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Review article

Comprehensive review on molecular mechanisms of neuropathic pain

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Key words: Neuropathic pain, synaptic plasticity, glia, ion channels.

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Abstract

Neuropathic pain is caused by functional abnormalities of structural lesions in the peripheral or central nervous system. Various diseases, such as postherpetic neuralgia, trigeminal neuralgia, diabetic neuropathy, spinal cord injury, cancer, stroke, and degenerative neurological diseases are responsible for producing neuropathic pain. Its symptoms include spontaneous and stimulus evoked painful sensations such as mechanical hyperalgesia, thermal and cold allodynia. Several maladaptive mechanisms underlying these symptoms have been identified in recent years which include peripheral sensitization of nociception, abnormal excitability of afferent neurons, central sensitization comprising pronociceptive facilitation, dis-inhibition of nociception, central reorganization processes, alterations in various ion channels. This review focuses on these pathophysiological principles, focussing on specific cellular and molecular changes that affect membrane excitability and are responsible for neuropathic pain.

Introduction

The International Association for the Study of Pain defines pain as an unpleasant sensory and emotional experience usually associated with actual or potential tissue damage. Pain is generally categorized according to several variables based on its duration (acute, chronic), and pathophysiologic mechanisms (physiologic, inflammatory/nociceptive, neuropathic). Acute pain is a result of traumatic tissue injuries which have limited duration and involves temporal reductions in intensity. Chronic pain may be defined as discomfort persisting 3–6 months beyond the expected period of healing. Physiologic pain involves non-traumatic discomfort of very short duration. This alerts the individual to the potentially injurious environmental stimulus and initiates withdrawal reflexes that prevent or minimize tissue injury. Nociceptive/ inflammatory pain is defined as noxious perception resulting from cellular damage due to surgical, traumatic, or disease-related injuries [1].

Neuropathic pain is distinguished from other pain conditions where the pain begins with disease of non-neural tissues. Neuropathic pain is defined by the International Association for the Study of Pain as "pain initiated or caused by a pathologic lesion or dysfunction" in peripheral nerves and central nervous system [2]. Neuropathic pain is usually considered as constant, burning, electrical, lancinating and shooting [3]. Neuropathic pain originates within the nervous system itself, rather than activating the peripheral nociceptors. Negative symptoms, which are first indication of damage to the somatosensory system, include deficits of different somatosensory qualities such as tactile hypoesthesia, thermal hypoesthesia, pinprick hypoalgesia, and loss of vibratory sensation. These symptoms are usually uncomfortable but not painful. These symptoms arise due to direct insult to primary sensory neurons. This may produce cell death or compromise transduction or conduction or transmission of sensory information [4]. Spontaneous positive sensations evoked by stimulus include hyperalgesia and allodynia, paresthesia and dysesthesia, paroxysmal pain [5].

An estimate of prevalence of pain with neuropathic characteristics lies between 6.9% and 10%. Prevalence of chronic pain with neuropathic characteristics varies from 3-17% and neuropathic pain associated with particular conditions, including postherpetic neuralgia (3.9-42.0/100,000 person-years), trigeminal neuralgia (12.6-28.9/100,000 person-years), painful diabetic peripheral neuropathy (15.3-72.3/100,000 person-years), glossopharyngeal neuralgia (0.2-0.4/100,000 person-years) [6].

Anatomical overview of pain pathways

Nociceptive stimuli activate two types of nociceptors. These include low threshold nociceptors connected to fast conducting A-delta pain fibers, and high threshold nociceptors that conduct impulses in slow (unmyelinated) C-fibers [8]. Noxious information is conveyed from peripheral nociceptors to the dorsal horn via C-fibres and A-delta fibers. Second-order spinal neurons send impulses via neospinothalamic and paleospin othalamic tracts. These cells activate motor and sympathetic efferents within the spinal cord. Ascending fibre stake signals to brainstem, midbrain, central gray, and thalamus. Projections are then made with the frontal and limbic cortex. Descending fibers originating

from cortex, hypothalamus, and brainstem project to the spinal cord to facilitate pain transmission [1].

