Modulation of Pain and Itch by Spinal Glia

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Abstract Chronic pain and itch are a pathological operation of the somatosensory system at the levels of primary sensory neurons, spinal cord and brain. Pain and itch are clearly distinct sensations, and recent studies have revealed the separate neuronal pathways that are involved in each sensation. However, the mechanisms by which these sensations turn into a pathological chronic state are poorly understood. A proposed mechanism underlying chronic pain and itch involves abnormal excitability in dorsal horn neurons in the spinal cord. Furthermore, an increasing body of evidence from models of chronic pain and itch has indicated that synaptic hyperexcitability in the spinal dorsal horn might not be a consequence simply of changes in neurons, but rather of multiple alterations in glial cells. Thus, understanding the key roles of glial cells may provide us with exciting insights into the mechanisms of chronicity of pain and itch, and lead to new targets for treating chronic pain and itch.

Keywords Neuropathic pain · Chronic itch · Microglia · Astrocytes · Spinal dorsal horn

Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (IASP Taxonomy). Itch is also an unpleasant sensation that elicits the desire or reflex to scratch, and normally serves as a self-protection mechanism from harmful external agents such as parasites. Internal and external stimuli that cause pain and itch sensations stimulate the similar somatosensory pathways from peripheral nerve endings of primary afferent sensory neurons to the spinal dorsal horn (SDH) and the brain. However, pain and itch are clearly different sensations, and we can easily and distinctly recognize them. Recent evidence has indicated that there are distinct neuronal circuitries for pain and itch in the peripheral and central nervous system (CNS) [1–3].

Under pathological conditions, such as nerve damage in cancer, diabetes and traumatic injury for pain, and skin diseases like atopic dermatitis for itch, it has been proposed that aberrant modulation of pain and itch signaling at the levels of primary afferents and the SDH might be involved in their chronic states. An increasing body of evidence from models of chronic pain and itch has revealed that synaptic alterations in the SDH might not be a consequence simply of changes in neurons, but rather of multiple alterations in glial cells. In this review, we highlight recent advances in our understanding of the mechanisms that underlie chronic pain and itch with a specific focus on the role of microglia and astrocytes, respectively, in the SDH.

Glial Cells in the CNS

Glia (the Greek word for “glue”) were first found in the mid-nineteenth century as non-neuronal cells in the CNS by the German anatomist Rudolph Virchow [4]. However,

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it has now become apparent that glia are much more than “glue”, and are active players with crucial roles in controlling neuronal functions [5, 6]. Glial cells express a number of neurotransmitter receptors for receiving inputs from neighboring cells, including neurons, and also a number of biologically active substances for giving signals to the cells. Glial cells are mainly classified into three populations: astrocytes, oligodendrocytes and microglia. The former two cell types are derived from neural stem cells [7], whereas microglia originate from precursor cells in the yolk sac during early embryonic development [8, 9]. In the CNS, astrocytes are the main population of glia and are morphologically classified into protoplasmic astrocytes in the grey matter and fibrous astrocytes in the white matter [10]. Protoplasmic-type cells ensheath the synapses and make contact with blood vessels, and fibrous-type cells make contact with the nodes of Ranvier [10]. Recent studies have also indicated the presence of a diverse cell population in astrocytes, with distinct properties in different brain regions [11, 12]. Astrocytes occupy nonoverlapping spatial territories, and their processes are intimately associated with synapses [10, 13]. The processes of one astrocyte contact tens of thousands of synapses through structures termed the tripartite synapse to recognize the structural and functional relationship between the astrocyte and the pre- and postsynaptic terminals [13, 14]. Astrocytes do not produce action potentials, but instead increase intracellular Ca^{2+} levels in response to activation of neurotransmitter receptors by their ligands (such as glutamate, dopamine, noradrenalin, serotonin, or ATP) [15, 16]. Stimulated astrocytes then evoke various cellular responses including production and release of gliotransmitters (ATP, D-serine, and glutamate) and trophic factors, which can modulate synapse formation, maturation, efficacy, and plasticity [10, 15, 17]. A growing body of evidence using astrocyte-specific molecular and genetic manipulations has revealed that astrocytes play a pivotal role in neuronal function under physiological and pathological conditions [10, 12, 15, 17–20].

