J Pharmacol Sci **122**, 244 – 250 (2013)

Journal of Pharmacological Sciences

Current Perspective

T-type Calcium Channels: Functional Regulation and Implication in Pain Signaling

Fumiko Sekiguchi[1] and Atsufumi Kawabata[1,*]

[1]*Division of Pharmacology and Pathophysiology, Kinki University School of Pharmacy, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502, Japan*

Received May 8, 2013; Accepted June 14, 2013

Abstract. Low-voltage-activated T-type Ca^{2+} channels (T-channels), especially $Ca_v3.2$ among the three isoforms ($Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$), are now considered to play pivotal roles in processing of pain signals. $Ca_v3.2$ T-channels are functionally modulated by extracellular substances such as hydrogen sulfide and ascorbic acid, by intracellular signaling molecules including protein kinases, and by glycosylation. $Ca_v3.2$ T-channels are abundantly expressed in both peripheral and central endings of the primary afferent neurons, regulating neuronal excitability and release of excitatory neurotransmitters such as substance P and glutamate, respectively. Functional upregulation of $Ca_v3.2$ T-channels is involved in the pathophysiology of inflammatory, neuropathic, and visceral pain. Thus, $Ca_v3.2$ T-channels are considered to serve as novel targets for development of drugs for treatment of intractable pain resistant to currently available analgesics.

Keywords: $Ca_v3.2$, T-type calcium channel, neuropathy, visceral pain, hydrogen sulfide

1. Introduction

T-type Ca^{2+} channels (T-channels) are activated at near-resting membrane potential and play a crucial role in the neuronal firing. T-channels consist of a single pore-forming α_1-subunit, differing from high-voltage-activated (HVA) Ca^{2+} channels (L-, N-, P/Q-, and R-type Ca^{2+} channels) that are heteromultimers comprising an α_1-subunit together with ancillary β, γ, and $\alpha_2\delta$ subunits. Three α_1-subunit genes, *CACNA1G*, *CACNA1H*, and *CACNA1I*, encode α_{1G} ($Ca_v3.1$), α_{1H} ($Ca_v3.2$) and α_{1I} ($Ca_v3.3$) isoforms of the T-channel family, respectively (1). T-channels regulate neuronal excitability in the central and peripheral nervous systems, and hormone secretion under physiological conditions, whereas altered functions of T-channels are linked to pathophysiologies such as absence epilepsy, caridiovascular diseases, cancer, and pain (2). In 2001, Todorovic and his co-workers (3), for the first time, provided clear evidence for the involvement of T-channels in pain signaling, and thereafter a number of researchers including our group have demonstrated that $Ca_v3.2$ and possibly $Ca_v3.1$ play a pivotal role in nociceptive processing in health and disease (4). We have demonstrated that $Ca_v3.2$ T-channels are functionally upregulated by endogenous substances including hydrogen sulfide (H_2S) and participate in not only somatic but also visceral pain signaling (5 – 8). Here we describe the molecular characteristics and functional modulation of T-channels, especially $Ca_v3.2$, and review their impacts on peripheral and central processing of somatic and visceral pain signals.

*Corresponding author. kawabata@phar.kindai.ac.jp
Published online in J-STAGE on August 1, 2013
doi: 10.1254/jphs.13R05CP

Invited article

2. Functional modulation of T-type Ca^{2+} channels

2.1. Histidine at position 191 in $Ca_v3.2$ T-channels, a high affinity metal binding site, as a target for selective modulation of the channel functions by endogenous and exogenous substances

$Ca_v3.2$ T-channels are much more sensitive to inhibition by metals, such as zinc, copper, and nickel, than $Ca_v3.1$ or $Ca_v3.3$. It is now clear that a histidine residue at position 191 (His^{191}) in the second extracellular loop of domain I of $Ca_v3.2$ is a critical determinant for the trace metal inhibition and is not conserved in $Ca_v3.1$ or $Ca_v3.3$ (2) (Fig. 1). $Ca_v3.2$ T-channels appear to be

