signaling pathway of bladder afferents, specifically in response to proton stimulation. The findings of our earlier work indicated that female mice displayed greater sensitivity to intravesical acetic acid stimulation than male mice did. This resulted in a significantly heightened bladder reflex, characterized by hyperactivity. Therefore, the findings of this study indicate that increased expression of the ASIC2 and ASIC3 genes in DRGs could contribute to the increased sensitivity of the female bladder to acidic stimuli.

Various ASIC channels have been identified in the urinary tract epithelial cells of rats, mice, pigs, and humans. The activation of these channels leads to the generation of ionic currents and inward currents of calcium, which are similar to the responses observed in primary sensory neurons [57]. The aforementioned properties potentially serve as the fundamental mechanisms responsible for the release of neurotransmitters (ATP, NO, and ACh) or other factors (prostaglandins) that induce changes in the extracellular levels of neurotransmitters within the bladder mucosa [75–78]. These alterations subsequently impact afferent nerve activity and bladder function. In summary, the precise role of ASIC in the bladder, pertaining to urine storage and voiding, remains inconclusive. Consequently, further experimentation is warranted to elucidate this matter.

In addition, Nicolas Montalbetti [12] reported that the injection of cyclophosphamide (CYP) into ASIC3-deficient mice caused abnormal pelvic pain at a single point in time, whereas pelvic sensitization was only slightly altered in wild-type mice. The observed variation in mechanical pelvic sensitization between wild-type mice treated with CYP and ASIC3 KO animals may be related to the heightened sensitivity of bladder C injury receptors. The removal of ASIC3 from the sensory neurons of the bladder hinders their capacity to generate action potentials when exposed to extracellular acidification. The importance of ASIC3 in the detection of bladder injury is crucial since it regulates the excitability and sensitivity of C-fiber injury receptors. The absence of ASIC3 results in increased sensitivity of neurons at more advanced levels and interneurons within the spinal cord but also intensifies the reaction to damage. Therefore, ASIC3 signaling inhibits the nociceptive pathway of the bladder. The results of this study provide evidence that protons and their corresponding ASIC3 receptors play a role in the modulation of nerve endings, specifically in regulating the excitability and sensitization of injury receptors. These findings suggest that the absence of this regulatory mechanism can result in abnormal firing patterns following injury, leading to increased sensitivity of afferent pathways, excessive activation of the injurious pathway in chemical cystitis, and the subsequent development of pain. Nevertheless, additional investigations at the molecular level are required to elucidate the mechanisms underlying the heightened neuronal excitability resulting from the loss of proton signaling in the context of CYP-induced injury. These findings challenge the prevailing notion that the expression and activation of ASICs contribute to pain, as previously reported in prior studies [79]. Furthermore, these results also contradict the observed increase in bladder ASIC2a and ASIC3 expression in patients with BPS/IC.

4. ASICs and bladder disease

4.1. ASICs and BPS/IC

BPS/IC mainly presents as a disease with persistent or recurrent pain accompanied by at least one other symptom, such as urinary frequency. Therefore, we focus here on the mechanisms involved in the occurrence of pain in patients with ASIC and BPS/IC.

Many chemical mediators, such as neurotrophic factors, cytokines, chemokines and neuropeptides, are produced in the voiding reflex pathway in patients with cystitis. These chemical mediators also regulate the expression of ASICs. Kimberly Corrow [59] examined the expression of ASIC1, ASIC2a and ASIC3 in the bladders of female rats treated with cyclophosphamide for 48 h. They found that the mRNA and/or protein expression of ASIC2a and ASIC3 was significantly elevated in the uroepithelium and subepithelial plexus. In contrast, there were no significant changes in the mRNA expression of ASIC1 in the uroepithelium or in the forced urethra. Stephan Kellenberger [13] analyzed the mRNA levels of ASIC in the bladders of patients with BPS and their results also showed that ASIC2a and ASIC3 were significantly upregulated in patients with BPS, but no differences were detected in the levels of ASIC1a.

Under cystoscopy, there are two subtypes of BPS/IC: classical IC with Hunner's ulcers (ulcerated lesions) and nonclassical BPS/IC without ulcers. In a subsequent study by Yukio Homma [15], specimens from 50 subjects were analyzed, and the results showed that ASIC1 was significantly elevated in nonulcerated lesions of the classical IC but not in those of the nonclassical IC. This finding is inconsistent with the results of the two studies mentioned above. This discrepancy may be partly due to different selection criteria.

All of these results suggest a close relationship between the BPS/IC and ASIC subunits, in which ASIC2a and ASIC3 in the bladder mucosa may be important (Table 1).

