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REVIEWS

Role of T-Type Ca^{2+} Channels in Painful Diabetic Neuropathy

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Numerous investigations implicate pronounced changes in the functioning of T-type Ca^{2+} channels localized on the somata of primary nociceptor units in the development and maintenance of painful diabetic neuropathy. This review highlights the role of T-type Ca^{2+} channels of nociceptive afferents in the processing of pain signals under diabetic conditions, as well as suggests a promising therapeutic strategy to treat painful diabetic neuropathy.

Keywords: diabetic neuropathy, pain, primary nociceptive afferents, calcium channels of the T-type

INTRODUCTION

According to reports of the International Diabetes Federation, about one-tenth of the world population will suffer from diabetes by 2035, and the majority of those patients will live in low- and middle-income countries. In line with this prognosis, the prevalence of diabetes in Ukraine has reached epidemic proportions; the respective population increased by almost 60% during the last 11 years. At least 55% of the funds allocated to patients with diabetes in Ukraine are spent for the treatment of diabetic complications [1], including painful diabetic neuropathy (PDN). Diabetic neuropathy is one of the earliest, most frequent, and troublesome complications of diabetes; it occurs in about 60 to 70% of diabetic patients [2], among which approximately one-third suffer from severe burning, "electric," or stabbing pain [3–5]. Thus, diabetic neuropathy constitutes a major public health problem for diabetic patients. Ukraine is a country with a high incidence of PDN; however, basic research in the field of PDN in this country is hampered by the lack of resources and state-of-the art techniques. The awareness, understanding, and treatment of diabetic neuropathies are limited since their molecular mechanisms are still poorly understood, despite the growing number of studies throughout the world. Well-targeted basic research in the field of diabetic neuropathy is desperately needed for the development and faster introduction of new treatment strategies.

Upregulation of T-Type Calcium Channels in Primary Nociceptors Contributes to PDN

PDN is associated with alterations in the neuronal excitability of primary nociceptors and efficacy of synaptic transmission between these units and a population dorsal horn (DH) neurons of the spinal cord [6–8]. Low voltage-activated T-type Ca^{2+} channels (T-channels) are abundantly expressed in primary nociceptors [9–11]; these channels are responsible for the regulation of both neuronal excitability [8, 12, 13] and presynaptic release of glutamate [10, 14, 15]. Numerous pharmacological studies supported the role played by T-channels of primary nociceptors in the course of processing of pain signals [6, 7, 11, 16–19]. Besides, both global knock-out [20] and selective knock-down [21, 22] of $Ca_V3.2$ T-type channels in sensory neurons have

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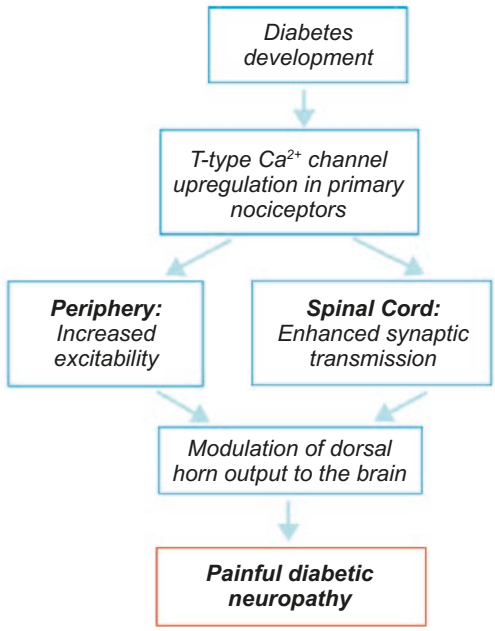
strongly validated the involvement of these channels in nociceptive signaling. Thus, T-channels represent a possible attractive pharmacological target for the treatment of pain syndromes resistant to currently available analgesics [6, 7, 11, 17, 23]. Numerous investigations implicate pronounced changes in the functioning of T-channels in the somata of peripheral nociceptors within the development and maintenance of PDN. Moreover, selective local knock-down [22, 24] or pharmacological blocking of T-type channels *in vivo* [24, 25] effectively reversed mechanical and thermal hyperalgesia in diabetic neuropathy of type 1 and type 2 diabetes. However, the exact mechanisms that link diabetes-induced T-channel upregulation in nociceptive neurons with PDN remain elusive, in particular due to a lack of necessary experimental approaches to study T-channel functioning in afferent fibers. Suggested mechanisms of how upregulation of T-type current intensifies pain perception include lowering the threshold for action potential (AP) generation in primary nociceptors [8], promoting spontaneous activity of secondary sensory neurons [26] and facilitating synaptic transmission between DRG neurons and DH neurons [10]. The recently established role of Cav3.2 T-type channels in electrical firing of secondary excitatory neurons located in lamina II of the spinal cord gray matter suggests that lowering the threshold of AP generation and increased probability of burst firing in these neurons may also contribute to PDN pathogenesis [27]. A scheme summarizing the involvement of T-type channels in PDN is presented in Fig. 1.

