Targeting the NMDA Receptor Subunit NR2B for the Treatment of Neuropathic Pain

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Summary: Neuropathic pain is generally defined as a chronic pain state resulting from peripheral or central nerve injury, or both. An effective treatment for neuropathic pain is still lacking. The NMDA receptor, one type of the ionotropic glutamate receptors, is known to be important for triggering long-lasting changes in synapses. NMDA receptor-dependent synaptic plasticity plays roles not only in physiological functions such as learning and memory, but also in unwanted pathological conditions such as chronic pain. This review addresses recent progress on NMDA receptors in neuropathic pain, with particular emphasis on the NR2B-subunit-containing receptors. The expression and function of NMDA receptors in synaptic plasticity in the pain transmission pathway from dorsal root ganglia to the anterior cingulate cortex is reviewed, and preclinical and clinical investigations of selective NMDA receptor in neuropathic pain are discussed. The NMDA receptors, in particular NR2B-containing NMDA receptors, serve as promising targets for treatment of neuropathic pain.
Key Words: Neuropathic pain, glutamate, NR2B subunit, NMDA receptor, anterior cingulated cortex, Ro25-6981.

INTRODUCTION

Integrative approaches including the use of human brain imaging and genetically manipulated mice have consistently suggested that chronic pain is due to long-term plastic changes along sensory pathways. Plastic changes not only take place in peripheral nociceptors, spinal dorsal horn, subcortical nuclei, but also in cortical nuclei that are involved in the process of noxious information. The mechanisms involve the recruitment of silent fibers, silent synapses, long-term potentiation of excitatory transmission, loss of inhibitory control, phenotype switch, and structural sprouting, among others. Because of these basic neurobiological changes, the pain transmission pathway loses its selectivity, and thus non-noxious or moderate sensory stimuli such as gentle touch or mechanical pressure become extremely painful. Understanding neuropathic pain therefore requires understanding of plastic changes in somatosensory pathways, and targeting central plasticity is becoming a major direction for identifying pain-relieving medications.[1,2]

Signaling molecules that are involved in injury-related plasticity may provide potential drug targets for treating neuropathic pain. Among several candidates, the NMDA receptor, one of the major glutamate receptors that is important for synaptic plasticity, has been intensively investigated by both academic and pharmaceutical companies.[3–6] The aim of this review is to address recent progress in studying NMDA receptors in neuropathic pain. We first review the basic properties of the NMDA receptor and its role in synaptic plasticity, and then review NMDA receptor-dependent plasticity at different levels of pain transmission, from spinal cord to anterior cingulate cortex (ACC). Finally, selective NMDA receptor antagonists serving as potential drug targets and their preclinical and clinical investigations in neuropathic pain are discussed.

THE NMDA RECEPTOR

Basic properties

Glutamate is a major excitatory neurotransmitter in the CNS that exerts its effect on ionotropic glutamate receptors (i.e., NMDA, AMPA, and kainate receptors). Of

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these, NMDA receptors have received particular attention because of their pivotal roles in excitatory synaptic transmission and plasticity, and in various brain processes from memory formation to chronic pain.[3,7] Most glutamate-mediated excitatory postsynaptic potentials are comprised of a fast non-NMDA receptor-mediated component and a slower NMDA-receptor-mediated component. In addition, NMDA receptors are distinguished from AMPA and kainate receptors in high Ca^{2+} permeability, Mg^{2+} blockade at resting membrane potential, and requirement of a coagonist, glycine, for activation.[8]

NMDA receptors consist of heterotetrameric assemblies of different subunits within a repertoire of three subtypes, NR1, NR2, and NR3. There are eight different NR1 subunits generated by alternative splicing from a single gene, four different NR2 subunits (A, B, C, and D) and two NR3 subunits (A and B). Functional NMDA receptors in mammalian cells require the heteromeric combination of at least one NR1 and one NR2 subtype (tetramers incorporating two NR1 and two NR2 subunits of the same or different subtypes).[8] Some cells express NR3, which coassembles with NR1 or NR1 and NR2 to form NR1–NR3 or NR1–NR2–NR3 tetrameric complexes, activated by glycine instead of glutamate.[9]

Whereas NR1 distributes ubiquitously in the CNS, NR2 subunits exhibit regional distributions, and the amount of expression is developmentally related. In the neonatal brain, NR2B and NR2D subunits are highly expressed and over the course of development they are substituted or replaced by NR2A and NR2C. Functional properties of NMDA receptors, such as channel deactivation time, single channel conductance, and Mg^{2+} blockade, are determined by subunit composition. For example, heteromers containing NR1 plus NR2B mediated a current that decays three to four times more slowly than receptors composed of NR1 plus NR2A; diheteromeric NMDA receptors containing NR2A or NR2B subunits has higher conductance and sensitivity to Mg^{2+} than those of NR2C- or NR2D-containing receptors.[8]

