Chapter

Peripheral Sensitization

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Abstract

Peripheral sensitization indicates increased responsiveness and reduced threshold of nociceptive neurons in the periphery to the stimulation, which usually occurs after peripheral tissue injury and inflammation. As an integral part of pain, peripheral sensitization and its mechanisms have received much attention, and numerous types of neurotransmitters and chemicals related to peripheral sensitization were investigated. We developed an animal model of peripheral sensitization, and it provides evidence that some neurotransmitters, such as glutamate and substance P, release from adjacent peripheral nerves contributing to the peripheral sensitization of pathological pain. In this chapter, we reviewed the advances in peripheral sensitization, and it will provide a basis for new targets to attenuate pain of peripheral origin.

Keywords: pain, peripheral sensitization, tissue injury, inflammation, neurotransmitter, ion channel

1. Introduction

Peripheral sensitization refers to reduced threshold and augmented response of the sensory nerve fibers in the peripheral to external stimulus, which is manifested as enhanced stimulus-dependent pain called primary hyperalgesia [1]. Commonly, peripheral sensitization occurs following peripheral nerve injury, tissue injury, and inflammation. Tissue injury may accompany the injury of peripheral nerve endings to some content. The endogenous chemicals released from the site of tissue injury or inflammation can activate and sensitize the peripheral sensory neurons, resulting in peripheral sensitization [2, 3]. Similar sensitization phenomenon taking place in the central nervous system is called central sensitization, which may be initially induced by peripheral sensitization [4, 5]. The peripheral sensitization and central sensitization together produce neuropathic pain and inflammatory pain reflected as allodynia and hyperalgesia. However, there is no satisfactory therapy for the management of allodynia and hyperalgesia.

Peripheral sensitization increases the release of the neurotransmitters from the peripheral endings and the terminals of the spinal cord, aggravating the neurogenic inflammation and nociception. Pain usually starts with the activation of peripheral sensory neurons which subsequently process and convey nociceptive message to spinal cord and brain regions. That is, to some extent, the inhibition of peripheral sensitization may prevent the subsequent central events. For the pain management, local drug delivery can focus on the specific peripheral mechanisms including transduction and transmission of nociceptive signaling to limit both peripheral and central sensitization processes [6]. These facts increase the necessity to investigate the exclusive mechanisms of peripheral sensitization. Thus, in this chapter, we

focus on the advances in peripheral sensitization, and it may contribute to the improvements of new therapies relieving pain of peripheral origin.

2. Peripheral nociceptors

Nociception is a process that different stimuli (thermal, mechanical, and chemical) are detected by the peripheral nerve fibers called nociceptor, through which the noxious stimuli are transduced into action potentials and conducted to the spinal cord and brain [7]. Unlike other sensory modalities that respond to innocuous stimulus such as touch, nociceptors are only activated by noxious stimuli that could be harmful to the organism. The nociceptor consists of three parts: the axon, cell body, and central terminals. The cell bodies of the nociceptors are located in the dorsal root ganglia (DRG) for the body and the trigeminal ganglia (TG) for the maxillofacial region, and they are always connected to the afferent fibers. The site where the terminals of the fibers respond to peripheral stimuli is known as the receptive field. According to the type of the afferent fibers, the nociceptor can be divided into myelinated Aδ fibers and unmyelinated C fibers. Many of the unmyelinated fibers respond to a wide range of noxious stimuli [8]. Nociceptors can send and receive the messages from both the central and peripheral terminals [5, 7]. Following injury and inflammation, the nociceptors may become sensitized by pro-nociceptive mediators, such as prostaglandins, bradykinin, substance P (SP), extracellular ATP, and protons [9]. The activation of the nociceptors is related to the site of the stimuli application and stimuli modality including chemical, thermal (hot and cold), and mechanical modalities [8]. Several changes in nociceptors may account for the peripheral sensitization. First, the thresholds of primary afferent Aδ and C fibers lower in response to innoxious stimuli. Second, Aδ and C fibers at the site of tissue injury or inflammation exhibit enhanced responses to supra-threshold mechanical or heat stimuli. Third, adjacent receptive fields of Aδ and C fibers increase innervation to the injured site [10].

3. Challenges in developing effective drugs

Although considerable progress has been made in investigating the role of peripheral sensitization in nociceptive processing during the past decades, pain researches bear burdens in translation from pre-clinical studies to successful clinical intervention. Several reasons may explain why the effective analgesics develop slowly. First, the complicated mechanisms of different patients in distinct pain states and the diversity types and functions of mediators in different pain pathways may be barriers in developing effective therapies [7, 11]. Second, currently available analgesic drugs targeting pain mechanisms produce serious side-effects and unsatisfactory efficacy [2, 11, 12]. As is known to all, the most popular analgesia drug, opioid, is hampered by desirable side-effects such as tolerance, respiratory depression, and addiction [13, 14]. The transient receptor potential vanilloid 1 (TRPV1) antagonists have side-effects such as loss of the noxious heat sensation, increased burn risk, and hyperthermia [15]. Obviously, these challenges drive us to find drugs targeting selectively on modulation of peripheral mechanisms and not crossing the blood-brain-barrier, through which the side-effects may be avoided. To reduce potential systemic side-effects and improve compliance, there is a growing interest in "targeted peripheral analgesics" for further investigation and clinical use [6].

4. Animal model

Several animal models of various pain states have been established to investigate the mechanisms of peripheral sensitization, for example, inflammation pain models, neuropathic pain evoked by disease or damage to peripheral nerves, and post-operative pain models. Animal models of inflammatory pain have used a number of different irritants that are injected into skin, paw, muscle, joint, and visceral organ. Carrageenan, complete Freund's adjuvant (CFA), and capsaicin are commonly used inflammatory irritants to induce hyperalgesia. Common nerve injury models include: (1) ligating or transecting the spinal nerves, such as spinal nerve ligation model (SNL); (2) ligating or lesioning the sciatic nerve, such as chronic constriction injury (CCI); and (3) ligating distal branches (peroneal and tibial) of the sciatic nerve (spared nerve injury) [16].