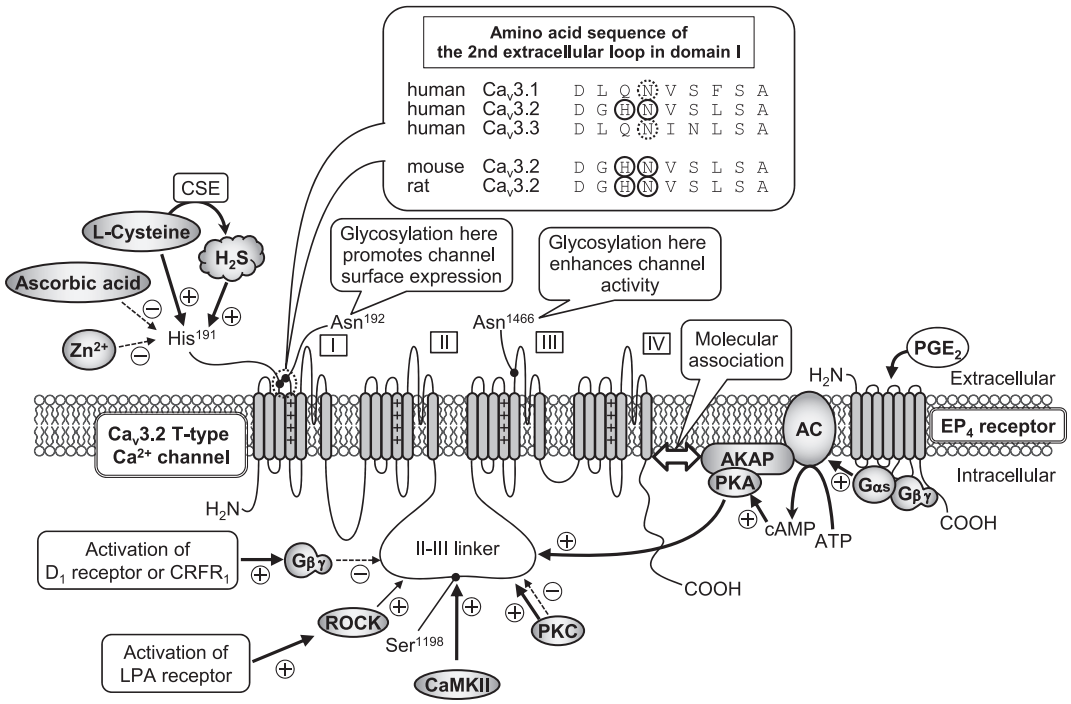

Fig. 1. Modulation of $Ca_v3.2$ T-channels. I, domain I; II, domain II; III, domain III; IV, domain IV; AKAP, A-kinase anchoring protein; AC, adenylyl cyclase; cAMP, cyclic AMP; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; PKA, protein kinase A; PKC, protein kinase C; PGE_2, prostaglandin E_2; ROCK, Rho-associated protein kinase; H_2S, hydrogen sulfide; CSE, cystathionine-γ-lyase; $CRFR_1$, corticotropin-releasing factor receptor 1; LPA, lysophosphatidic acid.

tonically exposed to inhibition by Zn^{2+} in cultured cells and possibly in mammalian tissues under physiological conditions, and Zn^{2+}-chelating agents or substances including L-cysteine and H_2S that interact with Zn^{2+} selectively enhance the channel functions of $Ca_v3.2$ among the three T-channel isoforms, leading to nociceptor sensitization or hyperalgesia in vivo (4, 6, 9, 10). H_2S, a diffusible gasotransmitter, is synthesized from L-cysteine by multiple enzymes such as cystathionine-γ-lyase (CSE) (Fig. 1), playing various roles in health and disease (11). Given that His^{191} is present in the extracellular loop of $Ca_v3.2$, H_2S, rather than L-cysteine, might function as an endogenous regulator of $Ca_v3.2$. On the contrary, ascorbic acid (vitamin C) selectively suppresses $Ca_v3.2$ via metal-catalyzed oxidation of the Zn^{2+}-binding His^{191} of $Ca_v3.2$ (4, 12), leading to inhibition of $Ca_v3.2$-mediated hyperalgesia (13). Importantly, His^{191} of $Ca_v3.2$ is conserved through different species including human, mouse and rat (Fig. 1), serving as a possible target for selective modulation of $Ca_v3.2$.

