Current Topics

Ion Channels as Therapeutic Targets for the Immune, Inflammatory, and Metabolic Disorders

Review

Involvement of Voltage-Gated Calcium Channels in Inflammation and Inflammatory Pain

Fumiko Sekiguchi, Maho Tsubota, and Atsufumi Kawabata*

Laboratory of Pharmacology and Pathophysiology, Faculty of Pharmacy, Kindai University; 3–4–1 Kowakae, Higashi-Osaka, Osaka 577–8502, Japan.

Received January 22, 2018

Voltage-gated calcium channels (VGCCs) are classified into high-voltage-activated (HVA) channels and low-voltage-activated channels consisting of $Ca_v3.1$–3.3, known as T ("transient")-type VGCC. There is evidence that certain types of HVA channels are involved in neurogenic inflammation and inflammatory pain, in agreement with reports indicating the therapeutic effectiveness of gabapentinoids, ligands for the $\alpha_2\delta$ subunit of HVA, in treating not only neuropathic, but also inflammatory, pain. Among the Ca_v3 family members, $Ca_v3.2$ is abundantly expressed in the primary afferents, regulating both neuronal excitability at the peripheral terminals and spontaneous neurotransmitter release at the spinal terminals. The function and expression of $Ca_v3.2$ are modulated by a variety of inflammatory mediators including prostanoids and hydrogen sulfide (H_2S), a gasotransmitter. The increased activity of $Ca_v3.2$ by H_2S participates in colonic, bladder and pancreatic pain, and regulates visceral inflammation. Together, VGCCs are involved in inflammation and inflammatory pain, and $Ca_v3.2$ T-type VGCC is especially a promising therapeutic target for the treatment of visceral inflammatory pain in patients with irritable bowel syndrome, interstitial cystitis/bladder pain syndrome, pancreatitis, *etc.*, in addition to neuropathic pain.

Key words voltage-gated calcium channel (VGCC); inflammatory pain; neuropathic pain; visceral pain; $Ca_v3.2$ T-type calcium channel

1. INTRODUCTION

Calcium influx through voltage-gated calcium channels (VGCCs) plays extensive roles in various biological events, including smooth muscle and myocardial contraction, endocrine secretion, neurotransmitter release, neuronal excitation, and also in cell proliferation and gene expression.[1,2] VGCCs are classified into high-voltage-activated (HVA) channels, including $Ca_v1.1$–1.4 and $Ca_v2.1$–2.3 and low-voltage-activated (LVA) channels consisting of $Ca_v3.1$–3.3, known as T ("transient")-type VGCC. $Ca_v1.1$–1.4, known as L-type VGCC, contain main pore-forming α_1 subunits, α_{1S}, α_{1C}, α_{1D} and α_{1F}, respectively, whereas $Ca_v2.1$, $Ca_v2.2$ and $Ca_v2.3$, known as P/Q-type, N-type and R-type VGCCs, contain α_{1A}, α_{1B} and α_{1E}, respectively. T-type VGCCs, *i.e.*, $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$, contain α_{1G}, α_{1H} and α_{1I}, respectively. Each α_1 subunit of Ca_v1 and Ca_v2, but not Ca_v3, forms molecular complexes with auxiliary subunits, β, $\alpha_2\delta$ and γ, which regulate the cell surface expression and function of the channels.[1,2] In inflammation, the activity of VGCCs may be altered by changes in their expression or in trafficking to the plasma membrane, and/or by post-translational molecular modification, such as phosphorylation/dephosphorylation, ubiquitination/deubiquitination and/or oxidation/reduction, contributing to the development or exacerbation of inflammation and/or related sensations of pain.[3–6] Here, we focus on the role of VGCCs, especially $Ca_v3.2$ T-type VGCC, in inflammatory events including pain.

2. ROLE OF HIGH VOLTAGE-ACTIVATED CALCIUM CHANNELS IN INFLAMMATION AND INFLAMMATORY PAIN

Neurogenic inflammation is induced, in general, by excessive excitation of primary sensory nerves followed by the release of neuropeptides, such as calcitonin gene-related peptide (CGRP) and substance P, from their peripheral endings. The released neuropeptides may cause or promote inflammatory events including vasodilatation and increased vascular permeability.[7–9] VGCCs, in addition to transient receptor potential vanilloid 1 (TRPV1) and TRP ankyrin 1 (TRPA1) channels, participate in the excessive excitation of primary afferents responsible for neurogenic inflammation.[4,6,9–11] Migraine is well known to involve neurogenic inflammation, *i.e.*, the excessive release of neuropeptides; in particular, CGRP, from the peripheral terminals of trigeminal nerves, is a critical component in the pathogenesis of migraine.[8] Among HVA calcium channels, $Ca_v2.1$ (P/Q-type VGCC) appears to play a role in a certain type of migraine. For example, gain-of-function mutations in *CACNA1A*, a gene encoding the pore-forming α_1 subunit of $Ca_v2.1$, are implicated in familial hemiplegic migraine type 1 (FHM-1),[12] and associated with an increased open channel probability, as well as channel activation at a lower voltage.[11] Interestingly, the trigeminal ganglia isolated from $Ca_v2.1$ α_1 R192Q mutant knock-in mice expressing a human mutation causing FHM-1 show increases in basal and bradykinin-stimulated CGRP release,[13] and are characterized

