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Protein Kinases as Potential Targets for the Treatment of Pathological Pain

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Abstract Pathological pain or clinical pain refers to tissue injury-induced inflammatory pain and nerve injury-induced neuropathic pain and is often chronic. Pathological pain is an expression of neural plasticity that occurs both in the peripheral nervous system (e.g., primary sensory nociceptors), termed peripheral sensitization, and in the central nervous system (e.g., dorsal horn and brain neurons), termed central sensitization. Our insufficient understanding of mechanisms underlying the induction and maintenance of injury-induced neuronal plasticity hinders successful treatment for pathological pain.

The human genome encodes 518 protein kinases, representing one of the largest protein families. There is growing interest in developing protein kinase inhibitors for the treatment of a number of diseases. Although protein kinases were not favored as targets for analgesics, studies in the last decade have demonstrated important roles of these kinases in regulating neuronal plasticity and pain sensitization. Multiple protein kinases have been implicated in peripheral and central sensitization following intense noxious stimuli and injuries. In particular, mitogen-activated protein kinases (MAPKs), consisting of extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), are downstream to many kinases and are activated in primary sensory and dorsal horn neurons by nociceptive activity, growth factors and inflammatory mediators, contributing to the induction and maintenance of pain sensitization via posttranslational, translational, and transcriptional regulation. MAPKs are also activated in spinal glial cells (microglia and astrocytes) after injuries, leading to the synthesis of inflammatory mediators/neuroactive substances that act on nociceptive neurons, enhancing and prolonging pain sensitization. Inhibition of multiple kinases has been shown to attenuate inflammatory and neuropathic pain in different animal models. Development of specific inhibitors for protein kinases to target neurons and glial cells will shed light on the development of new therapies for debilitating chronic pain.

Keywords Neural plasticity · MAP kinases · Phosphorylation · Primary sensory neurons · Spinal cord · Glia

1
Pathological Pain and Neural Plasticity

1.1
Physiological and Pathological Pain

During evolution, living organisms develop a specialized apparatus called a nociceptor, detecting harmful stimuli from the environment. Intense noxious stimulation of high-threshold nociceptors will elicit a pain sensation. Nociception is initiated from the action potential generated in the peripheral nociceptor terminal, conducted via thin unmyelinated C fibers and myelinated Aδ fibers to the spinal cord dorsal horn, thalamus, and cortex. This acute pain sensation in normal conditions is called physiological pain, and has a protective role that warns of potential tissue damage in response to a noxious stimulus. Many pain transduction molecules have recently been identified, including the thermal receptors TRVP1 (transient receptor potential ion channel family; also called vanilloid receptor 1, VR1), TRPV2, TRPM8, and TRPA1, the mechanoreceptors DEG (degenerin), DRASIC (dorsal root acid-sensing ion channel), and TREK-1 (TWIK-related K^+ channel-1), and the chemical receptor P2X3 (Scholz and Woolf 2002).

In contrast to physiological pain, pathological pain or clinical pain is caused by tissue and nerve injuries. Pathological pain is usually chronic and mainly divided into inflammatory pain and neuropathic pain. Inflammatory pain is a pain related to peripheral tissue damage/inflammation (e.g., arthritis pain). In animal models this pain is usually produced by injection of irritative sub-

stances such as formalin, carrageenan, or complete Freund's adjuvant (CFA) into a hindpaw or joint of rats and mice (Stein et al. 1988), with a duration ranging from hours to days and weeks. Neuropathic pain is a pain caused by damage or dysfunction of the peripheral nervous system (PNS) and CNS. In animal models neuropathic pain is often induced by a partial lesion of the sciatic nerve and its branches in rats and mice, and this pain can last for many weeks to several months (Bennett and Xie 1988; Kim and Chung 1992; Decosterd and Woolf 2000). In addition, animal models have been developed for cancer pain and postoperative pain (incisional pain), which share some features with inflammatory and neuropathic pain but also have their distinct characteristics (Brennan et al. 1996; Mantyh et al. 2002). Pathological pain is typically characterized by hyperalgesia (increased responsiveness to noxious stimuli) and allodynia (painful responses to normally innocuous stimuli), as well as by spontaneous pain. Pain hypersensitivity is not only produced in the injured tissue or territory (innervated by the injured nerve), but also spread to the adjacent noninjured regions or the extra-territory.

1.2 Neural Plasticity: Peripheral and Central Sensitization

Pathological pain is caused by altered sensitivity in both the PNS and CNS. It is an expression of neural plasticity, an adaptation of nervous system in response to external stimuli. This plasticity also occurs in other parts of the nervous system during learning and memory and drug addiction (Ji et al. 2003).

During tissue injury and inflammation, inflammatory mediators (IFMs) such as prostaglandin E_2 (PGE_2), 5-HT, bradykinin, ATP, protons, nerve growth factor (NGF), lipids, and proinflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are released from inflammatory cells, nerve terminals, and surrounding non-neural tissues, or from damaged axons and their enclosing Schwann cells. The soma and axons of primary sensory neurons express receptors for these IFMs, and activation of the receptors leads to the activation of multiple intracellular signaling pathways, increasing the sensitivity and excitability of nociceptors. A sensitivity increase in the PNS (e.g., primary sensory neurons) is called peripheral sensitization. Various types of ion channels such as TRPV1, TRPV2, TRPM8, DEG, P2X3, acid-sensitive channel (ASIC), and tetrodotoxin (TTX)-resistant sodium channels (TTX_R, including $Na_v1.8$ and 1.9), are expressed in primary sensory neurons in the dorsal root ganglion (DRG) or trigeminal ganglion. The sensitivity of these channels can be regulated by IFMs. Current studies focus on TRPV1 and TTX_R, which are known to be essential for the induction of peripheral sensitization and pain hypersensitivity (Julius and Basbaum 2001).

Activation of peripheral nociceptors by high-threshold and persistent noxious stimuli (heat, mechanical, electrical) or by IFM (chemical stimuli) also results in an activity- or use-dependent neuronal plasticity in the CNS. This

plasticity modifies the performance of nociceptive pathway by enhancing and prolonging the responses to subsequent peripheral stimuli. These changes in the spinal cord, as well as in the brain, are referred to central sensitization (Woolf and Salter 2000). Although central sensitization is often but not always initiated by peripheral sensitization, it is responsible for the pain after injury by normally innocuous low-threshold afferent inputs (allodynia) and the spread of pain hypersensitivity to regions beyond injured tissue. In chronic pain conditions, when injury-induced peripheral sensitization becomes less significant after wound healing, central sensitization could still be self-maintained, contributing importantly to the persistence of chronic pain. Current studies on central sensitization focus on dorsal horn neurons. Spontaneous activity is generated in primary sensory neurons after tissue injury, leading to the release of the neurotransmitter glutamate from central terminals of these neurons and subsequent activation of postsynaptic glutamate receptors in dorsal horn neurons. While glutamate AMPA (*S*-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor is important for normal synaptic transmission, *N*-methyl-D-aspartate (NMDA) receptor is essential for synaptic plasticity after tissue injury, underlying central sensitization. Further, injury also releases the neuropeptide substance P and the neurotrophin BDNF (brain-derived neurotrophic factor), acting on neurokinin-1 (NK-1) and tyrosine kinase receptor (TrkB), respectively in dorsal horn neurons, also contributing to central sensitization (Woolf and Salter 2000; Ji et al. 2003).

