1. Introduction

Pain is an unpleasant sensation known to most people and can be divided into two categories: physiological (acute) and pathological (chronic) pain. Physiological pain is transient and necessary for the alarm system that warns us and helps to protect the body from tissue damage, while pathological pain is usually persistent and unnecessary for survival via pathologically altered pain pathways. Pathological pain is characterized by spontaneous pain, hyperalgesia (a heightened response to a noxious stimulus), and allodynia (a painful response to a usually innocuous stimulus), which spread to adjacent non-injured regions. Current therapy for pathological pain is applied as a symptomatic treatment with analgesics that have generally been screened for use in physiological pain. However, pathological pain, especially neuropathic pain, which is based on a maladaptive plasticity caused by damage to peripheral or central nerves, is often resistant to existing analgesics. To develop novel therapeutic strategies for pathological pain, a better understanding of the molecular and cellular mechanisms underlying pathological pain is required to fundamentally inhibit its induction and maintenance.

Pathological pain is believed to result from the increased sensitivity of nociceptive primary afferent neurons (peripheral sensitization) and hyperexcitability of nociceptive neurons in the dorsal horn of the spinal cord (central sensitization) (1). Peripheral nerve injury or inflammation can cause increased sensitivity of nociceptive primary afferent neurons and can convert non-nociceptive neurons (Aβ fibers) to nociceptive ones. Peripheral sensitization is involved in the recruitment and activation of peripheral immune cells, such as macrophages, neutrophils, T lymphocytes, and mast cells, at the nerve lesion site and in the dorsal root ganglion, as well as of the pe...
2. Activation of spinal microglia and astrocytes in pathological pain

Microglia are bone marrow-derived hematopoietic cells that invade the central nervous system (CNS) during embryonic development. Activated microglia exhibit a morphological change from a ramified shape to an amoeboid shape, an increase in proliferation, and an up-regulation of complement receptor 3, also known as CD11b/CD18, and Iba1. They also undergo functional changes including migration, phagocytosis, and the production and release of diverse factors including proinflammatory cytokines, chemokines, growth factors, nitric oxide, and prostaglandins. Astrocytes are the most abundant cells in the CNS and modulate synaptic transduction through astrocyte-neuron interactions. Activated astrocytes exhibit hypertrophic morphology with thick processes and up-regulation of glial fibrillary acidic protein (GFAP) and S100B expression. Garrison and colleagues first reported that astrocytes in the spinal cord are activated by chronic constriction injury of the sciatic nerve (CCI), an animal model of neuropathic pain (6). Activation of spinal astrocytes spreads to the spinal lamina both rostrally and caudally from where the nociceptive primary afferent neurons enter the spinal cord. Subsequent studies have demonstrated that both spinal microglia and astrocytes are activated in diverse models of pathological pain (7 – 10). However, the time courses of spinal microglial and astrocytic activation are different following nerve injury. It has been shown using OX-42, a microglial activation marker, and GFAP, an astrocytic activation marker, that early spinal microglial activation and delayed spinal astrocytic activation follow nerve injury/inflammation (9, 11, 12). Even after microglial activation begins to decrease, astrocytic activation persists, as does neuropathic pain (13). This temporal pattern is further confirmed by sequential activation of mitogen-activated protein kinase (MAPK) / extracellular signal-regulated kinase (ERK) in microglia and then in astrocytes following spinal nerve ligation (SNL) (14). Similarly, early phosphorylation of p-38 MAPK in microglia, followed by slow and persistent phosphorylation of c-Jun N-terminal kinase (JNK) in astrocytes, was observed in the spinal cord following SNL (15). Pharmacologically, minocycline, a tetracycline antibiotic that has been used as a microglial inhibitor, prevents the induction of pathological pain, while it typically does not reverse established allodynia and hyperalgesia following nerve injury/inflammation (9, 16). However, there are many reports that inhibiting microglial function reversed established neuropathic pain even several weeks after injury (17, 18). Astrocytic inhibitors, such as fluorocitrate, propentofylline, and L-α-amino adipate, which disrupt the function of astrocytes, as well as microglia, by inhibiting glial metabolism, attenuate both the induction and maintenance of allodynia and hyperalgesia in pathological pain models (9, 10, 15, 19). Furthermore, intrathecal infusion of a JNK peptide inhibitor prevented and reversed SNL-induced mechanical allodynia (15). Taken together, these findings suggest that early activation of microglia and subsequent long-term activation of astrocytes contribute to induction and maintenance of the pathological pain. However, we found that intrathecal ATP-induced long-lasting allodynia persisted even when the activation of spinal astrocytic activation began to decrease, which suggests that the later phase of the maintenance of the persistent pain state may be not mediated by spinal astrocytic activation (9).