* To whom correspondence should be addressed. e-mail: kawabata@phar.kindai.ac.jp

© 2018 The Pharmaceutical Society of Japan

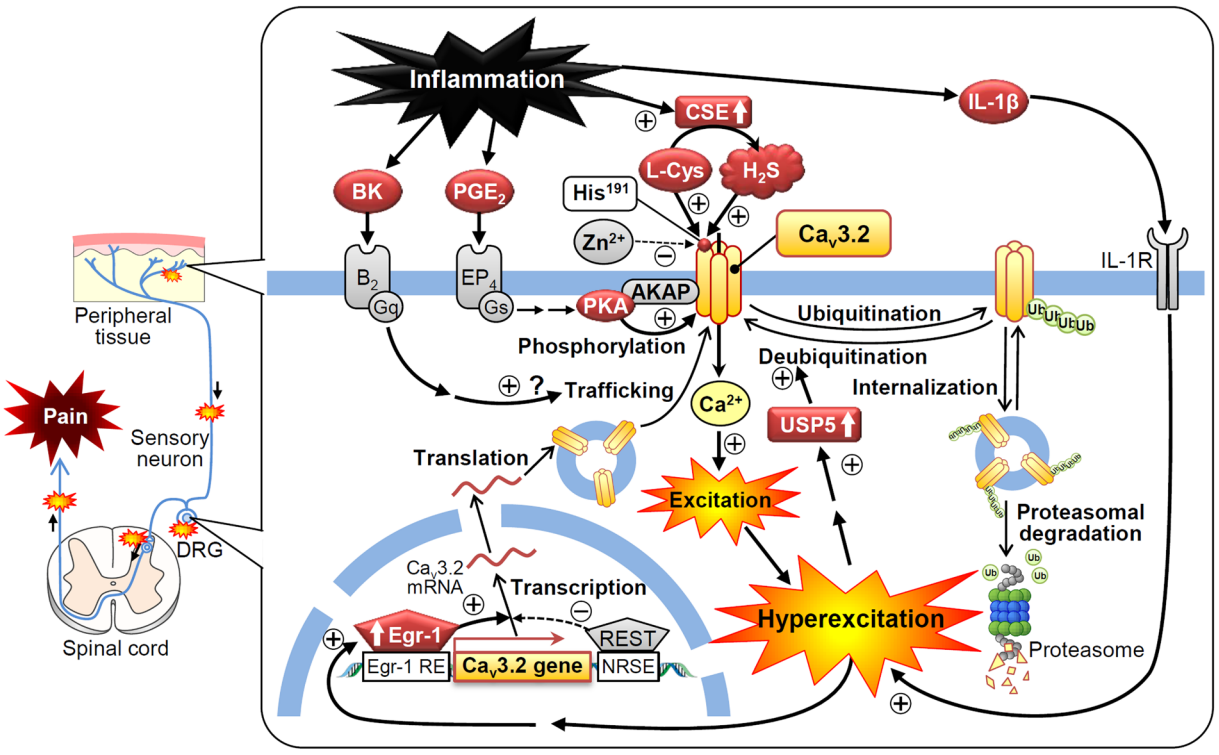


Fig. 1. Modulation by Inflammatory Mediators of the Function and Expression of $Ca_v3.2$ T-Type VGCC in the Primary Sensory Neurons

DRG, dorsal root ganglion; BK, bradykinin; CSE, cystathionine-γ-lyase; L-Cys, L-cysteine; IL-1β, interleukin-1β; IL-1R, IL-1 receptor; USP5, ubiquitin-specific protease 5; Egr-1, early growth response-1; Egr-1 RE, Egr-1 response element; REST, repressor element-1 silencing transcription factor; NRSE, neural restrictive silencer element; AKAP, A-kinase-anchoring protein. (Color figure can be accessed in the online version.)

by the enrichment of activated macrophages and constitutive overexpression of mRNAs for IL-1β, IL-6, IL-10 and tumor necrosis factor α (TNFα).[14] $Ca_v2.1$ thus regulates the release of neurotransmitters including glutamate and CGRP from the peripheral and central endings of trigeminal nerves, thereby participating in the pathogenesis of migraine.[13,15]

Transcripts for all L-type VGCC isoforms, $Ca_v1.1$–$Ca_v1.4$, are detectable in lymphocytes, although their functions remain largely unclear.[2] Type-2 helper T cells express $Ca_v1.2$ channels, which play a role in calcium signaling and cytokine production responsible for allergic asthma.[16,17] Functional $Ca_v1.2$ is also expressed in microglial cells, resident immune cells in the central nervous system (CNS).[18] In murine microglial BV2 cells known to express $Ca_v1.2$, the high-K^+-induced increase in intracellular calcium levels and lipopolysaccharide (LPS)-induced production of TNFα and nitric oxide (NO) are partially suppressed by nifedipine, an L-type VGCC blocker, suggesting the involvement of $Ca_v1.2$ in CNS inflammation.[18] There is also evidence that $Ca_v2.2$, an N-type VGCC expressed in microglia, is involved in the pathophysiology of neuropathic pain after spinal nerve injury.[19] Further, expression of the $\alpha_2\delta$ subunit of HVA calcium channels in the spinal dorsal horn is upregulated in rats with arthritis caused by complete Freud's adjuvant (CFA), and repeated treatment with gabapentin, an $\alpha_2\delta$ ligand, diminishes the joint inflammation-induced spinal microglial activation and thermal hyperalgesia.[20]

3. ROLE OF LOW VOLTAGE-ACTIVATED CALCIUM CHANNELS (T-TYPE VGCC) IN INFLAMMATORY PAIN

3.1. Functional Regulation of T-Type VGCC by Inflammatory Mediators Unlike HVA calcium channels, LVA calcium channels, *i.e.*, T-type VGCCs, consist of a pore-forming α_1 subunit without auxiliary subunits, β, $\alpha_2\delta$ or γ, so that gabapentinoids, $\alpha_2\delta$ ligands, are incapable of reducing the activity of T-type VGCCs. Among the three Ca_v3 family members of T-type VGCCs, $Ca_v3.2$ channels are abundantly expressed in the primary sensory neurons. They regulate the neuronal excitation at their peripheral axons or endings, as well as spontaneous neurotransmitter release at their central endings, thereby participating in nociceptive processing, particularly in neuropathic pain.[3,4,21–24] The function and expression of $Ca_v3.2$ are regulated by a variety of inflammatory mediators[3,4,25] and by the neuronal activity itself[26,27] (Fig. 1). Each of $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ can each be phosphorylated and functionally upregulated by protein kinase A (PKA).[28,29] We have shown that prostaglandin E_2 (PGE_2) enhances the phosphorylation and channel activity of $Ca_v3.2$ *via* the EP_4 receptor/cyclic AMP/PKA pathway in an A-kinase-anchoring protein (AKAP)-dependent manner, contributing to the development of inflammatory hyperalgesia[30] (Fig. 1). There has also been a report showing that bradykinin, an inflammation mediator, increases the population size of small-diameter dorsal root ganglion (DRG) neurons displaying T-type currents *via* B_2 receptors, suggesting the involvement of increased membrane trafficking of $Ca_v3.2$ channels[31] (Fig. 1). Hydrogen sulfide (H_2S), a gasotransmitter, is generated from L-cysteine

by multiple enzymes including cystathionine-γ-lyase (CSE) (Fig. 1), thus playing a dual role in inflammatory events.[32] In fact, systemic inflammation caused by LPS treatment or severe burn injury causes upregulation of CSE in the liver followed by increased plasma levels of H_2S (Fig. 1), which in turn aggravates systemic inflammation.[33,34] We have shown that NaHS and/or Na_2S, H_2S donors, enhance the activity of T-type VGCC in $Ca_v3.2$-transfected HEK293 cells,[35] as well as in neuronal precursor-like NG108-15 cells[36] and neuroendocrine-like differentiated human prostate cancer LNCaP cells,[37] both of which naturally express $Ca_v3.2$. Interestingly, intraplantar administration of NaHS, an H_2S donor, causes $Ca_v3.2$-dependent mechanical hyperalgesia/allodynia in rats and mice,[36,38,39] while an inhibitor of CSE prevents the development of inflammatory hyperalgesia following the intraplantar injection of LPS.[36] Therefore, H_2S formed by CSE is involved in inflammation and also contribute to inflammatory pain in a $Ca_v3.2$-dependent manner (Fig. 1).

