Glutamate Receptor Phosphorylation and Trafficking in Pain Plasticity in Spinal Cord Dorsal Horn

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Abstract

Glutamate is the major excitatory neurotransmitter in the central nervous system. Considerable evidence suggests that both ionotropic and metabotropic glutamate receptors are involved in pain hypersensitivity. However, glutamate receptor-based therapies are limited by side effects because the activities of glutamate receptors are essential for many important physiological functions. Here, we highlight recent key findings in molecular and cellular mechanisms of glutamate receptor regulation and their roles in triggering and sustaining pain hypersensitivity. Targeting these molecular mechanisms could form the basis for new therapeutic strategies for the treatment of chronic pain.

INTRODUCTION

Nociception refers to the neural processes for encoding and processing noxious stimuli. While acute nociception is protective as a warning system, chronic pain lacks obvious protective function and can be disabling, severe and intractable. Clinical manifestations of chronic pain include spontaneous pain, pain elicited by innocuous stimuli (allodynia) or exaggerated pain response to noxious stimuli (hyperalgesia) (see definitions on IASP homepage www.iasp-pain.org). Neuroplastic changes in both peripheral and central nervous systems are the key cellular and molecular substrates for the generation and maintenance of chronic pain (Woolf and Salter 2000; Basbaum *et al.*, 2009; Latremoliere and Woolf 2009; Sandkuhler 2009).

Because glutamate receptors are critical for neuroplasticity in nociceptive networks, there have been considerable efforts to develop therapeutic approaches that suppress the function of these receptors. These approaches have had limited clinical success largely due to limitations from the blockade of the many normal functions of glutamate receptors in the central nervous system. Recent understanding to the molecular mechanisms that regulate glutamate receptor function and signaling provide new opportunities to selectively interfere with those mechanisms that produce pain hypersensitivity.

Nociceptive transmission normally starts from the peripheral endings of those primary sensory afferents which are activated by noxious stimuli. Action potentials generated in

these nociceptive afferents are propagated into the spinal cord dorsal horn or the corresponding region in the trigeminal nuclear complex in the brain stem. At the central terminals of primary afferents, both nociceptive and non-nociceptive, glutamate is the major fast neurotransmitter which is released and mediates rapid excitatory post-synaptic potentials in dorsal horn neurons. In the presynaptic terminals glutamate is taken up into synaptic vesicles in neurons by vesicular glutamate transporter (VGLUTs) (Santos *et al.*, 2009). VGLUTs mediate the transport of glutamate from the cytoplasm into synaptic vesicles, driven primarily by the electrical component of H^+ electrochemical gradient generated by H^+ -ATPase (Santos *et al.*, 2009). There are three distinct isoforms of VGLUTs, with similar transport characteristics but exhibit an essentially complementary pattern of expression in primary sensory neurons (Todd *et al.*, 2003; Neumann *et al.*, 2008; Seal *et al.*, 2009). VGLUT1 is predominately expressed in the terminals of heavily myelinated A β fibers which mediate innocuous mechanical sensation. VGLUT1 positive afferents terminate in lamina III/IV and the inner part of lamina II (Ili) in dorsal horn, forming synapses with PKC γ interneurons in dorsal horn (Todd *et al.*, 2003; Neumann *et al.*, 2008). VGLUT2 is predominately expressed in terminals of thinly myelinated A δ fiber and unmyelinated C-fibers, including peptidergic and non-peptidergic, IB4 positive C-fibers. VGLUT2 positive afferents predominately mediate noxious sensations, and terminate in lamina I and II in dorsal horn (Todd *et al.*, 2003; Alvarez *et al.*, 2004). The most recently discovered glutamate transporter, VGLUT3, shows much more restricted distribution. VGLUT3 is expressed in a subpopulation of unmyelinated, low threshold mechanoreceptor, which is non-peptidergic and IB4 negative (Seal *et al.*, 2009). These afferents terminate in lamina I and Ili in dorsal horn (Seal *et al.*, 2009) and under physiological circumstances appear to mediate the sensation of pleasant touch (Loken *et al.*, 2009). Following inflammation or nerve injury, however, this population of neurons may be involved in mechanical allodynia, as VGLUT3 null mutant mice do not develop mechanical allodynia with inflammation or peripheral nerve injury (PNI) (Seal *et al.*, 2009).

Once glutamate is released it acts on post-synaptic glutamate receptors localized on second-order neurons in the dorsal horn. Three main classes of ligand-gated, ionotropic glutamate receptors (iGluRs), the α -amino-3-hydroxy-5-methyl-4-izoxazolepropionic acid receptor (AMPA), the kainate receptor (KAR), the *N*-methyl-D-aspartate receptor (NMDAR), and three groups of the G-protein coupled, metabotropic glutamate receptors (mGluRs), groups I–III, are involved in initiating and maintaining neuroplasticity (Woolf and Salter 2000; Latremoliere and Woolf 2009; Larsson 2009; Sandkuhler 2009; Larsson and Broman 2010). In the nociceptive system, glutamate receptors are expressed at peripheral, spinal and supraspinal levels. In this review, we focus on the glutamate receptors in primary sensory afferents and spinal dorsal horn neurons, with particular emphasis on the molecular and cellular mechanisms underlying the spinal synaptic plasticity in inflammatory or neuropathic pain. The roles of glutamate receptors in supraspinal nociceptive networks are reviewed in detail elsewhere (Bleakman *et al.*, 2006; Goudet *et al.*, 2009; Zhuo 2009).

SYNAPTIC PLASTICITY AT GLUTAMATERGIC SYNAPSES IN THE DORSAL HORN

All synaptic transmission from primary sensory afferents to second-order neurons is excitatory, involving iGluRs and mGluRs, at time scales from milliseconds to tens of seconds (Fig 1) (Woolf and Salter 2000;Latremoliere and Woolf 2009;Larsson 2009;Larsson and Broman 2010). Glutamate released from primary afferents binds to receptors strategically localized on second order neurons to produce fast excitatory post-synaptic potentials (fast EPSPs), mediated by AMPARs and KARs, on the scale of milliseconds (Fig 1A). NMDARs contribute little to the fast excitatory transmission, or to acute pain, as under normal circumstance NMDARs are down regulated at resting potential by Mg^{2+} block at the channel pore (Mayer *et al.*, 1984).