Table 1. Causes of neuropathic pain [7]

Peripheral Causes	Central Causes
Alcoholic polyneuropathy	**Spinal Root/Dorsal Root Ganglion:** Tumor, Trigeminal neuralgia, Arachnoiditis
Chemotherapy induced polyneuropathy	**Spinal Cord:** Trauma including compression, Syringomyelia and intrinsic tumors, Multiple sclerosis
Complex Regional Pain Syndromes (CRPS)	**Vascular:** Infarction, hemorrhage Spinal dysraphism Vitamin B12 deficiency HIV
Painful diabetic neuropathy	**Brain Stem:** Laterally medullar syndrome, multiple sclerosis, tumors
Phantom limb pain	**Thalamus:** Infarctions, tumors, hemorrhage, surgical lesions
	Sub-cortical and cortical: Infarction, trauma
Post herpetic neuralgia	Parkinson's disease related pain
Posttraumatic neuralgias	Post ischemic myelopathy

Several mechanisms contribute to the neuropathic pain. The most widely studied neuronal mechanisms are hyper-excitability, sensitization of primary sensory neurons (peripheral sensitization) and enhancement of excitatory synaptic transmission in spinal cord, brainstem, and cortical neurons (central sensitization). Other neuronal mechanisms include dis-inhibition (reduced inhibitory synaptic transmission), descending pathway facilitation (eg, from the brainstem to the spinal cord), and long-term potentiation (LTP) in the cortex and spinal cord [9].

As neuropathic pain is emerging as a disease of global burden, various mechanisms and pathophysiological principals related to neuropathic pain need to be understood. Developing drugs for the treatment of neuropathic pain targeting multiple mechanisms depends on an improved understanding of pain mechanisms. So, this review focus on deficits or maladaptive changes in the peripheral, central and autonomic nervous system responsible for neuropathic pain: sensitization of nociceptors, abnormal ectopic excitability of affected neurons, pronociceptive facilitation at the spinal dorsal horn, disinhibition of nociception at the spinal inhibitory network, sympathetically maintained pain, and CNS reorganization processes.

Central mechanisms

Central sensitization is usually initiated by nociceptive stimuli and afferent fibres co-release glutamate and neuropeptides which lead to postsynaptic calcium increase. After nerve injury, Aβ fibers undergo phenotypic changes including increased expression of neuropeptides and they acquire the capacity to trigger central sensitization [10]. Various mechanisms have been implicated in central sensitization: alteration in glutamatergicneuro-transmitters mediated hypersensitivity, dis-inhibition, glial-neuronal interactions.

Nociceptive signaling initiated in peripheral sensory neurons enters the spinal cord dorsal horn and is carried to supraspinal structures such as the brain stem, thalamus, somatosensory cortex, insular cortex and anterior cingulate cortex [11]. Neuropathic pain occurs due to long-term plastic changes along sensory pathways. In the nociceptive transmission pathway, plasticity is responsible for cellular mechanism in neuropathic pain. Plastic changes occur not only in peripheral nociceptors, spinal dorsal synapses, and subcortical nuclei, but also in cortical nuclei that are involved in the processing of noxious information. After the nerve injury, synaptic potentiation in the spinal cord and cortical areas along with abnormal peripheral activity leads to the neuropathic pain.

Central Sensitization Triggers

The major players in the synaptic changes underlying central sensitization are N-Methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and metabotropic G-protein coupled glutamate receptor subtypes (mGluR) glutamate receptors, substance P, Neurokinin1 receptor, Brain-derived neurotrophic factor (BDNF) and Tropomyosin receptor kinase B (TrkB) receptor, ephrinB and EphBR, Ca2+/calmodulin-dependent protein kinase II (CaMKII), Protein Kinase A (PKA), Protein Kinase C (PKC), src, Extracellular signal–regulated kinase (ERK) and cAMP response element binding protein (CREB) and Voltage gated potassium channel, Kv4.2 [12,13].

Glutamate is the fast transmitter of primary afferent neurons which binds to several receptors on postsynaptic neurons in the dorsal horn of spinal cord including ionotropic AMPA, NMDA, Kainate receptors and mGluR. Activation of NMDA receptor is an essential step in central sensitization mediated by C-fibres [14].

Substance P (SP), released along with glutamate from unmyelinated peptidergic nociceptors, is also involved in the generation of central sensitization [15]. It binds to neurokinin-1 (NK1) G-protein–coupled receptor which is expressed by spinothalamic, spinoparabrachial neurons and contributes to the C-fiber–evoked synaptic potentials as well as to intracellular signaling [16].

Calcitonin gene-related peptide (CGRP) potentiates the effects of SP and participates in central sensitization through postsynaptic CGRP1 receptors, which further activate PKA and PKC and enhances the release BDNF from trigeminal nociceptors [17,18].

BDNF, a neurotrophic factor and synaptic modulator synthesized from nociceptor neurons and released into the spinal cord plays role in the production of central sensitization [19]. BDNF promotes NMDA receptor-mediated C-fiber–evoked responses and causes activation of

several signaling pathways in spinothalamic track neurons, including ERK and PKC on binding to its high-affinity trkB receptor [20].

The inflammatory kininbradykinin produced in the spinal cord in response to peripheral noxious stimuli acts through its Gq-coupled B_2 receptor, which is expressed by dorsal horn neurons and promotes synaptic strength by activating PKC, PKA, and ERK [21].