Both upregulation of the T-channel density and changes in their biophysical properties have been shown in primary nociceptors isolated from animals suffering from PDN [8, 12, 22, 25, 28–32]. Moreover, as was mentioned above, selective knock-down [22, 24] or pharmacological blocking of T-channels [24, 25] reversed pathological changes in the sensory processes under conditions of neuropathies related to type 1 and type 2 diabetes. Thus, it appears that activation of different metabolic [33, 34] and peripheral signaling cascades [35–38] are eventually responsible for the modulation of T-channels in primary nociceptors in both type 1 and type 2 PDNs. Regardless of the precise mechanisms of this modulation [11, 16, 17] upregulation of T-channels in nociceptive DRG neurons appears to be causally linked to the maintenance of PDN [22, 24, 25], representing a possible perfect target for its treatment. Nevertheless, particular molecular and cellular mechanisms that link the T-channel upregulation with the pathological pain processing in PDN are practically unknown, preventing the development of targeted therapies devoid of side effects. In this short review, we further consider possible molecular mechanisms involved in diabetes-induced T-channel upregulation and the role of T-channels in central axons and terminals of the primary nociceptors in gating pain transmission to the DH neuronal networks during PDN.



F i g. 1. Involvement of the T-type Ca^{2+} channels in painful diabetic neuropathy.

Potential Role of T-Type Channels in Neurotransmitter Release in Central Terminals of the Nociceptive DRG Neurons

To date, the majority of studies have used the cell bodies of primary sensory neurons *in vitro* as a model system for examining functions, modulation, and dysfunction of T-channels [6, 7, 11, 16–19]. Although convenient, this model suffers from significant limitations in representing physiological processes that take place in sensory axons and in central and peripheral terminals, i.e., in the cell structures critically important for detection and processing of sensory information. In the variety of different neuronal cell types, including primary nociceptive neurons, T-channels act as a major Ca^{2+} entry pathway within the range of membrane potential close to its resting value [31].

These channels support low-threshold exocytosis in neuroendocrine cells [39–43] and contribute to presynaptic release of neurotransmitters [14, 15]. These channel structures are expressed in presynaptic active zones [14] and are associated with some of the proteins of the vesicle release machinery [44–47]. T-channels were recently shown to be expressed in the superficial DH parts and co-localized with markers of primary nociceptors [10, 19]. It was also shown recently that the Cav3.2 subtype of T-channels is abundantly expressed in thin unmyelinated fibers of the dorsal roots [19]. It is important that these channels are present in presynaptic terminals within the DH [19]. The presence of T-channels in the presynaptic areas substantiates their emerging role in neurotransmitter release in the central terminals of nociceptive DRG neurons. This role is additionally indirectly supported by electrophysiological results demonstrating T-channel-dependent regulation of miniature spontaneous excitatory activity in DH neurons [38] (including those projecting to the supraspinal structures [10]). These findings also suggest that presynaptic T-channels are essential for pain processing at the spinal cord level. However, the roles of presynaptic T-channels of DRG neurons in presynaptic $[Ca^{2+}]_i$ signaling and in the regulation of synaptic transmission to the DH neurons, as well as molecular and cellular mechanisms of their involvement in pain processing, are still not well understood. Importantly, the exact mechanisms that causatively link diabetes-induced T-channel upregulation in nociceptive neurons [12, 22, 28–32] with diabetic neuropathic pain [22, 24, 25] also remain elusive.