Importantly, NMDARs are also down regulated biochemically by biasing of kinase/phosphatase regulatory systems towards dephosphorylation under basal physiological conditions (Huang *et al.*, 2001;Pelkey *et al.*, 2002). However, NMDARs are recruited into synaptic transmission under high frequency stimulation, producing EPSPs on a scale of tens of milliseconds. The recruitment of NMDARs involves disinhibition of the Mg^{2+} block and receptor phosphorylation (Fig 1B).

Slow excitatory depolarizing synaptic potentials (slow EPSPs) are also critically dependent upon relief of the Mg^{2+} block from the NMDAR channel pore. Slow EPSPs are evoked by post-synaptic G-protein coupled receptor (GPCR) activation. Upon sustained, intense noxious stimuli, primary sensory afferents release peptide neuromodulators, such as substance P (Ikeda *et al.*, 2006) and CGRP (Schaible *et al.*, 1994) together with glutamate presynaptically. This sustained release of peptides and glutamate leads to activation of post-synaptic GPCRs, including group I mGluRs. The binding of ligand to GPCRs induces conformational changes in the transmembrane and intracellular domains of the receptor, leading to its interaction with heterotrimeric G protein. The activated G protein subunits then associate with downstream effectors to effect membrane potential by inhibiting K^+ channels or facilitating a non-selective cation conductance, leading to slow EPSPs lasting up to tens of seconds (Fig 1C). The slow EPSPs provide substantial opportunities for temporal summation of fast EPSPs, and the cumulative depolarization of the dorsal horn neuron leads to relief of the Mg^{2+} block in the NMDAR channel (Mayer *et al.*, 1984). The sustained depolarization furthermore recruits voltage-gated Ca^{2+} currents, triggering plateau potentials mediated by Ca^{2+} -activated non-selective cation channels (Morisset and Nagy 1999). Thus, post-synaptic Ca^{2+} concentration increases substantially, resulting in subsequent activation of multiple intracellular signaling pathways, leading to increased neuronal excitability and enhanced synaptic transmission. Importantly, the fast excitatory transmission mediated by AMPARs is also facilitated by signaling initiated and maintained by NMDARs. The end result of these processes is central sensitization- the enhancement in the activity of neurons and circuits in the nociceptive pathway. Thus, previously subthreshold synaptic inputs are recruited to generate increased or augmented responses (Woolf and Salter 2000;Salter 2005;Latremoliere and Woolf 2009).

The synaptic potentiation in dorsal horn underlying central sensitization may be homosynaptic or heterosynaptic (Woolf and Salter 2006). One form of homosynaptic potentiation in spinal cord is wind up, in which the action potential discharge elicited by a train of identical low frequency C-fiber strength stimuli gets larger on each successive stimulus. Windup results from the activation of NK1 and CGRP1 receptors that produce a cumulative membrane depolarization of slow EPSPs and subsequent activation of NMDARs by suppression of Mg^{2+} block as discussed above. The degree of relief of the Mg^{2+} blockade increases with increasing depolarization and thus windup is a feedforward process of progressive enhancement of current flow through NMDARs with repetitive synaptic inputs. Windup is transient, as the facilitation is lost quickly when the synaptic inputs stop and the membrane potential returns to the resting level within seconds of the end of noxious stimulation.

A distinct form of homosynaptic potentiation occurs in nociceptive dorsal horn neurons. This form of synaptic enhancement persists for hours or days after nociceptive synaptic stimulation and is thus a form of long-term potentiation (LTP)(Woolf and Salter 2000;Sandkuhler 2007). LTP is observed in NK1-expressing neurons in spinal lamina I and can be evoked by both brief periods of high frequency electrical stimulation (~100HZ) (Randic *et al.*, 1993) or low frequency electrical stimulation (i.e. 2HZ) (Ikeda *et al.*, 2006) at C-fiber strength (Sandkuhler 2007;Sandkuhler 2009). NMDAR (Ikeda *et al.*, 2003) and group I mGluR (Azkue *et al.*, 2003) activation is involved in the induction of LTP. Homosynaptic plasticity is bi-directional and under certain circumstances glutamatergic synapses in the dorsal horn may show long-term depression (LTD) (Sandkuhler 2007;Larsson 2009;Sandkuhler 2009;Larsson and Broman 2010).

In contrast to homosynaptic plasticity, in which the efficacy only of activated synapses is changed, the most prominent form of plasticity at excitatory synapses in the dorsal horn is heterosynaptic potentiation, in which activity in one set of synapses enhances activity in non-activated synapses(Woolf and Salter 2006). In fact, heterosynaptic potentiation appears to be the dominate form of use-dependent, persistent plasticity contributing to central sensitization in spinal cord nociceptive pathways (Latremoliere and Woolf 2009). Activation of group I mGluRs and subsequent release of intracellular Ca^{2+} from endoplasmic reticulum appears to be critical for spreading the facilitation from active synapses to neighboring non-active synapses (Fagni *et al.*, 2000;Adwanikar *et al.*, 2004).

Persistent potentiation of transmission at glutamatergic synapses in spinal nociceptive pathways, either homosynaptic potentiation or heterosynaptic potentiation, is critical for pain hypersensitivity. Behaviorally, homosynaptic potentiation contributes to primary hyperalgesia, whereas heterosynaptic potentiation contributes to secondary hyperalgesia and allodynia (Latremoliere and Woolf 2009). Thus, understanding the mechanisms which produce persistent synaptic potentiation in nociceptive pathways provides the opportunity to advance new therapeutic approaches.

A large body of evidence indicates that persistent synaptic plasticity at glutamatergic synapses in the CNS is critically dependent on post-translational modification of glutamate receptors by phosphorylation and alterations in the trafficking of these receptors are critical

processes (Woolf and Salter 2006; Latremoliere and Woolf 2009; Larsson 2009; Larsson and Broman 2010). Here we focus on recent discoveries on the role of glutamate receptor phosphorylation and trafficking in excitatory synaptic plasticity in nociceptive pathways in the dorsal horn. Interfering with these fundamental regulatory mechanisms has been found to suppress pain hypersensitivity in models of inflammatory and neuropathic pain, and as such, these targeting these regulatory mechanisms may provide new strategies for treating chronic pain.