2 Protein Kinases and Pain Sensitization

Nearly 50 years ago, protein phosphorylation was identified as a regulatory mechanism for the control of glycogen metabolism. It took many years before its general significance came to be appreciated. Protein kinases almost regulate every aspect of cell life from growth to death. They mediate most of the signal transduction in eukaryotic cells by regulating substrate activity via phosphorylation. Protein kinases are among the largest families of genes and have been intensively studied. The human genome encodes 518 protein kinases, accounting for 1.7% of all human genes (Manning et al. 2002). These kinases share a catalytic domain conserved in sequence and structure but also notably different in how their catalysis is regulated. The ATP binding pocket together with less-conserved surrounding pockets has been the focus of inhibitor design. Multiple protein kinases have been implicated in pain sensitization (Table 1). Importantly, inhibitors of protein kinases do not affect physiological pain: the normal pain threshold in response to mechanical or thermal stimulus remains unchanged. The serine-threonine kinases consist of most protein kinases, and many of serine-threonine kinases are involved in pain regulation (Table 1). There are also 90 tyrosine kinases in the human

Table 1 A list of protein kinases that are known to be involved in inflammatory pain (IP), neuropathic pain (NP), peripheral sensitization (PS), and central sensitization (CS). See Sects. 2.1–2.3 for more details

	IP	NP	PS	CS
Ser/Thr kinases				
PKA	Yes	Yes	Yes	Yes
PKA, R1β	Yes	No	Yes	Yes
PKB (AKT)	N.T.	N.T.	Yes	Yes
PKC	Yes	Yes	Yes	Yes
PKCε	Yes	Yes	Yes	N.T.
PKCγ	Yes	Yes	N.T.	Yes
PKG	Yes	N.T.	Yes	Yes
PKG-I	Yes	N.T.	N.T.	Yes
CaM kinase II	Yes	Yes	Yes	Yes
MEK/ERK1/2	Yes	Yes	Yes	Yes
p38 MAPK	Yes	Yes	Yes	Yes
p38-β	N.T.	N.T.	N.T.	Yes
JNK /MAPK	N.T.	Yes	N.T.	N.T.
Rho kinase (ROCK)	Yes	Yes	N.T.	N.T.
IκB kinase (IKK)	Yes	Yes	N.T.	N.T.
Casein kinase 2	Yes	N.T.	N.T.	Yes
Cdk5	N.T.	N.T.	N.T.	Yes
Tyrosine kinases				
TrK A	Yes	Yes	Yes	N.T.
TrK B	Yes	Yes	N.T.	Yes
EphB	Yes	N.T.	N.T.	Yes
Src	N.T.	N.T.	N.T.	Yes
Other kinases				
PI3K	N.T.	N.T.	Yes	Yes

N.T., not tested

genome including receptor tyrosine kinases and nonreceptor tyrosine kinases (Manning et al. 2002); several tyrosine kinases are implicated in pain facilitation. In particular, we will discuss the roles of mitogen-activated protein kinases (MAPKs) in regulating pathological pain (see Sect. 2.3), because there is increasing evidence suggesting that this family of serine/threonine kinases is critical for the induction and maintenance of pain hypersensitivity after injuries (Ji and Woolf 2001).

2.1 Serine-Threonine Protein Kinases

Protein Kinase A (PKA) This is one of the most classic kinases. Although protein kinase A (PKA) is typically activated by cyclic AMP (cAMP), cAMP's actions are not exclusively mediated by PKA. cAMP could activate Epac, a guanine nucleotide exchange factor, leading to the activation of p44/42 MAPK and the ε-isoform of PKC. PKA has been strongly implicated in peripheral and central sensitization. The adenyl cyclase–cAMP–PKA pathway mediates sensitization of the peripheral terminals of nociceptors induced by PGE_2, a major inflammatory mediator (Aley and Levine 1999). PKA is also required for the second phase nociceptive response to formalin and capsaicin-produced mechanical allodynia, two pain models that are thought to result from central sensitization (Coderre and Yashpal 1994; Sluka and Willis 1997). PKA also contributes to the sensitization of spinal-thalamic projection neurons in the lamina I of the spinal cord following tissue injury (Willis 2002). These NK-1-expressing projection neurons are essential for injury-induced pain hypersensitivity (Mantyh et al. 1997). In mice with a null mutation of the neuronal-specific isoform of PKA's type I regulatory subunit (RIβ), a selective deficit is found in the development of inflammation and tissue injury-produced pain (Malmberg et al. 1997a).

Protein Kinase C PKC is activated by diacylglycerol (DAG) and Ca^{2+}. There is substantial evidence supporting a role of spinal PKC in regulating pain hypersensitivity in different pain models (Mao et al. 1992; Coderre 1992; Sluka and Willis 1997). PKC is also required for the sensitization of spinal-thalamic projection neurons in the lamina I following intense C fiber stimulation (Willis 2002). There is an increase in spinal cord membrane-bound PKC following nerve injury (Mao et al. 1992). In particular, the γ-isoform of PKC is expressed predominantly in inner lamina II neurons of the dorsal horn; there is reduced neuropathic and inflammatory pain but preserved acute nociceptive pain in mice lacking PKCγ (Malmberg et al. 1997b). PKCγ expression is further induced in the spinal cord by inflammation and nerve injury (Martin et al. 1999). The ε-isoform of PKC, in contrast, is expressed in primary sensory neurons and plays a major role in peripheral sensitization of nociceptor terminals in inflammatory and neuropathic pain conditions (Khasar et al. 1999).

Calcium/Calmodulin-Dependent Kinase This kinase (CaMK-II) has been intensively studied in hippocampal neurons and plays an essential role in synaptic plasticity and learning and memory. The active form of αCaMK-II autophosphorylated at threonine 286, a critical site for CaMK-II activation, has been shown to enhance excitatory synaptic transmission in dorsal horn neurons (Kolaj et al. 1994). The superficial dorsal horn is densely stained with an

anti-αCaMK-II antibody (Fang et al. 2002; Zeitz et al. 2004). Recent studies show that CaMK-II is required for central sensitization and neuropathic pain sensitization (Fang et al. 2002; Garry et al. 2003). Disruption of CaMK-II's docking to the NMDA receptor may be important for the activation of this receptor and the subsequent sensitization of pain behavior after nerve injury (Garry et al. 2003). However, in mice with a point mutation in the αCaMK-II gene at threonine 286, inflammatory pain and neuropathic pain remain unchanged (Zeitz et al. 2004). The role of another isoform, CaMK-IV, in pain regulation is unclear, although it is expressed in 30% of DRG neurons and implicated in the activation of cAMP-response element binding protein (CREB), an important transcription factor for many neuronal genes (Ji et al. 1996).

Protein Kinase G (PKG) Ca^{2+} influx through the NMDA receptor activates the nitric oxide/cGMP/PKG pathway. Neuronal nitric oxide synthase (NOS) is Ca^{2+}/calmodulin-dependent. Nitric oxide (NO) serves as a diffusible and retrograde messenger. The major target of NO in the CNS appears is a soluble guanylyl cyclase (sGC) leading to the production of cyclic guanosine monophosphate (cGMP) and subsequent activation of PKG (Meller and Gebhart 1993). Nerve injury upregulates NOS in DRG neurons (Zhang et al. 1993). PKG appears to play a role in peripheral and central sensitization (Meller and Gebhart 1993; Sluka and Willis 1997; Aley et al. 1998). PKG-I is expressed in the spinal cord, and mice lacking PKG-I show reduced inflammatory hyperalgesia with preservation of acute thermal nociception (Tegeder et al. 2004a). In *Aplysia* sensory neurons, PKG-I couples to the ERK (extracellular signal-regulated kinase) pathway and contributes to axotomy-induced long-term hyperexcitability (Sung et al. 2004).

Other Kinases The transcription factor nuclear factor NF-κB plays an important role in inflammatory responses by inducing the transcription of genes encoding many inflammatory mediators. NF-κB is normally retained in cytoplasm by IκB inhibitor proteins. The phosphorylation of IκB by its kinase, IKK, results in IκB degradation. IκB degradation enables nuclear translocation of NF-κB for gene transcription. Specific inhibition of IKK is shown to reduce both inflammatory and neuropathic pain (Tegeder et al. 2004b). Rho kinase (ROCK) is regarded as a promising drug target for neurological disorders (Mueller et al. 2005). ROCK inhibitors were shown to suppress inflammatory and neuropathic pain (Inoue et al. 2004; Tatsumi et al. 2005). Casein kinase 2 (CK2) is a widely expressed protein kinase and involved in central sensitization and inflammatory pain (Li et al. 2005). Although cyclin-dependent kinase 5 (Cdk5) is typically involved in cell division, recent evidence also suggests a role of this kinase in regulating neural plasticity in the brain. Intrathecal administration of roscovitine, a Cdk5 inhibitor, attenuates formalin-induced nociceptive response in rats (Wang et al. 2005).