Potential mechanisms for nitric oxide (NO) synthesized by either neuronal or inducible NO synthases in the dorsal horn include the Cyclic guanosine monophosphate (cGMP) synthesis cascade, nitrosylation of membrane channels, adenosine diphosphate-ribosylation, and production of reactive species [22,23].

Synaptic plasticity

Synaptic plasticity contributing to central sensitization has been studied extensively in the spinal cord, anterior cingulate gyrus, prefrontal cortex, amygdala, and periaqueductal gray [24,25].

Synaptic plasticity in the spinal cord dorsal horn

Primary afferent fibers form synapse with dorsal horn sensory neurons in spinal cord. Now, dorsal horn neurons send ascending projecting fibers and make synapses with neurons located at supraspinal sites, such as the thalamic nuclei. These ascending pathways send sensory information from periphery to the brain. The spinal cord dorsal horn is the first relay for pain transmission in the CNS. Glutamate is a main neurotransmitter between primary afferent fibers and dorsal horn neurons and postsynaptic responses. In synapses receiving low threshold sensory inputs, postsynaptic NMDA receptors are found. In synapses receiving low or moderate intensity of sensory inputs, AMPA receptors are found, while in synapses receiving high threshold inputs, both glutamatergic AMPA and KA receptors are found present. After injury, increased release of neurotransmitters from nociceptors depolarizes postsynaptic neurons to activate silent NMDA receptors. Hyperalgesia is reported due to increase in calcium influx which further promotes synaptic connections between nociceptors and dorsal horn pain transmission neurons. Spinal cord central sensitization is dependent on NMDA-mediated elevations of cytosolic Ca^{2+} in the postsynaptic neuron. Activation of metabotropic glutamate and substance P receptors on the postsynaptic neuron also contribute to sensitization by cytosolic calcium release. Downstream activation of signaling pathways and second messenger systems, such as kinases (Mitogen-activated protein kinases (MAPK), PKA, PKC, Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), Src), further increases excitability of these neurons, by promoting NMDA receptor function [26,27]. The mechanism of LTP induction involves the activation of NMDA receptors, neurokinin 1 receptors and the downstream MAP kinase pathway [28].

Synaptic plasticity in the anterior cingulate cortex (ACC)

ACC is a major forebrain area for pain perception [29]. Both long term potentiation and depression of excitatory transmission can be induced in ACC. Both NR2A and NR2B subunit of NMDA receptors, and various downstream signaling pathways such as calmodulin (CaM), calcium-stimulated AC1 and AC8, Ca2+/calmodulin-dependent protein kinase IV (CaMKIV), and MAPK are involved in LTP in the ACC [30,31]. Peripheral inflammation caused the upregulation of NR2B in the ACC, causes inflammation-related persistent pain. Pre-synaptic release of glutamate and postsynaptic AMPA responses in ACC neurons are increased after peripheral nerve injury [32].

Dis-inhibition: Loss of GABAergic and glycinergic control

GABAergic or glycinergic inhibitory inter-neurons are widely distributed in the superficial dorsal horn and are the basis of the gate control theory of pain, which states that loss of function of these inhibitory inter-neurons often result in increased pain [33].

The γ-aminobutyric acid (GABA) pathway forms a major inhibitory neurotransmitter system in the CNS. Depression of spinal inhibitory mechanisms is important for increased excitatory transmission and central sensitization. Peripheral nerve injury results in loss of GABA-mediated inhibitory currents, decreased extracellular levels of GABA, decrease in dorsal horn levels of the GABA synthesizing enzyme glutamic acid decarboxylase [34,35] and decreased GABA receptor levels in the spinal cord due to degeneration of the primary afferent neuron terminals on which the receptor is present [36].

GABAergic and/or glycinergic inhibition is important factor in maintenance of information processing by preventing the generation of synchronized wave activity in the CNS. Synchronous activation of dorsal horn network lead to pain that disrupts the innervations patterns of peripheral nerves or dorsal roots [37].

Peripheral nerve injury or inflammation reduces the expression of the potassium-chloride exporter KCC2, which increases the intracellular Cl^- concentration and further alleviate the inhibitory effects of GABAergic or glycinergic transmission [38,39]. Down regulation of Potassium-chloride transporter (KCC2) results in a shift in the Cl^- gradient which activates GABA-A receptors depolarization and enhance excitability and increase pain transmission [40]. Dis-inhibition also occurs through modulation of glycinergic signaling involving spinal cord action of prostaglandins [41]. Tissue injury causes spinal release of the prostaglandin E_2, which acts on Prostaglandin E2 receptor 2 (EP2) receptors expressed by excitatory inter-neurons and projection neurons in the superficial dorsal horn. Stimulation of the cAMP-PKA pathway phosphorylates GlyRa3, glycine

receptor subunits, causing the neurons unresponsive to the inhibitory effects of glycine [9].