IONOTROPIC GLUTAMATE RECEPTORS IN NOCICEPTION AND PAIN

Ionotropic glutamate receptors are comprised of three subgroups: AMPARs, KARs and NMDARs. At the core, each of these receptors is a tetramer of the pore-forming subunits, with the four subunits co-translationally assembled as a dimer of dimers in the functional receptor. Associated with the pore-forming core is a diversity of transmembrane and intracellular proteins to produce a multiprotein receptor complex. The associated proteins participate in receptor trafficking, control receptor activity and pharmacological characteristics, and mediate signaling downstream of the receptor. The core subunits of ionotropic glutamate receptor share a common structure: a long extracellular N-terminal domain, three transmembrane segments, a reentrant pore loop, and an intracellular C-terminal domain. The N-terminal domain and the extracellular loop form the ligand-binding pocket and the carboxyl tail regulates receptor trafficking, localization and phosphorylation (Kew and Kemp 2005; Sobolevsky *et al.*, 2009). The nomenclature for iGluRs recently put forward by the International Union of Basic and Clinical Pharmacology (IUPHAR) will be used here (Collingridge *et al.*, 2009).

AMPA receptors: nociception and pain plasticity

AMPA receptors are encoded by four receptor subunit genes, *GRIA1*, *GRIA2*, *GRIA3* and *GRIA4*. The functional AMPA channel is a tetramer with the assembly of one or more of these subunits. In adult animals, nearly all GluA2 subunits undergo post-transcriptional RNA editing, which significantly reduces the Ca²⁺ permeability of GluA2-containing AMPARs. AMPARs lacking edited GluA2, in contrast, are Ca²⁺ permeable and exhibit inward rectification (Fleming and England 2010). Mice deficient of *GRIA1* have reduced Ca²⁺ permeable AMPARs with decreased AMPA currents, whereas *GRIA2* deficient mice have increased Ca²⁺ permeable AMPARs and increased AMPA currents (Hartmann *et al.*, 2004).

AMPARs are expressed in both peripheral and spinal nociceptive pathways (Fig 2). In DRG neurons, AMPAR subunits are expressed in the cell bodies and in the peripheral or central axons (Sato *et al.*, 1993; Willcockson and Valtschanoff 2008). Presynaptic AMPA receptors at the central terminals of primary sensory afferents have been implicated in presynaptic inhibition of glutamate release (Lee *et al.*, 2002).

Postsynaptically, GluA1 and GluA2 subunit-containing AMPARs are expressed virtually in all the glutamatergic synapses in the dorsal horn, with the highest levels in laminae I-II, whereas GluA3 and GluA4 subunits are highly expressed in the ventral horn, with limited expression in superficial laminae (Nagy *et al.*, 2004a; Polgar *et al.*, 2008; Antal *et al.*,

2008; Todd *et al.*, 2009). At the cellular level, inhibitory neurons preferentially express GluR1 subunits, whereas almost all the excitatory neurons express GluR2-containing Ca^{2+} impermeable AMPARs (Latremoliere and Woolf 2009).

Pharmacological studies support a role of AMPARs in inflammatory pain or pain induced by tissue injury. For example, intrathecal application of AMPAR antagonists or Ca^{2+} permeable AMPAR antagonists significantly attenuates mechanical allodynia induced by burnt injury, thermal and mechanical hyperalgesia induced by intraplantar injection of inflammatory reagents carrageenan and complete Freund's adjuvant (CFA), and mechanical hyperalgesia induced by post-surgery pain (Sorkin *et al.*, 2001; Park *et al.*, 2008; Larsson 2009). Genetic studies also indicate that GluA1 and GluA2 containing-AMPARs are involved in inflammatory pain. In acute inflammatory pain induced by the first phase of formalin test or by capsaicin, both *GRIA1* and *GRIA2* null mutant mice have increased nocifensive behaviors (Hartmann *et al.*, 2004), suggesting GluA1 and GluA2 containing AMPARs are involved in the tonic pre-synaptic inhibition of neurotransmitter release from primary sensory afferents (Lee *et al.*, 2002). While in the second phase of formalin-test, *GRIA1* null mutant mice have reduced responses, and *GRIA2* null mutant mice have increased responses. In a more chronic inflammatory pain model, the CFA model, mice with null mutation of *GRIA2* but not *GRIA1* have increased thermal and mechanical hyperalgesia. These results indicate Ca^{2+} permeable AMPARs are essential for inflammation induced tonic or chronic pain (Hartmann *et al.*, 2004). Importantly, *GRIA1*, *GRIA2* and *GRIA3* null mutant mice have normal basal neurotransmission, and the basal thermal and mechanical thresholds are intact in each phenotype with the null mutant of each respective gene (Hartmann *et al.*, 2004).

Electrophysiological studies indicate that AMPAR-mediated spinal synaptic transmission in dorsal horn neuron is enhanced by inflammation, and this enhancement is suppressed by Ca^{2+} permeable AMPA channel blockers (Voitenko *et al.*, 2004; Katano *et al.*, 2008; Luo *et al.*, 2008). What are the molecular mechanisms underlying AMPAR-mediated enhanced synaptic efficacy? AMPARs can be phosphorylated at serine-threonine residues in the carboxyl tail of the subunits to upregulate channel activity or to change synaptic targeting of AMPARs (Lee 2006), phosphorylation of the GluA1 at the PKC/CaMKII site Ser831 or the PKA site Ser845 in the dorsal horn is observed after either plantar incision or inflammation induced by capsaicin or CFA (Fang *et al.*, 2003a; Nagy *et al.*, 2004a; Lu *et al.*, 2008). Phosphorylation at Ser831 increases single-channel conductance of homomeric GluA1 (Oh and Derkach 2005), whereas phosphorylation at Ser845 increases mean open probability of the channel and promotes surface insertion of GluA1 containing receptor (Esteban *et al.*, 2003). Therefore, phosphorylation of GluA1 subunit of AMPAR not only changes channel activity *per se*, but also regulates receptor trafficking. Such recruitment of GluA1 subunit into dorsal horn synapses is observed following various forms of inflammation (Galan *et al.*, 2004; Katano *et al.*, 2008; Park *et al.*, 2008; Pezet *et al.*, 2008); (Larsson and Broman 2008), which is consistent with the increased phosphorylation of spinal GluA1-S845 following inflammation (Fang *et al.*, 2003a; Fang *et al.*, 2003b). It is known that synaptic recruitment of GluA1 is a major mechanism contributing to synaptic potentiation (Kessels and Malinow 2009). Thus, AMPA synaptic transmission is potentiated in dorsal horn neurons following inflammation through phosphorylation of GluA1 subunits.