Finally, it is worth discussing phosphatidylinositol 3-kinase (PI3K) and the PI3K pathway. Although PI3K is a lipid kinase that phosphorylates the D-3 position of phosphatidylinositol lipids to produce PI(3,4,5)P_3, acting as a membrane-embedded second messenger (Toker and Cantley 1997), PI3K behaves like a protein kinase. Another reason to include PI3K is that the downstream protein kinase Akt (protein kinase B) is a serine/threonine kinase and is postulated to mediate most of PI3K's effects. Activation of Akt is typically used to examine the activation of PI3K pathway (Zhuang et al. 2004). PI3K is a major pathway activated by growth factors; therefore, NGF can strongly activate the PI3K pathway in DRG neurons. PI3K is also activated in DRG neurons by C fiber activator capsaicin due to intracellular Ca^{2+} increase. PI3K inhibitors prevent heat hyperalgesia by both NGF and capsaicin. The PI3K is especially important for the induction of heat hyperalgesia (Zhuang et al. 2004).

2.2 Tyrosine Kinases

Receptor Tyrosine Kinases The receptors for growth factors are tyrosine kinases that are autophosphorylated upon ligand binding. In particular, NGF and BDNF mediate their effects largely via TrkA and TrkB, respectively. Although NGF is required for the survival of sensory neurons during fetal development, it is not necessary for the survival of mature sensory neurons. Rather it maintains the phenotype of sensory neurons in the adult. NGF plays an essential role in peripheral sensitization; local and systemic injection of NGF produces hyperalgesia. Although BDNF is synthesized in primary sensory neurons, it can be released from central terminals in the spinal cord following intense noxious stimulation and plays an important role in central sensitization. Intrathecal infusion of a scavenger for TrkA and TrkB (BDNF receptor) or a general Trk inhibitor k252a reduces both inflammatory and neuropathic pain (McMahon et al. 1995; Mannion et al. 1999; Ji and Strichartz 2004). A recent study has also shown that receptor tyrosine kinase EphB is required for central sensitization and the development of inflammatory pain (Battaglia et al. 2003).

Non-receptor Tyrosine Kinases Among this large group of signaling molecules, the Src family is the best known, which includes Src, Fyn, Lck, Lyn, and Yes. Src is activated by receptor tyrosine kinases as well as by G protein-coupled receptor (GPCR) and PKC. Activation of Src is implicated in p44/42 MAPK activation (Kawasaki et al. 2004). Salter and his collaborators have shown an important role of Src in regulating the activity of NMDA receptors, an essential mediator of central sensitization (Salter and Kalia 2004). Thus, Src inhibitor was shown to suppress central sensitization (Guo et al. 2002). It is not clear whether other nonreceptor tyrosine kinases also contribute to pain sensitization.

2.3 Mitogen-Activated Proteins Kinases (MAPKs)

The MAPKs are a family of evolutionally conserved molecules that play a critical role in cell signaling. This family includes three major members—extracellular signal-regulated kinase (ERK or p44/42 MAPK), p38, and c-Jun N-terminal kinase (JNK)—that represent three different signaling cascades. MAPKs transduce a broad range of extracellular stimuli into diverse intracellular responses by both transcriptional and nontranscriptional regulation (Widmann et al. 1999). Early studies indicated a critical role of ERK in regulating mitosis, proliferation, differentiation, and survival of mammalian cells during development. Recent evidence shows that ERK also plays an important role in neuronal plasticity and inflammatory responses in the adult. p38 and JNK are typically activated by cellular stress (ultraviolet irradiation, osmotic shock, heat shock), lipopolysaccharide (LPS), and certain proinflammatory cytokines such as TNF-α and IL-1β (Widmann et al. 1999; Ji and Woolf 2001). Therefore, these two kinases, especially JNK, are also called stress-activated protein kinase (SAPK), contributing importantly to inflammatory responses and neuronal degeneration. All the family members are activated by different upstream MAPK kinases (MKKs). The corresponding MKKs for ERK, p38, and JNK are MKK1/2 (also called MEK1/2), MKK3/6, MKK4/7. The ERK5 is the least-known member of MAPK family and is activated by MKK5. MKKs are activated by MAPK kinase kinase. Studies on MAPKs greatly benefit from specific inhibitors available to explore the function of each pathway and from phosphorylation-specific antibodies available to investigate the activation of each pathway. Like other kinase inhibitors, MAPK inhibitors do not affect basal pain perception.

2.3.1 ERK/MAPK (p44/42 MAPK) Pathway

ERK is the first and the most studied member of the MAPK family. It was originally identified as a primary effector of growth factor receptor signaling, a cascade that involves sequential activation of Ras, Raf (MAPK kinase kinase), MEK (MAPK kinase), and ERK (MAPK). However, the activation of the ERK cascade is not restricted to growth factor signaling. ERK is activated by persistent neural activity and pathological stimuli. A growing body of evidence demonstrates an involvement of ERK in neuronal plasticity, such as learning and memory, as well as pain hypersensitivity (Widmann et al. 1999; Ji and Woolf 2001; Ji et al. 2003).

Activation of ERK in Primary Sensory Neurons There is an activity-dependent ERK activation in DRG neurons. Depolarization of adult DRG neuronal cultures induces a very rapid and transient ERK phosphorylation (pERK). This

transient pERK induction is also observed in vivo following peripheral noxious stimuli, reaching peak at 2 min and almost returning to the baseline after 10 min (Dai et al. 2002). In addition to neuronal activity, NGF, capsaicin, and epinephrine can all activate ERK in a subset of DRG neurons. ERK is not only induced in the soma of DRG neurons but also in peripheral axons of DRG neurons (Dai et al. 2002; Zhuang et al. 2004). Intraplantar injection of capsaicin increases pERK-labeled nerve fibers in the epidermis within 2 min. Inhibition of the ERK pathway by MEK inhibitors attenuates heat hyperalgesia by capsaicin and NGF as well as mechanical hyperalgesia by epinephrine (Aley et al. 2001; Dai et al. 2002; Zhuang et al. 2004). ERK is likely to mediate heat hyperalgesia by inducing TRPV1 sensitization (Dai et al. 2002; Zhuang et al. 2004).

Activation of ERK in Dorsal Horn Neurons We have shown that ERK activation in spinal dorsal horn neurons is nociceptive activity-dependent (Ji et al. 1999). Injection of the C fiber nociceptor activator capsaicin into a hindpaw of rats induces a remarkable ERK phosphorylation. pERK is induced in dorsal horn neurons as early as 1 min after C fiber activation. This activation exactly follows the rule of spinal topographic organization: pERK-labeled neurons are found in the medial superficial dorsal horn of the spinal cord on the stimulated side where primary nociceptive afferents from the hindpaw terminate. The pERK can only be induced by thermal noxious (heat and cold) and mechanical noxious (prick) stimulus, but not by innocuous stimulus (light touch) (Ji et al. 1999). It appears that the duration of noxious stimulation is also important; a very brief noxious stimulation (<10 s) may not induce pERK. Noxious stimulation also induces ERK activation in the trigeminal spinal nucleus (Huang et al. 2000).

Moreover, pERK can be induced in a spinal cord slice preparation, where different types of afferent fibers in the attached dorsal root can be electrically stimulated (Ji et al. 1999; Lever et al. 2003). A bath application of capsaicin to spinal slices, which will activate TRPV1 receptors in presynaptic C fiber terminals to stimulate the release of neurotransmitter acting on postsynaptic receptor, strongly activates ERK in superficial dorsal horn neurons in spinal slices (Fig. 1a, b). Using this simple and reliable in vitro (or ex vivo) model, we have investigated the molecular mechanisms involved in the regulation of C fiber-induced ERK activation (Kawasaki et al. 2004). We found that multiple neurotransmitter receptors, including NMDA, AMPA, and metabotropic glutamate receptors, substance P NK-1 receptor, and TrkB receptor all contribute to C fiber-evoked ERK activation (Kawasaki et al. 2004; Fig. 1c).