3.2. Molecular Mechanisms for Altered Function and Expression of $Ca_v3.2$ T-Type VGCC There exists a histidine residue at position 191 (His^{191}) in the second extracellular loop of domain I in $Ca_v3.2$, but not in $Ca_v3.1$ or $Ca_v3.3$. In distinct species of mammals, the channel function of $Ca_v3.2$ is suppressed by extracellular Zn^{2+} that binds to the His^{191} of $Ca_v3.2$. Therefore, exogenously applied zinc chelators or Zn^{2+}-sensitive endogenous compounds such as L-cysteine and H_2S enhance the channel activity of $Ca_v3.2$ by cancelling Zn^{2+} inhibition[4,23,24,40] (Fig. 1). It is noteworthy that systemic or local administration of zinc chelators and dietary zinc deficiency cause visceral pain/hyperalgesia in a $Ca_v3.2$-depedent manner.[27,41]

The transcriptional expression of $Ca_v3.2$ is positively and negatively regulated by early growth response-1 (Egr-1) and repressor element-1 silencing transcription factor (REST), respectively[24,42] (Fig. 1). For example, human prostate LNCaP cells, once differentiated into neuroendocrine-like cells, overexpress $Ca_v3.2$ through the upregulation of Egr-1 and downregulation of REST, which may contribute to the acquisition of castration resistance.[37] It is likely that Egr-1-dependent overexpression of $Ca_v3.2$ is involved in inflammatory pain, since Egr-1 is a zinc finger transcriptional factor that is rapidly induced by inflammatory stimuli.[43] $Ca_v3.2$ expressed on the plasma membrane is ubiquitinated and subjected to proteasomal degradation, which is negatively regulated by ubiquitin-specific protease 5 (USP5), a specific deubiquitinating enzyme for $Ca_v3.2$ (Fig. 1). The upregulated USP5-induced increase in $Ca_v3.2$ protein expressed on the membrane surface in the primary afferents appears to contribute to the pathogenesis of neuropathic pain in mice.[44] Similarly, intraplantar administration of CFA causes USP5-dependent $Ca_v3.2$ overexpression in the DRG neurons, leading to inflammatory pain[44] (Fig. 1). Of particular interest is the finding that the USP5-dependent increase in the protein levels of $Ca_v3.2$ in the primary sensory neurons can be caused by increased neuronal activity[26] (Fig. 1). Molecular mechanisms for the activity-dependent upregulation of USP5 largely remain unclear, although IL-1β has been identified as a key upstream mediator for the $Ca_v3.2$-USP5 interaction.[45] Taken together, the accelerated function and increased expression of $Ca_v3.2$ in the primary afferents appear to be involved in inflammatory pain.

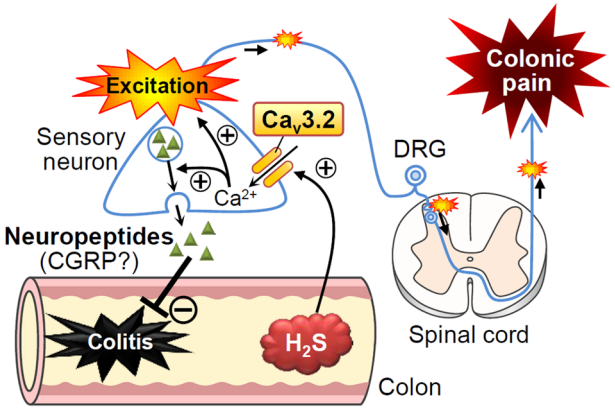


Fig. 2. Role of $Ca_v3.2$ T-Type VGCC in Colitis and Colonic Pain

DRG, dorsal root ganglion; CGRP, calcitonin gene-related peptide. (Color figure can be accessed in the online version.)

4. ROLE OF VOLTAGE-GATED CALCIUM CHANNELS IN VISCERAL INFLAMMATION AND PAIN

4.1. Colitis and Colonic Pain $Ca_v1.2$ L-type VGCCs are abundantly expressed in smooth muscle cells and regulate smooth muscle contraction in the gastrointestinal tract.[46] Direct measurements of calcium currents in single smooth muscle cells isolated from distinct species have shown a 50–70% decreases in calcium currents following colonic inflammation, whereas in animals with experimental colitis, the protein or gene expression of $Ca_v1.2$ in colonic myocytes does not clearly change.[47–49] Src-mediated tyrosine phosphorylation of $Ca_v1.2$ enhances the channel activity, and the phosphorylation of $Ca_v1.2$ is attenuated in colonic inflammation.[48,49] Collectively, post-translational modification of $Ca_v1.2$ in smooth muscle cells is considered to play a role in the abnormal gastrointestinal smooth muscle motility following colonic inflammation, and may contribute to the intestinal dysmotility in patients with inflammatory bowel diseases (IBD).[49]

We have shown that repeated intracolonic administration of the H_2S donor NaHS attenuates the colitis induced by trinitrobenzene sulfonic acid (TNBS) in rats,[50] in agreement with independent evidence,[51] and that the NaHS-induced mucosal cytoprotection is abolished by the ablation of sensory neurons or by T-type VGCC blockade.[50] Given the overexpression of $Ca_v3.2$ in the DRG following TNBS treatment,[50] we hypothesize that luminal H_2S causes $Ca_v3.2$-dependent enhancement of the excitability of the afferent neurons, which might, in turn, evoke the release of cytoprotective neuropeptides (Fig. 2). This hypothesis is supported by previous reports suggesting the mucosal cytoprotection exerted by neuropepitdes such as CGRP in the gastrointestinal tract.[52–55]

VGCCs regulate the neuronal excitability and neurotransmitter release in the primary afferent neurons, and may play a role in the pathogenesis of colonic pain and stool abnormality in patients with IBD and irritable bowel syndrome (IBS). In TNBS-treated rats, $Ca_v1.2$ and $Ca_v2.3$ are upregulated in the DRG, and the colonic hypersensitivity to distension stimuli is reduced by intrathecal administration of nimodipine, an L-type VGCC blocker, and/or SNX-482, an R-type VGCC blocker.[56] There is also evidence that pregabalin, a ligand of the $\alpha_2\delta$ auxiliary subunit for HVA calcium channels, suppresses colonic hypersensitivity in TNBS-treated rats[57,58] and

