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Activation of JNK pathway in persistent pain

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ABSTRACT

The c-Jun N-terminal kinase (JNK) is a stress-activated member of MAP kinase family. JNK activation has been strongly implicated in inflammatory responses, neurodegeneration, and apoptosis. Recent evidence shows that JNK pathway is also transiently activated in primary sensory neurons after tissue or nerve injury, which is required for the development of hyperalgesia and allodynia. In particular, JNK is persistently activated in astrocytes of the spinal cord after nerve injury, and this activation can maintain central sensitization and mechanical allodynia. In this mini-review, we will provide evidence for the involvement of JNK pathway in regulating persistent pain sensitization. We will also discuss possible upstream signaling mechanisms that cause JNK activation and downstream signaling mechanisms by which JNK modulates pain sensitivity. Thus, targeting JNK pathway might be a useful strategy to treat both neurodegeneration and chronic pain.

Chronic pain, resulting from tissue injury (inflammatory pain) or nerve damage (neuropathic pain) or tumor growth (cancer pain), is a real clinical challenge [10,21,29]. Although it is generally believed that chronic pain is caused by neural plasticity that occurs both in the peripheral nervous system (peripheral sensitization) and central nervous system (central sensitization), the mechanisms underlying the induction and maintenance of chronic pain are incompletely understood [15,45]. Inflammation is a driving force for the pathogenesis of chronic pain by producing multiple inflammatory mediators such as prostaglandin (PGE_2), nerve growth factor (NGF), and proinflammatory cytokines (e.g., tumor necrosis factor-α (TNF-α) and interleukin-1beta (IL-1β)) in inflamed or damaged tissues [16,36]. These inflammatory mediators are not only produced by peripheral tissues, but also by glial cells (e.g., microglia and astrocytes) in the central nervous system. Recently, accumulating evidence supports an important role of spinal microglia and astrocytes in promoting chronic pain [8,14,40,42].

In particular, mitogen-activated protein kinases (MAPKs) are activated in spinal glia after nerve injury and play an important role in chronic pain sensitization by signaling to the inflammatory mediators. First, MAPK pathways are activated by different inflammatory mediators. Second, activation of MAPK pathways also increases the synthesis of multiple inflammatory mediators. This positive feedback loop leads to more production of inflammatory mediators [16]. There are three major members in the MAPK family, extracellular signal regulated kinase (ERK), p38, and c-Jun N-terminal kinases (JNK) [19]. Recent studies have demonstrated that ERK and p38 MAPKs play important roles in regulating persistent pain sensitization via both neuronal and glial mechanisms [16,18,31,35].

Compared to ERK and p38, less is known about JNK control of pain. JNK is activated by upstream kinase MKK4/7, following cellular stress (e.g., heat shock), direct DNA damage, and reactive oxygen species, proinflammatory cytokines, rise of intracellular Ca^{2+} and under several neurodegenerative conditions [4]. JNK plays an important role in inducing neuronal apoptosis; inhibition of JNK pathway protects against cerebral ischemia and other neurodegenerative conditions [4]. Peripheral neuropathy is a common neurological symptom in AIDS patients. Treatment of neonatal dorsal root ganglion (DRG) neurons with HIV envelope glycoprotein gp120 produces apoptosis, which can be blocked by inhibiting JNK pathway [2]. The JNK family consists of three genes, jnk1, jnk2 and jnk3. JNK3 is found primarily in brain and has different functions from JNK1 or JNK2 [24]. In a sciatic axotomy model of neuronal injury, death of neonatal DRG neurons is reduced by JNK3 deficiency [22]. Accumulating evidence suggests that JNK also plays a role in the development and maintenance of chronic pain.