2.2. The domain II-III linker of T-channels as a target for protein kinases and G protein β and γ ($G_{\beta\gamma}$) subunits downstream of G protein–coupled receptors (GPCRs)

The intracellular linker between domains II and III (II-III linker) of T-channels is considered a target site for modulation of the channel functions by various protein kinases including Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), protein kinase A (PKA), PKC, and Rho-associated protein kinase (ROCK) (Fig. 1) (2, 14). A serine residue at position 1198 (Ser^{1198}) within the II-III linker of $Ca_v3.2$ appears to be a key site for phosphorylation and functional potentiation of the channels by CaMKII (14), whereas precise phosphorylation sites in the II-III linker for other protein kinases have not been identified. There is evidence that PKC and PKA enhance the activities of $Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$ expressed in mammalian cell lines (2, 14). However, several papers show suppression of T-channel activity by PKC (4, 14) (Fig. 1), implying that effects of PKC on T-channels might vary with cell types, PKC isoforms, or other experimental conditions. On the other hand, PKA activation of T-channels appears to mediate various cellular responses to activation of G_s protein–coupled receptors (2, 14). Recently, we have demonstrated the molecular

association between $Ca_v3.2$ and A-kinase anchoring protein 79/150 (AKAP79/150) and shown that activation of PKA following stimulation of EP_4 receptors, G_s protein–coupled receptors, by prostaglandin E_2 (PGE_2) causes AKAP-dependent phosphorylation and functional upregulation of $Ca_v3.2$ T-channels in NG108-15 cells (15) (Fig. 1). AKAP-dependent facilitation of T-channel currents by cyclic AMP has also been demonstrated in isolated rat dorsal root ganglion (DRG) neurons (15), implying potential roles of PKA-mediated activation/sensitization of T-channels in pain signaling. $G_{12/13}$ protein–dependent activation of RhoA and ROCK following stimulation of lysophosphatidic acid (LPA) receptors, a family of GPCRs, causes a shift of the activation and inactivation curves to the more depolarized potentials for $Ca_v3.2$, although it mediates reversible inhibition of $Ca_v3.1$ and $Ca_v3.3$ (2, 14). The physiological or pathological roles of 'sensitization' of $Ca_v3.2$ following LPA receptor activation (Fig. 1) remain unclear, although LPA has been linked to neuropathic pain (14). The II-III linker of T-channels is also a target for functional modulation by $G_{\beta\gamma}$ subunits downstream of GPCRs (Fig. 1). Stimulation of dopamine D_1 receptors expressed in adrenocarcinomal H295R cells causes direct interaction of $G_{\beta 2\gamma 2}$ subunits with $Ca_v3.2$, leading to inhibition of the channel functions (14). Similarly, activation of corticotropin-releasing factor receptor 1 ($CRFR_1$) inhibits $Ca_v3.2$, but not $Ca_v3.1$ and $Ca_v3.3$, through a cholera toxin–sensitive, $G_{\beta\gamma}$-mediated pathway (2, 14).

2.3. T-channel modulation by asparagine-linked glycosylation

Modulation of $Ca_v3.2$ T-channels by asparagine *N*-linked glycosylation at positions 192 (Asn^{192}) and 1466 (Asn^{1466}) was recently reported (16). Glycosylation at Asn^{192} promotes channel expression of $Ca_v3.2$ at the cell surface, whereas glycosylation at Asn^{1466} enhances the channel activity (Fig. 1). Strikingly, N-linked glycosylation of $Ca_v3.2$ also underlies glucose-dependent potentiation of the channel functions, which might be implicated in diabetic neuropathy. It has yet to be studied whether N-glycans regulate functions of $Ca_v3.1$ or $Ca_v3.3$, although those two Asn residues at corresponding positions are conserved in all three T-channel isoforms (Fig. 1).