3. Signals involved in the activation of spinal astrocytes

Recent evidence suggests that the activation of spinal microglia following nerve injury/inflammation is caused by CX3CL1 (fractalkine) and CCL2 (MCP-1), which are produced and released from injured or intensely excited sensory neurons, probably in an activity-dependent manner. These two chemokines act on their receptors, CX3CR1 and CCR2, respectively, the expressions of which are enhanced in activated microglia (4, 5, 20, 21). Indeed, intrathecal administration of CCL2 (MCP-1) evoked mechanical allodynia (20), and CCR2-deficient mice showed a reduced pain response and spinal microglial activation in a neuropathic pain model (22). Furthermore, some toll-like receptors (TLRs), such as TLR2, TLR3, and TLR4, play crucial roles in microglial activation following peripheral nerve injury (2, 5, 23). It has
been shown that fibronectin, an endogenous TLR4 ligand, is induced following peripheral nerve injury, which leads to the up-regulation of P2X4, a purinoceptor expressed in microglia (17, 24). Thus, these “neuron-to-microglia” signals have been well documented (3 – 5) (Fig. 1).

In contrast, the signals that activate spinal astrocytes following nerve injury/inflammation are still unclear. Astrocytes possess functional receptors for nociceptive neurotransmitters and neuromodulators, such as glutamate, substance P, calcitonin gene-related peptide (CGRP), ATP, and prostaglandins, which may lead to morphological changes, GFAP up-regulation, and proliferation of spinal astrocytes in response to intense and persistent noxious stimuli. Indeed, it has been reported that an NMDA-receptor antagonist prevented spinal astrocytic activation and mechanical allodynia following CCI (8). Prolonged neuronal depolarization following

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**Fig. 1.** A summary of the roles of spinal astrocytes and microglia in pathological pain. Following peripheral nerve inflammation or injury, nociceptive neurotransmitters or neuromodulators (glutamate, substance P, CGRP, etc.) are released from the central nerve terminals of the primary afferent neurons and act on spinal astrocytes as neuron-to-astrocyte signals. Simultaneously, neuron-to-microglial signals, such as CXC3L1 and CCL2, are produced and released from injured or uninjured primary afferent neurons and activate spinal microglia. Then, proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and IL-18, are produced and released from activated microglia; and they act on their receptors expressed on neighboring spinal astrocytes as microglia-to-astrocyte signals. Proinflammatory cytokines and chemokines, such as CCL2, are produced and released from activated astrocytes, act on their receptors expressed on superficial dorsal horn neurons as astrocyte-to-neuron signals, and facilitate pain transmission by enhancing the function of NMDA and AMPA receptors. The glial glutamate transporters GLT-1 and GLAST are down-regulated in spinal astrocytes following peripheral nerve inflammation/injury. Decreased glutamate uptake may enhance glutamatergic synaptic neurotransmission and subsequent glutamate-related neuronal adaptation (central sensitization) related to pathological pain. AQP4: aquaporin 4, ERK: extracellular signal-regulated kinase, GlyT: glycine transporter, JNK: c-Jun N-terminal kinase, MMP: matrix metalloprotease, TLRs: toll-like receptors.
intense and persistent nerve injury elevates the extracellular concentration of K+ ions, which may contribute to spinal astrocytic activation. The K+ ions are taken up by neighboring astrocytes, which depolarize and swell as a result of osmotic water influx. It has been shown that aquaporin 4, the most abundant water channel in the CNS, is up-regulated in spinal astrocytes following spinal cord injury and is involved in pathological pain through astrocytic swelling–induced glutamate release (25). Given that microglial activation precedes astrocytic activation in diverse models of pathological pain, as described above, it is possible that early factors released from activated microglia lead to astrocytic activation. One candidate “microglia-to-astrocyte” signal is the proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, which are produced and released from activated microglia. Astrocytes possess receptors for these cytokines and are activated in response to proinflammatory cytokines. In addition, Miyoshi et al. reported that peripheral nerve injury up-regulated IL-18 in activated microglia and the IL-18 receptor in activated astrocytes. Functional blockade of IL-18 signaling attenuated both mechanical allodynia and GFAP up-regulation in spinal astrocytes, suggesting that IL-18/IL-18 receptor is a strong candidate for the “microglia-to-astrocyte” signals in pathological pain (26) (Fig. 1).