Most studies on JNK pathway in DRG neurons are related to apoptosis [2,22,41]. Interestingly, activation of DRG JNK only causes death of neonatal neurons but not adult neurons, indicating that adult neurons have a downstream block to apoptosis [41]. After L5-

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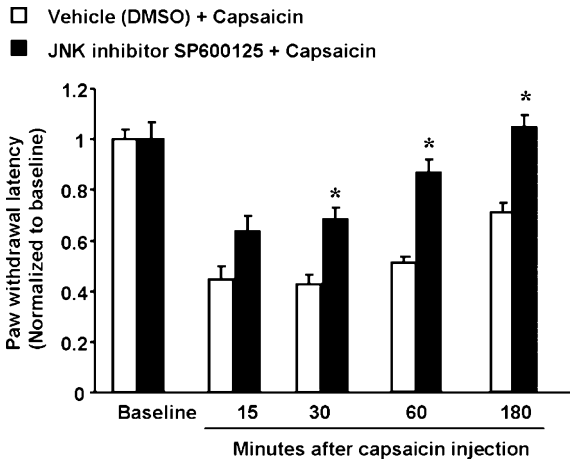

Fig. 1. Prevention of capsaicin-induced heat hyperalgesia by JNK inhibition. Intraplantar injection of the JNK inhibitor SP600125 (5 μg in 20 μl) inhibits capsaicin-induced heat hyperalgesia. Capsaicin (15 μg in 5 μl) was injected into the same site of hindpaw 20 min after SP600125 injection. The heat hyperalgesia was measured using Hargreaves apparatus (radiant heat) and paw withdrawal latencies from 15 to 180 min after capsaicin stimulation were determined. $^{*}P < 0.05$, compared to corresponding vehicle control (10% DMSO), $n = 6$, t-test. The baseline paw withdrawal latency is adjusted to 12–15 s.

spinal nerve ligation (SNL) in adult rats, there is a rapid (<1 day) but transient (<10 days) JNK activation in L5-DRG neurons [49]. This activation does not cause apoptosis, because death of DRG neurons is not noticeable in the first several weeks after nerve injury [39]. After nerve injury, JNK activation primarily occurs in small diameter C-fiber neurons [31,49]. This transient JNK activation is important for the early development of neuropathic pain, because DRG infusion of the peptide JNK inhibitor D-JNKI-1 prevents the development of SNL-induced mechanical allodynia for a week but does not reverse mechanical allodynia [49].

Although JNK activation in DRG neurons is very prominent in nerve injury conditions, acute inflammation may also cause moderate and transient activation of JNK [9]. Intraplantar injection of capsaicin induces a rapid (within 15 min) heat hyperalgesia that recovers after 3 h. This hyperalgesia is prevented by the JNK inhibitor SP600125 (Fig. 1). JNK activation in nociceptor terminals/axons after skin injury is likely to cause the acute hyperalgesia. Moreover, acute hyperalgesia induced by intraplantar endothelin-1, complete Freund's adjuvant, is also suppressed by SP600125 [9,30].

Nerve injury is known to activate both microglia and astrocytes in the spinal cord, but with different time courses. The persistent expression of astrocytic markers correlates well with the persistence of neuropathic pain symptom such as mechanical allodynia [5,8,14]. Intrathecal injection of L-alpha-aminoadipate, a relatively specific cytotoxin for astrocytes, was shown to reverse nerve injury-induced mechanical allodynia, supporting a role of spinal astrocytes in maintaining neuropathic pain symptoms [49].

In contrast to transient JNK activation in the DRG, spinal nerve ligation (SNL) induces persistent activation (>3 weeks) of JNK in the spinal cord [49]. Although both JNK1 and JNK2 are constitutively expressed in the spinal cord, only phosphorylated JNK1 (pJNK1, active form) is expressed in the spinal cord and increased after SNL. Double immunofluorescence has shown that JNK1 is expressed in spinal astrocytes, because JNK1 is colocalized with GFAP (astrocytic marker) but not with NeuN (neuronal marker) or OX-42 (microglial marker) [49]. In primary astrocyte cultures, pJNK1 is the predominant isoform compared to pJNK2 [14]. In the intact spinal cord, pJNK is found almost exclusively in astrocytes, though not all astrocytes expressed pJNK. JNK activation also occurs in spinal astrocytes after partial sciatic nerve injury [26]. Further, we found a marked activation of JNK1 in spinal astrocytes in another chronic pain condition following adjuvant-induced inflammation (Gao and Ji, unpublished data).