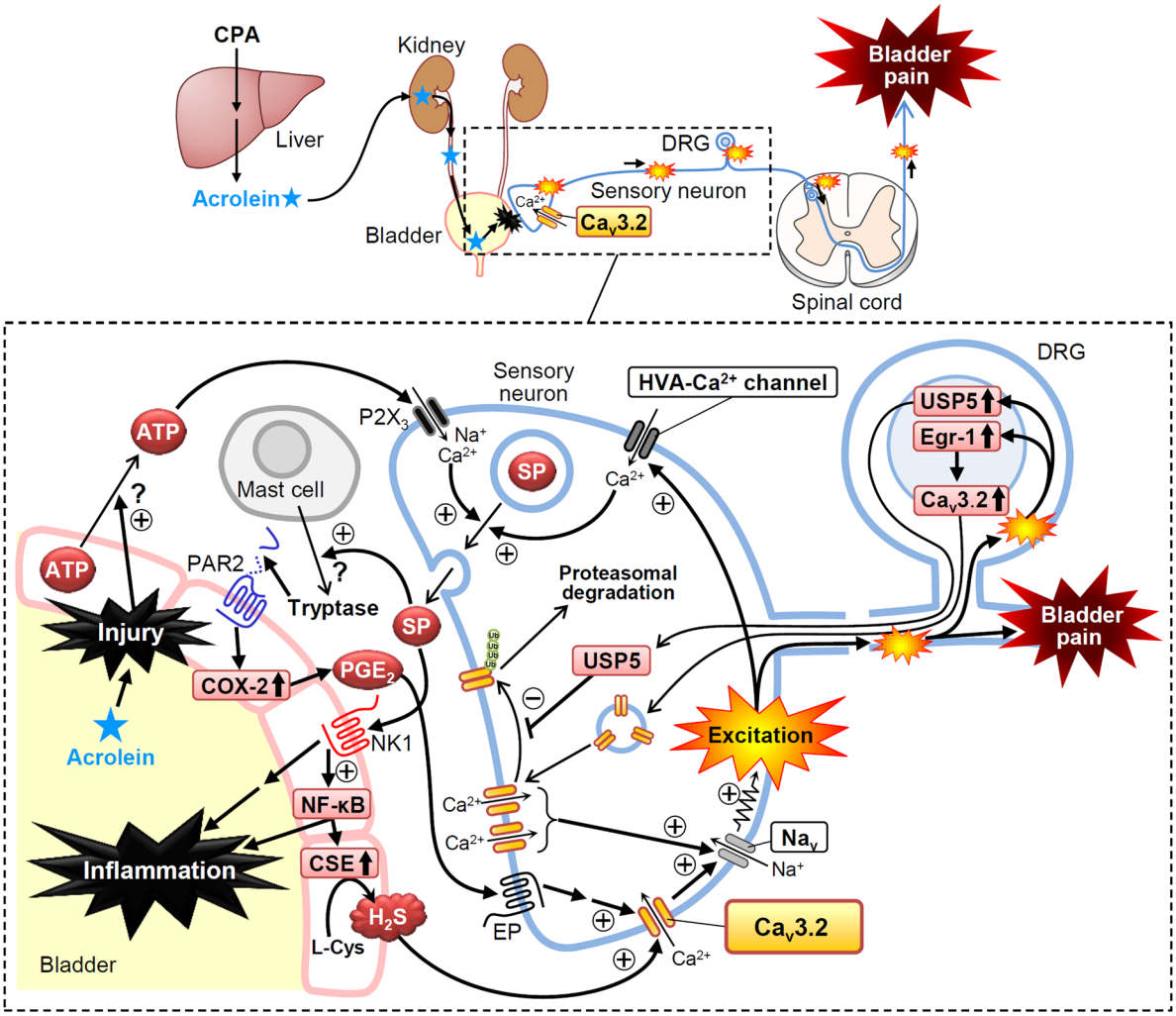


Fig. 3. Role of $Ca_v3.2$ T-Type VGCC in Cystitis and Bladder Pain

DRG, dorsal root ganglion; CSE, cystathionine-γ-lyase; L-Cys, L-cysteine; USP5, ubiquitin-specific protease 5; Egr-1, early growth response-1; SP, substance P; PAR2, proteinase-activated receptor 2; PGE_2, prostaglandin E_2. (Color figure can be accessed in the online version.)

in IBS patients.[59] Apart from the pronociceptive role of HVA calcium channels, we have reported that intracolonic administration of NaHS induces $Ca_v3.2$ T-type VGCC-dependent colonic pain-like nociceptive behavior and referred hyperalgesia in conscious mice; it also induces the phosphorylation of extracellular signal-regulated kinase (ERK) in the spinal cord at T13-L1 and L5-S1 levels, a prompt marker for nociception, in anesthetized mice.[60,61] In addition, we have demonstrated that selective T-type VGCC blockers strongly suppress the visceral pain following intracolonic NaHS in mice.[60,62] Subsequently, an independent group has demonstrated the critical role of $Ca_v3.2$ in colonic hypersensitivity to distension stimuli in rats treated with butyrate, an animal model for IBS.[63] Collectively, $Ca_v3.2$ expressed in the peripheral terminals of primary afferents, once stimulated with H_2S, enhances the neuronal excitability, leading to colonic pain, while at the same time it exerts mucosal cytoprotection during colitis (Fig. 2).

4.2. Cystitis and Bladder Pain The most well-known syndrome of lower urinary tract dysfunction is overactive bladder (OAB), which is defined by complaints of "urinary urgency, usually associated with urinary frequency, with or without urgency incontinence." On the other hand, interstitial cystitis (IC)/bladder pain syndrome (BPS) is characterized by bladder pain and lower urinary tract symptoms including increased urinary frequency and urinary urgency, with or without urothelial inflammation, as recognized by a well-demarcated reddish mucosal lesion (*i.e.*, Hunner's lesion).[64] The bladder pain and increased urinary frequency in IC/BPS patients may be associated with nociceptor sensitization,[65] so that VGCCs could play a role in the pathogenesis of IC/BPS. However, the role of HVA calcium channels in bladder pain remains largely unclear. The effectiveness of $\alpha_2\delta$ ligands including gabapentinoids on IC/BPS-related bladder pain is controversial and questionable.[66–68] Nevertheless, some fundamental studies have provided evidence for the involvement of spinal $Ca_v2.1$ P/Q-type and $Ca_v2.2$ N-type, but not L-type, VGCCs in bladder nociception,[69] as well as the role of $Ca_v2.2$ N-type VGCC in bladder efferent cholinergic neurotransmission.[70] In contrast, we have shown the critical role of $Ca_v3.2$ T-type VGCCs in cystitis and related bladder pain in mice,[10,23] and this has been supported by independent findings that a selective T-type VGCC blocker increased both bladder capacity and voided volume in rats with bladder outlet obstruction.[70]

Several pathophysiologic mechanisms for IC/BPS, such as epithelial dysfunction, mast cell activation and neurogenic

inflammation, have been proposed, and animal studies have shown the involvement of various endogenous molecules, such as mast cell tryptase, proteinase-activated receptor-2 (PAR2), ATP, substance P, H_2S, *etc*.[10,23,71–73] (Fig. 3). Rodents treated with cyclophosphamide (CPA) mimic some of symptoms found in IC/BPS patients, including bladder pain and increased urinary frequency,[74,75] and have thus been used experimentally to clarify the mechanisms underlying the IC/BPS-related symptoms and to explore novel therapeutic targets for IC/BPS.[72] The CPA-induced cystitis/bladder pain is triggered by acrolein, a hepatic metabolite of CPA[76] (Fig. 3). In the bladder, acrolein, a toxic chemical, injures urothelial cells, leading to the release of ATP, which in turn stimulates the afferent neurons *via* activation of $P2X_3$ receptors[72] (Fig. 3). We have shown that selective T-type VGCC blockers and the knockdown of $Ca_v3.2$ restore the CPA-induced bladder pain-like nociceptive behavior and referred hyperalgesia in mice.[10,62] Most interestingly, CPA treatment causes $Ca_v3.2$ overexpression in DRG.[10,27] Given the CPA-induced upregulation of Egr-1 and USP5 in the DRG,[27] the CPA-induced $Ca_v3.2$ overexpression is attributable to Egr-1-dependent transcriptional upregulation and USP5-induced inhibition of proteasomal degradation of $Ca_v3.2$ (Fig. 3). The enhancement of $Ca_v3.2$ by endogenous H_2S is also considered to participate in the bladder pain (Fig. 3), considering that reversible and irreversible inhibitors of CSE, a H_2S-generating enzyme, prevent the CPA-induced nociceptive behavior and referred hyperalgesia and that CPA treatment augments the expression and activity of CSE in the bladder.[10,77] The role of the H_2S/$Ca_v3.2$ pathway in bladder pain is supported by evidence that intravesical injection of the H_2S donor NaHS causes prompt phosphorylation of ERK in the L6 spinal cord in anesthetized mice, an effect prevented by a T-type VGCC blocker.[10] The CPA-induced bladder pain and CSE upregulation involves substance P-induced NK_1 receptor activation[78] and nuclear factor-kappaB (NF-κB) signaling[77] (Fig. 3). It is noteworthy that intravesical injection of substance P causes $Ca_v3.2$-dependent referred hyperalgesia through NK_1 receptors in mice, accompanied by CSE upregulation in the bladder,[78] and that mast cell-neuron interactions in the bladder are critically important in the pathophysiology of IC/BPS.[79] PGE_2 generated by cyclooxygenase (COX)-2, possibly downstream of PAR2 activation by mast cell tryptase, also contribute to the CPA-induced $Ca_v3.2$-dependent bladder pain[72,80–82] (Fig. 3). The NF-κB/CSE/H_2S/$Ca_v3.2$ pathway is considered to mediate the neurogenic inflammation that is involved in IC/BPS (Fig. 3), since T-type VGCC blockers, knockdown of $Ca_v3.2$ and inhibitors of CSE or NF-κB prevent the CPA-induced bladder swelling, in addition to bladder pain.[10,27,77]