Microglial cells were described by Pio del Rio-Hortega (1932) as being the ‘third element’ distinct from neurons and astrocytes [21]. This cell type is the tissue-resident macrophage in the CNS, and constitutes 5%–10% of total cells in the adult CNS. Recent fate mapping studies have revealed that microglia arise from early yolk sac-derived precursors that leave the yolk sac and migrate to the neuroectoderm *via* the primitive bloodstream [22, 23]. The yolk sac-derived microglia are thought to remain throughout life and to be maintained by self-renewal in the healthy CNS, with little contribution from bone marrow (BM)-derived monocytes/macrophages [9, 24, 25]. In adults, microglia are ubiquitously distributed throughout the CNS and represent a morphologically unique type of cell which,

under normal conditions, have a small soma bearing thin and branched processes. Two photon *in vivo* imaging studies using a transgenic mouse line that expressed green fluorescent protein in microglia have revealed that microglia processes are highly dynamic in the brain and spinal cord [26–28]. The processes of microglia rapidly move toward the site of injury, an effect that is dependent on purinergic signaling [26, 29]. Furthermore, an increasing body of evidence has demonstrated that microglia directly appose synaptic regions (presynaptic terminals and dendritic spines) [30] and, in response to neuronal activity, steer their processes toward active synapses, which facilitates contact with highly active neurons [31]. Now, microglia in the CNS are increasingly recognized as being crucial for sculpting the structure of the CNS, refining neuronal circuitry and network connectivity, and contributing to plasticity.

Neuropathic Pain and SDH Microglia

One of the most debilitating chronic pain conditions is neuropathic pain, which is often a consequence of nerve injury or of diseases such as cancer, diabetes mellitus and trauma. Symptoms of neuropathic pain are spontaneous pain, hyperalgesia (the increased pain perception of noxious stimuli), and mechanical allodynia (pain hypersensitivity to normally innocuous stimuli). For these symptoms, especially mechanical allodynia, the currently available treatment options are very limited because nonsteroidal anti-inflammatory drugs, and even opioids at doses that do not produce significant side effects, are often ineffective. In animal models of neuropathic pain in which a peripheral nerve is damaged, microglia become activated in the SDH. Morphologically, their cell bodies become hypertrophic with thickened and retracted processes, and cell numbers are increased [32–36]. Molecularly, SDH microglia dramatically alter the expressions of many genes, including integrins, ionized calcium-binding adapter molecule-1 (Iba1), neurotransmitter receptors and intracellular kinases [32, 37]. Microglial proliferation may require signaling involving interferon- γ (IFN- γ) and colony stimulating factor 1 (CSF1); it has been reported that IFN- γ receptor-knockout mice display a suppression of the nerve injury-induced microglial proliferation [38]. CSF1 expression has been reported to be induced in injured dorsal root ganglion (DRG) neurons at an early phase, and conditional deficiency of CSF1 in DRG neurons and pharmacological blockade of CSF1 receptors reduce proliferation of microglia [39, 40]. Alternatively, in BM chimeric mice, circulating BM-derived monocytes/macrophages infiltrate the SDH parenchyma after nerve injury and differentiate into microglia-like cells [41–44]. However, a recent study

that evaluated this issue by irradiation with various doses and also by parabiosis, demonstrated that BM-derived cells do not contribute to the population of SDH microglia after nerve injury [35]. A recent study using genetic mouse models in which microglia and peripheral monocytes/macrophages are differentially labeled [36] supports the view that the nerve injury-induced SDH microgliosis is associated with a local expansion of resident microglia due to their proliferation activity in response to nerve injury [35].