Researchers [80] have reported that acid effectively stimulates ATP release from rat bladder mucosa expressing the ASIC subunit

and that this release may be mediated through transient receptor potential vanilloid 1 (TRPV1) and ASIC receptors. Rajni Sadananda [81] found, by exposing strips of porcine bladder mucosa to acid, that the ASIC receptor and (at lower pH) the TRPV1 receptor were also able to stimulate the release of ATP, which is in line with the results of a previous study. The researchers stimulated monolayer bladder urothelial cells from IC patients and control patients with 10–30 μM ATP and measured extracellular ATP levels via luciferin-luciferase. They reported that 30 μM ATP stimulation resulted in the release of more ATP from the IC epithelium than from control bladder urothelial cells, suggesting that the bladder epithelium of BPS/IC patients is more likely to release more ATP in response to ATP stimulation [82].

ATP is a cotransmitter with acetylcholine in parasympathetic nerves innervating the bladder, and extracellular ATP has been implicated in a number of sensory processes, including responses to pain and the regulation of visceral organ movements. ATP is a key modulator in the injurious pathway, and its release from injured tissues or sympathetic efferents sensitizes peripheral sensory neurons and increases the release of glutamate from the centrally terminal synapse. The exocytosis of ATP from presynaptic vesicles located in the central terminal can lead to an increase in the release of glutamate. This, in turn, can amplify the mechanisms of central sensitization that are believed to be the underlying cause of numerous chronic pain conditions. The sensory neurons predominantly involved in ATP signaling are characterized by the presence of purinergic receptors, namely, P2X3 and P2X2/3 [83]. Previous studies utilizing selective knockdown experiments or small-molecule inhibition have demonstrated that receptors containing P2X3 play crucial roles in the modulation of injurious signals. P2X3-containing receptors have been identified as potential targets for therapeutic interventions aimed at managing chronic pain [84].

P2X3, a ligand-gated ion channel receptor for ATP, is highly expressed in primary sensory neurons in the DRG, as well as in the bladder wall [85]. P2X3 receptors play a key role in primary sensory nerve-mediated nociceptive afferents [86] and P2X3 knockout mice were found to have elevated pain thresholds [87]. P2X3R activation dramatically increased intracellular calcium influx, increased the nerve cell amplitude of action potentials and enhanced the transmission of pain signals by primary sensory fibers. P2X3R overexpression mediates the release of inflammatory factors (TNF-α and IL-6), which sensitizes primary afferent injury receptors and promotes inflammatory responses and pain [88,89]. P2X3R-mediated ERK1/2 signaling triggers nociceptive sensitization, and ERK1/2 phosphorylation in the dorsal root ganglia can be used to observe immediate intracellular signaling activity after injurious stimulation [90]. Depolarization of neurons can, to some extent, increase the opening of calcium channels and increase the intracellular calcium concentration to activate intracellular signaling (protein kinase C (PKC) signaling pathway), thereby increasing neuronal activity and synaptic plasticity [91] (Fig. 3). Moreover, the protein expression of both P2X2 and P2X3 is high in the uroepithelium of the IC bladder, and changes in P2X2 and P2X3 expression may contribute to pain in IC patients [85]. In addition, studies have found heterologous co-expression of P2X3 and ASIC3 and P2X3 and ASIC3 can be coimmunoprecipitated from rat DRG membranes [92]. These findings suggest that ASIC3, P2X3, and ATP may be important for peripheral acidic- and purinergic-mediated signaling during injury.

Interstitial cystitis is associated with a lower urinary pH, and the use of alkaline solutions, such as sodium bicarbonate, can increase bladder capacity and relieve the symptoms of interstitial cystitis [93]. This finding is also consistent with reports that urine pH strongly affects pain symptoms in patients with IC [94]. Therefore, we can hypothesize that lower urine pH in patients with interstitial cystitis affects the ASIC subunit, leading to more ATP release and activating the ability of P2X3R to cause pain sensations. In addition, the role of calcium ions generated upon activation of the ASIC subunit is crucial, as calcium ions also modulate neuronal excitability and synaptic transmission in the injury sensory pathway and play an important role in the induction and expression of pain hypersensitivity [95]. A recent study confirmed that P2X3R is an independent prognostic factor for bladder pain syndrome/interstitial cystitis [96].

However, we should not ignore other relevant factors. First, many chemical mediators, including neurotrophic factors, cytokines, chemokines and neuropeptides, which may stimulate the transcription of ASIC, are produced in the micturition reflex pathway in patients with cystitis [97–99]. A recent study also revealed that arachidonic acid directly enhances the activation of ASIC subunits [100]. Among these mediators, many studies have demonstrated the involvement of nerve growth factor in altered bladder sensory function and the development of nociceptive sensitization in response to bladder inflammation. This finding is consistent with the idea that ASIC may contribute to the sensitization of peripheral injury receptors and lead to injury perception. Second, immune cells such as CD8$^+$ and CD4$^+$ lymphocytes and lymphoid cells can be found in the mucosa of patients with bladder pain syndrome [101], and studies have shown that immune cells can express ASICs. ASIC1b, ASIC3, and ASIC4 were identified at the mRNA level in T cells from multiple sclerosis mice, and protein blotting confirmed the presence of the ASIC1 protein in T cells [92]. Finally, TRPV1 serves as a class of

Table 1
Expression of ASIC channels in the bladder.