To investigate the functional significance of ERK activation, a MEK (ERK kinase) inhibitor (PD98059) was tested in the formalin model, where injection of diluted formalin into a hindpaw of rats elicits a pain behavior lasting for an hour. Intrathecal injection of PD98059 blocks the central sensitization-

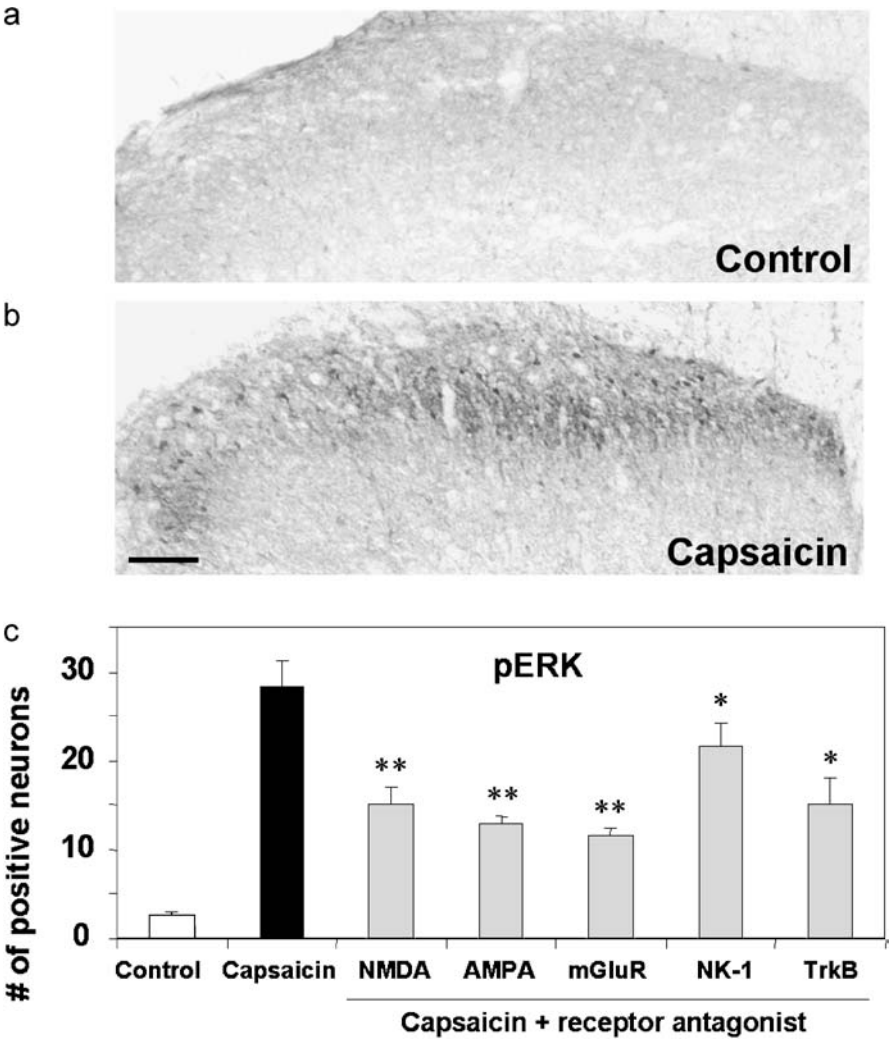

Fig. 1a–c ERK activation in the superficial dorsal horn of spinal slices as indicated by phospho ERK (pERK) immunostaining. **a** There are very few pERK-labeled neurons in the control slices. **b** A bath application of capsaicin (3 µM, 5 min) induces pERK in many neurons in the stimulated slices. Scale, 50 µm. **c** This panel shows the number of pERK-positive neurons in the laminae I–II of spinal slices after capsaicin stimulation in the presence of different receptor antagonists. Capsaicin-induced pERK is reduced by blocking NMDA (MK-801, 100 µM), AMPA (CNQX, 20 µM), mGluR (CPCCOEt, 1 µM), NK-1 (GR205171A, 100 µM), and TrkB receptors (k252a 100 nM). *, $p<0.05$; **, $p<0.01$, compared to capsaicin group, ANOVA. Mean±SEM ($n=5$). (See Kawasaki et al. 2004 for more details)

mediated second phase of the painful response to formalin injection (Ji et al. 1999). After this initial study, multiple studies in different animal models from different labs have confirmed that the ERK pathway contributes impor-

tantly to the development of central sensitization (Karim et al. 2001; Galan et al. 2003; Kawasaki et al. 2004; Yu and Chen 2005; Yu and Yezierski 2005). Injection of CFA into a hindpaw produces persistent (>2 weeks) inflammatory pain. CFA also induces sustained ERK activation in dorsal horn neurons, whereas capsaicin and formalin only induce transient ERK activation (Ji et al. 1999; Ji et al. 2002a). Intrathecal infusion of the MEK inhibitor U0126 reduces the late phase of inflammatory pain. This delayed action of ERK is likely to be caused by transcriptional regulation (Ji et al. 2002a). MEK inhibitors are also shown to alleviate neuropathic pain (Obata and Noguchi 2004; Zhuang et al. 2005).

Although overall pERK induction is persistent after CFA inflammation, the peak induction only lasts a few hours. The duration of ERK activation is controlled by phosphatases. pERK could be inactivated by MKP-1 (MAP kinase phosphatase-1) and PP2A (protein phosphatase 2A). Neuronal activity not only induces ERK activation but also rapidly increases the expression of immediate early gene MKP-1. Intrathecal injection of okadaic acid, a general PP2A inhibitor, enhances central sensitization by prolonging capsaicin-induced mechanical hyperalgesia and allodynia. Phosphatase inhibitor might prolong capsaicin-induced ERK activation, resulting in prolonged pain facilitation (Zhang et al. 2003; Ji and Strichartz 2004).

pERK Expression as a Marker for Nociceptive Activity and as an Assay for Screening Analgesic Compounds The expression of the immediate early-gene *c-fos* has been extensively used as a marker for demonstrating the activity of spinal nociceptive neurons (Hunt et al. 1987; Presley et al. 1990). Like the expression of c-Fos protein, pERK expression can also serve as a marker for neuronal activity following nociceptive input. Compared to c-Fos expression, pERK expression is more rapid and transient, following neuronal activity more closely. Importantly, pERK expression is functional, contributing critically to dorsal horn neuron sensitization. The function of ERK activation can be easily assessed by blocking this activation with specific MEK inhibitors. Further, pERK expression can be reliably studied in spinal slice preparation. Since many slices (>10) can be obtained from spinal lumbar enlargement of one rat and C fiber can be easily stimulated by bath capsaicin, pERK expression by capsaicin in spinal slices can be used for the screening of potential analgesic drugs (Ji 2004). Interestingly, pERK can be induced by tactile stimulation (touch) after nerve injury, which may underlie tactile allodynia after neuropathic pain.