Molecular Mechanisms of T-Type Channel Upregulation in PDN

The complexity of T-type channels regulation under diabetic conditions ensues from pathological changes in homeostasis of the diabetic patients. The primary pathological shift in homeostasis leading to upregulation of T-type channels is the development of hyperglycemia. The latter causes overexpression of the channels at the plasma membrane of neurons through increased N-glycosylation of the channel alpha-subunits, as well as of auxiliary subunits [33, 34, 48, 49] (for a review, see [50]). Secondary pathological changes in homeostasis induced by hyperglycemia (such as ischemia, hypoxia, acidosis, and inflammation) have not been evaluated as factors of T-type channels upregulation in diabetes. In the corresponding model experiments, however, hypoxia was shown to upregulate transcription of T-type channel genes [51]. Inflammation may also cause an increase in T-type channel gene transcription through activation of transcription factor Egr1 [52, 53], increase of T-type channel expression at the plasma membrane through activation of its deubiquitination [38], and enlargement of the population of DRG neurons expressing functional T-type channels [54]. Moreover, hyperglycemia and inflammation affect the redox status in the cells, while T-type channel activity significantly depends on the local redox state in the channel site [55, 56]. Diabetes causes an increase in the intracellular resting Ca^{2+} concentration [57], which should upregulate stationary ("window") T-type current through CaMKII phosphorylation of the channels; this shifts the activation curve to more negative potentials [58]. Stationary T-type current increases the spontaneous excitability of neurons [26]; so, its increase in nociceptive and other types of DRG neurons under diabetes conditions may provoke spontaneous pain sensations [12, 31]. Upregulation of T-type current in DRG neurons may also be caused by activation of PKA and PKC [59], which proceeds in diabetes through upregulation of the cytokine production in immune cells by advanced glycation end-products (AGE) [60] and via the following cascades: PGE2- PGE2-EPs-cAMP/PKA [61], HMGB1/AGE-RAGE-PKC [62], and bradykinin-PKC [63]. Methylglyoxal accumulation in diabetes also induces PKA activation through the methylglyoxal–TRPA1-AC1-PKA pathway [64]. It is worth mentioning that some of these pathways also sensitize TRPV1 receptors in DRG neurons, in such a way synergically enhancing pain perception. Thus, a number of well-established and putative pathways may contribute to diabetes-induced T-type current upregulation observed in PDN.

Gene Therapy of PDN

Painful diabetic neuropathy is a chronic and often intractable pathological state. Effective clinical interventions for this disease are very limited, and gene therapy [65] represents a novel and promising therapeutic strategy for PDN, especially in combination with simple and minimally invasive delivery methods (such as subcutaneous or intrasciatic injections). At present, there are several organic T-channel inhibitors and antisense oligodeoxyribonucleotide (AS ODN) constructs that are capable of providing analgesia in diabetic

neuropathy upon either intrathecal or systemic delivery [11, 12, 16, 22, 24, 25, 33, 66], making T-channels of DRG neurons important targets for further drug development. However, these already developed approaches are characterized by lack of molecular or cellular target specificity. They are based on complicated and/or substantially invasive procedures and provide insufficient inhibition and only short duration of therapeutic effects and are, thus, not ideal for the respective treatment. To address these numerous problems, we suggest to use cell-specific viral delivery of short-hairpin RNA (shRNA) expression cassettes as a delivery tool instead of AS ODN. This approach also supports more efficient and long-lasting transduction into target tissues. The $Ca_v3.2$ isoform of T-channels accounts for up to 90% of T-type current in nociceptive DRG neurons [22, 31, 67]; the presence of these channels is upregulated under diabetic conditions [8, 12, 22, 25, 28–32]. Thus, a knock-down of the $Ca_v3.2$ isoform as the most specific molecular target by means of RNA interference seems to be the most efficient approach for normalizing the T-current in diabetic conditions and for decreasing the PDN symptoms. An adeno-associated virus 6 (AAV6) vector with shRNA backbone having a siRNA sequence against $Ca_v3.2$ channels might be used to obtain long-term, cell-specific, and stable gene RNA interference with very limited accompanying toxicity.