3. Roles of T-channels in processing of pain signaling

3.1. Somatic pain processing by T-channels

After the first report concerning nociceptor sensitization by activation of T-channels (3), a critical role of $Ca_v3.2$ in sensory neurons was demonstrated by its gene silencing using the antisense oligodeoxynucleotides (17). Further, a study using knockout mice (18) demonstrated involvement of $Ca_v3.1$ and $Ca_v3.2$ in nociceptive processing, particularly signaling of inflammatory pain (18). Generally, it is considered that $Ca_v3.2$ T-channels regulate cellular excitability in the peripheral nerve endings of nociceptors, while N-type Ca^{2+} channels regulate release of neurotransmitters such as glutamate and substance P in the central terminals of the nocicepor neurons in laminae I and II of the spinal dorsal horn (19) (Fig. 2). However, the most recent evidence shows that $Ca_v3.2$ channels also regulate low-threshold exocytosis in cultured cells in an action potential-independent manner (20) and that presynaptic $Ca_v3.2$ regulates spontaneous excitatory synaptic neurotransmission in the spinal dorsal horn (21). Thus, $Ca_v3.2$ is now considered to play important roles in nociceptive processing at both peripheral and central endings of the primary afferent neurons (Fig. 2). This notion is consistent with our finding that not only intraplantar but also intrathecal administration of NaHS, a donor of H_2S, caused hyperalgesia in rats, an effect blocked by a T-channel blocker or by silencing of the $Ca_v3.2$ gene (6). Activation/sensitization of T-channels by endogenous H_2S formed by CSE may be involved in inflammatory hyperalgesia because inhibitors of T-channels and CSE, an H_2S-forming enzyme, prevent the development of inflammatory hyperalgesia caused by intraplantar administration of lipopolysaccharide (LPS) in rats (5). It is also to be noted that H_2S is capable of activating TRPA1 channels in addition to $Ca_v3.2$ (22) and that peripheral H_2S-induced mechanical hyperalgesia and allodynia require activation of both $Ca_v3.2$ and TRPA1 (23) (Fig. 2). AKAP-dependent functional upregulation of $Ca_v3.2$ by PKA following EP_4-receptor activation mediates PGE_2-induced mechanical hyperalgesia (15), although sensitization of TRPV1 channels by PKC following EP_1-receptor activation participates in the PGE_2-induced thermal hyperalgesia (24) (Fig. 2). Thus, $Ca_v3.2$ expressed in the nociceptors appears to play a role in the development of inflammatory pain or hyperalgesia. There is also some evidence for pro-nociceptive roles of $Ca_v3.2$ at the supraspinal levels (25, 26). A study shows that $Ca_v3.2$-dependent activation of ERK in the anterior nucleus of the paraventricular thalamus contributes to the development of acid-induced chronic hyperalgesia (25). It has also been demonstrated that $Ca_v3.2$ is involved in the generation of burst firing of both nociceptive and tactile reticular thalamic neurons (26).

3.2. Involvement of T-channels in neuropathic pain

Accumulating evidence strongly suggests involvement of T-channels in neuropathic pain (4). There is evidence

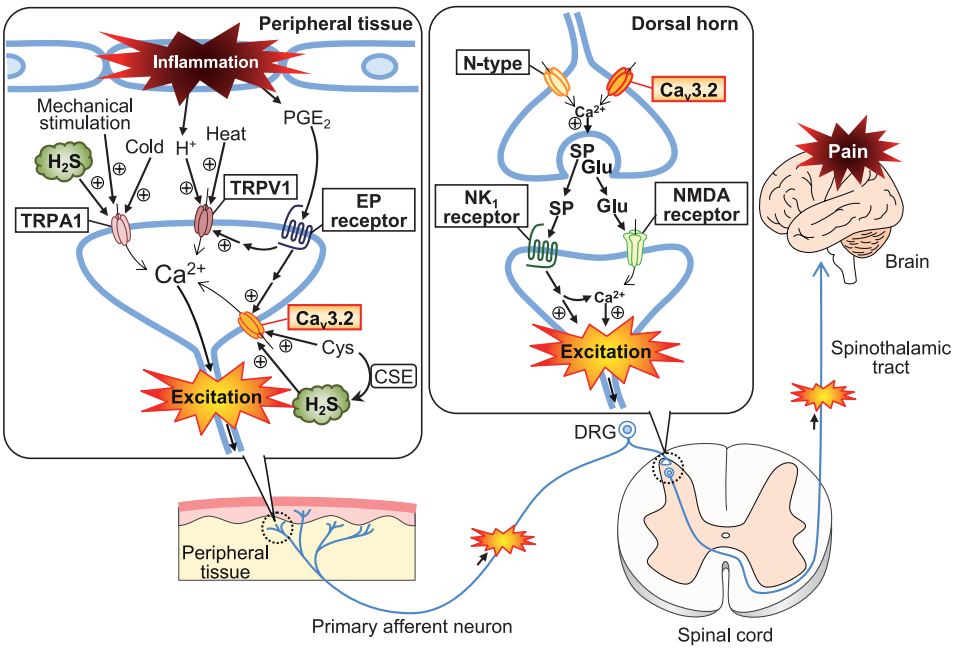

Fig. 2. Roles of $Ca_v3.2$ T-channels in the peripheral and central terminals of the primary afferent neurons. DRG, dorsal root ganglion; N-type, N-type Ca^{2+} channels; TRPV1, transient receptor potential vanilloid 1; TRPA1, TRP ankyrin 1; PGE_2, prostaglandin E_2; Cys, L-cysteine; Glu, L-glutamate; SP, substance P.