The causal role of microglia in the pathogenesis of neuropathic pain was first uncovered by studies investigating the role of the ionotropic purinergic receptor subtype P2X4 receptor (P2X4R) [45, 46]. P2X4R ion-channel is formed by three subunits [47, 48] and has almost equal permeability to Na^+ , K^+ and Ca^{2+} [49–51]. After nerve injury, SDH microglia upregulate expression of P2X4R, and pharmacological blockade and genetic knockout of P2X4R suppress the nerve injury-induced mechanical allodynia [45, 52, 53]. From the findings that intrathecal administration of P2X4R-stimulated cultured microglia to normal rats induces allodynia, it was indicated that P2X4R-expressing microglia are not only necessary but sufficient to produce mechanical allodynia [37, 45]. In other models (experimental autoimmune neuritis and herpetic pain), upregulation of P2X4R in SDH microglia has also been observed, the time course of which corresponds with mechanical allodynia [54, 55]. A recent study has reported that in female mice, there is no upregulation of spinal P2X4R expression after peripheral nerve injury (PNI) and no effect of pharmacological blockade of spinal P2X4Rs on the PNI-induced mechanical allodynia; instead, adaptive immune cells, likely T lymphocytes, might be involved [56], proposing that microglial pain processing involving P2X4R after PNI may only be essential in male mice. A similar sex difference has been reported in the cases of Toll-like receptor 4 [57] and p38 mitogen-activated protein kinase [58], both of which in spinal microglia have been shown to contribute to neuropathic pain in male [57, 59–61]. However, the upregulation of P2X4R in SDH microglia has also recently been shown in female mice of a herpetic pain model [55]. Thus, further work needs to be performed to clarify the sex-dependent contribution of SDH microglia in other models of neuropathic pain.

Microglia-selective upregulation of P2X4R expression is derived by transcriptional regulation. One of the key factors is interferon-regulatory factor 8 (IRF8), a member of the IRF family (IRF1–9), which is expressed in immune cells such as lymphocytes and dendritic cells [62]. Within the SDH, expression of IRF8 is upregulated exclusively in microglia after nerve injury [63]. In IRF8-expressing cultured microglia, expression of genes encoding P2X4R and other genes associated with reactive states is increased.

In IRF8-knockout mice, the nerve injury-induced P2X4R upregulation and mechanical allodynia are suppressed. Furthermore, IRF8 is a critical regulator of IRF5 expression [64, 65] that is also induced selectively in microglia after nerve injury [65]. Interestingly, IRF5 directly binds to the promoter region of the *P2rx4* gene to induce its expression. IRF5-knockout mice indeed show a reduction in the nerve injury-induced spinal P2X4R upregulation and allodynia [65]. Therefore, P2X4R-expressing microglia produced by nerve injury might be defined by activity of the IRF8–IRF5 transcriptional axis [65] (Fig. 1).

Several studies have proposed that possible sources of extracellular ATP in the SDH might be the primary sensory terminals [66–68], SDH neurons [66, 69, 70] or astrocytes [71]. In a recent study, it has been found that mice lacking vesicular nucleotide transporter (VNUT; also known as SLC17A9), a secretory vesicle protein responsible for the storage and release of ATP [72], have reduced nerve injury-induced allodynia [73]. Furthermore, using mouse lines with a conditionally disrupted VNUT gene in neurons and glial cells, the study revealed that VNUT expressed in SDH neurons, but not in astrocytes, microglia or primary afferents, contributes to the neuropathic allodynia and ATP release. In that study, VNUT-dependent exocytic ATP release from SDH neurons appears to be a crucial mechanism for neuropathic pain.

One of the mechanisms by which activated microglia alter the pain processing network within the SDH might

Fig. 1 Role of P2X4R-expressing spinal microglia in neuropathic pain. After nerve injury, microglia become hypertrophic and undergo proliferation. The activated microglia upregulate P2X4R expression through an IRF8–IRF5 transcriptional axis. IRF8 induces IRF5 expression, and then IRF5 directly binds to the promoter region of the *P2rx4* gene and induces expression of P2X4R mRNA. P2X4R is activated by extracellular ATP released from SDH neurons and, in turn, releases bioactive diffusible factors, such as BDNF. BDNF downregulates KCC2, which causes depolarization of these neurons following stimulation by GABA and glycine. The resultant hyperexcitability in the SDH pain network induced by microglial factors may be responsible for neuropathic pain.

involve microglia-derived signaling molecules, such as brain-derived neurotrophic factor (BDNF) [52, 74]. BDNF induces an altered transmembrane anion gradient in some lamina I SDH neurons by downregulating the $K^+ Cl^-$ cotransporter 2 (KCC2) [75], which causes changes in GABA- and glycine-evoked responses from inhibitory to excitatory, and mechanical allodynia [75]. BDNF also potentiates NMDA receptors through activation of TrkB and phosphorylation of the GluN2B subunit by the Src-family tyrosine kinase Fyn, and this process is primed by Cl^- -dependent disinhibition [76]. Other candidate signaling molecules are proinflammatory cytokines, such as IL-1 β , which modify excitatory or inhibitory synaptic transmission and neuronal activity in the superficial SDH neurons [77, 78] (Fig. 1).