Human clinical trials have suggested the safety and tolerability of the recombinant adeno-associated virus (AAV) technique in gene therapy for several CNS disorders [68, 69]. If we take into account the AAV6 high affinity for and efficient transduction within peripheral axons [70, 71], its ability to drive long-term (several months) expression of transgenes [72, 73], and its apparent lack of immunogenicity [74, 75], AAV6 seems to be a perfect vector for peripheral pain gene therapy. Considering specific tropism of the AAV6 serotype to a restricted part (about 15% of the entire population of DRG neurons) of small C-fiber nociceptive neurons, in which upregulation of T-channels seems to be causally linked to painful symptoms of diabetic neuropathy [22, 24, 25, 37], we expect that it will be possible to alleviate symptoms of PDN without (or with minimum) side effects by delivery of a virus vector with shRNA backbone having siRNA sequence against $Ca_v3.2$ channels. It is also important that AAV6 can be successfully delivered via sciatic nerve injections [70, 71], injections into branches of the latter [71], or even subcutaneous injections [70]. This situation substantially simplifies possible treatment procedures. Recent findings with respect to intrathecal administration of AAV2/5, which target sodium channels in DRG neurons, is a proof-of-principle demonstration for a novel therapy aimed at preventing diabetic neuropathic pain [76]. In conclusion, we consider AAV6-mediated cell-specific delivery of siRNA constructs, which target T-channels in nociceptive DRG neurons, to be a novel simple clinically relevant and long-lasting treatment of diabetic neuropathic pain.

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This is a review paper, and confirmation of the correspondence of the study to the existing ethical norms for experimental works is not necessary.

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REFERENCES

1. M. Tronko, “Pateints’ hope for science and government,” National Academy of Medical Sciences of Ukraine, 1–3 (2013).
2. C. Gooch and D. Podwall, “The diabetic neuropathies,” *Neurologist*, **10**, 311–322 (2004).
3. N. A. Calcutt and M. M. Backonja, “Pathogenesis of pain in peripheral diabetic neuropathy,” *Curr. Diab. Rep.*, **7**, 429–434 (2007).
4. B. C. Callaghan, H. T. Cheng, C. L. Stables, et al., Diabetic neuropathy: clinical manifestations and current treatments. *Lancet Neurol.* **11**, 521–534 (2012).
5. A. M. Vincent, B. C. Callaghan, A. L. Smith, et al., “Diabetic neuropathy: cellular mechanisms as therapeutic targets,” *Nat. Rev. Neurol.*, **7**, 573–583 (2011).
6. S. G. Waxman and G. W. Zamponi, “Regulating excitability of peripheral afferents: emerging ion channel targets,” *Nat. Neurosci.*, **17**, 153–163 (2014).
7. E. Bourinet, C. Altier, M. E. Hildebrand, et al., “Calcium-permeable ion channels in pain signaling,” *Physiol. Rev.*, **94**, 81–140 (2014).
8. D. E. Duzhyy, V. Y. Viatchenko-Karpinski, E. V. Khomula, et al., “Upregulation of T-type Ca^{2+} channels in long-term diabetes determines increased excitability of a specific type of capsaicin-insensitive DRG neurons,” *Mol. Pain*, **11**, 1–18 (2015).
9. K. E. Rose, N. Lunardi, A. Boscolo, et al., “Immunohistological demonstration of Cav3.2 T-type voltage-