for upregulation of T-channel current density in small DRG cells isolated from rats with neuropathic pain due to chronic constrictive injury (CCI) of the sciatic nerve (27) and that genetic silencing of $Ca_v3.2$ channels reverses CCI-induced neuropathic pain (17). We have demonstrated upregulation of $Ca_v3.2$ at protein levels in the DRG of rats with neuropathy induced by spinal nerve injury and that pharmacological inhibition or genetic silencing of $Ca_v3.2$ reverses the established neuropathic hyperalgesia/allodynia (28). Interestingly, the neuropathic pain caused by spinal nerve injury is also abolished by two distinct inhibitors of CSE, an H_2S forming enzyme, suggesting that in addition to the upregulation of $Ca_v3.2$, sensitization of $Ca_v3.2$ by CSE-derived endogenous H_2S through interaction with Zn^{2+}, as described in Section 2.1. and shown in Fig. 1, contributes to the neuropathic pain (28). In addition to $Ca_v3.2$, $Ca_v3.1$ and $Ca_v3.3$ may be involved in the pathophysiology of neuropathic pain, considering evidence that neuropathic pain induced by L5 spinal nerve ligation is attenuated in $Ca_v3.1$-defective mice (29) and that $Ca_v3.3$, as well as $Ca_v3.2$, is upregulated at mRNA levels in the spinal cord of the rats with neuropathy induced by chronic compression of DRG (30).

Many anticancer agents, such as vincristine, paclitaxel, and oxaliplatin, often cause painful peripheral neuropathies that limit their use in cancer therapy. Involvement of T-channels in cancer chemotherapy-induced neuropathy was first suggested by a report showing that systemic administration of ethosuximide, a classic T-channel inhibitor, alleviated mechanical and cold allodynia/hyperalgesia in rats with paclitaxel- or vincristine-induced neuropathy (31). We have confirmed that paclitaxel-induced neuropathic hyperalgesia is abolished by more selective inhibitors of T-channels and by $Ca_v3.2$ gene silencing, although expression levels of $Ca_v3.2$ in DRG do not change in this neuropathic pain model (32), differing from the spinal nerve injury–induced neuropathic pain models in which $Ca_v3.2$ is dramatically upregulated in DRG (28). It is also noteworthy that the paclitaxel-induced neuropathy is reversed by an inhibitor of CSE, suggesting involvement of functional upregulation of the CSE/H_2S/$Ca_v3.2$ pathway. Strikingly, we have detected significant decrease in levels of ascorbic acid (vitamin C), known to selectively inhibit $Ca_v3.2$ (see Fig. 1), in the hindpaw tissue of rats with paclitaxel-induced neuropathy (13). We have also shown that intraplantar injection of ascorbic acid or topical application of disodium isostearyl 2-*O*-L-ascorbyl phosphate (DI-VCP), a skin-permeable ascorbate derivative, abolishes the H_2S-induced hyperalgesia and paclitaxel-induced neuropathy and also partially inhibits the spinal nerve injury–induced neuropathy (13). These findings are consistent with some clinical evidence for the decreased plasma vitamin C levels in post-herpetic neuralgia patients (33, 34) and for the therapeutic value of ascorbic

acid in patients with post-herpetic neuralgia (33, 35) or with complex regional pain syndrome after wrist fractures (36). Together, it is likely that vitamin C acts as a modulator of $Ca_v3.2$ T-channels to suppress excessive sensitization of nociceptors.

There is also much evidence for involvement of T-channels in diabetic neuropathy. Upregulation of T-channel current density has been shown in DRG cells isolated from diabetic Bio-Bred/Worchester (BB/W) rats and from rats with streptozotocin (STZ)-induced diabetic neuropathy (4). Presynaptic $Ca_v3.2$ responsible for excitatory neurotransmission in the spinal dorsal horn is also functionally upregulated in the animal model of painful diabetic neuropathy (21). It has also been reported that in vivo silencing of $Ca_v3.2$ in DRG reverses mechanical and thermal hyperalgesia in STZ-induced diabetic neuropathy rats (4). Thus, the functional upregulation of $Ca_v3.2$ in the sensory neurons appears to cause diabetes-induced hyperexcitability and excessive neurotransmission in the peripheral and spinal endings of the nociceptors, respectively, contributing to the pathogenesis of diabetic neuropathy. Of interest is that N-linked glycosylation of $Ca_v3.2$ responsible for its surface expression and enhanced activity (see Fig. 1) is linked to glucose elevations (16), as mentioned in Section 2.3, which might be involved in the etiology of diabetic painful neuropathy.