From these findings, microglia are a potential target for drug discovery. Recently, the novel P2X4R antagonist NP-1815-PX (5-[3-(5-thioxo-4H-[1,2,4]oxadiazol-3-yl)phenyl]-1H-naphtho[1,2-b][1,4]diazepine-2,4(3H,5H)-dione) was discovered [55]. This compound is easily dissolved in water and inhibits rodent and human P2X4Rs with a high potency. Among the P2XRs tested, this compound was selective for P2X4R, and produced an antiallodynic effect in pathological chronic pain models without any alterations in acute physiological pain responses or motor coordination, which suggests a predicted therapeutic benefit of this antagonist.

Chronic Itch and SDH Astrocytes

Chronic itch is a debilitating symptom of skin diseases like atopic dermatitis, other systemic disorders, such as liver and kidney diseases or HIV/AIDS, and metabolic disorders [79–82]. Under such conditions, itch becomes more severe and chronic, which leads to excessive, repetitive scratching, thereby causing skin lesions that can worsen the itch sensation and lead to further scratching (the vicious itch–scratch cycle) [79, 80]. Chronic itch affects millions of individuals worldwide [83], but identifying the optimal treatment for it is a major clinical challenge.

Advances in understanding the mechanisms of acute and chronic itch have been achieved by the development of animal models. For chronic itch, several models have been established, most of which focus on cutaneous skin diseases, including atopic dermatitis, contact dermatitis and xerosis (dry skin) [84–86]. Recently, several studies have uncovered that glial cells in the SDH are crucial for itch chronicity. Astrocytes in the SDH show enlarged cell bodies and extensively arborized processes in a model of atopic dermatitis [87], which are well-known histological alterations of reactive astrocytes [88]. Such changes in SDH astrocytes have also been found in models of contact

dermatitis [87] and dry skin [89]. Thus, reactive astrogliosis in the SDH occurs under chronic itch conditions. It appears that astrocytic activation is not induced in all SDH segments because reactive astrocytes were observed mainly in cervical and trigeminal segments that correspond to the nape of neck, rostral back, ear and face where the itchy mice have extensively scratched and skin lesions have developed [87]. Thus, reactive astrocytes may be restricted to the segments corresponding to the lesioned itchy skin.

A clinically important element of chronic itch is the vicious cycle of itching and scratching. Interestingly, the time course of activation of SDH astrocytes seems to match that of the development and progression of the itch–scratch cycle [87]. Furthermore, preventing scratch-induced skin damage by trimming the toenails or by using an Elizabethan collar led to a recovery from their skin lesions, a suppression of reactive astrocytes, and a reduction in scratching [87, 89]. Thus, it is possible that astrocytic activation might require a signal from the skin. Tominaga *et al.* have demonstrated that hyperinnervation of primary afferent C fibers expressing the transient receptor potential cation channel subfamily V member 1 (TRPV1) to the epidermis occurs in lesioned itchy skin of CV-NC/Nga mice [90]. In patients with atopic dermatitis, the density of epidermal nerve fibers is higher in the skin [91, 92]. Pharmacological ablation of TRPV1-positive C fibers suppresses glial fibrillary acidic protein upregulation [87]. Now, what biologically active substances derived from primary afferents activate SDH astrocytes is a major issue for future investigations.