In addition to phosphorylation of GluA1 subunits, inflammation also induces rapid phosphorylation of GluA2 subunits at Ser880 site, in a PKC-dependent manner (Park *et al.*, 2009). In transgenic mice expressing mutant *GRIA2* which could not be phosphorylated at GluA2-Ser880, CFA-induced pain hypersensitivity is attenuated (Park *et al.*, 2009) (Fig 2B). Furthermore, inflammation also induces GluA2 subunit internalization, as the amount of GluA2 subunits is decreased in the PSD fraction but increased in endocytic vesicle fractions of the dorsal horn (Katano *et al.*, 2008; Park *et al.*, 2008; Park *et al.*, 2009). The internalization could be initiated by inflammation-induced GluA2 phosphorylation at Ser880 (Park *et al.*, 2009), which results in the subsequent disruption of GluA2 binding to its synaptic anchoring protein, glutamate receptor interacting protein (GRIP) (Park *et al.*, 2009), leading to subsequent GluA2 subunit endocytosis (Chung *et al.*, 2000). Alternatively, impaired association of GluA2 with its scaffolding protein, N-ethylmaleimide-sensitive fusion (NSF) protein, could also lead to GluA2 internalization as NSF, which is required for the retention of GluA2 on synaptic membranes, is reduced in the PSD fraction of the dorsal horn following inflammation (Katano *et al.*, 2008). Insertion of GluA1 subunits and internalization of GluA2 subunits together significantly increase Ca^{2+} -permeable AMPARs at spinal synapses (Vikman *et al.*, 2008), resulting in increased synaptic AMPA transmission in dorsal horn neurons, leading to inflammation-induced pain hypersensitivity (Fig 2B).

For pain hypersensitivity induced by PNI, pharmacological studies indicate that intrathecal administration of AMPAR antagonists or selective Ca^{2+} permeable non-NMDAR antagonists suppresses PNI-induced thermal hyperalgesia (Sorkin *et al.*, 2001; Garry *et al.*, 2003b). Consistent with involvement of AMPARs, thermal hyperalgesia is attenuated by a myristoylated peptides which disrupts the interaction of GluA2 with GRIP (Garry *et al.*, 2003b). In contrast to thermal hyperalgesia, mechanical allodynia induced by PNI is resistant to AMPAR antagonists (Sorkin *et al.*, 2001). Furthermore, neither GluA1-Ser831 nor GluA1-Ser845 is enhanced following PNI (Lu *et al.*, 2008). GluA1 and GluA2/3 subunit immunoreactive labeling is increased in the ipsilateral superficial dorsal horn following PNI (Harris *et al.*, 1996). The levels of GluA2 and GRIP protein are also increased in the ipsilateral dorsal horn following PNI (Garry *et al.*, 2003b; Tomiyama *et al.*, 2005). However, whether PNI induces AMPAR subunit trafficking in dorsal horn synapses remains to be investigated.

Converging evidence indicates that AMPA receptor phosphorylation is involved in inflammation but not PNI-induced pain hypersensitivity. Following inflammation, AMPAR-mediated synaptic transmission is enhanced in spinal dorsal horn neurons due to the phosphorylation of AMPARs, the insertion of GluA1 subunits into synapses and the increase of Ca^{2+} -permeable AMPARs at synapses. In contrast to inflammation, the cellular mechanisms of AMPAR receptor regulation in PNI-induced pain hypersensitivity need further investigation.

Kainate receptors: emerging role in nociception

The kainate family receptors (KARs) are encoded by five subunit genes, *GRIK1-5*. The subunits are assembled in various combinations to form the functional tetramer receptor. GluK1-3 are low affinity subunits and GluK4-5 are high affinity subunits. Compared with

AMPA receptors and NMDARs, the understanding of KARs in nociception is largely unexplored, mainly due to the relative lack of specific pharmacological tools. However, emerging evidence suggests that KARs, especially GluK1-containing KARs, play an important role in nociception.

Kainate receptors are expressed in both peripheral and spinal nociceptive pathways (Fig 2). In DRG neurons, the GluK1 subunits are expressed primarily in small-diameter non-peptidergic TRPV1 positive neurons, as well as the central axons of non-peptidergic primary afferents in superficial dorsal horn (Lee *et al.*, 2001). In the spinal cord dorsal horn, GluK1 subunits are localized at spinothalamic tract neurons (Hegarty *et al.*, 2007). An increase in GluK1-containing KARs is observed in spinal cord dorsal horn in models of inflammatory pain (Guo *et al.*, 2002b) (Fig 2B). Mice deficient in *GRIK1* exhibit reduced inflammation-induced nociceptive behaviors. As both phases of formalin-induced activities are suppressed in *GRIK1* null mutant mice, GluK1 is involved in both acute and tonic pain hypersensitivities (Ko *et al.*, 2005).

With the development of relatively more specific GluK1 antagonists, pharmacological studies support that KARs could be the new target for inflammatory pain treatment. Several studies demonstrate that systemic or spinal inhibition of GluK1-containing KARs reduces pain hypersensitivity induced by formalin, capsaicin, carrageenan and CFA (Guo *et al.*, 2002b; Dominguez *et al.*, 2005; Jones *et al.*, 2006). In a primate PNI model, electrophysiology studies indicate that responses of spinothalamic neurons to thermal and mechanical stimuli are blocked by the GluK1 antagonist, LY382884 (Palecek *et al.*, 2004). However, the behavioral effect of LY382884, or any other selective GluK1 antagonist, on PNI-induced pain hypersensitivity has not been investigated yet.

Thus, there is evidence indicates that KARs, especially GluK1 containing KARs, are involved in pain hypersensitivity induced by inflammation. In contrast, whether KARs are involved in PNI-induced pain hypersensitivity is still an open question. As receptor phosphorylation and trafficking are the major molecular mechanisms underlying AMPAR receptor-mediated dorsal horn synaptic potentiation following inflammation, it is not unreasonable that KARs could also be regulated by phosphorylation and trafficking after PNI.

NMDA receptors: nociception and pain plasticity

NMDARs are encoded by 7 subunit genes, *GRIN1*, *GRIN2A-2D*, and *GRIN3A-GRIN3B*. The functional NMDA tetramer channel has two obligatory GluN1 glycine binding subunits, and two GluN2 or GluN3 L-glutamate binding subunits (Furukawa *et al.*, 2005; Paoletti and Neyton 2007). Binding of two glutamates and two glycines leads to conformational changes that open the channel conductance pathway and subsequent channel activation. The subunit composition determines the pharmacological and biophysical characteristic of NMDARs. For example, GluN1/GluN2A diheteromeric receptors exhibit relatively fast deactivation compared with receptors containing other GluN2 subunits (Kew and Kemp 2005). In contrast to AMPARs and KARs, NMDARs have high Ca^{2+} permeability with slow activation/deactivation kinetics.

