Neuropathic pain
Review

Transient receptor potential vanilloid subtype 1 depletion mediates mechanical allodynia through cellular signal alterations in small-fiber neuropathy

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Abstract
Transient receptor potential vanilloid subtype 1 (TRPV1) is a polymodal nociceptor that monitors noxious thermal sensations. Few studies have addressed the role of TRPV1 in mechanical allodynia in small-fiber neuropathy (SFN) caused by sensory nerve damage. Accordingly, this article reviews the putative mechanisms of TRPV1 depletion that mediates mechanical allodynia in SFN. The intraepidermal nerve fibers (IENFs) degeneration and sensory neuronal injury are the primary characteristics of SFN. Intraepidermal nerve fibers are mainly C-polymodal nociceptors and A δ-fibers, which mediated alldynic pain after neuronal sensitization. TRPV1 depletion by highly potent neurotoxins induces the upregulation of activating transcription factor 3 and IENFs degeneration which mimics SFN. TRPV1 is predominately expressed by the peptidergic than nonpeptidergic nociceptors, and these neurochemical discrepancies provided the basis of the distinct pathways of thermal analgesia and mechanical allodynia. The depletion of peptidergic nociceptors and their IENFs cause thermal analgesia and sensitized nonpeptidergic nociceptors respond to mechanical allodynia. These distinct pathways of noxious stimuli suggested determined by the neurochemical-dependent neurotrophin cognate receptors such as TrkA and Ret receptors. The neurogenic inflammation after TRPV1 depletion also sensitized Ret receptors which results in mechanical allodynia. The activation of spinal TRPV1(+) neurons may contribute to mechanical allodynia. Also, an imbalance in adenosinergic analgesic signaling in sensory neurons such as the downregulation of prostatic acid phosphatase and adenosine A 1 receptors, which colocalized with TRPV1 as a membrane microdomain also correlated with the development of mechanical allodynia. Collectively, TRPV1 depletion–induced mechanical allodynia involves a complicated cascade of cellular signaling alterations.

Keywords: P2X3, TrkA receptor, Ret receptor, Prostatic acid phosphatase, Adenosine A 1 receptor, Calcitonin gene–related protein, Activating transcription factor 3

1. Introduction
The transient receptor potential vanilloid subtype 1 (TRPV1), also known as capsaicin receptor, is encoded in humans by the trpv1 gene. The TRP family of receptors (including several subtypes) and their physiological function are well established in the literature. TRPV1 was first cloned by Caterina et al. and has been a well-known receptor. Transient receptor potential vanilloid subtype 1 acts as a thermoreceptor. Transient receptor potential vanilloid subtype 1 is expressed by small-diameter nociceptors, and TRPV1 depletion has specifically been reported to result in thermal analgesia. The molecular mechanisms of TRPV1-mediated thermal analgesia are related to the cytotoxicity induced by increased calcium permeability and the influx of Ca 2+ into TRPV1(+) nociceptors. Patients with small-fiber neuropathy (SFN) experience various nociceptive sensations such as reduced noxious thermal sensation (thermal analgesia) and mechanical...
hypersensitivity (mechanical allodynia). This suggests that TRPV1 (+) nociceptors regulate both thermal and mechanical nociception in SFN. This review article discusses the molecular mechanisms of TRPV1 associated with mechanical allodynia.