Anatomical reorganization

Following a peripheral nerve injury, the central axons of injured Aβ-fibers sprouts from deeper laminae of the dorsal horn (laminae II and IV) into lamina II of the dorsal horn, which is normally restricted to C-fiber and Aδnociceptors. This synaptic rearrangement means that second-order dorsal horn neurons that normally receive high threshold sensory input, now receive inputs from low threshold mechanoreceptors. Such misinterpretation of information within the spinal cord results in low threshold nociceptive sensory information leading to the hypersensitivity after peripheral nerve injury [42,43].

Spinal dynorphin

Nerve injury leads to a prominent increase in spinal dynorphin (a dorsal horn protein) expression in inter-neurons. Spinal dynorphin causes spinal release of glutamate and prostaglandins. Dynorphin binds to the NMDA receptor, increases intracellular calcium and initiate transmitter release [44].

Descending Modulatory Mechanisms

Peripheral nerve damage causes primary hyperalgesia and allodynia developed at the beginning but it needs facilitation from rostroventromedial medulla (RVM) for maintenance. Descending modulatory pathways also influence dorsal horn sensitization mechanisms involved in neuropathic pain. Cortical, thalamic, and periaqueductal inputs converge on the RVM. This center gives rise to both inhibitory and excitatory inputs to the dorsal horn via an ipsilateral pathway in the dorsolateral funiculus [3].

Central Immune Mechanisms

Glial cells (microglia and astrocytes) also contribute to the central sensitization process during injury. Under normal conditions, microglia functions as resident macrophages of the central nervous system and found distributed within the grey matter of the spinal cord and serve as sentinels of injury. After peripheral nerve injury, microglia accumulates in the superficial dorsal horn within the termination point of injured peripheral nerve fibers. The activated microglia releases signaling molecules, cytokines (such as Tumor necrosis factor α (TNF-α), interleukin-1β and 6 (IL- 1 β and 6)), which promotes neuronal central sensitization and nerve injury-induced persistent pain [45].

Activation stages of glia

Glial reaction is upregulation of glial markers and causes morphological changes in glia (hypertrophy and upregulation of the microglial markers i.e. Glial fibrillary acidic protein, GFAP).

Upregulation of glial receptors such as adenosine triphosphate receptors, chemokine receptors and Toll-like receptors lead to activation and phosphorylation of intracellular signaling pathway, mitogen-activated protein kinase pathways which lead to upregulation of glial mediators, such as cytokines, chemokines, and growth factors. After release, these glial mediators can interact with neurons to elicit pain via central sensitization [2].

In neuropathic pain, peripheral neurons transmit signals to spinal dorsal horn neurons by releasing various neurotransmitters such as calcitonin gene-related protein, substance P, glutamate, and adenosine triphosphate (ATP). Other neurotransmitters which are involved locally in the dorsal hornare GABA, glycine, serotonin. These neurotransmitters cause microglial activation which lead to neuropathic pain [46].

ATP is important for microglia-neuron communication [47]. Microglia expresses a variety of purinergic receptors, P2 receptors: metabotropic P2Y receptors, P2Y1, 2, 4, 6, and 12 receptors and ionotropic P2X receptor; P2X4 and P2X7R subtypes [48]. Only P2Y12 receptor is involved in the development of tactile allodynia associated with peripheral nerve injury [49].

ATP/P2X4-mediated activation of microglia triggers a mechanism of dis-inhibition. ATP induced activation of P2X4 receptors causes the release of BDNF from microglia which acts upon TrkB receptors on lamina I projection neurons, to generate a change in the Cl^- gradient, which shifts the action of GABA from hyperpolarization to depolarization [50].

Toll-like receptors (TLRs) regulates innate immunity and are involved in glial activation. TLRs are trans-membrane signaling proteins which are expressed in peripheral immune cells and glia. Genetic or pharmacological inhibition of TLR2, TLR3 or TLR4 function results in decreased microglial activation and hypersensitivity due to peripheral nerve injury [51].

Chemokine receptors contribute to the pathogenesis of pain via modulating glial activation and neural plasticity. Chemokine activates multiple receptors, CX3C chemokine receptor 1(CX3CR1) appears to be the only known receptor for CX3CL1 (chemokine (C-X3-C motif) ligand 1 protein in humans encoded by the CX3CL1 gene)and is exclusively expressed in microglia and is upregulated in neuropathic pain. Activated microglia also increase synthesis and secretion of various cytokines and chemokines, including IL-1β, IL-6, tumor necrosis factor-α (TNF-α), Prostaglandin E2 (PGE_2), and nitric oxide. Further, p38 activation turns on the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which leads to the expression of IL- 1β, IL6, and Cyclooxygenase 2 [52].