To better understand the mechanisms of transmission between peripheral sensory nerve endings, we have successfully established an electrophysiological animal model in which antidromic electrical stimulation of a sensory nerve excites the adjacent primary afferents from the different spinal segments [17]. In this model, two adjacent cutaneous branches of spinal dorsal rami in the thoracic segments, T9 and T10, were dissociated and transected proximally from the spinal cord in anesthetized rats. Then antidromic electrical stimulation with 0.5 ms pulse duration, 20 Hz frequency, and 1 mA intensity was applied to T9 spinal nerve branch, and the nerve activities of T10 nerve branch were recorded [18]. All recorded afferent neurons from the T10 cutaneous branch were classified as Aβ, Aδ, and C fibers according to the conduction velocity and the receptive properties [19]. The discharges of the isolated Aβ, Aδ, and C fibers of the T10 cutaneous branch were significantly enhanced by antidromic electrical stimulation of the adjacent T9 spinal nerve branch [18]. It is in line with our previous studies that antidromic stimulation of T9 spinal nerve branch can activate and sensitize Aβ, Aδ, and C fibers obtained from the adjacent T10 dorsal cutaneous branch [20–23]. The activation and sensitization of these fibers not only occur between the peripheral sensory nerve terminals, but also conduct nociceptive impulses to the central nerve system [17, 24]. The increase in neural discharges of the peripheral fibers caused by the electrical stimulation of adjacent cutaneous nerves mimicked the effects of released chemicals at the peripheral nerve endings [25]. On the basis of this model, the effects of antidromic electrical stimulation of spinal cutaneous branches on the discharge activities of remote mechanoreceptive units were observed. It was found that antidromic stimulation of either T8 or T9 dorsal cutaneous branch significantly increased the discharge activities of the remote T12 nerve, and the increasing time after the electrical stimulation was delayed as the distance increased between the stimulated branch and the recorded one [26].

In these experiments, both nerve branches of the adjacent segments were isolated from the central nervous system; the activation of nerve fibers at one segment by antidromic electrical stimulation affects an adjacent segment suggesting that electrical and chemical signals are transmitted from the stimulated nerves to the recorded fibers without any involvement of the central nervous system. Electrical stimulation of the nerve directly induces the release of chemicals; these chemicals and substances through diffusion produce afferent impulses of adjacent nerve endings and also cause release of neurotransmitters at the adjacent peripheral nerve endings, a process known as axon reflex [17, 23, 26, 27].

5. Mechanisms of peripheral sensitization

There are two processes implicated in peripheral sensitization: (1) early post-translational changes in the peripheral terminals of nociceptors, for example, the

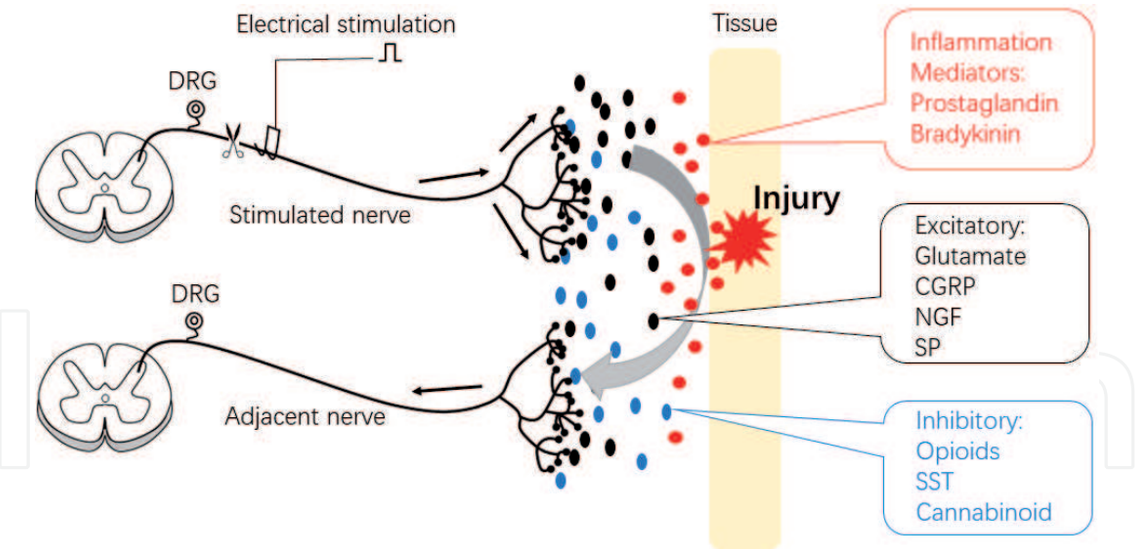

Figure 1.
After tissue injury, chemicals such as inflammatory mediators and neurotransmitters released from the injury site or the nerve endings activate the receptors and channels on the adjacent peripheral nerve terminals, subsequently resulting in peripheral sensitization. In our electrophysiological model, antidromic electrical stimulation of one nerve branch induces the release of neurotransmitters and modulators into the peripheral tissues to mimic tissue injury condition. The released chemicals diffuse to the adjacent peripheral nerve terminals and induce peripheral sensitization. Abbreviations: CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; NGF, nerve growth factor; SP, substance P; SST, somatostatin.

phosphorylation of the ion channels prolongs depolarization and enhances response by lowering the open threshold or prolonging the open time of channels; (2) altered gene expression, changing transcription or translation of certain protein [10, 28]. For instance, deletion or silencing of calcitonin gene-related peptide alpha (αCGRP) gene expression drastically reduces TRPV1 potentiation in peptidergic nociceptors by abrogating its Ca^{2+}-dependent exocytotic recruitment [29].