1.1. Roles of transient receptor potential vanilloid subtype 1 in small-fiber neuropathy models

The skin is innervated by intraepidermal nerve fibers (IENFs) which respond to external stimuli, and skin denervation is a common clinicopathological manifestation of SFN.10,18,20,26,35,63,147 Neuroanatomically, these IENFs are mainly C-polymodal nociceptors and Aδ-fibers, which mediated algodysne pain confirmed by neuropharmacological blockade.50,57,93,126 evoked potentials recordings, 57,93,145 and genetic expression.57 Particularly, the C-polymodal nociceptors and TRPV1 (+) neuronal soma in the dorsal root ganglia (DRG).50 The benefit of these animal models is that they allow the simultaneous anatomical, these IENFs are mainly C-polymodal nociceptors and reinnervation of CGRP ( (+) nociceptors coexpress TRPV1 in the DRG of rodents,53,125 and the reinnervation of CGRP (+) IENFs reverses thermal allodynia after TRPV1 deletion.51,53 which may due to TRPV1 deletion after the expression of neuronal phenotypes and neurophysiological functions.151,152 By contrast, denervation of substance P (+) IENFs reportedly results in a loss of thermal response ability because of the low density of IENFs and high colocalization (40%–60%) with TRPV1.53,125 These peptidergic neuronal soma exhibit the same pathology as their peripheral IENFs.51 In addition to thermal transmission, the depletion of these peptidergic neurons after TRPV1 depletion improved periodontitis,13 arthritis,41 and orthodontic force–derived mechanical irritation157 by altered inflammatory response. Collectively, the elimination of peptidergic neurons after TRPV1 deletion may alter systemic neurophysiological function which involved inflammatory responses.

Pathological evidence indicates that the ratio of TRPV1 colocalized with different phenotypic nociceptors is a critical factor for different neuropathic behaviors. For example, TRPV1 has limited expression by nonpeptidergic (also called purinergic) P2X3 nociceptors, and specifically, P2X3(+) nociceptors sensitized by highly potent neurotoxins respond to mechanical allodynia15,62 because of the burst release of adenosine triphosphate (ATP) from denervated skin and injured DRG tissues.50 The other desensitized nonpeptidergic nociceptors that coexpress with TRPV1 also exhibit similar neuropathic behaviors. For example, the downregulation of the prostastic acid phosphatase (PAP), which is highly coexpressed with P2X3(+) nociceptors (up to 87%) and approximately 50% coexpressed with TRPV1, correlates with the development of mechanical allodynia.65,162 In addition to TRPV1 (+) neuronal soma and their peripheral IENFs correlated to the noxious stimulation, presynaptic TRPV1 (+) central terminals on the spinal cord suggested modulated the postsynaptic current activities.55,105 This neurophysiological modulation by TRPV1 is further confirmed by c-fos activation on postsynaptic spinal interneurons after TRPV1 activation at the periphery.46,119 The expression of TRPV1 (+) central terminals and interneurons showed their neurophysiological plasticity that suggested commonly contribute to painful sensation, which was regulated by the pathways from the ventromedial medulla and thalamus in brainstem and diencephalon, respectively, to spinal TRPV1 (+) interneurons.22 Particularly, TRPV1 activation expressed by spinal GABAergic interneurons mediated mechanical allodynia by the disinhibition of long-term depression in the spinothalamic tract.75 Collectively, topographical TRPV1 expression played an important role in plastic neuronal activity for pain transmission.

Notably, the neurophysiological functions of small-diameter nociceptors depend on the regulation of neurotrophin signals; the reinnervation of CGRP ( +) peptide IENFs normalizes the thermal noxious sensation that underlies the activation of NGF-TrkA signaling.48,52,53 TrkA is the high-affinity receptor of NGF that regulates the neurophysiological functions of peptidergic nociceptors. NGF-TrkA signaling also regulates the neurophysiological functions of nonpeptidergic PAP (+) nociceptors through high PAP/TrkA colocalization ratios (approximately 70%).162 However, 2 studies have reported that the activation...
of NGF-TrkA signaling has no effect on TRPV1 recovery, despite TRPV1/TrkA colocalization ratios of approximately 30%–50%.\textsuperscript{52,79} Furthermore, NGF has been found to normalize neuropathic manifestations through distinct neurotrophin receptor–dependent pathways.\textsuperscript{52} For example, NGF has paradoxical effects, such as upregulation of TrkA, the peptidergic nociceptor–dependent neurotrophin receptor, and down-regulation of the nonpeptidergic nociceptor–dependent Ret receptor.\textsuperscript{52} Evidence from pharmacological interventions indicates that the activation of Ret receptors mediates both thermal and mechanical noxious sensations and that TrkA receptors have no effect on mechanosensations. Notably, the expression of different neurotrophin cognate receptors such as activating transcription factor 3 (ATF3) is associated with intranuclear signaling, suggesting that these injury-dependent molecules expressed by different neurotrophin cognate receptors are critical factors in the development of neuropathic pain.