2.3.2 p38 MAPK Pathway

p38 is typically activated by cellular stress and inflammatory mediators. p38 activation can also be activity-dependent. Systematic p38 inhibitors produce antiinflammatory effects in animal models (Ji and Woolf 2001). Interestingly,

phospholipase A2 (PLA2) is downstream to p38. The activation of PLA2 leads to the generation of arachidonic acid for prostaglandin production, eliciting pain (Svensson et al. 2005). Activated p38 is also translocated to the nucleus phosphorylating the transcriptional factors and induces gene expression. The biosynthesis of TNF-α and IL-1β, as well as many other inflammatory mediators, is positively regulated by p38 (Ji and Woolf 2001).

p38 Activation in the DRG and Spinal Cord Phospho-p38 (p-p38), the active form of p38, is normally expressed in 10%–15% of DRG neurons that are primary C fiber nociceptors (Ji et al. 2002b; Obata et al. 2004). p38 is activated in DRG neurons following peripheral inflammation and nerve injuries (Kim et al. 2002; Ji et al. 2002b; Jin et al. 2003; Schafers et al. 2003; Obata and Noguchi 2004). However, total p38 (nonphosphorylated and phosphorylated) levels do not increase after injury, indicating that the increase in p-p38 is caused by increased phosphorylation, rather than elevated substrate (Ji et al. 2002b). After nerve injury, p38 is activated not only in DRG neurons with axonal injury, but also in adjacent neurons without axonal injury (Obata et al. 2004). While TNF-α contributes to an early activation of p38 after nerve injury, NGF, via retrograde transport, is important for persistent p38 activation after inflammation and nerve injury (Ji et al. 2002b; Schafers et al. 2003; Obata et al. 2004). Unlike ERK, p38 is not activated in spinal cord neurons in either control or pathological pain conditions (Jin et al. 2003; see further discussion in Sect. 3.4).

p38 and Pathological Pain The pyridinyl imidazole compounds SB203580, SB202190, and PD169316 are regarded as specific inhibitors for p38. SB203580 is the most frequently used p38 inhibitor. It does not inhibit the phosphorylation of p38 MAPK, but rather binds to the ATP pocket in the enzyme, inhibiting activity of the enzyme. CNI-1493 is a potent antiinflammatory agent and was initially used as a monocyte synthesis inhibitor to block glial activation and later recognized as a p38 inhibitor (Milligan et al. 2003). FR167653 is another p38 inhibitor. Unlike SB203580, CNI-1493 and FR167653 can block the phosphorylation of p38 (Koistinaho and Koistinaho 2002; Obata et al. 2004). To examine whether p38 activation in the DRG is involved in the generation of inflammatory pain, SB203580 was administered into the intrathecal space to target p38 activity in the DRG. This inhibitor reduced inflammation-induced heat hyperalgesia and suppressed CFA-induced TRPV1 upregulation, but had no effect on CFA-induced inflammation (Ji et al. 2002b). Intrathecal injection of p38 inhibitors SD-282 and SB203580 also reduced substance P- and NMDA-induced pain hypersensitivity and suppressed COX-2 upregulation and PGE_2 release in the spinal cord (Svensson et al. 2003a; Svensson et al. 2003b). p38 inhibitor can also alleviate neuropathic pain. Intrathecal SB203580 prevents the development mechanical allodynia after spinal nerve ligation (Jin et al. 2003; Schafers et al. 2003; Tsuda et al. 2004). SB203580, CNI-1493, FR167653, and SD-282 further reverses neuropathic pain after spinal nerve ligation, sciatic

inflammatory neuropathy (SIN), and diabetic neuropathy (Jin et al. 2003; Milligan et al. 2003; Obata et al. 2004; Sweitzer et al. 2004). The mechanisms of p38 regulation of pain sensitization are discussed in Sects. 3.1 and 3.4.

2.3.3
JNK/MAPK Pathway

JNK is the least studied member of MAPK family regarding its role in pain regulation. JNK can be activated by cell stress such as heat shock, direct DNA damage, and generation of reactive oxygen species and plays an important role in the induction of apoptosis. JNK has three isoforms: JNK1, JNK2, and JNK3, and JNK3 is closely correlated with neuronal function. Activated JNK phosphorylates the transcription factors c-Jun and ATF-2 (Widmann et al. 1999). Nerve injury induces JNK activation in DRG neurons (Obata et al. 2004; Zhuang et al. 2006). JNK is also induced in glial cells in the spinal cord by nerve lesion. Intrathecal injection of the JNK inhibitor SP600125 and D-JNKI-1 could prevent and reverse neuropathic pain (Ma and Quirion 2002; Obata et al. 2004; Zhuang et al. 2006; also see Sect. 3.4).

3
Mechanisms of Pain Sensitization by Protein Kinases

3.1
Peripheral Mechanisms

3.1.1
Induction of Peripheral Sensitization: Posttranslational Regulation

As discussed in Sect. 1.2, TRPV1 and TTX_R sodium channels are expressed in nociceptive primary sensory neurons and play a pivotal role in the induction of peripheral sensitization (Fig. 2). TRPV1 is essential for the generation of heat hyperalgesia. Inflammatory heat hypersensitivity following bradykinin, NGF, CFA, and carrageenan is significantly reduced in TRPV1 knockout mice (Caterina et al. 2000; Chuang et al. 2001). TTX_R sodium channels are crucial for the generation of hyperexcitability of sensory neurons. Knockdown of $Na_v1.8$ with antisense oligodeoxynucleotides results in decreased inflammatory pain and neuropathic pain (Porreca et al. 1999). Hypersensitivity of TRPV1 and TTX_R following stimulation of inflammatory mediators underlies the induction of peripheral sensitization (Julius and Basbaum 2001).

Several protein kinases such as PKA, PKC, CaMK-II, PI3K and ERK are implicated in TRPV1 sensitization. PKA and PKC are also involved in regulating the sensitivity of TTX_R. A membrane translocation of PKCε appears to be important for its activation of TTX_R. The PI3K pathway could contribute to peripheral sensitization by inducing the trafficking and membrane insertion

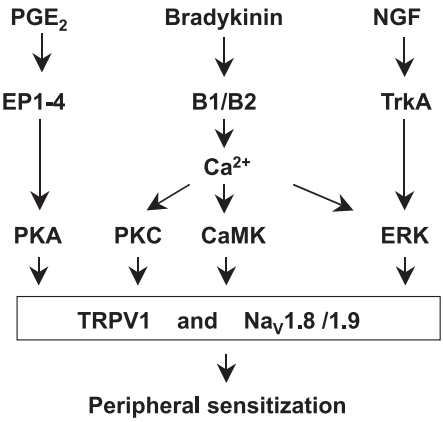

Fig. 2 Induction of peripheral sensitization by protein kinases in nociceptors. Following tissue injury, inflammatory mediators PGE_2, bradykinin, and NGF activate corresponding G protein-coupled receptors EP1–4 and B1/B2, and tyrosine kinase receptor TrkA on nociceptor terminals, axons, and soma, leading to the activation of PKA, PKC, CaMK-II, and ERK. These protein kinases increase the sensitivity of TRPV1 and TTX-resistant sodium channels $Na_V1.8/1.9$ by posttranslational regulation, causing peripheral sensitization

of critical ion channels (Gold et al. 1998; Julius and Basbaum 2001; Ji and Strichartz 2004; Zhuang et al. 2004). Since the action of these kinases could happen within minutes and occur in nociceptor terminals, it should involve posttranslational regulation of protein kinases.

3.1.2 Maintenance of Peripheral Sensitization: Transcriptional and Translational Regulation

Tissue and nerve injuries induce gene transcription and protein synthesis in primary sensory neurons in the DRG, contributing to persistent pain hypersensitivity. For example, peripheral inflammation increases the transcription of substance P, calcitonin gene-related protein (CGRP), and BDNF. After nerve injury, these genes are also induced in noninjured DRG neurons that are adjacent to injured DRG neurons. NGF is believed to be critical for the expression of these genes (reviewed in Ji and Strichartz 2004). Since p38 and ERK can be activated by NGF; these two MAPKs appear to mediate NGF-induced expression of substance P, CGRP, and BDNF via the transcription factor CREB. The cAMP-response element (CRE) sites are found in many genes expressed in the DRG including substance P, CGRP, and BDNF (Ji and Strichartz 2004).

In addition to transcriptional regulation, MAPK also mediates gene expression via translational regulation. Peripheral inflammation and NGF induce increase in TRPV1 protein levels but not in TRPV1 messenger RNA (mRNA) levels in the DRG. CFA increases p-p38 and TRPV1 expression in C fiber noci-

ceptors, and p-p38 is heavily colocalized with NGF receptor TrkA and TRPV1. Further, intrathecal inhibition of p38 blocked inflammation-induced upregulation of TRPV1. p38 is likely to regulate the translation of TRPV1 via translation initiation factor eIF-4E in a NGF-dependent way (Ji et al. 2002b). NGF levels increase in the inflamed paw after CFA injection. NGF is taken up by nerve terminals and retrogradely transported to neuronal soma in the DRG, inducing p38 activation and TRPV1 translation. Finally, TRPV1 is anterogradely transported from DRG cell body to peripheral nerve terminals, contributing to persistent inflammatory heat hyperalgesia (Ji et al. 2002b). The NGF–p38–TRPV1 cascade in also important for heat hyperalgesia after nerve injury, in which NGF is produced from injured axons after Wallerian degeneration and taken up by adjacent intact axons (Obata et al. 2004).