4.3. Pancreatitis and Related Pain Acute pancreatitis often occurs secondarily to biliary diseases and alcohol abuse, and is characterized by inflammation in the pancreatic tissue (and in other organs in severe cases) and upper abdominal pain. Chronic pancreatitis represents the progressive destruction of the pancreas and the slow development of pancreatic fibrosis, accompanied by persistent pancreatic pain. Pancreatitis involves neurogenic inflammation caused by neurotransmitters released from the peripheral terminals of primary afferents.[83] There is clinical evidence that the short-term use of pregabalin, which inhibits HVA calcium channel activity, reduces both pancreatic pain and opiate use in patients with chronic pancreatitis.[84] Fundamental studies have shown the increased protein expression of the $\alpha_2\delta_1$ subunit of HVA calcium channels in the spinal cord of rats with chronic pancreatitis caused by dibutyltin dichloride (DBTC), and that the repeated systemic administration of gabapentin attenuates the DBTC-induced pancreatic pain and $\alpha_2\delta_1$ upregulation.[85] On the other hand, we have demonstrated that T-type VGCC blockers and knockdown of $Ca_v3.2$ restore the pancreatic pain, but not the inflammatory symptoms, in mice with acute pancreatitis caused by repeated administration of cerulein.[62,86,87] A CSE inhibitor suppresses the pancreatic-related pain, and the protein expression and enzymatic activity of CSE increase in the pancreatic tissue after repeated cerulein treatment.[86,87] Collectively, the CSE/H_2S/$Ca_v3.2$ pathway plays a critical role in the acute pancreatitis-related pain,[23] which is similar to the mechanisms involved in cystitis-related bladder pain (Fig. 3). The pronociceptive role of the H_2S/$Ca_v3.2$ pathway in the pancreas is supported by evidence that injection of NaHS, an H_2S donor, induces T-type VGCC-dependent prompt phosphorylation of ERK and delayed expression of Fos protein in the spinal dorsal horn of mice or rats.[86–88]

5. CONCLUSION

Accumulating evidence suggests the role of VGCCs in the development of neurogenic inflammation associated with migraine or cystitis and in inflammatory pain. Gabapentinoids, such as pregabalin and gabapentin, which target the $\alpha_2\delta$ subunit of HVA calcium channels, should be effective on not only against neuropathic pain but also inflammatory pain. Preclinical studies have contributed to the development of several selective T-type VGCC blockers and have demonstrated their effectiveness of these in distinct animal models for neuropathic pain.[4,62,89] Nonetheless, the therapeutic usefulness of T-type VGCC blockers as analgesics in humans, particularly for the treatment of neuropathic pain, still remains controversial.[90–93] Since $Ca_v3.2$ T-type VGCC plays a key role in visceral pain in the colon, bladder and pancreas, as described so far, it is of particular interest to test the effectiveness of T-type VGCC blockers on visceral pain in patients with IBS, IC/BPS, pancreatitis, *etc*. The development of $Ca_v3.2$-selective blockers will shed light on the validity of $Ca_v3.2$ as a therapeutic target for the clinical treatment of inflammatory pain, in addition to neuropathic pain.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. International union of pharmacology. XLVIII. Nomenclature and structure–function relationships of voltage-gated calcium channels. *Pharmacol. Rev.*, **57**, 411–425 (2005).
2) Zamponi GW, Striessnig J, Koschak A, Dolphin AC. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. *Pharmacol. Rev.*, **67**, 821–870 (2015).
3) Todorovic SM, Jevtovic-Todorovic V. T-type voltage-gated calcium channels as targets for the development of novel pain therapies. *Br. J. Pharmacol.*, **163**, 484–495 (2011).
4) Sekiguchi F, Kawabata A. T-type calcium channels: functional