The different phenotypes of small-diameter nociceptors have a wide spectrum of profiles colocalized with TRPV1 that exhibit distinct neurophysiological functions, such as the induction of both thermal analgesia and mechanical allodynia after TRPV1 depletion, which is mediated by 2 distinct noxious pathways regulated by specific neurotrophins and their cognate receptors.\textsuperscript{52}

1.3. Correlation of neuronal soma injury after transient receptor potential vanilloid subtype 1 depletion with neurotrophic receptor expression and pain development

Transient receptor potential vanilloid subtype 1 depletion and the degeneration of IENFs cause a cascade of responses in the neuronal soma. For example, ATF3 is a member of the ATF/CREB transcription factor superfamily.\textsuperscript{46} Activating transcription factor 3 (ATF3) is associated with intranuclear signaling, suggesting that these injury-dependent molecules expressed by different neurotrophin cognate receptors are critical factors in the development of neuropathic pain.\textsuperscript{52}

Recently, comprehensive studies have demonstrated increased ATF3 upregulation in DRG neurons following various nerve injuries and exposure to noxious stimuli,\textsuperscript{12,25,32,41,112,127,156,161} ATF3 upregulation by small-diameter nociceptors is specific and differential, and the phenotypic profiles of ATF3 provide molecular explanations for manifested behaviors. For example, ATF3 has been reported to be preferentially upregulated by small-diameter nociceptors, suggesting the susceptibility and topographical relationship of ATF3 to neuropathic pain.\textsuperscript{13,50,52,162} Although ATF3 is preferentially expressed by small-diameter nociceptors after TRPV1 depletion, this ATF3 upregulation is distinct. For example, ATF3 was reported to be predominantly expressed by nonpeptidergic nociceptors such as P2X3 (+) and PAP (+) nociceptors rather than by CGRP (+) peptidergic nociceptors.\textsuperscript{50,162} In addition, ATF3 upregulation was linked to skin denervation and phenotypic changes in nonpeptidergic nociceptors. Another study reported opposite results; ATF3 was reported to be predominantly expressed by small-diameter nociceptors, suggesting the susceptibility and topographical relationship of ATF3 to neuropathic pain.\textsuperscript{13,50,52,162} Although ATF3 is preferentially expressed by small-diameter nociceptors after TRPV1 depletion, this ATF3 upregulation is distinct. For example, ATF3 was reported to be predominantly expressed by nonpeptidergic nociceptors such as P2X3 (+) and PAP (+) nociceptors rather than by CGRP (+) peptidergic nociceptors.\textsuperscript{50,162} In addition, ATF3 upregulation was linked to skin denervation and phenotypic changes in nonpeptidergic nociceptors. Another study reported opposite results; ATF3 was predominantly expressed by peptidergic CGRP (+) nociceptors rather than isolecitin B4 (+) nonpeptidergic neurons.\textsuperscript{108}

Transient receptor potential vanilloid subtype 1 depletion by neurotoxicity is a comprehensive neuropathological effect of the skin denervation response to transcription factors in neuronal soma, suggesting that ATF3 activity as a pain indicator directly reflects nerve injury and that expression by nonpeptidergic nociceptors is necessary for pain development. However, ATF3 has also been documented to act as a simplified marker for injury rather than a pain marker.\textsuperscript{134} Furthermore, extracellular signaling is coordinated with intracellular ATF3 responses. The enhancement of extracellular purinergic signaling occurs in parallel with TRPV1 depletion and the burst release of ATP from injured DRG tissues.\textsuperscript{50} Notably, extracellular ATP also acts as a gliotransmitter to mediate gial activation in pain development\textsuperscript{90,98} through communication with microglia and astrocytes and neuronal interactions, which are also factors affecting pain development.\textsuperscript{50} Thus, the burst release of ATP from injured tissues is also associated with the neuroinflammation that mediates neuropathic manifestations.