3.2 Spinal Mechanisms

3.2.1 Induction of Central Sensitization: Posttranslational Regulation

Glutamate is a predominant excitatory neurotransmitter in all nociceptors. Mild noxious stimulation generates fast excitatory postsynaptic potentials, lasting milliseconds. This fast synaptic transmission is mediated by ionotropic glutamate AMPA and kainate receptors. Additional activation of intracellular signaling cascades is required for the development of central sensitization (Woolf and Salter 2000; Ji and Woolf 2001).

Activation of second messenger systems, especially protein kinases, can phosphorylate AMPA and NMDA receptors via posttranslational regulation, enhancing synaptic transmission and producing central sensitization (Fig. 3). For example, a PKC-mediated phosphorylation of NMDA receptor in dorsal horn neurons removes its voltage-dependent Mg^{2+} block, which in turn enables glutamate to generate a greater inward current through the NMDA ion channel at resting membrane potentials (Chen and Huang 1992). Tyrosine kinase Src enhances NMDA current via phosphorylation of NR2A/B subunit of NMDA receptor. The tyrosine phosphorylation appears to increase channel open time and kinetics. Noxious simulation and inflammation induce the phosphorylation of NMDA receptor subunits in dorsal horn neurons (Zou et al. 2000; Guo et al. 2002; Salter and Kalia 2004). CaMK and PKA have been implicated in the phosphorylation of AMPA receptors, leading to an increase of AMPA current (Ji et al. 2003).

Recently it was shown that painful stimulation induces trafficking and insertion of AMPA receptor subunits to the plasma membrane of spinal cord neurons (Galan et al. 2004). CaMK and ERK appear to mediate membrane insertion of AMPA receptors during synaptic plasticity. ERK could also regulate the activity of $K_V4.2$ potassium channels, increasing the excitability of dor-

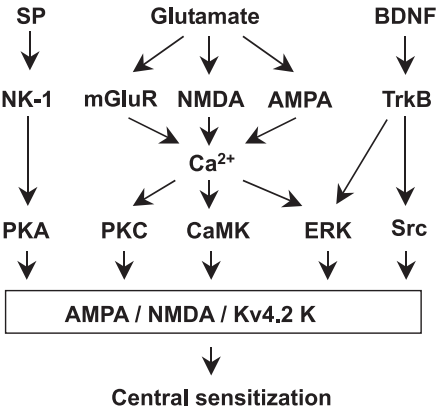

Fig. 3 Induction of central sensitization by protein kinases in dorsal horn neurons. Injury-evoked spontaneous activity induces the release of the neurotransmitters glutamate and the neuromodulators substance P and BDNF from primary afferents in the dorsal horn, activating corresponding ionotropic NMDA and AMPA receptors, metabotropic mGluR receptors, and tyrosine kinase TrkB receptors, leading to subsequent activation of PKA, PKC, CaMK-II, ERK, and Src in postsynaptic dorsal horn neurons. These protein kinases increase the sensitivity of AMPA and NMDA receptors and suppress the activity of $K_V4.2$ potassium channels by posttranslational regulation, causing central sensitization

sal horn neurons (Hu et al. 2003). In addition to posttranslational regulation, nociceptive activity also induces a rapid increase of CaMK-II protein (within 10 min), which may involve translational regulation (Fang et al. 2002).

3.2.2 Maintenance of Central Sensitization: Transcriptional Regulation

Intense noxious stimulation, inflammation, and nerve injury produce an increase in the expression of immediate early genes (e.g., *c-fos, Zif268, Cox-2)* and later response genes (e.g., prodynorphin, NK-1, and TrkB) in the dorsal horn of spinal cord. A continuous production of the protein products of these genes could maintain central sensitization (reviewed in Ji et al. 2003). Upon activation, pERK is translocated to the nucleus of dorsal horn neurons. ERK activation is likely to maintain pain hypersensitivity via regulating gene expression. Inhibition of ERK activation blocks inflammation-induced upregulation of *c-fos*, prodynorphin, NK-1, as well as CREB phosphorylation (Ji et al. 2002a; Kawasaki et al. 2004). pERK is shown to activate CREB via a CREB kinase RSK2 (Fig. 4). CREB-binding site CRE has been identified in the promoter regions of numerous genes expressed in the dorsal horn, including those mentioned above. It is of particular interest that ERK activation is downstream to many other kinases, such as PKA, PKC, PI3K, Trk, and Src (Kawasaki et al. 2004; Fig. 4). Convergence of multiple signal pathways on ERK

activation indicates a pivotal role of the ERK pathway in intracellular signal transduction.

Recently the concept for the "memory of pain" has been proposed to explain the persistence of pain. Studies on neural plasticity in the spinal cord and in the hippocampus reveal similar mechanisms for central sensitization

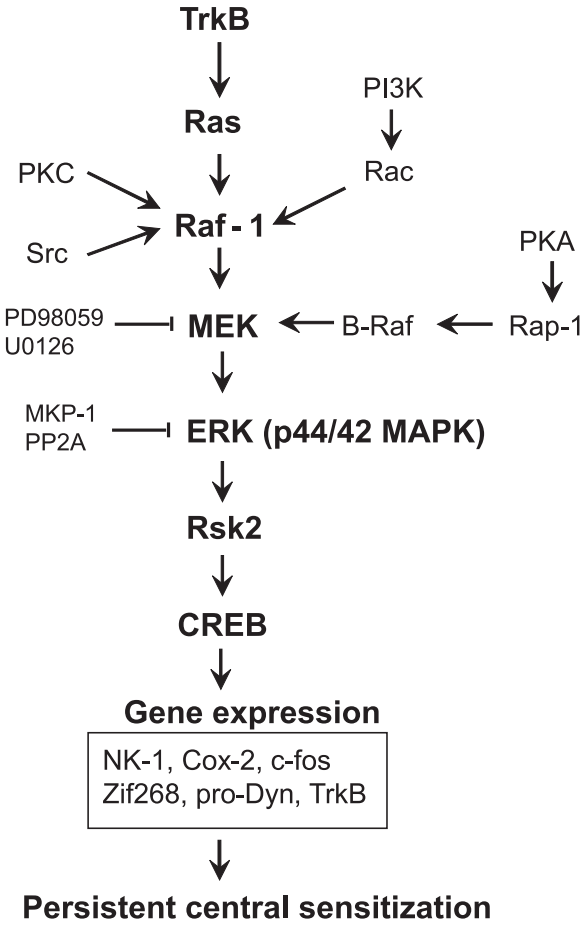

Fig. 4 Maintenance of central sensitization by protein kinases in dorsal horn neurons. ERK is classically activated by the Ras–Raf–MEK pathway following stimulation of growth factor receptors (TrkB). Several kinases such as PKA, PKC, Src, and PI3K can converge on ERK activation. Meanwhile, ERK is inhibited by the phosphatases MKP-1 and PP2A. MEK is inhibited by the inhibitors PD98059 and U0126. Upon phosphorylation, pERK activates the transcription factor CREB via CREB kinase Rsk2, leading to the transcription of CRE-mediated genes including immediate early genes *Zif268*, *Cox-2*, *c-fos* and late response genes *NK-1*, *TrkB*, and *prodynorphin* in dorsal horn neurons. Central sensitization is maintained by the protein products of these genes

and long-term potentiation (LTP), which are believed to underlie generation of pain hypersensitivity and memory, respectively (Sandkuhler 2000; Willis 2002; Ji et al. 2003). LTP is also induced in dorsal horn neurons following intense noxious stimulation (Sandkuhler 2000). Whereas long-term memory requires gene transcription, short-term memory only requires posttranslational modifications. This same dichotomy appears to apply to central sensitization-mediated pain hypersensitivity: persistent pain (chronic pain) but not acute pain requires gene transcription (Ji et al. 2003). In particular, the transcription factor CREB is believed to play an essential role in long-term neuronal plasticity in both hippocampal and dorsal horn neurons. CREB can maintain long-term neural plasticity not only by inducing gene transcription but also by forming new synapses (Lonze and Ginty 2002).