regulation and implication in pain signaling. *J. Pharmacol. Sci.*, **122**, 244–250 (2013).
5) Huang J, Zamponi GW. Regulation of voltage gated calcium channels by GPCRs and post-translational modification. *Curr. Opin. Pharmacol.*, **32**, 1–8 (2017).
6) Roca-Lapirot O, Radwani H, Aby F, Nagy F, Landry M, Fossat P. Calcium signalling through L-type calcium channels: role in pathophysiology of spinal nociceptive transmission. *Br. J. Pharmacol.*, **175**, 2362–2374 (2018).
7) Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. *Nat. Rev. Drug Discov.*, **13**, 533–548 (2014).
8) Iyengar S, Ossipov MH, Johnson KW. The role of calcitonin gene-related peptide in peripheral and central pain mechanisms including migraine. *Pain*, **158**, 543–559 (2017).
9) Corrigan F, Mander KA, Leonard AV, Vink R. Neurogenic inflammation after traumatic brain injury and its potentiation of classical inflammation. *J. Neuroinflammation*, **13**, 264 (2016).
10) Matsunami M, Miki T, Nishiura K, Hayashi Y, Okawa Y, Nishikawa H, Sekiguchi F, Kubo L, Ozaki T, Tsujiuchi T, Kawabata A. Involvement of the endogenous hydrogen sulfide/$Ca_v3.2$ T-type Ca^{2+} channel pathway in cystitis-related bladder pain in mice. *Br. J. Pharmacol.*, **167**, 917–928 (2012).
11) Pietrobon D. Calcium channels and migraine. *Biochim. Biophys. Acta*, **1828**, 1655–1665 (2013).
12) Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SM, Lamerdin JE, Mohrenweiser HW, Bulman DE, Ferrari M, Haan J, Lindhout D, van Ommen GJ, Hofker MH, Ferrari MD, Frants RR. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca^{2+} channel gene CACNL1A4. *Cell*, **87**, 543–552 (1996).
13) Ceruti S, Villa G, Fumagalli M, Colombo L, Magni G, Zanardelli M, Fabbretti E, Verderio C, van den Maagdenberg AM, Nistri A, Abbracchio MP. Calcitonin gene-related peptide-mediated enhancement of purinergic neuron/glia communication by the algogenic factor bradykinin in mouse trigeminal ganglia from wild-type and R192Q $Ca_v2.1$ Knock-in mice: implications for basic mechanisms of migraine pain. *J. Neurosci.*, **31**, 3638–3649 (2011).
14) Franceschini A, Vilotti S, Ferrari MD, van den Maagdenberg AM, Nistri A, Fabbretti E. TNFα levels and macrophages expression reflect an inflammatory potential of trigeminal ganglia in a mouse model of familial hemiplegic migraine. *PLOS ONE*, **8**, e52394 (2013).
15) Pellacani S, Sicca F, Di Lorenzo C, Grieco GS, Valvo G, Cereda C, Rubegni A, Santorelli FM. The revolution in migraine genetics: from aching channels disorders to a next-generation medicine. *Front. Cell. Neurosci.*, **10**, 156 (2016).
16) Robert V, Triffaux E, Paulet PE, Guery JC, Pelletier L, Savignac M. Protein kinase C-dependent activation of $Ca_v1.2$ channels selectively controls human T_H2-lymphocyte functions. *J. Allergy Clin. Immunol.*, **133**, 1175–1183.e12 (2014).
17) Rosa N, Triffaux E, Robert V, Mars M, Klein M, Bouchaud G, Canivet A, Magnan A, Guery JC, Pelletier L, Savignac M. The β and $\alpha_2\delta$ auxiliary subunits of voltage-gated calcium channel 1 (Ca_v1) are required for TH2 lymphocyte function and acute allergic airway inflammation. *J. Allergy Clin. Immunol.*, (2017), in press.
18) Espinosa-Parrilla JF, Martinez-Moreno M, Gasull X, Mahy N, Rodriguez MJ. The L-type voltage-gated calcium channel modulates microglial pro-inflammatory activity. *Mol. Cell. Neurosci.*, **64**, 104–115 (2015).
19) Saegusa H, Tanabe T. N-type voltage-dependent Ca^{2+} channel in non-excitable microglial cells in mice is involved in the pathophysiology of neuropathic pain. *Biochem. Biophys. Res. Commun.*, **450**, 142–147 (2014).
20) Yang JL, Xu B, Li SS, Zhang WS, Xu H, Deng XM, Zhang YQ. Gabapentin reduces CX3CL1 signaling and blocks spinal microglial activation in monoarthritic rats. *Mol. Brain*, **5**, 18 (2012).
21) Weiss N, Zamponi GW. Control of low-threshold exocytosis by T-type calcium channels. *Biochim. Biophys. Acta*, **1828**, 1579–1586 (2013).
22) Todorovic SM, Jevtovic-Todorovic V. Neuropathic pain: role for presynaptic T-type channels in nociceptive signaling. *Pflugers Arch.*, **465**, 921–927 (2013).
23) Terada Y, Kawabata A. H_2S and pain: a novel aspect for processing of somatic, visceral and neuropathic pain signals. *Handbook Exp. Pharmacol.*, **230**, 217–230 (2015).
24) Fukami K, Sekiguchi F, Kawabata A. Hydrogen sulfide and T-type Ca^{2+} channels in pain processing, neuronal differentiation and neuroendocrine secretion. *Pharmacology*, **99**, 196–203 (2017).
25) Iftinca MC, Zamponi GW. Regulation of neuronal T-type calcium channels. *Trends Pharmacol. Sci.*, **30**, 32–40 (2009).
26) Stemkowski P, Garcia-Caballero A, Gadotti VM, M'Dahoma S, Huang S, Black SAG, Chen L, Souza IA, Zhang Z, Zamponi GW. TRPV1 nociceptor activity initiates USP5/T-type channel-mediated plasticity. *Cell Reports*, **17**, 2901–2912 (2016).
27) Ozaki T, Matsuoka J, Tsubota M, Tomita S, Sekiguchi F, Minami T, Kawabata A. Zinc deficiency promotes cystitis-related bladder pain by enhancing function and expression of $Ca_v3.2$ in mice. *Toxicology*, **393**, 102–112 (2018).
28) Kim JA, Park JY, Kang HW, Huh SU, Jeong SW, Lee JH. Augmentation of $Ca_v3.2$ T-type calcium channel activity by cAMP-dependent protein kinase A. *J. Pharmacol. Exp. Ther.*, **318**, 230–237 (2006).
29) Chemin J, Mezghrani A, Bidaud I, Dupasquier S, Marger F, Barrere C, Nargeot J, Lory P. Temperature-dependent modulation of Ca_v3 T-type calcium channels by protein kinases C and A in mammalian cells. *J. Biol. Chem.*, **282**, 32710–32718 (2007).
30) Sekiguchi F, Aoki Y, Nakagawa M, Kanaoka D, Nishimoto Y, Tsubota-Matsunami M, Yamanaka R, Yoshida S, Kawabata A. AKAP-dependent sensitization of $Ca_v3.2$ channels *via* the EP_4 receptor/cAMP pathway mediates PGE_2-induced mechanical hyperalgesia. *Br. J. Pharmacol.*, **168**, 734–745 (2013).
31) Huang D, Liang C, Zhang F, Men H, Du X, Gamper N, Zhang H. Inflammatory mediator bradykinin increases population of sensory neurons expressing functional T-type Ca^{2+} channels. *Biochem. Biophys. Res. Commun.*, **473**, 396–402 (2016).
32) Li L, Hsu A, Moore PK. Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation—a tale of three gases! *Pharmacol. Ther.*, **123**, 386–400 (2009).
33) Li L, Whiteman M, Moore PK. Dexamethasone inhibits lipopolysaccharide-induced hydrogen sulphide biosynthesis in intact cells and in an animal model of endotoxic shock. *J. Cell. Mol. Med.*, **13** (8B), 2684–2692 (2009).
34) Zhang J, Sio SW, Moochhala S, Bhatia M. Role of hydrogen sulfide in severe burn injury-induced inflammation in mice. *Mol. Med.*, **16**, 417–424 (2010).
35) Sekiguchi F, Miyamoto Y, Kanaoka D, Ide H, Yoshida S, Ohkubo T, Kawabata A. Endogenous and exogenous hydrogen sulfide facilitates T-type calcium channel currents in $Ca_v3.2$-expressing HEK293 cells. *Biochem. Biophys. Res. Commun.*, **445**, 225–229 (2014).
36) Kawabata A, Ishiki T, Nagasawa K, Yoshida S, Maeda Y, Takahashi T, Sekiguchi F, Wada T, Ichida S, Nishikawa H. Hydrogen sulfide as a novel nociceptive messenger. *Pain*, **132**, 74–81 (2007).
37) Fukami K, Sekiguchi F, Yasukawa M, Asano E, Kasamatsu R, Ueda M, Yoshida S, Kawabata A. Functional upregulation of the $H_2S/Ca_v3.2$ channel pathway accelerates secretory function in neuroendocrine-differentiated human prostate cancer cells. *Biochem. Pharmacol.*, **97**, 300–309 (2015).
38) Maeda Y, Aoki Y, Sekiguchi F, Matsunami M, Takahashi T, Nishikawa H, Kawabata A. Hyperalgesia induced by spinal and peripheral hydrogen sulfide: Evidence for involvement of $Ca_v3.2$ T-type calcium channels. *Pain*, **142**, 127–132 (2009).