Intracellular signal cascades of ATF3 involve activation of the c-Jun/c-Jun N-terminal kinase (JNK) signal pathway during axonal transportation activity, which may mediate neuronal stress signals.\textsuperscript{31} In addition to ATF3, activating transcription factor 2 (ATF2) acts as a responding molecule in pathological manifestations.\textsuperscript{158} It is activated by JNK in diabetic neuropathy.\textsuperscript{39} Although few studies have reported an association between ATF2 expression and TRPV1 expression,\textsuperscript{90} ATF2 is a potential upstream molecule that regulates TRPV1–mediated inflammatory pain.

1.4. Transient receptor potential vanilloid subtype 1 depletion results in neuropathoinflammatory pain

Skin denervation and neuronal soma injury are typical neuropathological characteristics of SFN. They result in neurogenic inflammation, also referred to as neuroinflammation, which is associated with neuropathic behavior. Tumor necrosis factor-α (TNFα) is a major pleiotropic cytokine that mediates neuro-inflammation through activation of the TNF receptor 1 (TNFR1) ligand receptor.\textsuperscript{143,163} TNFα and TRPV1 are considered 2 critical mediators of inflammatory pain, suggesting that functional TRPV1 is required for the development of inflammatory pain mediated by TNFα.\textsuperscript{120} Blocking TNFα/TNFR1 signaling is a new therapeutic strategy for inflammatory diseases and is particularly effective in alleviating injury-induced neuropathic pain.\textsuperscript{23,28,31,110} Notably, one study reported that Ret receptor–mediated nociceptive behavior was reversed by TNFR1 loss,\textsuperscript{158} suggesting that the interaction of TNFα with nociceptors rather than with TRPV1 is a more critical factor for the development of neuropathic behavior.\textsuperscript{96} However, colocalized studies of TNFR1 (+)/TRPV1 (+) neurons have demonstrated that TNFR1 may play a silent role in neuropathic behavior after TRPV1 depletion by the highly potent neurotoxin RTX. Instead, TNFα sensitizes Ret (+) neurons, which mediate mechanical allodynia after TRPV1 depletion by neurotoxins; for example, RTX induces a burst of TNFα that further sensitizes and upregulates Ret (+) neurons in addition to TRPV1.\textsuperscript{92} Moreover, TNFα–deficient mice have been found to exhibit fewer nonpeptidergic Ret (+) neurons, suggesting that Ret/TNFα signaling is required in neurotoxin–induced neuropathy, which is mediated by a TRPV1–independent pathway. One study also demonstrated that TNF receptor 2 (TNFR2), another receptor of TNFα, is a major responding effector that mediates TRPV1–dependent inflammatory pain.\textsuperscript{24} The collective evidence indicates that TNFα initiates upstream signaling and that, in concert with its cognate receptors, has a broad spectrum of roles in the development of neuropathic pain.

Interleukin-6 (IL-6) and interleukin-1β (IL-1β) belong to another category of cytokines involved in neuroinflammation. Reduction of IL-6 and IL-1β may relieve neuropathic pain through attenuated TRPV1 expression.\textsuperscript{3,5,86} In several neuropathic models, the parallel expression of TRPV1 and cytokines was correlated with pain development.\textsuperscript{3,28,95,96,109,139,144} These findings suggest...
that proinflammatory cytokine–mediated pain sensation requires TRPV1 activation.

TNFα neutralization is currently used for clinical treatment of autoimmune diseases,\(^{85}\) and TNFα-inhibitory drugs are also used to inhibit glial cell activation in neuropathic pain treatment.\(^{100}\) Neuroinflammation induces neuropathic behavior through TNFα-mediated activation of glial cells.\(^{7,135}\) TNFα sensitizes Ret (+) neurons, which mediate neuropathic behavior through an alternative pathway. Generally, glial cell line–derived neurotrophic factor (GDNF) is used as an analgesic agent that acts as a ligand to the Ret receptor, regulating its function and morphology.\(^{43,98,133}\) An NGF inducer, 4-methylcatechol (4-MC), acts as a ligand to the Ret receptor, regulating its function and morphology.\(^{43,98,133}\) An NGF inducer, 4-methylcatechol (4-MC),\(^{63,76,121}\) has been demonstrated to suppress neuropathic behavior and enhance neuroregeneration.\(^{51,54,63}\) However, NGF also induced neurogenic inflammation followed by mechanonociceptive behavior, which mediated by unsilencing nicotinic acetylcholine receptor subunit α-3 (CHRNA3) (+) peptidergic nociceptors\(^{124}\) and activated previous silent nociceptors.\(^{44}\) Noteworthy, the elimination of CHRNA3 (+) nociceptor after TRPV1 depletion\(^{72}\) sparing NGF-induced neurogenic inflammation.