Species	Bladder anatomy	ASIC expression
Mouse	bladder mucosa	ASIC1
	bladder muscle	ASIC1 and ASIC2
	subepithelial area	ASIC3
Rat	urothelium	ASIC2a and ASIC3
	subepithelial plexus	ASIC2a and ASIC3
Pig	bladder mucosa	ASIC1, ASIC2 and ASIC3
	lateral wall detrusor	ASIC1, ASIC2 and ASIC3
Human	bladder epithelium	ASIC1, ASIC2a and ASIC3
	subepithelial matrix	ASIC1, ASIC2a and ASIC3
	bladder epithelial cells	ASIC1, ASIC2a and ASIC3
	smooth muscle cells	ASIC1, ASIC2a and ASIC3
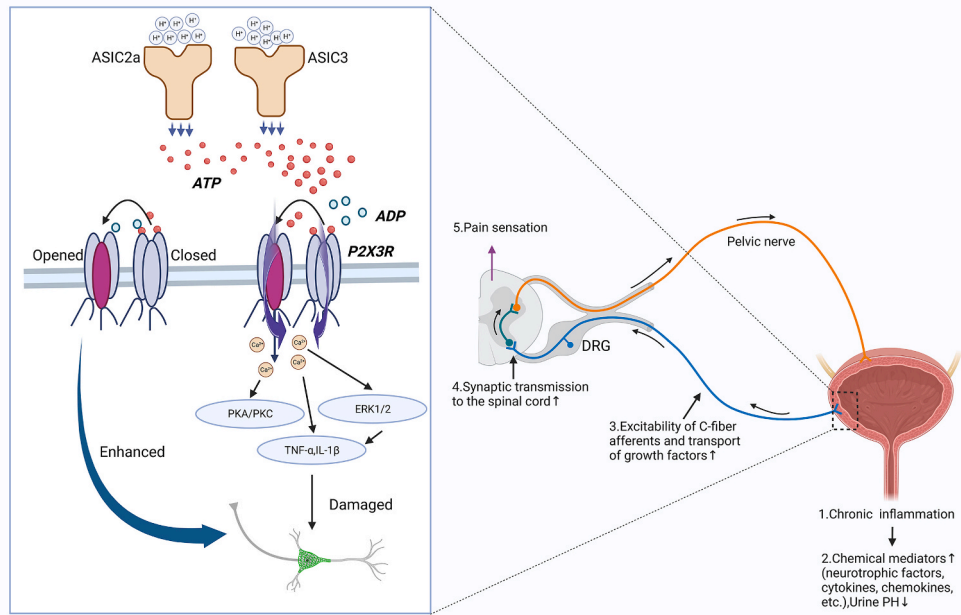
Fig. 3. Diagram showing the processes of chronic pain in IC/BPS patients whose pain is mediated by ASICs. Patients with IC/BPS experience a decrease in urine pH, which triggers the activation of ASIC in the bladder and increases ATP release. Next, ATP attaches itself to the extracellular domain of P2X3R. The transmembrane domain of P2X3R then opens, allowing calcium ion inflow and the opening of ion channels in the membrane. An increase in the intracellular calcium ion concentration causes pain by inducing intracellular signal transduction (PKC and ERK1/2), improving synaptic plasticity and neuronal excitability, and enhancing the transfer of primary sensory information.

receptor ion channel protein, and the channel also plays an important role in sensing and responding to heat, pain, and chemical stimuli.

4.2. ASICs and NLUTD

NLUTD due to SCI is a condition that affects both storage and voiding functions, including neurogenic detrusor overactivity (NDO) and voiding inefficiency caused by detrusor sphincter dyssynergia (DSD) as SCI progresses [102].

Chiara Traini [103] reported increased expression of ASIC1 in dome cells via immunohistochemical studies of bladder tissue from patients with clinically diagnosed NDO. In contrast, unchanged expression of ASIC1 in the urinary epithelium has been reported in other disorders affecting bladder sensitivity, such as BPS, and in a rat cystitis model induced by cyclophosphamide. This difference may depend on the different pathogeneses of NDO and BPS. Among them, NDO is usually associated with forced urethral hypertrophy, which is very rare in patients with BPS.