The MAPK family includes 3 major members: extracellular signal- regulated kinase 1, 2 and 5 (ERK1, ERK2 and ERK 5 respectively), p38, and c-Jun N-terminal kinases (JNK) which are activated in spinal microglia after nerve injury [53]. MAPK pathways play an important role in intracellular

signaling in neurons and glia, and for the generation of persistent pain [54].

Increased phosphorylation of p38 (P-p38) is observed in spinal cord microglia after nerve injury [55]. Nerve injury activates the upstream activator of JNK, transforming growth factor-activated kinase-1 (TAK1), and downstream effectors of JNK, c-Jun in spinal astrocytes. Among several JNK iso-forms (JNK 1,2,3), JNK1 is expressed in spinal astrocytes [56,57].

Glia produces several mediators such as cytokines, chemokines, growth factors, proteases and glutamate, ATP, D-serine, and PGE2 that facilitate neuronal and synaptic activity and pain sensitivity. Pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 are the glial mediators which get up regulated in spinal cord glia after nerve injury and inflammation. TNF-α is mainly produced by microglia and plays an essential role in the generation of central sensitization and persistent pain [58]. TNF-α induces rapid expression of various chemokine receptors such as CCL2, CXCL10, and CXCL1. Spinal injection of TNF-α-activate astrocytes resulting in persistent mechanical allodynia by releasing CCL2 [56]. IL-1β induced in astrocytes after inflammation and nerve injury is also produced by microglia and neurons in the spinal cord [59]. Nerve injury induces IL-18 expression in spinal microglia [60]. Nerve injury upregulate brain-derived neurotrophic factor in spinal microglia by activating P2X4 and p38 [61]. Basic fibroblast growth factor (bFGF) is also induced in reactive astrocytes of the spinal cord in the late phase of nerve injury [62]. Nerve injury also induces cathepsin S in spinal microglia and tissue type plasminogen activator in spinal astrocytes to enhance neuropathic pain [63]. Prokineticin1 and prokineticin2 belong to a new family of chemokine which signal through two G-coupled receptors (prokineticin-receptor1 and prokineticin-receptor2) involved in various biological activities such as angiogenesis, hematopoiesis, immune processes, inflammation and nociceptive transmission. Tissue inflammation particularly in peripheral nerve injury, cancer, bone metastasis increases the expression of prokineticin2 and of the prokineticin-receptor2 within dorsal root ganglia and spinal cord. Activation of the prokineticin receptors in the spinal dorsal horn and astrocytes contributes to central sensitization and maintains chronic and neuropathic pain. Prokineticin2 also act on prokineticin receptors present onmonocytes, macrophages and dendritic cells, promotes chemotaxis and releases inflammatory and pronociceptive cytokines [64].

As pain is carried by neurotransmission in neural circuit, glia must interact with neurons to facilitate pain sensitivity. Glial mediators can modulate both excitatory and inhibitory synaptic transmission. Facilitation by excitatory synaptic transmission occurs via postsynaptic and extra-synaptic mechanisms. TNF-α induces trafficking of GluR2- lacking AMPA receptors to the plasma membrane in spinal cord motor neurons after spinal cord injury [58].

Cytokines and chemokines induce central sensitization via extrasynaptic mechanisms. NMDA currents in lamina II neurons are enhanced by IL-1β, TNF-α, and CCL2. TNF-α increases NMDA receptor activity by phosphorylating ERK in dorsal horn neurons. IL-1β induces phosphorylation of the NR1 subunit in spinal cord neurons. Astrocytic D-serine increases NMDA currents after binding with glycine site of NMDA receptors. This astrocytic glutamate release can be detected as slow inward currents which are mediated by extrasynaptic NR2B receptors and induced in spinal dorsal horn neurons after inflammation [65].

Glial mediators such as BDNF, cytokines, chemokines, and PGE2 also modulate inhibitory synaptic transmission via pre, post and extrasynaptic mechanisms. At pre-synaptic level, IL-1β and IL-6 have shown to inhibit the frequency of spontaneous postsynaptic currents (sIPSCs) in spinal lamina II neurons. Post-synaptically, IL-1β reduces the sIPSC amplitude and PGE2 inhibits glycinergic neurotransmission in the dorsal horn via post-synaptic GlyR3 and the cAMP /PKA pathway [41]. Extra-synaptically, GABA and glycine currents are suppressed by IL-1βand IL-6.BDNF acts on spinal lamina I neurons to reverse GABA inhibition by altering chloride reverse potential. Finally, the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 also shows long-term neuronal plasticity in the pain circuit by phosphorylating the transcription factor CREB, leading to the transcription of CREB-mediated pronociceptive genes (eg, cyclooxygenase- 2, neurokinin-1) in spinal cord neurons [66].