Peripheral sensitization results from sensitization and excitation of the primary afferent neurons following tissue injury and inflammation. When a peripheral nerve is injured, the distal stump of injured axons undergoes Wallerian degeneration, i.e., breakdown of myelin sheaths, recruitment of inflammatory cells from the circulation, and over-production of growth factors and pro-inflammatory cytokines or mediators. These cytokines and mediators not only promote the regeneration of injured axons but also activate and sensitize nociceptors [30]. During the process of sensitization, the inflammation mediators either bind to different receptors or activate second messenger systems, resulting in the modification of the ion channels [31]. Primary targets of these mediators are ion channels; the activation of either the voltage-gated channel or the ligand-gated channel enhances the number of the action potentials, a process known as sensitization. A wide range of chemicals, such as nerve growth factors (NGF), bradykinin, SP, prostaglandins, opioids, and glutamate, contribute to the peripheral sensitization (**Figure 1**). The following section will address inflammatory mediators, ion channels, and neurotransmitter receptors involved in peripheral sensitization.

6. Inflammatory mediator

Bradykinin and prostaglandin have attracted much attention among inflammatory mediators. The inflammatory mediator prostaglandin E2, released from the inflamed tissue surrounding the terminals of sensory neurons or from endothelial cells after surgical trauma, contributes to the abnormal pain responses in

inflammation pain and neuropathic pain. Peripheral injection of nonselective and selective cyclooxygenase (COX) inhibitors attenuates neuropathic pain following partial sciatic nerve transection [32], indicating that pro-inflammatory prostaglandins are involved in the development of neuropathic pain. Both morphological and pharmacological evidence indicate that peripheral prostaglandins are involved in the maintenance of neuropathic pain following nerve injury [30]. In a study using a thoracic muscle incision model to characterize post-operative pain-related behaviors, tissue prostaglandin E2 increased in surgery animals compared with the sham-operated control animals under the same anesthesia, indicating that prostaglandin E2 is associated with post-operative pain [33]. Prostaglandin also participated in the long-lasting sensitization of nociceptors in acute inflammation induced by carrageenan in mice [34].

Bradykinin is an important neuropeptide released after tissue injury. Upon release, bradykinin affects nociceptive afferents through activation of two pharmacologically distinctive receptors designated B2 and B1, respectively [35, 36]. An increased B1 receptor gene expression was found in peripheral neural tissue in CFA-induced mechanical hyperalgesia, and the selective bradykinin B1 receptor antagonist BI113823 reduced CFA-induced mechanical hyperalgesia [37]. Activation of bradykinin receptors promoted nociceptor sensitization and hyperalgesia by activating the protein kinase C (PKC) second-messenger system [38]. A study on CCI model showed that there was an increase in the mRNA expression of both B1 and B2 receptors in lumbar DRG following CCI. Furthermore, pharmacological antagonists of these receptors alleviate pain hypersensitivity associated with nerve injury [39]. Bradykinin-mediated sensitization of heat responses in C mechanoheat-sensitive fibers of isolated rat skin-saphenous nerve was significantly attenuated by the COX-1 and COX-2 inhibitors [40].

7. Ion channels

The electrical activity of primary afferent neurons is primarily governed by the expression and function of ion channels that define the resting membrane potential, action potential initiation, depolarization and repolarization, refractory period between action potentials, and transmitter release from their terminals in the dorsal horn. In this part, we will review the voltage-gated channel transient receptor potential vanilloid (TRPV), voltage-gated sodium channels, and voltage-gated calcium channels (VGCCs), which may be dysregulated underlying peripheral sensitization.

7.1 The voltage-gated channel transient receptor potential vanilloid

The TRPV is one of the subfamilies of the transient receptor potential (TRP) through which the external stimuli are transduced to electrical impulses. Based on amino acid sequence homology, members of this family in the mammalian have been classified into six subfamilies: TRPA (ankyrin), TRPV (vanilloid), TRPM (melastatin), TRPC (canonical), TRPP (polycystin), and TRPML (mucolipin) [41]. TRP channels are tetramers composed of identical subunits, which have six transmembrane domains and cytoplasmic amino and carboxy termini [42].

7.1.1 TRPV1

TRPV1 is a non-selective cation ion channel which is largely located in small-diameter neurons with C fiber axons [43] in DRG innervating body and TG

innervating oral and maxillofacial regions. Similar to voltage-gated sodium channels, TRPV1 exhibits radial symmetry around a central ion channel which is formed by transmembrane segments 5–6 (S5–S6) and the intervening pore loop and is flanked by S1–S4 domains [42]. TRPV1 is a polymodal receptor which can be activated by a wide range of stimuli including capsaicin [44], other endogenous lipids, acidic pH [43], and noxious heat (>42°C) [44]. TRPV1 upregulation in sensory neurons is a key element in pain development and maintenance of several chronic pathological conditions. Recently, the abundance of the evidence suggests that the TRPV1 receptor is one of the key targets for developing new analgesics.

7.1.2 TRPV1 and pain

It is widely acknowledged that TRPV1 contributes to heat and mechanical sensitization. Injection of capsaicin into the skin in humans produces a burning sensation and flare response, the area of application becomes insensitive to mechanical and thermal stimulation, the area of flare exhibits a primary hyperalgesia to mechanical and thermal stimuli, and an area beyond the flare exhibits secondary allodynia [45, 46]. Through the activation of TRPV1 by the capsaicin and other pungent compounds, burning pain may be produced via depolarizing specific subsets of Aδ and C nociceptors [44]. The TRPV1 population is also required for the development of thermal and mechanical hyperalgesia after CFA injection [47, 48]. Knockout of TRPV1 or pretreatment with the TRPV1 antagonists, AMG9810 or 5′-iodoresiniferatoxin (5′-IRTX), significantly reduced complement C5a-induced mechanical sensitization, indicating that TRPV1 activity is required for maintaining C5a-induced mechanical hypersensitivity [49]. The TRPV1 can assess the physiological environment of the sensory nerve terminal and alter neuronal responsiveness in the context of tissue injury [43]. The mechanical allodynia and thermal hyperalgesia were alleviated in Pirt (a membrane protein which binds to TRPV1 to enhance its activity) knockout mice in CCI models, and the increase in TRPV1 expression was less in Pirt knockout mice in CCI models, suggesting that Pirt together with TRPV1 is involved in CCI-induced neuropathic pain [50].