NMDARs are expressed in both peripheral and central nociceptive pathways (Fig 2). Peripherally, NMDARs are abundantly expressed in both small and large DRG neuron cell bodies and their peripheral terminus and central processes (Sato *et al.*, 1993; Marvizon *et al.*, 2002; Carlton 2009). The messenger RNA of all four subtypes are detected in DRG neurons, with GluN2B and GluN2D most abundantly expressed in protein level (Marvizon *et al.*, 2002; Li *et al.*, 2006a). NMDARs are transported to central terminals of primary sensory afferents to regulate presynaptic substance P release (Liu *et al.*, 1997); (Chen *et al.*, 2010). A recent study suggested NMDAR-induced presynaptic SP release is dependent on NR2B subunits and is controlled by the balance of the activity of receptor tyrosine kinase and phosphatase (Chen *et al.*, 2010). Inflammation increases GluN1 subunit expression in unmyelinated axons (Du *et al.*, 2003). Messenger RNA and protein level of GluN2B subunits are increased in the DRG following inflammation (Li *et al.*, 2006), with increased NMDAR current density and increased sensitivity to GluN2B selective antagonist inhibition (Du *et al.*, 2003; Li *et al.*, 2006b).

In the dorsal horn/dorsal horn, NMDARs are expressed abundantly, with GluN1 and GluN2A subunits expressed throughout the gray matter, whereas GluN2B subunits are concentrated in laminae I–II (Nagy *et al.*, 2004b). An increase in synaptic GluN2B containing NMDARs in dorsal horn neurons is observed following inflammation and PNI (Hu *et al.*, 2006b; Iwata *et al.*, 2007; Katano *et al.*, 2008; Yang *et al.*, 2009).

Spatial/temporal deletion of the obligatory GluN1 subunit of NMDAR in lumbar dorsal horn neurons results in suppression of phase 2 nociceptive behaviors induced by intraplantar injection of formalin and inhibition of pain hypersensitivity induced by CFA (Weyerbacher *et al.*, 2009; Garraway *et al.*, 2009). GluN2B is the critical subunit for NMDAR-dependent pain hypersensitivity as spinal knockdown of *GRIN2B* suppresses the second phase of formalin-induced nociceptive behaviors (Tan *et al.*, 2005). Furthermore, pharmacological block of NMDARs with selective GluN2B antagonists attenuates nociceptive behavior, thermal hyperalgesia and mechanical allodynia in various models of inflammation, neuropathic and cancer pain (Malmberg *et al.*, 2003; Abe *et al.*, 2005; Narita *et al.*, 2007; Gu *et al.*, 2009; Qu *et al.*, 2009). Importantly, basal sensory thresholds and acute nociceptive pain are not affected by genetic or pharmacological inhibition of spinal NMDARs, indicating that NMDARs are essential for chronic but not acute pain.

Inflammation or peripheral nerve injury increases NMDAR-mediated synaptic transmission, resulting in increased amplitude of excitatory postsynaptic currents (EPSCs) and Ca^{2+} signals in dorsal horn neurons (Hu *et al.*, 2006b; Luo *et al.*, 2008). What is the molecular mechanism of NMDAR-mediated synaptic enhancement in dorsal horn? At resting membrane potentials, NMDARs are blocked by Mg^{2+} in the channel pore in a voltage-dependent manner. Inflammation or PNI-evoked presynaptic release of glutamate, SP and CGRP leads to membrane depolarization (Ikeda *et al.*, 2006), which relieves the Mg^{2+} blockade (Mayer *et al.*, 1984). The activated NMDARs then allow Ca^{2+} to enter into neurons. This rapid increase in intracellular Ca^{2+} is the initial trigger for activity-dependent plasticity. (Fig 1B, Fig 2)

When intracellular Ca^{2+} is increased beyond a certain level, multiple intracellular signaling pathways including PKA, PKC, Src family kinase (SFK) are activated, and NMDAR function is upregulated through serine-threonine or tyrosine phosphorylation (Latremoliere and Woolf 2009; Larsson 2009; Larsson and Broman 2010). (Fig 2B).

Multiple factors are released into the spinal dorsal horn in response to peripheral tissue inflammation and PNI, such as bradykinin (Kohno *et al.*, 2008) and interleukin 1β (Zhang *et al.*, 2008a; Zhang *et al.*, 2008b). These factors can directly or indirectly activate PKC and PKA in post-synaptic dorsal horn neurons, leading the phosphorylation of GluN1 subunits. PKC- and PKA-dependent phosphorylation of Ser896 and Ser897 in GluN1 subunits in dorsal horn neurons is observed in models of inflammatory, neuropathic, cancer and visceral pain (Ulfenius *et al.*, 2006; Tian *et al.*, 2008; Zhang *et al.*, 2008a; Zhang *et al.*, 2008b; Yang *et al.*, 2009). GluN1 phosphorylation increases the amplitude of NMDAR-mediated EPSCs by increasing the opening rate of NMDAR channels, or modulating the synaptic targeting of NMDARs (Chen and Roche 2007). Besides the GluN1 subunit, increased GluN2B-Ser1303 phosphorylation is observed in capsaicin-induced pain hypersensitivity, but the significance of this phosphorylation site remains to be investigated (Zhang *et al.*, 2005).