Astrocytes and spinal glial cellsprovide trophic support to neurons and maintain the homeostasis of K^+, glutamate in CNS and PNS and also insulate the neural circuit of pain by forming a structural barrier and keeping the circuit silent by releasing inhibitory mediators under physiological conditions. Chronic pain induced by nerve injury is associated with neuropathy and gliopathy. Astrocytes lose their ability to maintain the homeostasis of K^+ and glutamate which leads to neuronal heperexcitability. Dysfunction of astrocytic water channel (AQP4) will result in edema in the CNS and PNS. After gliopathy, glia do not insulate the pain circuit and serve as an amplifier of pain, by producing pro-inflammatory and pronociceptive mediators[67].

Peripheral mechanisms

Persistent pain after nerve injury results from alterations in the properties of peripheral nerves. Factors which contribute to the prolonged hypersensitivity after nerve injury include damage to nerve fibers leading to increased spontaneous firing or alterations in their conduction, changes in the neuronal excitability, changes in the gene expression and up or down regulation of neurotransmitter release [69]. Mechanical, thermal and chemical stimuli are converted into voltage changes in sensory neurons by ion channels that respond to specific environmental stimuli. After nerve injury, peripheral sensitization occurs due to reduced

thresholds resulting from activation of these transducer channels.

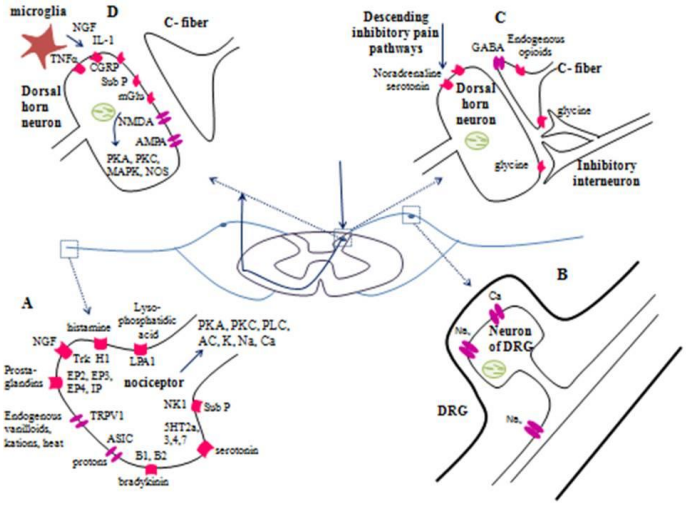

Figure1. Mechanism of neuropathic pain: A: Sensitization of nociceptors, B: Abnormal ectopic excitability of afferent neurons, C: Disinhibition of nociception at the spinal inhibitory network, D: Pronociceptive facilitation at the spinal dorsal horn [68].

Abnormal ectopic excitability of afferent neurons

Spontaneous sensations after peripheral nerve injury generates as a result of hyperexcitability in the primary sensory neuron which lead to ectopic action potential dischargeat the site of injury resulting in neuroma and is also responsible for spontaneous sensations and paresthesia, dysthesia and pain [70].

Alterations in ion channels

Neuropathic pain changes the expression, distribution, phosphorylation of various ion channels in sensory neurons and expression and function of receptors, enzymes, and voltage-dependent ion channels in peripheral nerves and dorsal root ganglion and also causes changes at synapses in the nociceptive pathway in the central nervous system and leads to changes in intrinsic membrane properties and the generation of membrane potential oscillations which results in rhythmic firing even in the absence of a stimulus. DRG neurons express many kinds of ion channels/receptors which have at least three functions: [2]

1) Transduction (e.g., transient receptor potential channels, sodium channels, acid-sensing ion channels, and ATP sensitive receptors in the peripheral terminals of DRG neurons transduce noxious stimuli into electric impulses),
2) Conduction (e.g., sodium and potassium channels involved in propagation of action potentials),
3) Modulation of synaptic transmission (e.g., voltage-gated calcium channels and glutamate receptors expressed in presynaptic terminals of the primary afferents in dorsal horn regulate the release of neurotransmitters)