7.1.3 Activation of the TRPV1

The activation of TRPV1 increases the calcium permeability of the receptor, priming membrane depolarization and subsequent sensory neuron activation [44, 51]. The TRPV1 receptor occupancy triggers Na^+ and Ca^{2+} influx, action potential firing, and the consequent burning sensation associated with spicy food or capsaicin-induced pain [44]. TRPV1 can be sensitized via the second messenger signaling cascade in response to various pro-inflammation mediators and chemicals like bradykinin, lipids, and prostaglandins [44]. A multitude of lipids modulate the TRP-channels through G-protein coupled receptor via different signaling pathways [52]. TRPV1 contributes to the persistence of remifentanil-induced both thermal and mechanical post-operative hyperalgesia through the trafficking of N-methyl-D-aspartate (NMDA) receptors via the activation of calmodulin-dependent protein kinase (CaMKII)-PKC but not protein kinase A (PKA) signaling pathways in DRG neurons [53]. Bradykinin sensitizes TRPV1 through enhancing the excitability of the peptidergic C-type nociceptor end and the neuronal exocytosis of large dense core vesicles containing αCGRP [54]. Tumor necrosis factor-α (TNF-α) can sensitize TRPV1 by promoting its expression, thus leading to mechanical allodynia and thermal hyperalgesia in vincristine-treated rats [55]. NGF causes a long-lasting sensitization of nociceptor endings, in particular to thermal and chemical stimuli, which can be attributed to up-regulated TRPV-1 receptors in sensory endings [56].

The phosphorylation of TRPV1 has been shown to cause sensitization of the channel. The first report of the co-expression pattern of two ligand-gated channels, TRPV1 and P2X3 in TG, demonstrating that pretreatment with αβ-meATP (a selective P2X3 agonist) results in phosphorylation and sensitization of TRPV1, thus contributes to the peripheral sensitization known to underlie masseter hyperalgesia [57]. Activation of the metabotropic glutamate receptor (mGluR) 1/5 leads to phosphorylation of a specific TRPV1 residue via PKC and A-kinase–anchoring protein (AKAP) 150 in trigeminal sensory neurons, and functional interactions between glutamate receptors and TRPV1 mediate mechanical hyperalgesia in the muscle tissue [58]. A recent study found that the temperature sensitivity of TRPV1 channels are enhanced by SUMOylation of TRPV1 protein at a C-terminal Lys residue, indicating that SUMOylation of TRPV1 is essential for the key mechanism underlying peripheral sensitization and the development of inflammatory thermal hyperalgesia [59].

7.1.4 Other TRP receptors

Sensory neurons also express other TRP receptors besides TRPV1. TRPA1 is initiated by noxious cold (17°C), natural oils such as cinnamaldehyde and mustard oil, and inflammatory mediators [60]. TRPA1 is demonstrated to be cold sensitive [61] and plays roles in both cold transduction and mechanotransduction in cutaneous sensory neurons. AKAP 79/150 facilitates phosphorylation and sensitization of TRPA1 in peripheral sensory neurons, resulting in persistent mechanical hypersensitivity [62]. Another study also indicated that TRPA1 activation could co-sensitize TRPV1 channels [60]. Similar to TRPV1, TRPM8 is temperature sensitive and partly expressed by the somatosensory neurons in the DRG and TG. TRPM8 is activated by cool/cold temperature starting in the innocuous range (18–23°C) and cooling compounds such as menthol and icilin [63]. TRPV3 and TRPV4 have also been cloned and are heat sensors. TRPV3 was found to be responsible for detecting innocuous warm temperature ranging from 31 to 39°C. TRPV3 knockout mice had strong deficit in response to innocuous heat sensitivity but not in other sensory modalities [64]. TRPV4, which is expressed in small or medium diameter neurons with overlap in expression with TRPV1 [65], is activated by phorbol ester, innocuous temperature with a threshold higher than 27°C, low pH, citrate, endocannabinoids, arachidonic acid metabolites, and nitric oxide (NO) [66]. The evidence showed that TRPV4 was implicated in the transduction of mechanical stimuli and the development of mechanical hyperalgesia [3].

7.2 The voltage-gated sodium channel

Voltage-gated sodium ion channels are integral membrane proteins comprising a pore-forming α-subunit and two accessory β-subunits [67]. To date, nine isoforms of α subunit voltage-gated sodium channel ($Na_v1.1$–1.9), which display various channel properties and selective tissue distribution, have been discovered. A variety of voltage-gated sodium channels are expressed in somatosensory neurons, including the tetrodotoxin-sensitive (TTX-S) channels $Na_v1.1$, 1.6 and 1.7 and the tetrodotoxin-resistant (TTX-R) channels $Na_v1.8$ and 1.9. Voltage-gated sodium channels are essential for the generation and conductance of action potentials and therefore a crucial factor in neuronal excitability. The functions of voltage-gated sodium ion channels are regulated by their expression level, channel properties, and subcellular distribution. If some drugs block sodium-channels, the conduction of action potentials will be prevented, for instance, the local anesthetics can be used to abolish pain due to blocking sodium channels. Acting as a broad acting sodium

blocker, phenytoin may inhibit overactivities of small fibers and reduce pain in small fiber neuropathic pain and diabetic neuropathic pain [68].