3.3 Brain Mechanisms

In addition to dorsal horn neurons, central sensitization also develops in rostroventral medial medulla (RVM) neurons, amygdala neurons, and cingulate cortex neurons following tissue injury (Urban and Gebhart 1999; Porreca et al. 2002). Inflammation induces phosphorylation of AMPA receptor (GluR1) in the RVM, in a PKC- and CaMK-dependent manner, which may regulate descending facilitation (Guan et al. 2004). The spino–parabrachio–amygdaloid pathway is implicated in nociception. Tissue injury induces an enhanced NMDA receptor function in rat amygdala neurons via PKA activation (Bird et al. 2005). The PKA pathway in the anterior cingulate cortex (ACC) also contributes to inflammatory pain, since calcium-dependent adenylate cyclase AC1 and AC8 are highly expressed in the ACC and inflammatory pain is attenuated in AC1 and AC8 knockout mice. The action of AC1 and AC8 appears to be mediated by the cAMP–PKA–CREB pathway (Wei et al. 2002a). Neuronal activity in the ACC could influence nociceptive transmission in the dorsal horn of the spinal cord by activating the endogenous facilitatory system.

Pain experience includes not only a sensory-discriminative component describing the quality, intensity, and spatiotemporal characteristics of the sensation, but also an emotional-affective component, referring to the unpleasantness or aversion of sensation. Emotional distress could be the most disruptive and undesirable feature of painful experiences. Physiological arousal and hypervigilance to pain cause a negative affect, such as fear, anxiety, anger, worry, aversion, and depression, which in turn could alter pain sensation. While current studies focus primarily on the sensory component of pain, mechanisms underlying the affective dimension of pain have recently received more attention. ACC is a part of the brain's limbic system and plays an important role in affect. ACC neurons respond to noxious stimuli with very large, often whole-body receptive fields, and acquire responses to environmental cues that predict a painful stimulus (Koyama et al. 1998). Formalin-induced conditioned place avoidance (F-CPA, noxious conditioning) has been established as an an-

imal model to examine affective pain and pain-associated memory. Lesions of ACC in animals produce severe deficits in avoidance conditioning (Johansen et al. 2001).

Molecular mechanisms underlying learning and memory have been intensively investigated in hippocampal neurons. It has been demonstrated that PKA, ERK, and CaMK can all converge on CREB phosphorylation, leading to long-term memory via gene transcription (Lonze and Ginty 2002). These mechanisms may also contribute to affective pain and pain-associated memory in ACC neurons. Activation of NMDA receptors results in Ca^{2+} influx and is required for formalin-induced affective pain (Lei et al. 2004). Ca^{2+}-dependent CaMK-IV is required for CREB activation in the ACC by noxious shock and for fear memory, but behavioral responses to acute noxious stimuli or tissue inflammation may not require CaMK-IV (Wei et al. 2002b). ERK activation has been implicated in several forms of learning and memory, including fear conditioning, spatial learning, conditioned taste aversion, etc. We found that pain-related aversion (F-CPA) induced ERK activation in the ACC, and that microinjection of the MEK inhibitor PD98059 in the ACC significantly inhibited affective pain as well as CREB phosphorylation following F-CPA (Zhang et al. 2005). These results indicate that the ERK/CREB signaling pathway, which has been implicated in the sensory component of pain in spinal neurons, may also participate in affective pain in ACC neurons.

3.4 Glial and Immuno–Mechanisms

Studies on pathological pain mostly focus on the responses of the neurons and neuronal-specific mechanisms of hypersensitivity and chronicity. However, glial cells express various receptors for neurotransmitters and neuromodulators (Watkins et al. 2001; Ji and Strichartz 2004). There is accumulating evidence indicating a role of spinal glial cells in the pathogenesis of pain. Both microglia and astrocytes are activated in inflammatory and neuropathic pain conditions. Multiple mediators such as proinflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α) and inflammatory enzymes (e.g., iNOS, COX-1, and COX-2) are synthesized in spinal astrocytes and microglia (DeLeo and Yezierski 2001; Watkins et al. 2001). There is increased synthesis of these mediators in activated glia following injuries; all of them are shown to produce pain sensitization. Spinal injection of the glial toxin fluorocitrate, glial modulator propentofylline, or nonspecific microglial inhibitor minocycline has been shown to reduce inflammatory and neuropathic pain (Meller et al. 1994; Watkins et al. 1997; Sweitzer et al. 2001; Raghavendra et al. 2003). In particular, several receptors such as ATP receptor P2X4, chemokine receptors CCR2 and CX3CR1, and Toll-like receptor TLR-4 are expressed in spinal microglia after nerve injury. Blockade of these receptors results in a reduction of neuropathic pain (Abbadie et al. 2003; Tsuda et al. 2003; Verge et al. 2004; Tanga et al. 2005).

It is not very clear how activation of these receptors in microglia leads to pain sensitization. We have proposed that activation of MAPKs may mediate pro-nociceptive effects of these receptors (Ji and Strichartz 2004). Nerve injury induces a drastic and widespread activation of p38 MAPK in the spinal cord. This activation begins at 12 h and peaks 3 days after nerve injury, in parallel with the time course of microglial activation. Moreover, p-p38 is completely colocalized with OX-42 (CD-11b), a hall marker for microglia (Jin et al. 2003). Inhibition of p38 activation in microglia attenuates nerve injury-induced me-

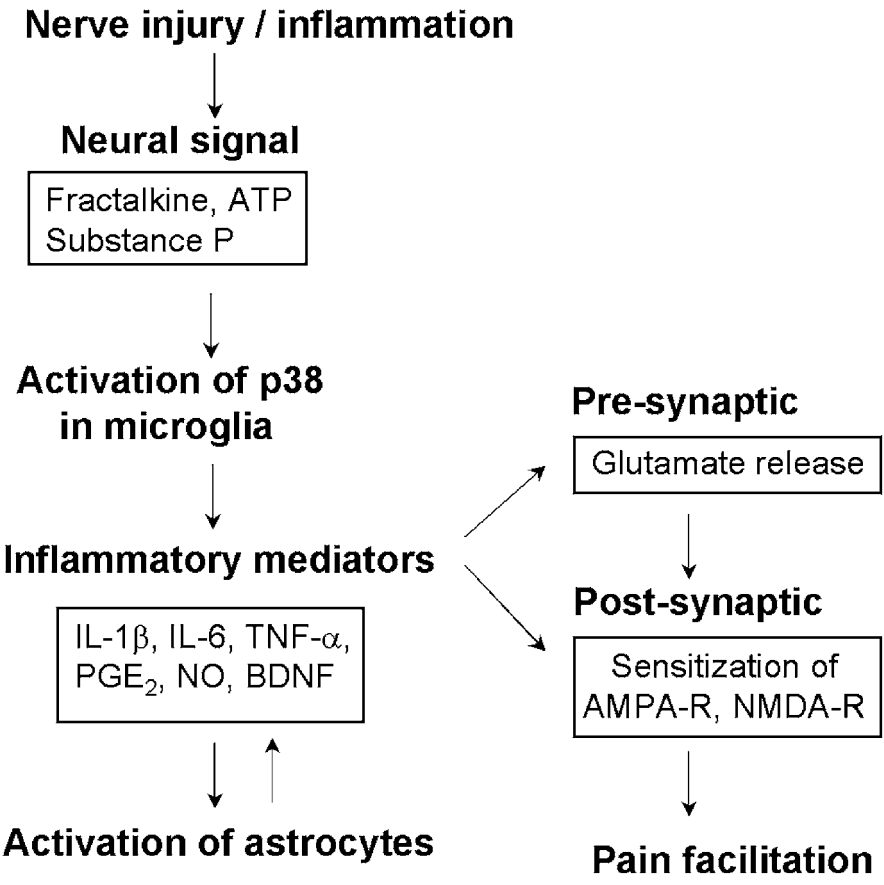

Fig. 5 Microglial regulation of pain facilitation via p38 MAPK. Inflammation and nerve injury release the signaling molecules fractalkine, ATP, and substance P from primary afferents, leading to p38 activation in spinal microglia. p38 activation results in the synthesis of multiple inflammatory mediators (e.g., IL-1β, IL-6, TNF-α, PGE_2, NO), enhancing and prolonging pain facilitation via both presynaptic (glutamate release) and postsynaptic (AMPA and NMDA receptor sensitization) mechanisms. Microglia may also synthesize neuromodulator BDNF to sensitize postsynaptic dorsal horn neurons