To determine the role of JNK in chronic pain, we used a potent and highly specific peptide inhibitor of JNK [4]. Most MAPK inhibitors are small molecules, designed to target the ATP binding sites of these kinases. However, the peptide inhibitor is derived from JNK binding domain of JNK-interacting protein-1 (JIP-1), and can block selectively the access of JNK to c-Jun and other substrates by a competitive mechanism. A TAT sequence (transporter sequence) is linked to the peptide, enabling the peptide membrane permeable. A convert to D-form amino acids further makes the peptide resistant to proteinases. This peptide inhibitor, named as D-JNKI-1 (D-form JNK inhibitor-1), is extremely potent in neuroprotection [4]. Spinal infusion of D-JNKI-1 prevents mechanical allodynia for more than 10 days [49]. Because JNK is also activated transiently in primary sensory neurons, the preventive effect of D-JNKI-1 in the first few days might be mediated by JNK activation in the DRG. However, the late-phase allodynia is predominantly, if not exclusively, mediated by JNK activation in the spinal cord. A bolus injection of D-JNKI-1, given 10 days after nerve injury, can block mechanical allodynia for more than 12 h. D-JNKI-1 effect is more potent and prolonged than that of SP600125 [49], which has been shown to suppress neuropathic pain in diabetes animals [6].

What are the upstream mechanisms that cause JNK activation? Full activation of JNK requires dual phosphorylation of threonine and tyrosine residues by the MAPK kinases (MAP2Ks), MKK4 and MKK7. These kinases are in turn activated by the MAPK kinase kinases (MAP3Ks), such as MLKs, ASK1, TAK1, MEKK1, and MEKK4. These MAP3Ks are activated by upstream kinases including cdc42, Rac1, PAK1 that link to a variety of cell receptors sensing stress and inflammation (Fig. 2) [3,7]. Therefore, a diversity of stimuli can acti-

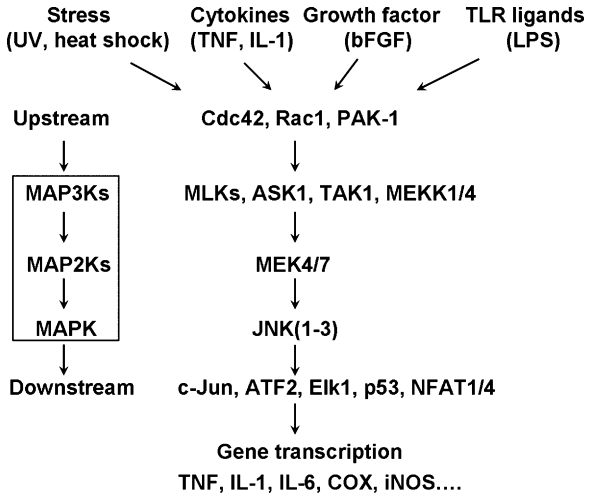

Fig. 2. Signal transduction of JNK pathway. JNK family consists of three genes: jnk1, jnk2, and jnk3. While JNK1 is the dominant form in the spinal cord (especially in astrocytes), other forms may exist in the DRG. The JNK pathway can be activated by the exposure of cells to a diversity of extracellular stimuli, such as stress (UV irradiation, heat shock), inflammatory cytokines (TNF-α, IL-1β), growth factors (bFGF, TGF), as well as by the activation of Toll-like receptors. The activated receptors are communicated to upstream kinases of the JNK/MAPK cascade, such as Cdc42, Rac1, PAK-1, p21Rho-GTPase. This MAPK cascade includes various members of the MAP3Ks (such as MLKs, ASK1, TAK1, MEKK1, and MEKK4) and the MAP2Ks MKK4 and MKK7 and leads to the activation of JNK. The activated JNKs translocate to the nucleus and phosphorylate a range of substrates, such as the transcription factors c-Jun, Elk-1, p53, ATF-2, c-Myc, and the NFAT family, leading to gene transcription. The protein products of these genes (e.g., TNF-α, IL-1β, COX1/2) will enhance pain sensitivity. In addition to transcriptional regulation, non-transcriptional regulation can also be involved.