gated calcium channel expression in soma of dorsal root ganglion neurons and peripheral axons of rat and mouse," *Neuroscience*, **250**, 263–274 (2013).
10. M. O. Jacus, V. N. Uebele, J. J. Renger, et al., "Presynaptic Cav3.2 channels regulate excitatory neurotransmission in nociceptive dorsal horn neurons," *J. Neurosci.*, **32**, 9374–9382 (2012).
11. S. M. Todorovic and V. Jevtovic-Todorovic, "T-type voltage-gated calcium channels as targets for the development of novel pain therapies," *Br. J. Pharmacol.*, **163**, 484–495 (2011).
12. M. M. Jagodic, S. Pathirathna, M. T. Nelson, et al., "Cell-specific alterations of T-type calcium current in painful diabetic neuropathy enhance excitability of sensory neurons," *J. Neurosci.*, **27**, 3305–3316 (2007).
13. S. M. Cain and T. P. Snutch, "Contributions of T-type calcium channel isoforms to neuronal firing," *Channels (Austin)*, **4**, 475–482 (2010).
14. Z. Huang, R. Lujan, I. Kadurin, et al., "Presynaptic HCN1 channels regulate Cav3.2 activity and neurotransmission at select cortical synapses," *Nat. Neurosci.*, **14**, 478–486 (2011).
15. A.-H. Tang, M. A. Karson, D. A. Nagode, et al., "Nerve terminal nicotinic acetylcholine receptors initiate quantal GABA release from perisomatic interneurons by activating axonal T-type (Cav3) Ca^{2+} channels and Ca^{2+} release from stores," *J. Neurosci.*, **31**, 13546–13561 (2011).
16. S. M. Todorovic and V. Jevtovic-Todorovic, "Neuropathic pain: role for presynaptic T-type channels in nociceptive signaling," *Pflugers Arch.*, **465**, 921–927 (2013).
17. S. M. Todorovic and V. Jevtovic-Todorovic, "Targeting of CaV3.2 T-type calcium channels in peripheral sensory neurons for the treatment of painful diabetic neuropathy," *Pflugers Arch.*, **466**, 701–706 (2014).
18. A. François, S. Laffray, A. Pizzoccaro, et al., "T-type calcium channels in chronic pain: mouse models and specific blockers," *Pflugers Arch.*, **466**, 707–717 (2014).
19. A. François, N. Schüetter, S. Laffray, et al., "The low-threshold calcium channel Cav3.2 determines low-threshold mechanoreceptor function," *Cell Rep.*, **10**, 370–382 (2015).
20. S. Choi, H. S. Na, J. Kim, et al., "Attenuated pain responses in mice lacking Ca(V)3.2 T-type channels," *Genes. Brain Behav.*, **6**, 425–431 (2007).
21. E. Bourinet, A. Alloui, A. Monteil, et al., "Silencing of the Cav3.2 T-type calcium channel gene in sensory neurons demonstrates its major role in nociception," *EMBO J.*, **24**, 315–324 (2005).
22. R. B. Messinger, A. K. Naik, M. M. Jagodic, et al., "*In vivo* silencing of the Ca(V)3.2 T-type calcium channels in sensory neurons alleviates hyperalgesia in rats with streptozocin-induced diabetic neuropathy," *Pain*, **145**, 184–195 (2009).
23. F. Sekiguchi and A. Kawabata, "T-type calcium channels: functional regulation and implication in pain signaling," *J. Pharmacol. Sci.*, **122**, 244–250 (2013).