3.3. Roles of T-channels in visceral pain processing

We have reported, for the first time, that $Ca_v3.2$ T-channels play a critical role in processing of visceral pain in the colon and pancreas (7, 8). Intracolonic (i.col.) administration of NaHS, an H_2S donor, to mice triggers visceral nociceptive behavior accompanied by referred hyperalgesia, which are suppressed by a T-channel inhibitor (7) and also by silencing of $Ca_v3.2$ (37). The nociceptive effect of i.col. NaHS/H_2S is blocked by pretreatment with $ZnCl_2$, and i.col. administration of Zn^{2+} chelators causes T-channel–dependent colonic pain and referred hyperalgesia (9). As described in Section 2.1., therefore, the luminal H_2S might interact with Zn^{2+} bound to His^{191} of $Ca_v3.2$ and cancel Zn^{2+} inhibition of the channel functions, leading to facilitation of $Ca_v3.2$ functions and subsequent colonic pain (see Fig. 1). The impact of $Ca_v3.2$ on colonic pain signaling is supported by a recent report from an independent group showing involvement of $Ca_v3.2$ in butyrate-induced colonic hypersensitivity to colorectal distention in the rat, a model for irritable bowel syndrome (IBS) (38). Similarly, activation/sensitization of $Ca_v3.2$ by endogenous H_2S formed by CSE plays critical roles in pancreatic pain accompanying cerulein-induced pancreatitis (8) and in bladder pain accompanying cyclophosphamide-induced cystitis (39). Interestingly, in these pancreatic and bladder pain models, CSE protein is upregulated in the pancreatic and bladder tissues, respectively (8, 40). Upregulation of $Ca_v3.2$ protein is also detectable in the DRG in the latter model (39). Collectively, $Ca_v3.2$ regulated by endogenous H_2S may serve as a novel therapeutic target for treatment of various types of visceral pain. Apart from its role in visceral nociceptive processing, excitation of sensory neurons following H_2S-induced $Ca_v3.2$ activation underlies bladder inflammation in mice treated with cyclophosphamide (39), but exerts colonic mucosal cytoprotection in rats with 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis (40). The $H_2S/Ca_v3.2$ system would thus appear to play bidirectional roles in inflammation, in addition to pronociceptive roles, through neuronal excitation in internal organs. In contrast to involvement of $Ca_v3.2$ in nociceptor excitation, there is evidence that thalamic $Ca_v3.1$ plays an anti-nociceptive role in visceral pain signaling (41). However, the roles of $Ca_v3.1$ in the primary afferents and of $Ca_v3.3$ are still largely open to question.

4. Conclusion

Among three T-channel isoforms, $Ca_v3.2$ has been best characterized in relation to pain signaling. As described so far, modulation of channel activity or subcellular location of $Ca_v3.2$ by extracellular or intracellular molecules is associated with the pathophysiology of persistent pain as well as epilepsy. Considering recent evidence that transcriptional expression of $Ca_v3.2$ is regulated positively by early growth response 1 (Egr1) and negatively by repressor element 1 (RE-1) protein-silencing transcription factor (REST) (42), abnormal regulation of transcriptional expression of $Ca_v3.2$ may also underlie chronic pain, as $Ca_v3.2$ protein is upregulated in the DRG of the rat with spinal nerve injury–induced neuropathic pain (28). Together, the development of selective inhibitors of $Ca_v3.2$ T-channels is an urgently needed for clinical application as analgesics or for research use as pharmacological tools. T-channel inhibitors with low blood–brain barrier (BBB) permeability might be useful as analgesics, since they might target T-channels expressed in the peripheral endings of nociceptors without causing central side effects. In addition, because the heart expresses both $Ca_v3.1$ and $Ca_v3.2$ isoforms, $Ca_v3.2$-selective T-channel inhibitors, if any, could reduce side effects on the heart. Thus, T-channel inhibitors with $Ca_v3.2$-selectivity and low BBB permeability would be ideal and useful as analgesics for intractable persistent pain resistant to gabapentin and pregabalin, currently available potent medicines

for neuropathic pain, that inhibit high-voltage–gated Ca^{2+} channels by interacting with $\alpha_2\delta$ subunits, but not T-channels.

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. 2005;57:411–425.