39) Okubo K, Matsumura M, Kawaishi Y, Aoki Y, Matsunami M, Okawa Y, Sekiguchi F, Kawabata A. Hydrogen sulfide-induced mechanical hyperalgesia and allodynia require activation of both $Ca_v3.2$ and TRPA1 channels in mice. *Br. J. Pharmacol.*, **166**, 1738–1743 (2012).
40) Nelson MT, Woo J, Kang HW, Vitko I, Barrett PQ, Perez-Reyes E, Lee JH, Shin HS, Todorovic SM. Reducing agents sensitize C-type nociceptors by relieving high-affinity zinc inhibition of T-type calcium channels. *J. Neurosci.*, **27**, 8250–8260 (2007).
41) Matsunami M, Kirishi S, Okui T, Kawabata A. Chelating luminal zinc mimics hydrogen sulfide-evoked colonic pain in mice: possible involvement of T-type calcium channels. *Neuroscience*, **181**, 257–264 (2011).
42) van Loo KM, Schaub C, Pernhorst K, Yaari Y, Beck H, Schoch S, Becker AJ. Transcriptional regulation of T-type calcium channel $Ca_v3.2$: bi-directionality by early growth response 1 (Egr1) and repressor element 1 (RE-1) protein-silencing transcription factor (REST). *J. Biol. Chem.*, **287**, 15489–15501 (2012).
43) Khachigian LM. Early growth response-1 in the pathogenesis of cardiovascular disease. *J. Mol. Med.* (Berl.), **94**, 747–753 (2016).
44) Garcia-Caballero A, Gadotti VM, Stemkowski P, Weiss N, Souza IA, Hodgkinson V, Bladen C, Chen L, Hamid J, Pizzoccaro A, Deage M, Francois A, Bourinet E, Zamponi GW. The deubiquitinating enzyme USP5 modulates neuropathic and inflammatory pain by enhancing $Ca_v3.2$ channel activity. *Neuron*, **83**, 1144–1158 (2014).
45) Stemkowski PL, Garcia-Caballero A, Gadotti VM, M'Dahoma S, Chen L, Souza IA, Zamponi GW. Identification of interleukin-1 beta as a key mediator in the upregulation of $Ca_v3.2$–USP5 interactions in the pain pathway. *Mol. Pain*, **13**, 1744806917724698 (2017).
46) Sanders KM, Koh SD, Ro S, Ward SM. Regulation of gastrointestinal motility—insights from smooth muscle biology. *Nat. Rev. Gastroenterol. Hepatol.*, **9**, 633–645 (2012).
47) Kinoshita K, Sato K, Hori M, Ozaki H, Karaki H. Decrease in activity of smooth muscle L-type Ca^{2+} channels and its reversal by NF-kB inhibitors in Crohn's colitis model. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **285**, G483–G493 (2003).
48) Kang M, Morsy N, Jin X, Lupu F, Akbarali HI. Protein and gene expression of Ca^{2+} channel isoforms in murine colon: effect of inflammation. *Pflugers Arch.*, **449**, 288–297 (2004).
49) Akbarali HI, Hawkins EG, Ross GR, Kang M. Ion channel remodeling in gastrointestinal inflammation. *Neurogastroenterol. Motil.*, **22**, 1045–1055 (2010).
50) Matsunami M, Kirishi S, Okui T, Kawabata A. Hydrogen sulfide-induced colonic mucosal cytoprotection involves T-type calcium channel-dependent neuronal excitation in rats. *J. Physiol. Pharmacol.*, **63**, 61–68 (2012).
51) Wallace JL, Vong L, McKnight W, Dicay M, Martin GR. Endogenous and exogenous hydrogen sulfide promotes resolution of colitis in rats. *Gastroenterology*, **137**, 569–578.e1 (2009).
52) Ichikawa T, Ishihara K, Kusakabe T, Hiruma H, Kawakami T, Hotta K. CGRP modulates mucin synthesis in surface mucus cells of rat gastric oxyntic mucosa. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **279**, G82–G89 (2000).
53) Kawabata A, Kinoshita M, Nishikawa H, Kuroda R, Nishida M, Araki H, Arizono N, Oda Y, Kakehi K. The protease-activated receptor-2 agonist induces gastric mucus secretion and mucosal cytoprotection. *J. Clin. Invest.*, **107**, 1443–1450 (2001).
54) Fiorucci S, Mencarelli A, Palazzetti B, Distrutti E, Vergnolle N, Hollenberg MD, Wallace JL, Morelli A, Cirino G. Proteinase-activated receptor 2 is an anti-inflammatory signal for colonic lamina propria lymphocytes in a mouse model of colitis. *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 13936–13941 (2001).
55) Akiba Y, Furukawa O, Guth PH, Engel E, Nastaskin I, Kaunitz JD. Sensory pathways and cyclooxygenase regulate mucus gel thickness in rat duodenum. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **280**, G470–G474 (2001).
56) Qian A, Song D, Li Y, Liu X, Tang D, Yao W, Yuan Y. Role of voltage gated Ca^{2+} channels in rat visceral hypersensitivity change induced by 2,4,6-trinitrobenzene sulfonic acid. *Mol. Pain*, **9**, 15 (2013).
57) Diop L, Raymond F, Fargeau H, Petoux F, Chovet M, Doherty AM. Pregabalin (CI-1008) inhibits the trinitrobenzene sulfonic acid-induced chronic colonic allodynia in the rat. *J. Pharmacol. Exp. Ther.*, **302**, 1013–1022 (2002).
58) Ohashi-Doi K, Gale JD, Kurebayashi Y. Pregabalin inhibits accelerated defecation and decreased colonic nociceptive threshold in sensitized rats. *Eur. J. Pharmacol.*, **643**, 107–112 (2010).
59) Houghton LA, Fell C, Whorwell PJ, Jones I, Sudworth DP, Gale JD. Effect of a second-generation $\alpha 2\delta$ ligand (pregabalin) on visceral sensation in hypersensitive patients with irritable bowel syndrome. *Gut*, **56**, 1218–1225 (2007).
60) Matsunami M, Tarui T, Mitani K, Nagasawa K, Fukushima O, Okubo K, Yoshida S, Takemura M, Kawabata A. Luminal hydrogen sulfide plays a pronociceptive role in mouse colon. *Gut*, **58**, 751–761 (2009).
61) Tsubota-Matsunami M, Noguchi Y, Okawa Y, Sekiguchi F, Kawabata A. Colonic hydrogen sulfide-induced visceral pain and referred hyperalgesia involve activation of both $Ca_v3.2$ and TRPA1 channels in mice. *J. Pharmacol. Sci.*, **119**, 293–296 (2012).
62) Sekiguchi F, Kawara Y, Tsubota M, Kawakami E, Ozaki T, Kawaishi Y, Tomita S, Kanaoka D, Yoshida S, Ohkubo T, Kawabata A. Therapeutic potential of RQ-00311651, a novel T-type Ca^{2+} channel blocker, in distinct rodent models for neuropathic and visceral pain. *Pain*, **157**, 1655–1665 (2016).
63) Marger F, Gelot A, Alloui A, Matricon J, Ferrer JF, Barrere C, Pizzoccaro A, Muller E, Nargeot J, Snutch TP, Eschalier A, Bourinet E, Ardid D. T-type calcium channels contribute to colonic hypersensitivity in a rat model of irritable bowel syndrome. *Proc. Natl. Acad. Sci. U.S.A.*, **108**, 11268–11273 (2011).
64) Ogawa T, Ishizuka O, Ueda T, Tyagi P, Chancellor MB, Yoshimura N. Current and emerging drugs for interstitial cystitis/bladder pain syndrome (IC/BPS). *Expert Opin. Emerg. Drugs*, **20**, 555–570 (2015).
65) Homma Y. Hypersensitive bladder: a solution to confused terminology and ignorance concerning interstitial cystitis. *Int. J. Urol.*, **21** (Suppl. 1), 43–47 (2014).
66) Rudick CN, Schaeffer AJ, Klumpp DJ. Pharmacologic attenuation of pelvic pain in a murine model of interstitial cystitis. *BMC Urol.*, **9**, 16 (2009).
67) Kwon WA, Ahn SH, Oh TH, Lee JW, Han DY, Jeong HJ. Effect of low-dose triple therapy using gabapentin, amitriptyline, and a nonsteroidal anti-inflammatory drug for overactive bladder symptoms in patients with bladder pain syndrome. *Int. Neurourol. J.*, **17**, 78–82 (2013).
68) Nickel JC, Crossland A, Davis E, Haab F, Mills IW, Rovner E, Scholfield D, Crook T. Investigation of a Ca^{2+} channel alpha2delta ligand for the treatment of interstitial cystitis: results of a randomized, double-blind, placebo controlled phase II trial. *J. Urol.*, **188**, 817–823 (2012).
69) Su X, Leon LA, Laping NJ. Role of spinal $Ca_v2.2$ and $Ca_v2.1$ ion channels in bladder nociception. *J. Urol.*, **179**, 2464–2469 (2008).
70) Igawa Y, Kumano S, Aizawa N, Saito Y, Ito H, Watanabe S, Takahashi N, Tajimi M, Nishimatsu H, Homma Y. Changes in the function and expression of T-type and N-type calcium channels in the rat bladder after bladder outlet obstruction. *J. Urol.*, **191**, 1159–1167 (2014).
71) Pang X, Marchand J, Sant GR, Kream RM, Theoharides TC, J. Increased number of substance P positive nerve fibres in interstitial cystitis. *Br. J. Urol.*, **75**, 744–750 (1995).
72) Nazif O, Teichman JM, Gebhart GF. Neural upregulation in interstitial cystitis. *Urology*, **69** (Suppl.), s24–s33 (2007).
73) Vesela R, Aronsson P, Andersson M, Wsol V, Tobin G. The poten-