7.2.1 $Na_v1.7$

$Na_v1.7$ is of high interest because it functions as a kind of non-opioid analgesics. $Na_v1.7$, encoded by a sodium channel voltage-gated IX alpha subunit gene (SCN9A), is highly enriched within DRG and TG peripheral sensory neurons, as well as sympathetic neurons and olfactory epithelia [67, 69]. In rodent, $Na_v1.7$ is expressed within the soma of small-diameter DRG neurons and along the peripheral and central C fibers from these cells [70]. However, it was found that human DRG had a high ratio of $Na_v1.7$ expression and low ratio of $Na_v1.8$ expression compared to mouse DRG, indicating that $Na_v1.7$ mRNA predominantly expressed voltage-gated sodium channels in human DRG tissue [71]. Expression of $Na_v1.7$ is also detected in the preterminal central branches and terminals in the dorsal horn, as well as at nodes of Ranvier in a subpopulation of small-diameter myelinated fibers [72].

7.2.2 $Na_v1.7$ and pain

$Na_v1.7$ serves a remarkable function in pain perception. The $Na_v1.7$ knockout animals lose acute noxious mechanical sensation and inflammatory pain [73]. Mice lacking $Na_v1.7$ in sensory neurons showed reduced hypersensitivity to selected neuropathic pain and inflammatory pain models [74]. It has been found that injection of carrageenan increases expression of $Na_v1.3$ and $Na_v1.7$ and TTX-S currents in DRG neurons [75]. Estradiol upregulates TG $Na_v1.7$ mRNA and protein expression, thus inducing sex-differences of nociception in temporomandibular disorders (TMD) and hyperalgesia of the inflamed temporomandibular joint (TMJ) [75]. Besides, $Na_v1.7$ may interact with other signaling systems, such as endogenous opioids which are upregulated in the absence of $Na_v1.7$ and thought to feedback onto DRG neurons and/or terminals to suppress their excitability [76]. The qPCR analysis revealed a significant and dose-dependent increase in $Na_v1.7$ mRNA expression after the treatment of paclitaxel, which is a widely used chemotherapeutic drug that induces neuropathy and neuropathic pain, and the transient Na^+ currents and action potential firing frequency in small-diameter human DRG neurons also increased [71].

7.2.3 SCN9A genes and $Na_v1.7$

Recently, the mechanisms underlying several human pain disorders have been identified to be related to inherited mutations in the sodium channel genes expressed in damage-sensing neurons. The $Na_v1.7$ is encoded by the SCN9A gene. Inherited primary erythromelalgia is resulted from mutations of the SCN9A gene, which causes a significant hyperpolarizing shift in voltage dependence of activation, facilitates channel opening, and increases the amplitude of current produced by $Na_v1.7$. Small fiber neuropathy (SFN) is a typical pain disorder with burning pain throughout the body, in which electrophysiological analysis of $Na_v1.7$ channels showed impaired slow inactivation, depolarized fast and slow inactivation, or enhanced resurgent currents [72]. By contrast, $Na_v1.7$ function mutations in human cause congenital inability to experience pain [77].

7.3 The voltage-gated calcium channel

The VGCCs are a family of membrane proteins which control the influx of calcium ions that trigger neurotransmitter release in response to the depolarization

of the presynaptic cell membrane. Calcium ions can not only alter membrane potential but also serve as important signaling entities [78]. VGCCs are expressed on virtually all excitable cells, and their activity is critical for neurotransmitters release, the regulation of neuronal excitability, and intracellular changes including gene induction [79]. VGCCs are classified into high voltage-activated (HVA) or low voltage-activated (LVA) channels based on their voltage dependence of activation [80]. HVA channels are subdivided further based on their pharmacological and biophysical characteristics into L ($Ca_v1.1$–1.3), N ($Ca_v2.2$), P/Q ($Ca_v2.1$), and R-type ($Ca_v2.3$) [81], and LVA channels are known as T-type channels. HVA channels are complexes of a pore-forming α1 subunit, a transmembrane disulfide-linked complex of α2 and δ subunits, an intracellular β subunit, and in some cases a transmembrane γ subunit [82], while T-type calcium channels are formed by a single α1 subunit [83]. These different subtypes of HVA and LVA channels correspond to ten different α1 subunits, three of which termed Ca_v1, Ca_v2, and Ca_v3 are key determinants of calcium channel subtype [84]. These channels are established and clinically validated drug targets for pain, and their roles and contributions to pain transmission have been extensively reviewed [85].

7.3.1 T-type calcium channels

T-type calcium channel family includes three subtypes, namely, $Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$. T-currents have a unique function in neuronal excitability. In comparison with HVA channels, T-type calcium channels can activate at much more negative membrane potentials, inactivate rapidly, deactivate slowly, have small single-channel conductance, and are insensitive to calcium ion antagonist drugs [82]. The large T-type currents are essential for light touch perception, long-term potentiation of synaptic transmission between nociceptive primary afferents, and superficial laminae SP-sensitive neurons of the dorsal horn [86, 87]. Reverse transcription (RT)–PCR and in situ hybridization analyses have shown that the most abundant T-type channels, $Ca_v3.2$, are expressed in small- and medium-diameter primary afferent neurons as well as neurons from the superficial laminae of the dorsal horn [88]. These $Ca_v3.2$ T-type channels in primary nociceptors are important regulators of afferent fiber excitability and contribute to peripheral sensitization [89].

7.3.2 $Ca_v3.2$ T-type calcium channels

The $Ca_v3.2$ subtype is a particularly attractive analgesic target. An increase in $Ca_V3.2$ T-type currents is associated with decreased nociceptive threshold, whereas inhibition of $Ca_v3.2$ channel activity mediates pain relief [90, 91]. A study indicated that intrathecal injection of $Ca_v3.2$ antisense oligonucleotide but not $Ca_v3.1$ or $Ca_v3.3$ antisense oligonucleotide resulted in about an 80% decrease in T-type calcium currents in DRG neurons, and only $Ca_v3.2$ antisense treatment attenuated nocifensive responses in both naïve and neuropathic pain rats [92]. Several studies validated the potential utility of blocking $Ca_v3.2$ T-type calcium channels to reduce nociception. For example, in a rat model of paclitaxel-induced peripheral neuropathy, T-type current amplitudes and density in DRG neurons were increased at day 7 after paclitaxel treatment and this was prevented by pretreatment of the specific $Ca_v3.2$ T-type calcium channel inhibitor ML218 hydrochloride [93]. Selective inhibition of $Ca_v3.2$ channels reversed hyperexcitability of peripheral nociceptors and alleviated thermal and mechanical hypersensitivity in rodent model of postsurgical pain [94]. However, $Ca_v3.2$ knock-out mice showed reduced sensitivity to noxious pain but not chronic neuropathic pain [95], which contrasts with the potent analgesic actions of intrathecally delivered $Ca_v3.2$ channel blockers in neuropathic pain models,

suggesting that there is compensation from other types of calcium channels in the afferent fibers of $Ca_v3.2$ null mice that maintain pain transmission [96].