Sodium channels

In thinly myelinated or unmyelinated Aδ and C fibers, altered levels of excitability result in lancinating and burning pain which is generated by sodium channels, which are differentially expressed and distributed and are abnormally active in neuropathic pain [71]. Sensory neurons contain two groups of voltage-gated sodium channels: fast acting tetrodotoxin sensitive (TTX-S) and slowly acting tetrodotoxin-resistant (TTX-R) channels. TTX-R channels are detected only in nociceptor sensory neurons and are considered to be involved in pathological pain states [72]. Voltage sensors of sodium channels are found present in the highly conserved S4 trans-membrane segments. Membrane depolarization produces changes in the trans-membrane electric field and then S4 segment spread outwards. This conformational change opens the pore. After activation, sodium channels quickly gets inactivated to prevent further ion flow through the pore and allow repetitive action potential firing of cells [73]. After nerve injury, concentration of sodium channels of either type increases at the site of the lesion and also in the whole axon [74]. Nine subtypes of voltage-gated sodium channels (NaV1.1 to NaV1.9) have been identified. NaV1.7, NaV1.8 and NaV1.9 are particularly involved in pain perception. Nav1.8 is basically expressed in A- and C-fiber nociceptors, Nav1.9 is found selective for a subset of C-fibers, whereas Nav1.1 and Nav1.6 are found mostly in non-nociceptive neurons [75,76]. Phosphorylation of Nav1.8 occurs after activation of the PKC/PKA intracellular pathways in presence of inflammatory mediators which alters its threshold and kinetic properties and is important in producing peripheral sensitization. If this phosphorylation occurs after peripheral nerve injury, it may potentially lead to an increase in TTX-resistant current and hyperexcitability in the uninjured afferents [77]. The expression of voltage gated sodium channels is also up-regulated by neurotrophins including nerve growth factor, brain-derived neurotrophic factor and glial-derived neurotrophic factor [78]. Inflammatory cytokines such as tumor necrosis factor α also up-regulate the expression of Nav1.3, Nav1.8, and Nav1.9 and increase both TTX-sensitive and –resistant currents in the DRG neurons [79]. These effects of neurotrophins and pronociceptive cytokines on sodium channel expression may be carried out through regulation of intracellular downstream signaling pathways of their receptors, including p38 and ERK1/2, mitogen-activated protein kinase [80].

Potassium channels (K^+)

Low voltage-activated potassium channels stabilize the membrane potential and regulate action potential number on depolarization but gets down-regulated by nerve injury Voltage-gated potassium channels are also required for action potential firing and are involved in spontaneous firing of action potentials after nerve injury [81,82].

Transient receptor potential ion channels

Transient receptor potential (TRP) ion channels are sensory transducers, which are expressed in nociceptive primary sensory neurons where they get involved in generating chemical and thermal stimulus evoked pain sensations [83]. Six TRPs (TRPV1, TRPV2, TRPV3, TRPV4, TRPM8 and TRPA1) are found to be expressed in primary afferent nociceptors, pain sensing neurons, where they act as transducers for thermal, chemical and mechanical stimuli. TRPV1 responds to noxious heat and capsaicin and produces the burning sensation whereas TRPA1 responds to cold temperatures and also produce a burning sensation. After nerve injury, the phenotype of cells expressing TRP channels changes so that TRPV1 and TRPA1 are also expressed by neurons of a non-nociceptive phenotype. Expression of TRPV1 is decreased in injured nociceptive neurons, while they are increased in the neighboring uninjured neurons [84]. After nerve damage, post-translational changes, trafficking and expression changes in TRPV1 occur including novel expression in large diameter, low threshold A-fibers which may indicate a phenotypic switch contributing to symptoms of neuropathic pain. TRPV1 is also expressed not only at its peripheral terminal but also in sensory nerve axons of peripheral nerves [85,86]. As TRPV1 activation threshold gets modified by inflammatory mediators from immune cells and injured nerves having macrophages and T-cells [87], it is possible that axonal TRPV1 along with peripheral terminal TRPV1 may also become sensitized. A reduction in TRPV1 thermal threshold to levels close to body temperature along the axon often lead to depolarization and generation of action potentials producing spontaneous pain [88]. TRPA1 expression is also increased in a subset of small diameter primary sensory neurons following nerve injury likely inducing cold hypersensitivity [89].

Various inflammatory markers sensitize TRPV1 so that it gets active at body temperature providing a mechanism for thermal hyperalgesia. TRPV1 is sensitized by a number of factors generated during inflammation including mild acidification (pH 6.5), bradykinin, prostaglandin E2, NGF and ATP [90]. Sensitization of TRPV1 by these factors occurs through PKA and PKC-dependent phosphorylation [91]. PGE2 increases the excitability of nociceptive neurons in response to noxious stimuli and sensitizes TRPV1 via specific PKA-dependent phosphorylation [92]. In the naïve state, TRPV1 is inhibited by endogenous phosphatidylinositol 4,5-bisphosphate by interacting with the C-terminal domain of the channel. Activation of phospholipase C through nerve growth factor or bradykinin signaling, depletes cellular phosphatidylinositol 4,5-bisphosphate (PIP2) and sensitizes the channel [93]. Prokineticin receptors, highly expressed in nociceptor endings and dorsal root ganglia, exert a tonic activation of TRPV1 and TRPA1contributing to peripheral sensitization [64].