In addition to serine-threonine phosphorylation, the function of NMDARs is also regulated by the balance of activity of the protein tyrosine kinase Src and striatal enriched tyrosine phosphatase (STEP) (Salter 2005). NMDAR tyrosine phosphorylation appears critical for triggering and maintaining pain hypersensitivities induced by inflammation or nerve injury (Guo *et al.*, 2002a; Liu *et al.*, 2008). There are 25 tyrosine residues in the carboxyl tail of the GluN2B subunit that could be tyrosine phosphorylated (Nakazawa *et al.*, 2001). The exact tyrosine phosphorylation sites involved in pain hypersensitivity are yet to be determined definitely, but Y1472 and Y1336 of the GluN2B subunit have been implicated (Abe *et al.*, 2005; Slack *et al.*, 2008; Peng *et al.*, 2010). Inflammation-induced spinal tyrosine phosphorylation is observed within minutes following inflammation, and lasts for days. Genetic deletion of the *Src* gene (Liu *et al.*, 2008) or pharmacological inhibition with the Src family kinase (SFK) inhibitor PP2 (Guo *et al.*, 2004; Liu *et al.*, 2008; Peng *et al.*, 2010) suppresses inflammation-induced tyrosine phosphorylation of spinal GluN2B and reverses pain hypersensitivity. Increased tyrosine phosphorylation of spinal GluN2B subunits is also observed following nerve injury (Abe *et al.*, 2005; Liu *et al.*, 2008). This enhancement lasts weeks after PNI, indicating that there is a sustained and ongoing increase of activity of Src relative to that of STEP (Liu *et al.*, 2008). Electrophysiology studies also support SFKs are involved in PNI-induced upregulation of NMDARs, as PP2 selectively suppresses the NMDAR-component EPSC evoked by A β -fiber stimulation in nerve-injured rats but has little effect on AMPA component EPSC (Hu *et al.*, 2006b). Furthermore, in *Src* null mutant mice (Liu *et al.*, 2008) or *Fyn* null mutant mice (Abe *et al.*, 2005), PNI-induced spinal GluN2B phosphorylation and mechanical allodynia are markedly attenuated, indicating both Src and Fyn maybe involved in PNI-induced NMDAR upregulation. Importantly, the basal sensory thresholds and the first phase formalin-induced behavior are unchanged in *Src* null mutant mice, indicating that Src is essential for chronic pain rather than acute pain (Liu *et al.*, 2008). Thus, biochemical, genetic, electrophysiological and behavioral evidence all point to a crucial role of SFK-mediated NMDAR upregulation in chronic pain hypersensitivity. Inflammation or nerve injury-induced activation of G protein coupled receptors (GPCRs),

such as group I mGluRs and EPI receptors or receptor tyrosine kinases, such as EphB2 receptors, and intracellular signaling pathways such as IP3 and PKC all converged in Src to upregulate NMDARs (Guo *et al.*, 2004; Abe *et al.*, 2005; Peng *et al.*, 2010) (Fig 3A). The phosphorylation of NMDARs by SFKs will increase channel open time and open probability, leading to increased NMDAR mediated synaptic currents (Salter 2005), thus resulting in pain hypersensitivity.

As Src-dependent phosphorylation of NMDARs is critically involved in both inflammatory pain and neuropathic pain, a 10-amino-acid peptide derived from Src unique domain was designed as a fusion with the protein transduction domain of HIV Tat protein (Src40-49Tat), rendering the peptide membrane permeant (Liu *et al.*, 2008). Src40-49Tat uncouples Src from the NMDA complex, therefore, inhibiting Src mediated NMDAR upregulation. Intravenous or intrathecal administration of Src40-49Tat reverses inflammation and PNI-induced mechanical, thermal and cold pain hypersensitivity, without changing basal sensory thresholds and acute chemical nociception. Furthermore, no confounding sedation, motor deficit or learning and memory impairment was observed at doses that suppress pain hypersensitivity (Liu *et al.*, 2008). As the peptide is derived from Src unique domain, it disrupts the binding of Src unique domain with the NMDAR complex through its adaptor protein, NADH dehydrogenase subunit 2, therefore avoiding potential confounding effects of general inhibition of the SFK family (Liu *et al.*, 2008). Thus, by uncoupling Src, from the NMDAR complex inhibits pain hypersensitivity while avoiding the deleterious consequence of directly blocking NMDARs (Bleakman *et al.*, 2006; Childers and Baudy 2007) (Fig 3B).

In addition to the phosphorylation of NMDAR subunits, the function and cell surface expression of NMDARs are also affected by membrane-associated guanylate kinase (MAGUK) proteins. The MAGUK proteins are intracellular scaffolding proteins which are comprised of a family of four main members, PSD-95, PSD-93, SAP102 and SAP 97. PSD-95 and PSD-93 are expressed in the superficial lamina of dorsal horn, and implied in nociception through their interaction with the C-termini of GluN2 subunits (Tao and Johns 2006). Spinal knockdown of PSD-93 (Zhang *et al.*, 2003) or PSD-95 (Tao *et al.*, 2001) markedly reduces thermal and mechanical hypersensitivity-induced by PNI or inflammation. Furthermore, PSD-93 null mutant mice or transgenic mice expressing a truncated version of PSD-95 are protected from pain hypersensitivity induced by inflammation or PNI (Tao *et al.*, 2003; Garry *et al.*, 2003a). Intriguingly, the molecular mechanisms underlying PSD-93 and PSD-95 in pain hypersensitivity are different. Mice with PSD-93 deletion exhibit a marked reduction in GluN2A and GluN2B tyrosine phosphorylation and decreased synaptic expression of NMDARs, accompanied with reduced NMDAR-dependent EPSC in brain and spinal cord (Tao *et al.*, 2003; Sato *et al.*, 2008; Liaw *et al.*, 2008). In contrast, the synaptic NMDAR expression and NMDAR-mediated EPSC are unaltered in PSD-95 truncated mice (Migaud *et al.*, 1998). Therefore, the anti-hyperalgesic effect observed by disrupting NMDAR-PSD95 is likely due to blocking the downstream effectors of NMDAR activation, rather than suppressing NMDAR activity *per se* (Tao *et al.*, 2008). One of the downstream effectors is nNOS, as disrupting PSD-95 association with nNOS also reduces thermal hyperalgesia induced by PNI (Florio *et al.*, 2009).

Collectively, the function of synaptic t NMDAR in spinal dorsal horn neurons is upregulated following inflammation or PNI, likely through serine/threonine phosphorylation of GluN1 subunits and/or tyrosine phosphorylation of GluN2B subunits. Selectively dissociating NMDARs from key enhancers of receptor function could be a new strategy to inhibit pain hypersensitivity. Alternatively, disrupting NMDAR interaction with scaffolding proteins and the downstream effectors could also provide novel targets for pain treatment.

METABOTROPIC GLUTAMATE RECEPTORS IN NOCICEPTION AND PAIN

The metabotropic GluRs (mGluRs) belong to the C-subfamily of G protein-coupled receptors (GPCRs) (Ritter and Hall 2009). The predicted mGluR topology includes a large N-terminal extracellular domain, three extracellular loops, three intracellular loops and a cytoplasmic C-terminus, separated by seven highly hydrophobic regions that span the lipid bilayer. The large N-terminal portion of the receptor forms a pocket which contains the glutamate-binding site, whereas the intracellular domains physically interact with the G-proteins to initiate signal transduction events (Ferraguti and Shigemoto 2006). Eight mGlu receptors have been identified and they are segregated into three groups: group I mGluRs (mGlu1 and mGlu5) are coupled to phospholipase C, and activation of this group of receptors generally increases neuronal excitability; group II (mGlu2 and mGlu3), and group III (mGlu4-mGlu8) mGluRs are negatively coupled to adenylate cyclase and activation of these two groups of receptors generally reduces neuronal excitability (Ferraguti and Shigemoto 2006). All three groups of the mGluRs are distributed throughout the central nervous system and are involved in various forms of pain hypersensitivity.