tial of non-adrenergic, non-cholinergic targets in the treatment of interstitial cystitis/painful bladder syndrome. *J. Physiol. Pharmacol.*, **63**, 209–216 (2012).
74) Okinami T, Imamura M, Nishikawa N, Negoro H, Sugino Y, Yoshimura K, Kanematsu A, Hashitani H, Ogawa O. Altered detrusor gap junction communications induce storage symptoms in bladder inflammation: a mouse cyclophosphamide-induced model of cystitis. *PLOS ONE*, **9**, e104216 (2014).
75) Olivar T, Laird JM. Cyclophosphamide cystitis in mice: behavioural characterisation and correlation with bladder inflammation. *Eur. J. Pain*, **3**, 141–149 (1999).
76) Wang CC, Weng TI, Wu ET, Wu MH, Yang RS, Liu SH. Involvement of interleukin-6-regulated nitric oxide synthase in hemorrhagic cystitis and impaired bladder contractions in young rats induced by acrolein, a urinary metabolite of cyclophosphamide. *Toxicol. Sci.*, **131**, 302–310 (2013).
77) Ozaki T, Tsubota M, Sekiguchi F, Kawabata A. Involvement of NF-κB in the upregulation of cystathionine-***g***-lyase, a hydrogen sulfide-forming enzyme, and bladder pain accompanying cystitis in mice. *Clin. Exp. Pharmacol. Physiol.*, **45**, 355–361 (2018).
78) Matsunami M, Okawa Y, Okubo K, Sekiguchi F, Kawabata A. Study on molecular mechanisms for interstitial cystitis using a substance P-induced delayed bladder pain model in mice: roles of endogenous hydrogen sulfide and $Ca_v3.2$ T-type Ca^{2+} channels. *J. Pharmacol. Sci.*, **118** (Suppl. 1), 193P (2012).
79) Theoharides TC, Pang X, Letourneau R, Sant GR. Interstitial cystitis: a neuroimmunoendocrine disorder. *Ann. N. Y. Acad. Sci.*, **840**, 619–634 (1998).
80) Wang ZY, Wang P, Bjorling DE. Role of mast cells and protease-activated receptor-2 in cyclooxygenase-2 expression in urothelial cells. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **297**, R1127–R1135 (2009).
81) Miki T, Matsunami M, Nakamura S, Okada H, Matsuya H, Kawabata A. ONO-8130, a selective prostanoid EP1 receptor antagonist, relieves bladder pain in mice with cyclophosphamide-induced cystitis. *Pain*, **152**, 1373–1381 (2011).
82) Tsubota M, Ozaki T, Hayashi Y, Okawa Y, Fujimura A, Sekiguchi F, Nishikawa H, Kawabata A. Prostanoid-dependent bladder pain caused by proteinase-activated receptor-2 activation in mice: Involvement of TRPV1 and T-type Ca^{2+} channels. *J. Pharmacol. Sci.*, **136**, 46–49 (2018).
83) Vera-Portocarrero L, Westlund KN. Role of neurogenic inflammation in pancreatitis and pancreatic pain. *Neurosignals*, **14**, 158–165 (2005).
84) Gurusamy KS, Lusuku C, Davidson BR. Pregabalin for decreasing pancreatic pain in chronic pancreatitis. *Cochrane Database Syst. Rev.*, **2**, CD011522 (2016).
85) Liao XZ, Zhou MT, Mao YF, Xu H, Chen H, Sun JH, Xiong YC. Analgesic effects of gabapentin on mechanical hypersensitivity in a rat model of chronic pancreatitis. *Brain Res.*, **1337**, 104–112 (2010).
86) Nishimura S, Fukushima O, Ishikura H, Takahashi T, Matsunami M, Tsujiuchi T, Sekiguchi F, Naruse M, Kamanaka Y, Kawabata A. Hydrogen sulfide as a novel mediator for pancreatic pain in rodents. *Gut*, **58**, 762–770 (2009).
87) Terada Y, Fujimura M, Nishimura S, Tsubota M, Sekiguchi F, Kawabata A. Roles of $Ca_v3.2$ and TRPA1 channels targeted by hydrogen sulfide in pancreatic nociceptive processing in mice with or without acute pancreatitis. *J. Neurosci. Res.*, **93**, 361–369 (2015).
88) Fukushima O, Nishimura S, Matsunami M, Aoki Y, Nishikawa H, Ishikura H, Kawabata A. Phosphorylation of ERK in the spinal dorsal horn following pancreatic pronociceptive stimuli with proteinase-activated receptor-2 agonists and hydrogen sulfide in rats: evidence for involvement of distinct mechanisms. *J. Neurosci. Res.*, **88**, 3198–3205 (2010).
89) Snutch TP, Zamponi GW. Recent advances in the development of T-type calcium channel blockers for pain intervention. *Br. J. Pharmacol.*, **175**, 2375–2383 (2018).
90) Lee M. Z944: a first in class T-type calcium channel modulator for the treatment of pain. *J. Peripher. Nerv. Syst.*, **19** (Suppl. 2), S11–S12 (2014).
91) Samour MS, Nagi SS, Mahns DA. $Ca_v3.2$-expressing low-threshold C fibres in human hairy skin contribute to cold allodynia—a non-TRPV1- and non-TRPM8-dependent phenomenon. *Pain*, **156**, 1566–1575 (2015).
92) Serra J, Duan WR, Locke C, Sola R, Liu W, Nothaft W. Effects of a T-type calcium channel blocker, ABT-639, on spontaneous activity in C-nociceptors in patients with painful diabetic neuropathy: a randomized controlled trial. *Pain*, **156**, 2175–2183 (2015).
93) Ziegler D, Duan WR, An G, Thomas JW, Nothaft W. A randomized double-blind, placebo-, and active-controlled study of T-type calcium channel blocker ABT-639 in patients with diabetic peripheral neuropathic pain. *Pain*, **156**, 2013–2020 (2015).