Neurotrophic factors, including brain-derived neurotrophic factor (BDNF) and nerve growth factor, are associated with NLUTD after spinal cord injury [67,104]. Neurotrophic factors are recognized as potential therapeutic targets, especially in NLUTD induced by spinal cord injury. Reduced ASIC mRNA expression in the DRGs of BDNF-deficient mice has been reported, suggesting that BDNF can maintain ASIC expression in sensory neurons. In an experiment, Naoki Wada [102] reported that ASIC2 and ASIC3 mRNA levels were increased in the L6-S1 DRGs of SCI mice compared with those in the L6-S1 DRGs of intact spinal cord mice. The expression of Asic2 and ASIC3 mRNAs was significantly reduced in anti-BDNF antibody-treated SCI mice. Anti-BDNF antibody-treated mice with spinal cord injury improved voiding ability, as evidenced by an increase in the volume of urine per micturition and an increase in voiding efficiency. These data suggest that in spinal cord injured mice, BDNF overexpression in the bladder and spinal cord is involved in the dysregulated activity of the external urinary sphincter (EUS) during the voiding phase, which correlates with the upregulation of ASIC mRNA in the DRG.

A study [16] assessed temporal changes in bladder and EUS activity and the expression of mechanosensitive channels in the lumbosacral DRG after SCI. In SCI mice, nonvoiding contractions were demonstrated at 2 weeks after spinal cord injury. SD was established after 4 weeks and significantly improved at 6 weeks. Compared with those in the spinal cord intact group, the mRNA levels of ASIC1 were elevated at 2, 4, and 6 weeks after spinal cord injury; the level of ASIC2 was elevated at 2 and 4 weeks and decreased at 6 weeks after spinal cord injury; and the level of ASIC3 was elevated at 4 weeks and decreased at 6 weeks after spinal cord injury. These results suggest that forced muscle overactivity is established at an early stage, whereas forced sphincter synergism disorder fully develops by 4 weeks and then improves at 6 weeks after SCI and that ASIC2 and ASIC3 may be involved in this process.

In addition, Daisuke Gotoh [105] investigated the effect of the soluble guanylate cyclase (sGC) activator BAY 60–2770 on neurogenic lower urinary tract dysfunction in SCI mice. The mice were divided into the following groups: the intact spinal cord group (Group A), the spinal cord injury + vehicle group (Group B), and the spinal cord injury + BAY 60–2770 group (Group C). They found that ASIC1, ASIC2, and ASIC3 mRNA expression in the L6-S1 DRGs of Group B was significantly greater than that in the L6-S1 DRGs of Group A and Group C. Compared with group C, the dysfunctions associated with both urethral hyperactivity and urethral sphincter synergism were significantly improved in Group B, which also suggests that ASICs are associated with NDO and DSD.

In conclusion, all of the above studies have shown that ASICs are closely related to neurogenic lower urinary tract dysfunction, but none of these studies have investigated and analyzed the role of different ASIC subtypes in the specific pathophysiology of voiding dysfunction after spinal cord injury; thus, more experimental studies are needed to clarify the exact mechanism by which ASICs affect low urinary tract symptoms.

5. Conclusion

In 1997, ASICs were first cloned and shown to be widely distributed in the nervous system. Since then, as research has continued, ASICs have been found to be widely distributed throughout the body, function as acid sensors, and are extensively involved in a variety of human pathophysiological processes.

This paper reviews the structure, function, and physiopathological roles of ASICs in the bladder and provides a basic systematic summary that reveals the possible mechanisms of ASICs in bladder pain syndromes and neurogenic lower urinary tract dysfunction. These findings provide information for future studies to explore the physiological and pathological significance of ASICs in the bladder and unexplored gap areas to facilitate targeted therapeutic options for various bladder disorders. These findings are important for further exploration of the physiological role of ASICs in vivo and the development of targeted ASIC drug therapies. However, little research has been conducted on the specific mechanisms by which ASICs function in the human body, and the next step of research should also focus on this topic so that ASICs can become important therapeutic targets for clinically relevant diseases.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

No data were used in this review.

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CRediT authorship contribution statement

Yang Zhang: Writing – original draft. **Di Dong:** Writing – original draft. **Jialong Zhang:** Conceptualization. **Kang Cheng:** Formal analysis. **Fang Zhen:** Project administration. **Mei Li:** Resources. **Binghai Chen:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

ASICs	Acid-sensitive ion channels
BPS	Bladder pain syndrome
IC	Interstitial cystitis
NLUTD	Neurogenic lower urinary tract dysfunction
SCI	Spinal cord injury
ENaC	Epithelial sodium channel
DEG	Degenerin
DRG	Dorsal root ganglion
WT	Wild-type
KO	Knockout
CYP	Cyclophosphamide

TRPV1	Transient receptor potential vanilloid 1
NDO	Neurogenic detrusor overactivity
DSD	Detrusor sphincter dyssynergia
BDNF	Brain-derived neurotrophic factor
PKC	Protein kinase C

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