Group I metabotropic glutamate receptors: nociception and pain plasticity

Group I metabotropic glutamate receptors, mGluR1 and mGluR5, are expressed in both peripheral and spinal nociceptive pathways (Fig 2). In peripheral sensory afferents, group I mGluR receptors are expressed in both myelinated and unmyelinated peripheral afferents. In DRG cell bodies, a proportion of mGluR5-positive neurons also express transient receptor potential cation channel TRPV1 (Walker *et al.*, 2000; Zhou *et al.*, 2001).

In the spinal cord, mGlu1 and mGlu5 receptors are expressed in both soma and dendrites of dorsal horn neurons, with mGluR5s localized in the superficial laminae, and mGluR1 α s expressed throughout the rest of the dorsal horn with high levels in lamina V (Wang and Tseng 2004; Pitcher *et al.*, 2007). The expression of group I metabotropic glutamate receptors is altered in models of inflammatory or neuropathic pain. In DRG and dorsal horn neurons, the expression level of mGluR5s is upregulated following inflammation, PNI and diabetic neuropathy (Hudson *et al.*, 2002; Pitcher *et al.*, 2007; Osikowicz *et al.*, 2009; Li *et al.*, 2009). Inflammation also leads to a recruitment of mGluR5s to the plasma membrane, and lateral movement of plasma membrane associated mGluR1 α s toward the synapse (Pitcher *et al.*, 2007).

Systemic or intrathecal administration of selective group I mGlu receptor antagonists attenuates pain hypersensitivity induced by inflammation, PNI and streptozotocin-induced diabetic neuropathy (Hudson *et al.*, 2002; Soliman *et al.*, 2005; Schkeryantz *et al.*, 2007; Osikowicz *et al.*, 2009). The systemic effect is likely mediated through both peripheral

and central mechanisms. The peripheral effect is demonstrated by subcutaneous intraplantar administration of group I mGluR antagonists into the rat hindpaw. Ipsilateral, but not contralateral, paw injection reduces nociceptive responses and mechanical or thermal hyperalgesia induced by inflammation and PNI (Walker *et al.*, 2000; Dogrul *et al.*, 2000; Bhawe *et al.*, 2001), indicating that these effects are mediated peripherally locally. However, intrathecal administration of selective mGlu1 or mGluR5 receptor antagonists also reduces pain hypersensitivity induced by inflammation (Soliman *et al.*, 2005) or PNI (Dogrul *et al.*, 2000). Although a supraspinal effect could not be excluded by intrathecal injection, spinal antisense knockdown of mGluR1 reduces pain hypersensitivity-induced by inflammation or PNI, further supporting that spinal mGluR1s are involved (Fundytus *et al.*, 2002; Noda *et al.*, 2003). Both pharmacological inhibition and genetic deletion of group I mGluRs reduces the second phase but not the first phase of formalin induced behaviors, indicating that mGluR1 and mGluR5 are selectively involved in chronic pain rather than acute pain (Karim *et al.*, 2001; Noda *et al.*, 2003).

Both peripheral and spinal mechanisms appear to contribute to group I mGluRs in regulating pain hypersensitivity. In primary sensory afferents, group I mGluRs are functionally coupled with TRPV1 receptors, and activation of TRPV1 in primary sensory afferents by inflammation or nerve injury leads to glutamate release from both peripheral and central terminals (Jin *et al.*, 2009). This will subsequently activate mGluR5s, which in turn sensitize TRPV1 receptors through PLC, PGE2 and PKA pathways (Hu *et al.*, 2002). TRPV1 receptor activation will further increase intracellular Ca^{2+} and depolarizing primary sensory afferents, leading to increased quantal release of glutamate (Hu *et al.*, 2002; Kim *et al.*, 2009) (Fig 2B). There are several lines of evidence supporting that mGluR5s are functionally linked with TRPV1 receptors in the nociceptor. For example, in spinal cord slices, the presynaptic quanta release of glutamate evoked by mGluR1 and mGluR5 agonists is blocked by capsazepine, a TRPV1 receptor antagonist (Park *et al.*, 2004; Kim *et al.*, 2009). Furthermore, pain hypersensitivity-induced by spinal application of a group I mGluR agonist is markedly reduced in *TRPV1* null mutant mice (Kim *et al.*, 2009). At the cellular level, activation of group I mGluR evokes extracellular Ca^{2+} influx in TRPV1 positive DRG neurons, and this effect is blocked by a mGluR5 receptor antagonist (Kim *et al.*, 2009). Therefore, converging evidence indicates that mGluR5 are functionally coupled with TRPV1 receptor to regulate primary sensory afferent activation and presynaptic neurotransmitter release.

Postsynaptically, activation of spinal group I mGluRs in dorsal horn neurons increases synaptic transmission through at least two pathways. First, activation of postsynaptic mGluR5 increases intracellular Ca^{2+} through IP_3 receptor, resulting in PKC activation and subsequent Src activation. NMDARs are then subsequently phosphorylated, resulting in increased synaptic transmission (Guo *et al.*, 2004) (Fig 2B). Secondly, activation of group I mGluRs leads to an activation of extracellular signal regulated kinase (ERK) (Adwanikar *et al.*, 2004; Hu *et al.*, 2007), and activation of ERK through mGluR5 will phosphorylate K^+ channel subunit Kv4.2 at the S616 phosphorylation site (Hu *et al.*, 2006a; Hu *et al.*, 2007), resulting in increased neuronal excitability (Fig 2B). Therefore, both presynaptic and postsynaptic group I mGluRs play important roles in developing and sustaining pain hypersensitivity.

Thus, group I mGlu receptors in both primary sensory neurons and dorsal horn neurons are involved in pain hypersensitivity induced by inflammatory and PNI. Presynaptically, mGluR5 functionally couples with TRPV1 to regulate primary sensory afferents activation and presynaptic glutamate release. Postsynaptically, activation of mGluR5 leads to increased neuronal excitability and synaptic transmission by K⁺ channel subunit Kv4.2 phosphorylation and Src-mediated NMDAR phosphorylation.