4. Astrocytic modulation of pathological pain

Astrocytic activation, as well as microglial activation, leads to the production and release of proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α; prostaglandins; nitric oxide; ATP; d-serine; and glutamate (5). These astrocytic mediators can modulate neuronal activity and enhance the pain pathway as “astrocyte-to-neuron” signals (Fig. 1). It has been reported that proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, can directly act on their receptors expressed on superficial dorsal horn neurons to enhance excitatory synaptic transmission and/or reduce inhibitory synaptic transmission (27). IL-1 receptor type I is co-localized with NR1, a subunit of the NMDA receptor, on spinal dorsal horn neurons. IL-1β is produced primarily in spinal astrocytes, but not in microglia and neurons, and it enhances NR1 phosphorylation in inflammatory pain models. Intrathecal administration of an IL-1–receptor antagonist (IL-1ra) has been shown to attenuate inflammatory hyperalgesia and NR1 phosphorylation (28). In at least some pathological pain models, the source of IL-1β is likely to be spinal astrocytes, although it has been reported that IL-1β is also produced in microglia in other pathological pain and neurodegenerative disease models. In regard to IL-1β production, matrix metalloprotease (MMP)-9 is up-regulated in primary sensory neurons following peripheral nerve injury, which contributes to microglial activation and the induction of pathological pain via the active cleavage of IL-1β in the early phase. In contrast, MMP-2 is persistently up-regulated in spinal astrocytes and contributes to the maintenance of pathological pain via active cleavage of IL-1β in the late phase (29). Furthermore, it has been shown that TNF-α released from astrocytes (astrocyte-conditioned medium) can enhance synaptic efficacy by increasing cell-surface expression of AMPA receptors (30). Recently, Gao et al. reported that SNL induces CCL2 (MCP-1) up-regulation in the spinal cord, as well as in primary sensory neurons, and that CCL2-positive spinal cells are astrocytes. CCR2, a CCL2 receptor, is up-regulated in neurons, as well as in microglia (22), following SNL. Furthermore, CCL2 has been shown to not only enhance spontaneous excitatory synaptic transmission, but also to potentiate NMDA- and AMPA-induced currents in spinal dorsal horn neurons (31). Taken together, CCL2/CCR2 is a new candidate for the “astrocyte-to-neuron” signals involved in central sensitization of pathological pain.