7.3.3 Other calcium channels

Primary afferent neurons express multiple types of VGCCs, P-, N-, L-, R-, T-type, and ancillary α2δ calcium channels have been most extensively studied with regard to chronic pain. N-type calcium channels are enriched at presynaptic nerve terminals where they trigger the release of neurotransmitters [97, 98], and inhibiting N-type channel activity results in reduced neurotransmission and thus analgesia [99, 100]. Moreover, $Ca_v2.2$ channel knock-out mice decreased pain responses in neuropathic and inflammatory pain [101–104]. Besides N-type channels, blockade of L-type and P/Q-type can also prevent and/or attenuate subjective pain as well as primary and/or secondary hyperalgesia and allodynia in a variety of experimental and clinical conditions [105].

The Ca_v α2δ subunit is an important accessory subunit for all HVA calcium channels, and numerous studies point to an important role of α2δ in neuropathic pain. The α2δ-1 mRNA and protein levels are dramatically up-regulated in DRG in several models of neuropathic pain [79], and this increase in α2δ-1 correlates with the onset of allodynia [106, 107]. In clinical practice, the Ca_vα2δ subunit is the key pharmacological target for gabapentinoids (highly effective in the treatment of neuropathic pain) such as gabapentin and pregabalin [108, 109].

8. Neurotransmitters and receptors

8.1 G-protein coupled receptors

G-protein coupled receptor (GPCR) plays an important role in peripheral sensitization. The heterotrimeric GPCRs are the largest, most diverse receptor families in the mammalian cells. GPCRs are integral membrane signaling proteins characterized by a seven-transmembrane-segment architecture. Upon activation of GPCRs, GPCRs associate with distinct classes of heterotrimeric G proteins, composed of α-, β-, and γ-subunits, and molecular cloning has now defined 34 genes encoding G-proteins in humans, 17 encoding α-, 5 encoding β-, and 12 encoding γ-subunits [110, 111]. According to the a-subunits, G proteins are classified into four major classes, namely, Gs, Gi/o, $Gq/_{11}$, and $G_{12/13}$. Stimulation of the Gs subfamily activates adenylyl cyclase whereas stimulation of the Gi subfamily leads to its inhibition. Stimulation of the Gq subfamily activates phospholipase C (PLC), and the G_{12} family is implicated in the regulation of small GTP binding proteins [110].

Through Gα and Gβγ, GPCRs are able to communicate with ligand- and voltage-dependent ion channels in pain pathways [112], including the TRP channels, acid-sensing ion channels (ASICS), and ATP-gated P2X channels, as well as voltage-gated sodium, calcium, and potassium channels [113]. The voltage-gated ion channels are finely tuned by GPCR in excitable cells, and these channels are key molecular transducers of electrical activities, allowing calcium signaling into the cells in response to action potentials or subthreshold depolarizations [114].

In chronic pain conditions, inflammatory mediators released by peripheral tissues and immune cells in response to injury act at GPCRs to sensitize peripheral nociceptors and therefore augment their responses to both noxious and

innocuous stimuli. GPCRs can block pain upon targeting opioid, cannabinoid, α2-adrenergic, muscarinic acetylcholine, GABA, Group II and III mGlu, and somatostatin receptors.

8.2 Glutamate

Glutamate is an important excitatory neurotransmitter in the nervous system. There are two classes of the receptors: ionotropic glutamate receptors (iGluRs) and mGluRs. iGluR is a ligand-gated ion channel, whose subtypes are named for the agonist that activates the receptors, including the NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate receptors (KA) [115]. mGluRs contain eight subtypes of receptors and are divided into three groups: Group I mGluRs (mGluR1 and mGluR5) are coupled to phospholipase C, activate PKC, and release Ca^{2+} from intracellular stores; Group II mGluRs (mGluR2 and mGluR3) and group III mGluRs (mGluR4, mGluR6-8) inhibit adenylyl cyclase activity [116, 117]. Both group II and group III mGluRs are mainly localized on presynaptic terminals.

All subtypes of iGluRs are found on DRG cells and can be transported into central and peripheral terminals by afferent axons. Besides, mGluRs have been identified on peripheral primary afferent fibers and are involved in the processing of peripheral nociception [118]. The excitation of the primary afferent neuron increases glutamate release in the peripheral and central ends of primary afferent neurons [119].

8.2.1 Glutamate and pain

Peripherally applied NMDA and non-NMDA receptor antagonists attenuate or block nociceptive behaviors in several animal models of inflammation pain [120–122]. The fact that injection of NMDA into the masseter muscle potently excites muscle afferent fibers and that local application of NMDA receptor antagonists abolishes glutamate-evoked increase in afferent discharge suggests that activation of peripheral NMDA receptors plays an important role in excitation of muscle afferent fibers [123]. A study showed that injection of glutamate into human masseter and temporalis muscles evoked pain and it could be decreased by co-injection of NMDA antagonist ketamine [124].