2 Iftinca MC, Zamponi GW. Regulation of neuronal T-type calcium channels. Trends Pharmacol Sci. 2009;30:32–40.

3 Todorovic SM, Jevtovic-Todorovic V, Meyenburg A, Mennerick S, Perez-Reyes E, Romano C, et al. Redox modulation of T-type calcium channels in rat peripheral nociceptors. Neuron. 2001; 31:75–85.

4 Todorovic SM, Jevtovic-Todorovic V. T-type voltage-gated calcium channels as targets for the development of novel pain therapies. Br J Pharmacol. 2011;163:484–495.

5 Kawabata A, Ishiki T, Nagasawa K, Yoshida S, Maeda Y, Takahashi T, et al. Hydrogen sulfide as a novel nociceptive messenger. Pain. 2007;132:74–81.

6 Maeda Y, Aoki Y, Sekiguchi F, Matsunami M, Takahashi T, Nishikawa H, et al. Hyperalgesia induced by spinal and peripheral hydrogen sulfide: evidence for involvement of $Ca_v3.2$ T-type calcium channels. Pain. 2009;142:127–132.

7 Matsunami M, Tarui T, Mitani K, Nagasawa K, Fukushima O, Okubo K, et al. Luminal hydrogen sulfide plays a pronociceptive role in mouse colon. Gut. 2009;58:751–761.

8 Nishimura S, Fukushima O, Ishikura H, Takahashi T, Matsunami M, Tsujiuchi T, et al. Hydrogen sulfide as a novel mediator for pancreatic pain in rodents. Gut. 2009;58:762–770.

9 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. 2011;181:257–264.

10 Nelson MT, Woo J, Kang HW, Vitko I, Barrett PQ, Perez-Reyes E, et al. Reducing agents sensitize C-type nociceptors by relieving high-affinity zinc inhibition of T-type calcium channels. J Neurosci. 2007;27:8250–8260.

11 Kimura H. Hydrogen sulfide: from brain to gut. Antioxid Redox Signal. 2010;12:1111–1123.

12 Nelson MT, Joksovic PM, Su P, Kang HW, Van Deusen A, Baumgart JP, et al. Molecular mechanisms of subtype-specific inhibition of neuronal T-type calcium channels by ascorbate. J Neurosci. 2007;27:12577–12583.

13 Okubo K, Nakanishi H, Matsunami M, Shibayama H, Kawabata A. Topical application of disodium isostearyl 2-O-L-ascorbyl phosphate, an amphiphilic ascorbic acid derivative, reduces neuropathic hyperalgesia in rats. Br J Pharmacol. 2012;166: 1058–1068.

14 Zhang Y, Jiang X, Snutch TP, Tao J. Modulation of low-voltage-activated T-type Ca^{2+} channels. Biochim Biophys Acta. 2013; 1828:1550–1559.

15 Sekiguchi F, Aoki Y, Nakagawa M, Kanaoka D, Nishimoto Y, Tsubota-Matsunami M, et al. AKAP-dependent sensitization of $Ca_v3.2$ channels via the EP_4 receptor/cAMP pathway mediates PGE_2-induced mechanical hyperalgesia. Br J Pharmacol. 2013; 168:734–745.

16 Weiss N, Black SA, Bladen C, Chen L, Zamponi GW. Surface expression and function of $Ca_v3.2$ T-type calcium channels are controlled by asparagine-linked glycosylation. Pflugers Arch. 2013. In press.

17 Bourinet E, Alloui A, Monteil A, Barrere C, Couette B, Poirot O, et al. Silencing of the $Ca_v3.2$ T-type calcium channel gene in sensory neurons demonstrates its major role in nociception. EMBO J. 2005;24:315–324.

18 Shin HS, Cheong EJ, Choi S, Lee J, Na HS. T-type Ca^{2+} channels as therapeutic targets in the nervous system. Curr Opin Pharmacol. 2008;8:33–41.

19 Zamponi GW, Lewis RJ, Todorovic SM, Arneric SP, Snutch TP. Role of voltage-gated calcium channels in ascending pain pathways. Brain Res Rev. 2009;60:84–89.

20 Weiss N, Hameed S, Fernandez-Fernandez JM, Fablet K, Karmazinova M, Poillot C, et al. A $Ca_v3.2$/syntaxin-1A signaling complex controls T-type channel activity and low-threshold exocytosis. J Biol Chem. 2012;287:2810–2818.

21 Jacus MO, Uebele VN, Renger JJ, Todorovic SM. Presynaptic $Ca_v3.2$ channels regulate excitatory neurotransmission in nociceptive dorsal horn neurons. J Neurosci. 2012;32:9374–9382.