5. Astrocytic glutamate and glycine transporters in pathological pain

Astrocytes play an essential role in synaptic transmission through rapid removal of extracellular amino acids, especially glutamate, from the synaptic cleft by high-affinity, Na+-dependent glutamate transporters (excitatory amino acid transporters, EAATs). This process maintains the extracellular glutamate concentration in the physiological range, preventing glutamate overexcitation and neurotoxicity that can occur under a variety of pathological conditions, and modulates glutamate-mediated neuronal plasticity. Furthermore, astrocytes are able to specifically metabolize incorporated glutamate into glutamine with the enzyme glutamine synthetase to return glutamine to neurons (the glutamate–glutamine shuttle). To date, five subtypes of EAATs have been cloned and characterized in neurons (EAAC1/EAAT3, EAAT4, and EAAT5) and glial cells (GLT-1/EAAT2 and GLAST/EAAT1). Among these, the glial EAATs, GLT-1 and GLAST, are localized almost exclusively in astrocytic processes in the vicinity of the synaptic cleft and represent the predominant route for the clearance of extracellular glutamate. In the spinal cord, glutamate plays a key role in normal pain transmission as a nociceptive transmitter and in the induction of central sensitization, a neuronal plasticity-based event. Several lines of evidence suggest that glutamate transporters have important roles in physiological and pathological pain (32). Pharmaco-
logical inhibition of glutamate transporters in the spinal cords of normal animals leads to spontaneous nociceptive behaviors and hyperalgesia to mechanical and thermal nociceptive stimuli through facilitation of spinal glutamatergic synaptic activity (33). These findings suggest that glutamate uptake through spinal EAATs plays an important role in maintaining normal pain transmission under physiological conditions. Furthermore, it has been shown that the down-regulation or functional deficiency of glial glutamate transporters in the spinal dorsal horn is associated with diverse models of pathological pain (32, 34, 35). We have examined the effects of GLT-1 gene transfer into the rat spinal cord using recombinant adenoviruses in inflammatory and neuropathic pain models. Intraspinal infusion of adenoviral vectors expressing the GLT-1 gene increased GLT-1 expression, and transgene expression was primarily localized to astrocytes, rather than to microglia or neurons. Spinal GLT-1 gene transfer had no effect on acute thermal and mechanical nociceptive responses but it prevented the induction of carrageenan-induced inflammatory hyperalgesia and partial sciatic nerve ligation–induced mechanical allodynia (36). Consistent with our findings, riluzole, which reduces extracellular glutamate, at least in part, by activating EAATs, inhibited the induction of inflammatory and neuropathic pain (34). Recently, it was reported that ceftriaxone, a β-lactam antibiotic shown to selectively up-regulate GLT-1 expression, prevented the induction of mechanical allodynia, as well as astrocytic activation, in neuropathic pain models (37). Taken together, these findings support the notion that the decreased expression or function of astrocytic EAATs in the spinal cord resulting from peripheral nerve injury/inflammation can enhance glutamatergic synaptic neuronal transmission and subsequent glutamate-related neuronal adaptation related to pathological pain. It has been reported that propentofylline, an atypical methylxanthine shown to attenuate astrocytic activation as well as pathological pain (10, 19), induced the expression of GLT-1 and GLAST in the spinal dorsal horn following spinal nerve transection (35). Furthermore, repeated administration of amitriptyline, a tricyclic antidepressant used as a first-line drug for the treatment of neuropathic pain, reversed the down-regulation of GLT-1 and GLAST in spared nerve injury–model rats (38). The anti-allodynic effects of tricyclic antidepressants are considered to be due to the inhibition of serotonin/noradrenaline reuptake at serotonergic and noradrenergic nerve terminals, but they may also be due, at least in part, to the up-regulation of EAATs in spinal astrocytes. Glutamate-receptor antagonists are effective in reducing pathological pain in animal models and clinical settings, but their usefulness is limited by adverse side effects. Thus, the upregulation or functional enhancement of EAATs in spinal astrocytes may be a better strategy for the prevention of pathological pain (Fig. 1).

Accumulating evidence suggests that the disinhibition of inhibitory glycnergic neurons in the spinal dorsal horn is implicated in the generation of pathological pain. Glycine transporters (GlyT)1 and GlyT2, which are located in astrocytes and glycnergic neurons, respectively, play important roles in supplying glycine to glycnergic neurons. It has been shown that both GlyT1 and GlyT2 inhibitors produce potent and long-lasting anti-allodynic effects in inflammatory and neuropathic pain models (39). GlyT1 is a promising candidate for therapy targeting spinal astrocytes for pathological pain relief (Fig. 1).

6. Conclusions

Most available analgesics for pathological pain are symptomatic, but there are few analgesics based on pathological mechanisms. In the past decade, many studies have focused on the role of spinal microglia and astrocytes in pain, and these cells have been recently recognized as key players in pathological pain. As described above, it is likely that early activation of microglia and subsequent long-term activation of astrocytes contribute to the induction and maintenance of the pathological pain, suggesting that drugs targeting astrocytes may be appropriate for reducing the persistent pain state. However, the role of microglia has been widely studied, while it still remains unclear how spinal astrocytes are activated following peripheral nerve inflammation/injury, and it is not known which molecules on astrocytes are suitable therapeutic targets for pathological pain (Fig. 1). Further understanding of the molecular and cellular mechanisms underlying the role of spinal astrocytes may therefore reveal potential therapeutic targets for pathological pain.

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