Glutamate not only plays an important role in nociception transmission, but also is involved in the inflammation pain and neuropathic pain. Glutamate levels and the number of glutamate receptors elevate during cutaneous or deep tissue inflammation. Peripheral inflammation increases the proportions of both unmyelinated and myelinated nerves expressing iGluRs [125]. Local injection of either NMDA or non-NMDA receptor antagonist significantly reduces thermal hyperalgesia induced by injection of carrageenan into the hind paw or injection of the kaolin/carrageenan into the knee joint, but without affecting joint edema [120]. Activation of group II mGluRs by mGluR2/3 agonists induces analgesia in inflammatory and neuropathic pain models [126, 127]. Activation of Group II mGluRs suppresses prostaglandin E2-induced sensitization of TRPV1 calcium responses in mice [128]. CFA-induced nociceptive behaviors were significantly alleviated by administration of L-AP4, group III mGluR agonist, suggesting that group III mGluRs negatively regulate nociceptive behaviors and pain transmission by lessening neuronal firing rates at the peripheral nerve in inflammation [117]. Group I mGluR antagonists and group II/III mGluR agonists attenuated the enhanced nociception and noxious stimulus-induced glutamate release in the spinal cord dorsal horn in rats of CCI model and

injection of CFA into hind paw, suggesting a possible mechanism for their anti-hyperalgesic effects [129].

8.2.2 Ionotropic glutamate receptors

Neurochemical studies indicate that neurotransmitters diffuse across the synaptic cleft (synaptic transmission) as well as diffuse through the extracellular space and affect nearby neurons (non-synaptic communication) in the central nervous system. This is confirmed in a study that the site of action for glutamate can be at the autologous or nearby nerve terminals, and activation of these receptors can lower the activation threshold and increase the excitability of primary afferents [130]. In our experiments [18], we set up repeated antidromic stimulation of T9 nerve branch and recorded the activities from T10 cutaneous nerve branch. Forty minutes after the first antidromic stimulation of the T9 nerve branch, either NMDA receptor antagonist dizocilpine maleate (MK-801) or non-NMDA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) is injected subcutaneously to the receptive field of T10 cutaneous branch, and the enhanced spontaneous discharges in the T10 cutaneous branch caused by stimulation of the T9 nerve branch were significantly blocked. The results indicate that peripheral iGluRs are involved in the activation of peripheral nerves following the antidromic stimulation, and the released glutamate diffuses to the adjacent sensory nerves and activates the adjacent afferents by binding to glutamate receptors located on the nerve terminals. No significant difference was found in effects on the nerve activities between the NMDA and non-NMDA iGluR antagonists, and injection of saline did not produce any effect on the increased discharges of the recorded nerve branch. These results provide us evidence that glutamate may contribute to interactions between peripheral nerve terminals via non-synaptic communication [131]. Cao et al. [132] summarized the evidence that glutamate released from the non-synaptic communication contributes to the nociception in peripheral: (1) electrical stimulation of peripheral nerve can result in the release of glutamate into peripheral tissues; (2) NMDA, AMPA, and KA receptors localize on a large population of myelinated and unmyelinated sensory axons in the peripheral nerves; (3) primary afferents can be excited by the exogenous glutamate and endogenous glutamate; and (4) no synaptic contacts have been reported between two peripheral nerves using morphological approaches.

8.2.3 Glutamate interacts with other receptors

Glutamate receptors may interact with other neurotransmitter receptors in the peripheral to regulate nociception. A study found that peripheral glutamate receptors and TRPV1 receptors may interact to modulate the peripheral sensitization in some deep craniofacial nociceptive afferents [133]. CaMKII, which is persistently activated after NMDA receptor stimulation and phosphorylation of TRPV1, is likely to mediate the interactions between peripheral NMDA and TRPV1 receptors [134]. Glutamate, SP, and CGRP together contribute to the heat hyperalgesia combined with inflammation in the TRPV1-Cre mice [135]. There was a novel concept that tramadol acts as an agonist of TRPV1 [136] and local administration of tramadol blocked the paw licking (nociceptive behavior) in mice induced by glutamate [137].

A few studies on interactions between glutamate and opioid in the periphery have been conducted. A behavioral study has demonstrated that local cutaneous injection of DAMGO, a μ-opioid ligand, ameliorates the nociceptive behaviors caused by local injection of glutamate [138]. Our previous study demonstrated that local application of morphine suppressed the glutamate-evoked excitatory

responses of Aδ and C fibers in the rat hairy skin, and this effect was reversed by pretreatment with the opioid receptor antagonist naloxone, suggesting that the effect of morphine on glutamate-evoked activities is mediated through activation of opioid receptors on the peripheral terminals of sensory neurons [139]. Glutamate is released from small diameter afferent fibers by heat stimulation in the periphery or local application of capsaicin, and the glutamate release is regulated by activation of opioid receptors on the peripheral endings of small-diameter afferent fibers [140].

Injection of SP significantly increases the afferent discharge of peripheral sensory nerve endings [25]. A radioimmunoassay study showed that SP contents in the skin and tissues increased after electroacupuncture [141], indicating that SP plays a direct role in the stimulation of skin sensory nerve endings. Our previous study provided electrophysiological evidence for an interaction between SP receptor and glutamate receptor on the fine fiber activities in rat hairy skin, which may be involved in the mechanisms of hyperalgesia. Sub-threshold doses of SP (1 μmol/L, 10 μL) injected subcutaneously into the dorsal hairy skin had no effect on the afferent discharges of either Aδ or C units, while local injection of the submaximal doses of glutamate (10 μmol/L, 10 μL) into the receptive fields increased the afferent discharges of 35% (11/31) of Aδ fibers and 33% (6/18) of C fibers. In addition, glutamate-induced excitatory response was significantly enhanced by coinjection of subthreshold doses of SP [142]. Effects of glutamate and SP on spinal dorsal horn neurons may result from co-release of these two mediators from the same dorsal root afferent terminals [143].