Recent studies have revealed the causal role of SDH astrocytes in chronic itch by investigating the transcription factor signal transducer and activator of transcription 3 (STAT3) [87] and the receptor toll-like receptor 4 (TLR4) [89]. Both are selectively activated and expressed in reactive astrocytes in models of chronic itch. Acute pharmacological inhibition of spinal STAT3 and conditional knockout of astrocytic STAT3 suppress scratching behaviors [87], implying that ongoing STAT3 activation is necessary for maintaining chronic itch. Liu *et al.* have shown that TLR4-knockout mice display reduced spontaneous scratching and touch-evoked scratching (alloknesis) in dry skin and contact dermatitis models [89]. Acute intrathecal treatment with a TLR4 antagonist and an astroglial toxin suppresses spontaneous scratching and alloknesis [89]. Thus, the above findings [87, 89] make a strong case that reactive astrocytes play a causal role in chronic itch.

A growing body of literature has revealed the existence of itch-specific neuronal circuitry in the nervous system [2, 3, 79, 85]. For example, mutant mice lacking gastrin-releasing peptide (GRP) receptors (GRPR) and mice with

Fig. 2 Role of SDH astrocytes in chronic itch. Under chronic itch conditions, scratching causes skin lesions, which in turn lead to activation of STAT3 in SDH astrocytes through primary afferent C fibers. STAT3 activation causes astrocytes to be reactive. TLR4 is also involved in activation of astrocytes. Reactive astrocytes produce and release LCN2. LCN2 then sensitizes an itch processing neuronal pathway involving GPR-GRPR signaling. Enhanced itch signaling leads to excessive scratching, worsening skin lesions, which maintains enhanced astroglial reactivity, and thereby contributes to the vicious cycle of itching and scratching, and to chronic itch.

ablated GRPR-expressing SDH neurons display a reduction in scratching behaviors without any changes in pain-related behavioral responses [93, 94], indicating GRP-GRPR signaling in the SDH as an itch-specific SDH circuit. This leads to the question of whether SDH astrocytes modulate this itch signaling. It has been reported that scratching evoked by intrathecal GRP is increased in chronic itch models, but the increase is abolished by pharmacological inhibition of astrocytes [87]. One candidate for astrocytic factors that modulate itch signaling might be lipocalin-2 (LCN2). Several studies have implicated LCN2 as a protein secreted from reactive astrocytes in response to neuronal inflammation [95–97] and in the modulation of neuronal excitability [98]. In models of chronic itch, LCN2 is upregulated in SDH astrocytes, which might require STAT3 [87]. Furthermore, the pivotal role of astrocytic LCN2 was demonstrated by SDH astrocyte-specific knock-down of LCN2 [87]. Intrathecal co-administration of LCN2 with GRP enhances GRP-evoked scratching. These results together indicate that LCN2 is an astrocytic factor that plays a pivotal role in the progression and maintenance of chronic itch (Fig. 2).

Conclusions

This review primarily focused on the role of microglia and astrocytes in chronic pain and itch, respectively. Of note, pharmacological, molecular, and genetic manipulations of the function or expression of microglial molecules, such as P2X4R [45, 53], and astrocytic STAT3 [87] and TLR4 [89], substantially influence chronic pain and itch behaviors and, importantly, have no effect on acute physiological

pain and itch behaviors under normal conditions. Thus, glial cells and their expressing molecules might be good targets for treating chronic pain and itch. However, several studies have shown that astrocytes in the SDH also become activated and contribute to the maintenance of mechanical pain hypersensitivity after nerve injury [99–102]. The functional difference of SDH astrocytes between chronic pain and itch conditions remains an open and important question, but there are a few possibilities. First, in contrast to neuropathic pain models [103], the primary afferent sensory neurons in the chronic itch models are not injured [87], suggesting that astrocytic activation in the SDH might be associated with injury of primary afferents in neuropathic pain models, but not in chronic itch models. Second, nerve injury causes not only reactive astrocytes, but also a dramatic activation of microglia [104], which is in contrast to chronic itch models [87]. Activated microglia are powerful modulators of the functions of astrocytes and neurons [105–107], and a recent study has demonstrated that a neurotoxic reactive phenotype of astrocytes is induced by activated microglia-secreted factors [108]. Therefore, it has been speculated that the reactive phenotypes of SDH astrocytes may be different between neuropathic pain and chronic itch conditions. Thus, it is expected that an increased understanding of the functions of SDH microglia and astrocytes will provide us with exciting insights into the mechanisms for chronicity of pain and itch, and also provide clues to develop novel therapeutic agents for treating chronic pain and itch.

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