22 Streng T, Axelsson HE, Hedlund P, Andersson DA, Jordt SE, Bevan S, et al. Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. Eur Urol. 2008;53:391–399.

23 Okubo K, Matsumura M, Kawaishi Y, Aoki Y, Matsunami M, Okawa Y, et al. Hydrogen sulfide-induced mechanical hyperalgesia and allodynia require activation of both $Ca_v3.2$ and TRPA1 channels in mice. Br J Pharmacol. 2012;166:1738–1743.

24 Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y, et al. Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. Mol Pain. 2005;1:3.

25 Chen WK, Liu IY, Chang YT, Chen YC, Chen CC, Yen CT, et al. $Ca_v3.2$ T-type Ca^{2+} channel-dependent activation of ERK in paraventricular thalamus modulates acid-induced chronic muscle pain. J Neurosci. 2010;30:10360–10368.

26 Liao YF, Tsai ML, Chen CC, Yen CT. Involvement of the $Ca_v3.2$ T-type calcium channel in thalamic neuron discharge patterns. Mol Pain. 2011;7:43.

27 Jagodic MM, Pathirathna S, Joksovic PM, Lee W, Nelson MT, Naik AK, et al. Upregulation of the T-type calcium current in small rat sensory neurons after chronic constrictive injury of the sciatic nerve. J Neurophysiol. 2008;99:3151–3156.

28 Takahashi T, Aoki Y, Okubo K, Maeda Y, Sekiguchi F, Mitani K, et al. Upregulation of $Ca_v3.2$ T-type calcium channels targeted by endogenous hydrogen sulfide contributes to maintenance of neuropathic pain. Pain. 2010;150:183–191.

29 Na HS, Choi S, Kim J, Park J, Shin HS. Attenuated neuropathic pain in $Ca_v3.1$ null mice. Mol Cells. 2008;25:242–246.

30 Wen XJ, Xu SY, Chen ZX, Yang CX, Liang H, Li H. The roles of T-type calcium channel in the development of neuropathic pain following chronic compression of rat dorsal root ganglia. Pharmacology. 2010;85:295–300.

31 Flatters SJ, Bennett GJ. Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy. Pain. 2004; 109:150–161.

32 Okubo K, Takahashi T, Sekiguchi F, Kanaoka D, Matsunami M, Ohkubo T, et al. Inhibition of T-type calcium channels and hydrogen sulfide-forming enzyme reverses paclitaxel-evoked neuropathic hyperalgesia in rats. Neuroscience. 2011;188:

148–156.
33 Chen JY, Chang CY, Feng PH, Chu CC, So EC, Hu ML. Plasma vitamin C is lower in postherpetic neuralgia patients and administration of vitamin C reduces spontaneous pain but not brush-evoked pain. Clin J Pain. 2009;25:562–569.
34 Chen JY, Chu CC, Lin YS, So EC, Shieh JP, Hu ML. Nutrient deficiencies as a risk factor in Taiwanese patients with postherpetic neuralgia. Br J Nutr. 2011;106:700–707.
35 Schencking M, Vollbracht C, Weiss G, Lebert J, Biller A, Goyvaerts B, et al. Intravenous vitamin C in the treatment of shingles: results of a multicenter prospective cohort study. Med Sci Monit. 2012;18:CR215–CR224.
36 Zollinger PE, Tuinebreijer WE, Breederveld RS, Kreis RW. Can vitamin C prevent complex regional pain syndrome in patients with wrist fractures? A randomized, controlled, multicenter dose-response study. J Bone Joint Surg Am. 2007;89:1424–1431.
37 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. 2012;119:293–296.
38 Marger F, Gelot A, Alloui A, Matricon J, Ferrer JF, Barrere C, et al. T-type calcium channels contribute to colonic hypersensitivity in a rat model of irritable bowel syndrome. Proc Natl Acad Sci U S A. 2011;108:11268–11273.
39 Matsunami M, Miki T, Nishiura K, Hayashi Y, Okawa Y, Nishikawa H, et al. 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. 2012;167:917–928.
40 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. 2012;63:61–68.
41 Kim D, Park D, Choi S, Lee S, Sun M, Kim C, et al. Thalamic control of visceral nociception mediated by T-type Ca^{2+} channels. Science. 2003;302:117–119.
42 van Loo KM, Schaub C, Pernhorst K, Yaari Y, Beck H, Schoch S, et al. 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. 2012;287:15489–15501.