24. A. L. Obradovic, S. M. Hwang, J. Scarpa, et al., "CaV3.2 T-type calcium channels in peripheral sensory neurons are important for mibefradil-induced reversal of hyperalgesia and allodynia in rats with painful diabetic neuropathy," *PLoS One*, **9**, e91467 (2014).
25. J. R. Latham, S. Pathirathna, M. M. Jagodic, et al.," Selective T-type calcium channel blockade alleviates hyperalgesia in ob/ob mice," *Diabetes*, **58**, 2656–2665 (2009).
26. M. Chevalier, P. Lory, C. Mironneau, et al., "T-type Cav3.3 calcium channels produce spontaneous low-threshold action potentials and intracellular calcium oscillations," *Eur. J. Neurosci.*, **23**, 2321–2329 (2006).
27. M. Candelas, A. Reynders, M. Arango-Lievano, et al., "Cav3.2 T-type calcium channels shape electrical firing in mouse Lamina II neurons," *Sci. Rep.*, **9**, 1–18 (2019).
28. M. M. Jagodic, S. Pathirathna, P. M. Joksovic, et al., "Upregulation of the T-type calcium current in small rat sensory neurons after chronic constrictive injury of the sciatic nerve," *J. Neurophysiol.*, **99**, 3151–3156 (2008).
29. X.-H. Cao, H. S. Byun, S.-R. Chen, et al., "Diabetic neuropathy enhances voltage-activated Ca^{2+} channel activity and its control by M4 muscarinic receptors in primary sensory neurons," *J. Neurochem.* **119**, 594–603 (2011).
30. S. A Shankarappa, E. S. Piedras-Rentería, and E. B. Stubbs, "Forced-exercise delays neuropathic pain in experimental diabetes: effects on voltage-activated calcium channels," *J. Neurochem.*, **118**, 224–236 (2011).
31. E. V Khomula, V. Y. Viatchenko-Karpinski, A. L. Borisyuk, et al., "Specific functioning of Cav3.2 T-type calcium and TRPV1 channels under different types of STZ-diabetic neuropathy," *Biochim. Biophys. Acta*, **1832**, 636–649 (2013).
32. E. V. Khomula, A. L. Borisyuk, V. Y. Viatchenko-Karpinski, et al., "Nociceptive neurons differentially express fast and slow T-type Ca(2+) currents in different types of diabetic neuropathy," *Neural Plast.*, **2014**, 938235 (2014).
33. P. Orestes, H. P. Osuru, W. E. McIntire, et al., "Reversal of neuropathic pain in diabetes by targeting glycosylation of Cav3.2 T-type calcium channels," *Diabetes*, **62**, 3828–3838 (2013).
34. N. Weiss, S. A. G. Black, C. Bladen, et al., "Surface expression and function of Cav3.2 T-type calcium channels are controlled by asparagine-linked glycosylation," *Pflugers Arch.*, **465**, 1159–1170 (2013).
35. S. Pathirathna, S. M. Todorovic, D. F. Covey, et al., "5alpha-reduced neuroactive steroids alleviate thermal and mechanical hyperalgesia in rats with neuropathic pain," *Pain*, **117**, 326–339 (2005).
36. M. T. Nelson, P. M. Joksovic, E. Perez-Reyes, et al., "The endogenous redox agent L-cysteine induces T-type Ca^{2+} channel-dependent sensitization of a novel subpopulation of rat peripheral nociceptors," *J. Neurosci.*, **25**, 8766–8775 (2005).
37. Y. Zhang, W. Qin, Z. Qian, et al., "Peripheral pain is enhanced by insulin-like growth factor 1 through a G protein-mediated stimulation of T-type calcium channels," *Sci. Signal.*, **7**, ra94 (2014).