Calcium channels

Activation of voltage-dependent calcium channels is crucial for neurotransmitter release. Enhanced expression of these voltage gated cation channels under pain-producing pathological states enhance synaptic vesicle release of pain-inducing transmitters such as glutamate, substance P and calcitonin gene-related peptide upon stimulation that activates inter-neurons and projection neurons, altering sensory excitability which further leads to pain sensations. Low-voltage T-type voltage gated cation channels are found in dorsal root ganglion, primary afferent cell bodies and in free nerve endings. They contribute to the initiation of the action potential in these locations and lower the required threshold for activation. By promoting synaptic excitation, enhanced T-type voltage gated cation channel (VGCC) activity favors the development of pain [94].

Peripheral immune mechanism

Cytokines and chemokines

Cytokines and chemokines are growth factor proteins secreted from leukocytes as part of the immune and inflammatory response and play important role in the pathogenesis of pain [95]. These factors can act on neurons to induce changes in gene expression, which lead to the abnormal electrical activity. Following nerve injury, tumor necrosis factor-α is released from Schwann cells and infiltrating and resident macrophages, and stimulates the sequential production and release of interleukin-1β and interleukin-6 (IL-6). IL-1β may be involved in a complex signaling cascade that leads to the production of pronociceptive compounds (such as nitric oxide, NGF, and prostaglandins) from immune cells or Schwann cells. These substances cause changes in gene expression and neuronal excitability in intact nociceptors. The gp130 cytokines, IL-6 have been shown to be crucial in the up-regulation of key modulators of sensory processing, such as BDNF, galanin and substance P following nerve injury. The chemokine CCL2 (MCP-1) is another injury-induced factor that accumulates within sensory neurons in models of neuropathic pain and contributes to macrophage recruitment [96].

Growth factors

Neurotrophins levels particularly nerve growth factor and cytokines are found increased at the injury site. The neurotrophins activate kinases, which change the expression, posttranslational modification and trafficking of TRPV1 and voltage gated sodium channels [97].

Conclusion and future perspective

Neuropathic pain constitutes a major clinical problem. Central sensitization, immune cells and proinflammatory mediators play a critical role in the generation of neuropathic pain after injury of the peripheral nervous system. In order

to provide better pain control, focus should be made on the mechanisms underlying the symptoms of neuropathic pain. Treatment strategies should address the multi-factorial nature of neuropathic pain, including the multiple levels of the mechanisms. This mechanism-based approach to the treatment of neuropathic pain is beneficial for improving the quality of life of patients with neuropathic pain. Current treatment strategies are dominated by opioids, NSAIDs, anticonvulsants, selective serotonin reuptake inhibitors. However, progress in evolving new approaches that target multiple mechanisms has been slow. But now, there has been a lot of innovation in developing new drugs working in ways that are different from how drugs have worked in the past. Approaches have been focused on developing cell and gene therapies, hitting chemokine receptors, glial cells and cytokines and anti-nerve growth factor drugs.

Abbreviations

CNS: Central Nervous System; LTP: Long Term Potentiation; NMDA: N-Methyl-D-aspartate; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF: Brain-derived neurotrophic factor; CaMKII: Ca2+/calmodulin-dependent protein kinase II;ERK: Extracellular signal–regulated kinase; CREB: cAMP response element binding protein; mGluR: metabotropic G-protein coupled glutamate receptor; TrkB: Tropomyosin receptor kinase B; PKA:Protein Kinase A; PKC: Protein Kinase C; SP: Substance P; NK1: Neurokinin 1; CGRP: Calcitonin gene-related peptide; NO: Nitric Oxide; cGMP: Cyclic guanosine monophosphate; MAPK: Mitogen-activated protein kinases; PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; ACC: anterior cingulate cortex; CaM: calmodulin; CaMKIV: Ca2+/calmodulin-dependent protein kinase IV; GABA: γ-aminobutyric acid; KCC2: Potassium-chloride transporter; EP2: Prostaglandin E2 receptor 2; RVM: Rostro Ventromedial Medulla; TNF-α: Tumor necrosis factor α; IL: interleukin; GFAP: Glial fibrillary acidic protein; ATP: Adenosine triphosphate; TLR: Toll-like receptors; CX3CR1: CX3C chemokine receptor 1; PGE2: Prostaglandin E2; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; JNK: c-Jun N-terminal kinases; TAK1: Transforming growth factor-activated kinase-1; bFGF: Basic fibroblast growth factor; sIPSC: Spontaneous postsynaptic currents; TTX-S: Tetrodotoxin sensitive; TTX-R: tetrodotoxin-resistant; NaV: Sodium channels; TRP: Transient receptor potential; PIP2: Phosphatidylinositol 4,5-bisphosphate; VGCC: Voltage gated cation channel.

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