Studies have also shown that 4-MC induces GDNF synthesis\(^{54}\) and normalizes the upregulation of Ret (+) neurons and neuropathic behavior.\(^{52}\) The normalization of TNFα and Ret receptors could be targeted in the development of a pharmacological intervention that could provide a new therapeutic direction in the treatment of SFN beyond glial cell activation. The signaling interactions between TNFα and Ret require further investigation.

### 1.5. Role of microdomains in transient receptor potential vanilloid subtype 1 signaling transduction

The cell membrane microdomain is a microstructure whose structural integrity is essential in functional physiology. The structural alteration of microdomains is correlated with neuronal pathogenesis, such as that of peripheral pathology\(^{24,84,148}\) and inhibits endocannabinoid-mediated analgesic systems.\(^{128}\) Microdomains have a complex composition of lipid derivatives\(^{132}\) and have received increasing attention for their relation to nociceptive modulation.\(^{18,37,82,86,132,136}\) Microdomains are also involved in the modulation of nociceptive development.\(^{96,132,150}\) Growing evidence suggests that TRPV1 is a microdomain component that modulates nociceptive transmission, particularly through interaction with nociceptive molecules.\(^{11,96,131,149}\) Microdomain signaling requires the hydrolysis of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2],\(^{149}\) a process also involved in PAP-mediated antinociception through the prevention of TRPV1-sensitized noxious sensations.\(^{55,149}\) Molecular intervention between TRPV1 and PAP involves a PI (4,5) P2 signal. TRPV1 depletion is associated with PAP downregulation, which results in higher PI (4,5) P2 availability, which acts as an agonist of TRPV1 to modulate TRPV1 activity.\(^{4,123}\) PI (4,5) P2 metabolism is also involved in inhibiting the mechanosensitive receptors modulated by TRPV1 activation.\(^{5}\) Furthermore, PAP downregulation is associated with an imbalance in analgesic adenosinergic signaling.\(^{65,162}\) For example, adenosine receptors mediate cellular analgesia through adenosine ligand–activated adenosine receptors.\(^{9,138,164}\) In particular, adenosine A1 receptor (A1R) activation plays a key role in SFN.\(^{55,67,190}\) Downregulation of PAP (+) neurons reduces their ectonucleotidase activity, which in turn reduces the hydrolysis of AMP to adenosine, resulting in the inhibition of cellular adenosinergic.\(^{55}\)

One study found that microdomain disruption by cholesterol depletion with methyl-β-cyclodextrin (MβC) preserves PAP-mediated antinociception through PI (4,5) P2 hydrolysis,\(^{90}\) indicating that TRPV1 and PAP are located in cholesterol-rich microdomains. These findings suggest that intracellular signal alterations also contribute to pain modulation. However, another study demonstrated that TRPV1 depletion had paradoxical