38. A. García-Caballero, V. M. Gadotti, P. Stemkowski, et al., "The deubiquitinating enzyme USP5 modulates neuropathic and inflammatory pain by enhancing Cav3.2 channel activity," *Neuron*, **83**, 1144–1158 (2014).
39. V. Carabelli, A. Marcantoni, V. Comunanza, et al., "Chronic hypoxia up-regulates alpha1H T-type channels and low-threshold catecholamine secretion in rat chromaffin cells," *J. Physiol.* **584**, 149–165 (2007).
40. V. Carabelli, A. Marcantoni, V. Comunanza, et al., "Fast exocytosis mediated by T- and L-type channels in chromaffin cells : distinct voltage-dependence but similar Ca^{2+}-dependence," *Eur. Biophys. J.*, **36**, 753–762 (2007).
41. E. Carbone, A. Giancippoli, A. Marcantoni, et al., "A new role for T-type channels in fast 'low-threshold' exocytosis," *Cell Calcium*, **40**, 147–154 (2006).
42. S. Mahapatra, C. Calorio, D. H. F. Vandael, et al., "Calcium channel types contributing to chromaffin cell excitability, exocytosis and endocytosis," *Cell. Calcium*, **51**, 321–30 (2012).
43. E. Carbone, A. Marcantoni, A. Giancippoli, et al., "T-type channels-secretion coupling: evidence for a fast low-threshold exocytosis," *Pflugers Arch.*, **453**, 373–383 (2006).
44. J. L. Weiss, H. Hui and R. D. Burgoyne, "Neuronal calcium sensor-1 regulation of calcium channels, secretion, and neuronal outgrowth," *Cell. Mol. Neurobiol.* **30**, 1283–1292 (2010).
45. N. Weiss and G. W. Zamponi, "Control of low-threshold exocytosis by T-type calcium channels," *Biochim. Biophys. Acta*, **1828**, 1579–1586 (2013).
46. N. Weiss, S. Hameed, J. M. Fernández-Fernández, et al., "A Ca(v)3.2/syntaxin-1A signaling complex controls T-type channel activity and low-threshold exocytosis," *J. Biol. Chem.*, **287**, 2810–2818 (2012).
47. N. Weiss, G. W. Zamponi and M. De Waard, "How do T-type calcium channels control low-threshold exocytosis?," *Commun. Integr. Biol.*, **5**, 377–380 (2012).
48. J. Lazniewska, Y. Rzhepetskyy, F. X. Zhang, et al., "Cooperative roles of glucose and asparagine-linked glycosylation in T-type calcium channel expression," *Pflugers Arch. Eur. J. Physiol.*, **468**, 1837–1851 (2016).
49. K. Ondacova, M. Karmazinova, J. Lazniewska, et al., "Modulation of Cav3.2 T-type calcium channel permeability by asparagine-linked glycosylation," *Channels*, **10**, 175–184 (2016).
50. J. Lazniewska and N. Weiss, "Glycosylation of voltage-gated calcium channels in health and disease," *Biochim. Biophys. Acta Biomembr.*, **1859**, 662–668 (2017).
51. R. Del Toro, K. L. Levitsky, J. López-Barneo, et al., "Induction of T-type calcium channel gene expression by chronic hypoxia," *J. Biol. Chem.*, **278**, 22316–22324 (2003).
52. K. M. J. Van Loo, C. Schaub, K. Pernhorst, et al., "Transcriptional regulation of T-type calcium channel CaV3.2: 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).
53. J. Mamet, M. Klukinov, T. L. Yaksh, et al., "Single intrathecal administration of the transcription factor decoy AYX1 prevents acute and chronic pain after incisional, inflammatory, or neuropathic injury," *Pain*, **155**, 322–333 (2014).
54. D. Huang, C. Liang, F. Zhang, et al., "Inflammatory mediator bradykinin increases population of sensory neurons expressing functional T-type Ca^{2+} channels," *Biochem. Biophys. Res. Commun.*, **473**, 396–402 (2016).
55. P. M. Joksovic, M. T. Nelson, V. Jevtovic-Todorovic, et al., "CaV3.2 is the major molecular substrate for redox regulation of T-type Ca^{2+} channels in the rat and mouse thalamus," *J. Physiol.*, **574**, 415–430 (2006).
56. M. T. Nelson, P. M. Joksovic, P. Su, et al., "Molecular mechanisms of subtype-specific inhibition of neuronal T-type calcium channels by ascorbate," *J. Neurosci.*, **27**, 12577–12583 (2007).
57. A. Verkhratsky and P. Fernyhough, "Mitochondrial malfunction and Ca^{2+} dyshomeostasis drive neuronal pathology in diabetes," *Cell Calcium*, **44**, 112–22 (2008).
58. J. T. Wolfe, H. Wang, E. Perez-Reyes, et al., "Stimulation of recombinant Ca(v)3.2, T-type, Ca(2+) channel currents by CaMKIIgamma(C)," *J. Physiol.*, **538**, 343–355 (2002).
59. J. Chemin, A. Mezghrani, I. Bidaud, et al., "Temperature-dependent modulation of CaV3 T-type calcium channels by protein kinases C and A in mammalian cells," *J. Biol. Chem.*, **282**, 32710–32718 (2007).
60. J. Kato, N. Agalave and C. Svensson, "Pattern recognition receptors in chronic pain: Mechanisms and therapeutic implications," *Eur J Pharmacol.*, **788**, 261–273 (2016).
61. H. Li, Y. Luo, Y. Xu, et al., "Meloxicam improves cognitive impairment of diabetic rats through COX2-PGE2-EPs-cAMP / pPKA pathway," *Mol. Pharm.*, **15**, 4121–4131 (2018).
62. S. M. Bestall, R. P. Hulse, Z. Blackley, et al., "Sensory neuronal sensitisation occurs through HMGB-1 – RAGE and TRPV1 in high-glucose conditions," *J. Cell Sci.*, **131**, cs215939 (2018).
63. L. Premkumar and G. Ahern, "Induction of vanilloid receptor channel activity by protein kinase C," *Nature*, **408**, 985–990 (2000).
64. R. Griggs, D. Santos, D. Laird, et al., "Methylglyoxal and a spinal TRPA1-AC1-Epac cascade facilitate pain in the db/db mouse model of type 2 diabetes," *Neurobiol. Dis.*, **127**, 76–86 (2019).
65. T. Federici and N. Boulis, "Gene therapy for peripheral nervous system diseases," *Curr. Gene Ther.*, **7**, 239–248 (2007).
66. A. Francois, N. Kerckhove, M. Meleine, et al., "State-dependent properties of a new T-type calcium channel blocker enhance Ca(V)3.2 selectivity and support analgesic effects," *Pain* **154**, 283–293 (2013).
67. E. M. Talley, L. L. Cribbs, J. H. Lee, et al., "Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels," *J. Neurosci.*, **19**, 1895–1911 (1999).