chanical allodynia (Jin et al. 2003; Tsuda et al. 2005). p38 activation in microglia is likely to induce the synthesis of inflammatory mediators as well as neuromodulator BDNF. The release of these neuroactive substances could diffuse to synaptic regions and sensitize pain transmission neurons via both presynaptic and postsynaptic mechanisms (Fig. 5). In addition to p38, ERK is also activated in spinal microglia after nerve lesion and contributes to neuropathic pain (Zhuang et al. 2005).

Compared to rapid activation of microglia, injury induces a delayed but persistent activation of astrocytes, suggesting a particular role of astroglia in the persistence of chronic pain. Nerve injury induces persistent activation of JNK in spinal astrocytes (Ma and Quirion 2002; Zhuang et al. 2006). Importantly, this activation is requried for the maintenance of neuropathic pain (Zhuang et al. 2006). Further, ERK is activated in spinal astrocytes at late times of nerve injury and contributes to late maintenance of neuropathic pain (Zhuang et al. 2005). Therefore, MAPKs could mediate different phases of chronic pain by regulating the activity of different subtypes of glial cells.

In addition to spinal glial cells, MAPKs can also be activated in glial cells in the sciatic nerve (Schwann cells) and DRG (satellite cells), as well as in inflammatory cells infiltrating the damaged tissue after injuries, increasing the sensitivity of sensory neurons by producing inflammatory mediators (Ji and Strichartz 2004).

4
Protein Kinase Inhibitors in Clinical Studies

A number of diseases including cancer, diabetes, and inflammation are associated with perturbation of protein kinase-mediated signal transduction. Chromosomal mapping has shown that 244 kinases map to disease loci or cancer amplicons (Manning et al. 2002). Many kinase inhibitors are developed to treat cancer in clinical trials. These include receptor tyrosine kinas inhibitors (e.g., EGF receptor inhibitors) and several MEK inhibitors such as CI-1040, PD0325901, and ARRY-142886, and Raf inhibitor BAY 43-9006 (Cohen 2002; Sebolt-Leopold and Herrera 2004). In the cancer field, protein kinase inhibitors are proving to be well-tolerated compared with conventional chemotherapeutic treatments. The MEK inhibitor PD184352 was shown to inhibit the growth of colon cancer that was implanted into mice, without causing obvious adverse side effects over several months treatment (Sebolt-Leopold et al. 1999). The lack of significant side effects of MEK inhibitors is surprising since the ERK cascade has been implicated in many cell processes including cell growth. However, the essential roles of the ERK cascade in proliferation and differentiation are only required during development, and this pathway might be far less crucial for normal function in adults. Since cancer pain is so devastating (Mantyh et al. 2002), MEK inhibitors could be assessed for anticancer pain effects while being tested for antitumor effects in clinical trials.

Several p38 MAPK inhibitors including SB281838, BIRB0796, Ro320-1195, and SCIO-469 are in clinical trials for rheumatoid arthritis (Cohen 2002; Watkins and Maier 2003; Nikas and Drosos 2004). Given the important role of p38 MAPK in several pathological pain conditions, these inhibitors should also be tested for clinical pain, if they are well tolerated. JNK pathway is essential for regulating neurotoxicity and apoptosis. CEP-1347, an inhibitor for the JNK upstream kinase MLK (mixed lineage kinase) was in clinical trial for neurodegenerative diseases (Cohen 2002). Given the fact that neuropathic pain is also regarded as a neurodegenerative condition and JNK inhibition attenuates neuropathic pain (Zhuang et al. 2006), CEP-1347 or JNK inhibitor (e.g., D-JNKI-1) could also be tested for neuropathic pain in clinical studies.

In addition to MAPK inhibitors, wortmannin, a selective inhibitor for PI3K pathway, is in clinical trials for the treatment of osteoporosis (Noble et al. 2004). Inhibitors for Rho kinase (ROCK) such as HA1077 (AT877, fasudil) are used to treat cerebral vasospasm. Indirubin is a CDK5 inhibitor used to treat neurodegenerative disorders (Noble et al. 2004). Since all the kinases are implicated in pain facilitation, these inhibitors could be potentially useful for the management of clinical pain.

Many specific protein kinase inhibitors that cannot be used as drugs for reasons of toxicity, pharmacology, or solubility—such as the MEK inhibitors PD98059 and SB203580—could be useful reagents for basic research. While current efforts on developing protein kinase inhibitors focus on small molecules, membrane permeable peptide inhibitors could provide another efficient way to block the function of protein kinases. Recently, a peptide JNK inhibitor, derived from JNK binding domain of JNK-interacting protein-1 (JIP-1), was designed to 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, making the peptide membrane permeable. This highly specific peptide inhibitor is an extremely potent neuroprotectant both in vitro and in vivo (Borsello et al. 2003; Borsello and Bonny 2004). It is also highly effective in suppressing neuropathic pain symptoms after nerve injury (Zhaung et al. 2006). Studies on kinase regulation of pathological pain will greatly benefit from the development of specific and potent kinase inhibitors.

5 Summary

There are over 500 protein kinases in the human genome. More than 20 protein kinase inhibitors are in clinical trials, and many others have entered clinical trials without their structure being disclosed, and a great many more are still in preclinical studies. Protein kinases are becoming the second largest group of drug targets after GPCRs, accounting for 20%–30% of drug discovery activity in many pharmaceutical companies. There is increasing evidence suggesting that

protein kinases play essential roles in regulating various kinds of pathological pain in animal models. Although many protein kinase inhibitors are in clinical trials for treating different diseases, especially cancer, they are not specifically being tested for clinical pain.

Animal studies have shown that kinase inhibitors do not affect basal pain perception, indicating that protein kinases are not very active in normal conditions. Although several kinases such as ERK, PI3K, and Cdk5 are important for cell growth during development, they play different roles in the adult by regulating neural plasticity. Importantly, protein kinases are activated after tissue injuries and contribute to the induction and maintenance of pathological pain by posttranslational, translational, and transcriptional regulation. Therefore, protein kinase inhibitors are different from traditional analgesics such as morphine. Instead of raising pain thresholds, they are antihyperalgesic and antiallodynic by "normalizing" pain sensitivity.

There is growing interest in MAPK cascades in the field of pain research for the following reasons:

- There are specific antibodies available to detect the activation of ERK, p38, and JNK. Potentially, phosphorylated MAPKs could be used as biomarkers for pathological pain.
- Compared to other kinases, there are relatively specific inhibitors available to study the function of MAPK pathways.
- MAPK pathways appear to be downstream to many other kinases.
- Multiple MAPK inhibitors are in clinical trials for treating different diseases.

Chronic pain (persistent pathological pain) has affected hundreds of millions people in the world. Although pathological pain is an expression of neural plasticity manifesting as peripheral and central sensitization, the activation of glial cells enhance and prolong neuronal sensitization. Since traditional painkillers were mainly designed to target neurons and are only partially effective, the development of glial- or neural/glial-targeting protein kinase inhibitors (e.g., MAPK inhibitors) could lead to more effective treatments for chronic pain.

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