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Figure 1. Mechanism of thermal analgesia and mechanical allodynia induced by transient receptor potential vanilloid subtype 1 (TRPV1) depletion in small-fiber neuropathy. TRPV1 receptors were depleted by highly selective neurotoxins such as resiniferatoxin (1) and induced thermal analgesia and mechanical allodynia through 2 distinct pathways. The depletion of TrkA receptors, which were expressed by peptidergic calcitonin gene–related peptides and substance P (+) neurons (2), resulted in thermal analgesia (3). By contrast, nonpeptidergic TRPV1 (+) neurons upregulated activating transcription factor 3 (ATF3) expression, which reflected underlying injury of neuronal soma (4), resulting in a burst of TNFα (5). TNFα had no effect on TNFR1 because it was depleted as a result of its high degree of colocalization with TRPV1 (6) but sensitized Ret receptors (7), leading to the development of mechanical allodynia (8). TRPV1 colocalized with prostaglandin E2 phosphatase and A1R within the same cell membrane microdomain. TRPV1 depletion caused an imbalance in analgesic adenosinergic signaling that induced the downregulation of prostaglandin E2 phosphatase (9), which resulted in reduced hydrolysis of adenosine by AMP (10). This in turn reduced the capacity of A1R-mediated cellular analgesia (11). Structural disruption of microdomains by cholesterol depletion is associated with reduced PI (4,5) P2 hydrolysis (12), which leads to mechanical allodynia (13).
effects on nociceptive transmission; for example, TRPV1 depletion from microdomains induced neuropathic pain. Microdomains labelled by flotillin 1 (FLOT1) and flotillin 2 (FLOT2) as well as microdomains within sensory neurons have an abundance of FLOT1 and FLOT2. A1R also coexpresses with FLOT1 and FLOT2, and these FLOT1 (+) and FLOT2 (+) neurons are depleted after TRPV1 depletion associated with the development of mechanical allodynia. These findings suggest that microdomains containing cellular analgesic molecules on sensory neurons act as functional units for pain transmission.

In addition, microdomains contain nociceptive receptors such as P2X3, and P2X3 may act as the downstream molecule of PI (4,5) P2. Moreover, PI (4,5) P2 modulation of P2X3 may occur through autocrine signaling because of the high colocalization of PAP and P2X3 in DRG neurons. These collective findings indicate that TRPV1 depletion initiates a cascade of signaling alterations caused by the neuronal injury response to degeneration of peripheral IENFs after neurotoxin-induced SFN.

1.6. Clinical implications for pain management of lipid derivatives of microdomains

Most SFN studies have focused on investigating the responses and contributions of sensitized small-diameter nociceptors after those nociceptors suffer injury and irritation. Microdomains act as functional units of nociceptive transmission and could contribute to the development of new first-line pharmacotherapeutic treatments. Microdomains are composed of cholesterol, sphingomyelin, and gangliosides, and studies have demonstrated that the TRPV1 activity is affected by altered ganglioside synthesis and sphingomyelin inhibition. Spingomyelin signaling modulates nociception through the activation of p75 neurotrophin receptors, and NGF regulates TrkA receptor activation through alteration of these lipid derivatives. In addition, some G protein–coupled receptors are intracellular components of microdomains and are involved in nociceptive development. The elimination of lipid metabolic constituents is a potential target for the pain management of lipid metabolism disorders related to microdomain–attributed peripheral neuropathy. Therefore, targeting the cytoplasm membranes surrounding microdomains could constitute a new therapeutic direction. Multiple doses of drugs may be required for the depletion of lipid derivatives because of the dynamic replenishment of cholesterol from intracellular stores.

2. Conclusions

Transient receptor potential vanilloid subtype 1 acts as a polymodal nociceptor that responds to different noxious stimuli. Commercial capsaicin dermal patch (Qutenza) with 8% (wt/wt) capsaicin has high efficacy for treatment SFN such as diabetic peripheral neuropathy. Local analgesia by a high concentration of capsaicin because of axonal degeneration by cytoskeleton disassembling and mitochondrial fission, which showed “defunctionalization” of nociceptor peripheral fibers including the transient loss of membrane potential, inability to axonal transport of neurotrophic factors, and reduction of IENFs. Noteworthy, administration routes determined the survival of TRPV1 (+) neuronal soma and nociceptive-related neuropeptides expression at different nervous tissues. It believed TRPV1 agonist administration through different routes had distinct effects on neurophysiological responses. This article reviews the role of TRPV1 in SFN. In particular, it discusses the depletion of TRPV1 by highly potent neurotoxins such as RTX, which induces a cascade of extracellular and intracellular signaling alterations caused by peripheral skin denervation and injury to central sensory neurons. Figure 1 summarizes the putative mechanisms of neuropathic manifestations induced by TRPV1 depletion.

Disclosures

The authors have no conflicts of interest to declare.

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