Group II and III metabotropic glutamate receptors: emerging role in nociception

Both group II and III mGluRs are predominately expressed presynaptically in primary sensory DRG neurons and axons. For group II mGluRs, most mGluR2/mGluR3-positive cells are small diameter, TRPV1-positive neurons, and the majority of them co-express with IB₄ and P2X₃ (Carlton *et al.*, 2009). For group III receptors, the presence of mGluR4 and mGluR7 have been confirmed in DRG neurons and the central terminals in lamina I and the outer zone of lamina II in the spinal cord (Carlton and Hargett 2007; Zhou *et al.*, 2007).

Group II and III mGluRs are negatively coupled to the cAMP pathway. Activation of these receptors in primary sensory afferents inhibits nociceptor sensitization and presynaptic neurotransmitter release, thus depressing synaptic transmission in dorsal horn (Gerber *et al.*, 2000; Du *et al.*, 2008; Carlton *et al.*, 2009) (Fig 2B). Pharmacological activation of group II and III mGluRs inhibits pain hypersensitivity in various models. For example, systemically or intraplantar administration of mGluR2/3 agonists reduces inflammation- and PNI-induced pain hypersensitivity (Du *et al.*, 2008; Osikowicz *et al.*, 2009; Chiechio *et al.*, 2009). Systemic or spinal administration of group III mGluRs also inhibits both phases of formalin-induced nociceptive behaviors and attenuates mechanical hyperalgesia induced by inflammation or PNI (Marabese *et al.*, 2007; Goudet *et al.*, 2008; Dolan *et al.*, 2009).

Collectively, these studies indicate that the activation of group II and III mGluRs localized at peripheral and central terminal of sensory afferents suppresses pain hypersensitivity induced by inflammation or PNI. Therefore, activation of group II and group III mGluRs could be novel targets for pain treatment. Further studies are necessary to understand the molecular mechanism of these two receptor groups in nociception regulation.

Conclusions

Glutamate receptors have been the target for the development of pain treatments for decades. However, despite much encouraging preclinical data, truly safe and effective glutamate-based treatments for the devastating condition of chronic pain are yet to be developed.

Recent advance in our understanding of glutamate receptor regulation in pain plasticity provides new insights for developing novel glutamate receptor based pain management strategies. There is now abundant evidence indicates that glutamate receptor phosphorylation and trafficking play important roles in pain hypersensitivity. In spinal dorsal horn synapses, inflammation-induced the serine/threonine phosphorylation of AMPAR subunits, the insertion of GluA1 subunits into the synapses and the internalization of the GluA2 subunits lead to upregulated AMPAR-mediated synaptic efficacy. Inflammation and PNI-induced membrane depolarization relieves the Mg²⁺ block of NMDARs and boosting up the synaptic efficacy in a non-linear manner. NMDARs are also upregulated through serine/threonine and

tyrosine phosphorylation, resulting in increased NMDAR mediated synaptic potentiation. Furthermore, sustained release of presynaptic glutamate activates post-synaptic group I mGluRs, resulting G protein activation. The activated G protein subunits then associate with downstream effectors to inhibit K^+ channels or to facilitate NMDARs through Src-mediated phosphorylation. The end result of AMPAR, NMDAR and group I mGluR activation is sustained increased neuronal excitability and enhanced synaptic transmission, which result in pain hypersensitivity following inflammation of PNI. Disrupting these cellular and molecular pathways provide new opportunities to inhibit pain without the side effects of blocking glutamate receptors *per se*.

A main challenge for the above mentioned approach is that protein kinases that upregulate glutamate receptors in dorsal horn synapse are also involved in many other physiological pathways. Therefore, general inhibition of these kinases may not be a practical solution. Several recent studies demonstrate an alternative approach to inhibit pain hypersensitivity is to disrupt the upstream regulatory signaling pathways or the downstream transduction pathways from glutamate receptors. Disrupting protein-protein interaction with peptides, peptidomimetics or small molecules is therefore, a potential strategy for developing glutamate receptor-based therapies. As we continue to discover molecular and cellular mechanisms of glutamate receptor regulation and their role in pain hypersensitivity, we expect to identify more promising targets for chronic pain management.

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Fig 1.

Schematic illustration of the synaptic transmission from primary sensory afferents to second-order neurons in the dorsal horn. **(A)** Under normal circumstances, the fast excitatory post-synaptic potentials (fast EPSPs) are mediated by AMPARs and KARs, on the scale of milliseconds. **(B)** NMDARs contribute little to fast excitatory transmission as NMDARs are down regulated at resting potential by the Mg^{2+} block at the channel pore and receptor dephosphorylation. However, NMDARs are recruited into synaptic transmission under high frequency stimulation, producing EPSPs on a scale of tens of milliseconds. The recruitment of NMDARs involves disinhibition of Mg^{2+} block and receptor phosphorylation. **(C)** Sustained release of glutamate leads to activation of post-synaptic group I mGluRs, resulting in G protein activation. The activated G protein subunits then associate with downstream effectors to affect membrane potential by inhibiting K^+ channels or facilitating non-selective cation channels, leading to slow EPSPs lasting up to tens of seconds.

Fig 2.

Glutamate receptors in spinal cord synapses. **(A)** Schematic illustration of the basal condition of the synapses between the central axon of primary sensory afferents and dorsal horn neurons. NMDARs are blocked by Mg^{2+} at resting potential. **(B)** Intense noxious stimulation increases synaptic transmission. Presynaptically, group I mGluRs, especially mGluR5 sensitize the TRPV1 through PKA-dependent phosphorylation, resulting in increased presynaptic release of glutamate. On contrast, activation of presynaptic group II and group III mGluRs inhibits presynaptic neurotransmitter release. Postsynaptically, the phosphorylation of AMPARs, the insertion of GluA1 subunits and the internalization of GluA2 subunits lead to upregulated AMPAR-mediated synaptic efficacy and increased Ca^{2+} permeable AMPARs at synapses. Membrane depolarization relieves the Mg^{2+} block of NDMARs. NMDARs are also upregulated by receptor phosphorylation. Group 1 mGluRs enhance synaptic transmission by indirectly phosphorylation of NMDARs and increasing neuronal excitability by inhibiting K⁺ channel.

Fig 3.

Src family kinases (SFKs) are key enhancers of NMDAR-mediated pain hypersensitivity.

(A) Inflammation or nerve injury activates multiple signaling pathways, including G-protein-coupled receptor (GPCR) pathways, such as mGluR5 or receptor tyrosine kinase pathways (RTK), such as EphB2, to activate SFKs which upregulates NMDAR function and results in pain hypersensitivity. **(B)** Uncoupling SFKs from the NMDAR complex could be a new strategy to inhibit pain without the deleterious consequence of directly blocking NMDARs.