8.3 Opioid

8.3.1 Opioid receptors

Peripheral nerve endings also express a variety of inhibitory neurotransmitter receptors such as opioid, GABA, and cannabinoid receptors. These receptors are related to peripheral sensitization and they may be targets for analgesia drug development. Opioid is known as the most powerful drug for severe pain, including three classic opioid receptors in the central nervous system: μ-(MOR), δ-(DOR), and κ-(KOR) receptors [144]. The existence of the three receptors was confirmed by the identification and sequence analysis of complementary DNA and the selective deletion of opioid receptor genes [145]. In peripheral, opioid receptors are present on the peripheral terminals of thinly myelinated and unmyelinated cutaneous sensory fibers [138]. Opioid agonists can attenuate the excitability of primary afferent neurons and the release of proinflammatory neuropeptides from central and peripheral terminals. Particularly within injured tissue, these events lead to antinociceptive and anti-inflammatory effects [146].

All opioid receptors are members of the rhodopsin class of GPCR, principally, although not exclusively, mediating their effects via the Gi/o pertussis toxin (PTX)-sensitive heterotrimeric G-protein family. After the ligand binds at the receptor, conformational changes allow intracellular coupling of mainly Gi/o proteins to the C-terminus of opioid receptors [147]. The μ-opioid agonists are still the gold standard for the treatment of moderate and severe pain. Agonists of μ-receptors exclusively coupled to inhibitory Gi/o proteins, which is important in anesthesia as they mediate the analgesic and sedative/hypnotic actions [148].

8.3.2 Opioids and pain

Both experimental and clinical studies suggest that peripheral analgesic effects of opioids are predominant under inflammatory conditions, leading to upregulation

of opioid receptors on peripheral sensory neurons and to local production of endogenous opioid peptides in immune cells [149–151]. The reason why opioids are predominantly functional under inflammatory conditions is that the opioid-producing cells are recruited in the inflamed tissue but not non-inflamed tissue [152]. In models of peripheral inflammation, local injection of low, systemically inactive doses of μ, δ, and κ-receptor agonists produced analgesia which was dose-dependent, stereospecific, and reversible by selective opioid antagonists [153]. In CFA-induced paw inflammation, MOR mRNA displayed a biphasic upregulation (at 2 h and 96 h), whereas mRNA for DOR remained unchanged, and KOR mRNA showed a peak at 12 h [154, 155]. In addition, human studies indicated that local application of opioid agonists are beneficial in patients with visceral and neuropathic pain as these drugs have analgesic efficacy and less side-effects because they do not readily cross the blood-brain barrier [48, 156].

8.3.3 Opioid-induced hyperalgesia

Unexpectedly, a large number of studies have demonstrated that opioids can elicit hyperalgesia and allodynia [157]. Opioid-induced hyperalgesia (OIH) may be associated with analgesic tolerance. OIH refers usually to the development of hypersensitivity to painful stimuli observed upon chronic opioid administration. Different mechanisms have been identified for this process including sensitization of primary afferent neurons and enhanced release of glutamate by these primary afferents, hyperexcitability of second order neurons to excitatory neurotransmitters, and up-regulation of nociceptive neuromodulators by descending pain controls [158, 159]. A lot of evidence suggests that MOR antagonists might reduce opioid analgesia [160]. However, the co-administration of methylnaltrexone bromide, a peripherally restricted MOR antagonist, was sufficient to abolish morphine tolerance and OIH without diminishing antinociception in perioperative and chronic pain models [161].

8.3.4 Other inhibitory receptors

Endogenous inhibitory receptors play a crucial part in the management of pain. Peripheral sensory neurons exhibit a large number of receptors that mediate inhibition of neuronal activity, and the agonists of these receptors produce antinociception. Application of either $GABA_A$ or $GABA_B$ receptor agonists attenuated the colonic afferent response to colon stretch. Conversely, $GABA_A$ and $GABA_B$ receptor antagonists increased the stretch response. These results suggest that GABA receptors are present and functional in the peripheral terminals of colonic afferents, and activation of these receptors via endogenous GABA release contributes to the suppression of colonic afferent excitability and visceral nociception without the central nervous system [162]. The antinociceptive effects of cannabinoids were confirmed in preclinical models of inflammatory, cancer, and neuropathic pain and in several human studies [163]. In an animal electrophysiological model similar to our previous studies [164], somatostatin inhibited the cross excitation between nerve terminals involved in peripheral hyperalgesia and had a peripheral analgesic effect [164]. The somatostatin and its receptors exerted a tonic inhibitory control over peripheral nociceptors, especially the peripheral nerve terminals of small-diameter cutaneous afferent fibers [165].

9. Conclusion

Based on the up-to-date studies in peripheral sensitization, we establish the essential roles of inflammation mediators, neurotransmitters, and their receptors in

this process, expecting to provide a new prospect of analgesics on peripheral targets in pain management. Noxious stimuli can excite the peripheral endings of primary sensory afferents, through activation of voltage-gated ion channels and/or ligand-gated receptors that increase the number of action potentials, leading to peripheral sensitization. Many inflammation mediators and neurotransmitters participate in the peripheral sensitization. Therefore, these chemicals provide enormous options for pain intervention of peripheral origin. Topically administered drugs such as lidocaine and capsaicin in patches, capsaicin in cream, and creams containing antidepressants (i.e., doxepin and amitriptyline) act locally in tissues through specific receptors and/or ion channels [166]. Topical drug delivery focuses on peripheral mechanisms and not only reaches greater concentrations in the region where it is applied, but also produces fewer side-effects along with greatly enhanced efficacy. Considering the unspecific and multifaceted function of chemicals involved in the peripheral sensitization, it is crucial to select the most suitable and specific targets to treat certain pain disease in clinic.

Beyond the peripheral sensitization, changes in the central nervous system neurons also play an essential role in the nociception process. Multiple lines of evidence show that central sensitization, produced following intense peripheral noxious stimuli, tissue injury, or nerve damage, is involved in diverse pain conditions, such as myofascial pain syndromes, idiopathic low back pain, and chronic pelvic pain [167]. Given the complexity and diversity of peripheral and central mechanisms of various pain conditions, it needs further investigation to figure out the specific mechanisms of pain symptoms and identify the most effective pain therapies in future.

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