68. M. G. Kaplitt, A. Feigin, C. Tang, et al., "Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial," *Lancet (London, England)*, **369**, 2097–2105 (2007).
69. C. Janson, S. McPhee, L. Bilaniuk, et al., "Clinical protocol. Gene therapy of Canavan disease: AAV-2 vector for neurosurgical delivery of aspartoacylase gene (ASPA) to the human brain," *Hum. Gene Ther.*, **13**, 1391–1412 (2002).
70. C. Towne, M. Pertin, A. T. Beggah, et al., "Recombinant adeno-associated virus serotype 6 (rAAV2/6)-mediated gene transfer to nociceptive neurons through different routes of delivery," *Mol. Pain*, **5**, 52 (2009).
71. S. M. Iyer, K. L. Montgomery, C. Towne, et al., "Virally mediated optogenetic excitation and inhibition of pain in freely moving nontransgenic mice," *Nat. Biotechnol.*, **32**, 274–278 (2014).
72. Y. Xu, Y. Gu, G.-Y. Xu, et al., "Adeno-associated viral transfer of opioid receptor gene to primary sensory neurons: a strategy to increase opioid antinociception," *Proc. Natl. Acad. Sci. USA*, **100**, 6204–6209 (2003).
73. H. Zheng, C. Qiao, C.-H. Wang, et al., "Efficient retrograde transport of adeno-associated virus type 8 to spinal cord and dorsal root ganglion after vector delivery in muscle," *Hum. Gene Ther.*, **21**, 87–97 (2010).
74. S. W. J. McPhee, C. G. Janson, C. Li, et al., "Immune responses to AAV in a phase I study for Canavan disease," *J. Gene Med.*, **8**, 577–588 (2006).
75. A. S. Beutler, "AAV provides an alternative for gene therapy of the peripheral sensory nervous system," *Mol. Ther.*, **18**, 670–673 (2010).
76. A. M. Tan, O. A. Samad, S. D. Dib-Hajj, et al., "Virus-mediated knockdown of Nav1.3 in dorsal root ganglia of STZ-induced diabetic rats alleviates tactile allodynia," *Mol. Med.*, **21**, 544–552 (2015).