Synaptic plasticity in the brain

Unlike AMPA receptor and kainate receptors, NMDA receptors are highly permeable to Ca^{2+}, a critical intracellular signaling molecule for triggering synaptic plasticity. The essential role of NMDA receptors in the induction of two forms of synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), is well established. Application of the NMDA receptor antagonist AP-5 has no effect on basal synaptic responses, but reliably blocks the LTP induction in hippocampal CA1 region and cingulate cortex.[10]

The key mechanism for the involvement of NMDA receptors in synaptic plasticity is its voltage-dependence. At resting membrane potentials, NMDA receptors are inactive because of blockade by extracellular Mg^{2+}, even in the presence of glutamate. Thus, to activate NMDA receptors at synapses, two events must occur simultaneously: 1) glutamate has to be released and bind to NMDA receptors and 2) the postsynaptic membrane has to be depolarized, so that the Mg^{2+} blockade can be removed. NMDA receptor-mediated Ca^{2+} influx then activates a series of signaling molecules within postsynaptic cells, including protein kinases, protein phosphatases, and immediate early gene proteins. The signaling pathways trigger the induction, as well as the subsequent maintenance of synaptic plasticity.[3,10]

Expression in somatosensory pain pathway

Nociceptive transmission starts from peripheral fibers of the dorsal root ganglia (DRG), then is conveyed to the spinal cord dorsal horn and, finally, to supraspinal structures such as brain stem, thalamus, somatosensory cortex, insular cortex, and cingulate cortex. At each relay, sensory synapses are under precise regulation, in order to provide appropriate behavioral responses. NMDA receptors are expressed in pain pathways from periphery to brain, and NMDA receptor-dependent long-term plastic changes along the pathway are believed to play a critical role in triggering neuropathic pain. As already noted, the functional NMDA receptor is composed of both NR1 and NR2 subunits. NR1 is widely distributed in the nervous system; the NR2 subunit is expressed differentially in the brain. For example, NR2A and NR2B are most abundant in forebrain and limbic areas, NR2C is expressed predominantly in the cerebellum, and NR2D is expressed mainly in midbrain and medullary neurons.[11,12] Here, therefore, we first review NMDA receptor expression in different regions of the pain transmission pathway, from the DRG to the anterior cingulate cortex.

NMDA receptors in peripheral nociceptive fibers may participate in neuropathic pain by mediating peripheral sensitization. It has been shown that NMDA receptors are expressed on the central and peripheral terminals of primary afferent neurons. For example, NMDA receptors on the central terminals of primary afferents regulate the release of substance P from unmyelinated C-fiber terminals.[13] Peripheral nociceptive fibers express NR2B, NR2C, and NR2D subunits, whereas NR2A subunits appear to be absent from the peripheral terminals of primary afferents.[14] The distribution of NR2 receptors might also differ, with NR1/NR2D or NR1/NR2C found only in C-fibers and NR1/NR2B found in both A-fibers and C-fibers.[14]

The spinal cord dorsal horn is the first relay station of nociceptive information from periphery to the brain. All NMDA receptor subunits (NR1 and NR2A–D) are expressed in the spinal dorsal horn; however, NR2B exhibits the largest expression among NR2 subunits, followed by decreasing proportions of NR2C, NR2A, and NR2D.[15] NR2A was found to be present in all parts of the gray matter

except lamina II, but NR2B was largely restricted to lamina II.[16] Other studies also showed that the NR2B subunit has a restricted distribution in the superficial dorsal horn.[17]

Abundant NMDA receptors are expressed in supraspinal pain-related structures, such as periaqueductal gray (PAG), rostral ventral medulla (RVM), thalamus, somatosensory cortex, insular cortex, and anterior cingulate cortex. For instance, in PAG and RVM, all receptor subtypes (NR1 and NR2A–D) are expressed.[18] In the human thalamus, the relative abundance of NMDA receptor transcripts was observed as NR2A > NR2B > NR2D > NR2C.[19] In pain-related cortical areas, NR2A and NR2B are predominantly expressed.[20]

Function in pain-related plasticity

Synaptic plasticity in the hippocampus is the cellular model for learning and memory, whereas synaptic plasticity in pain pathway may contribute to central sensitization and hyperalgesia.[21] The requirement of NMDA receptor activation in synaptic plasticity is common among many regions in the CNS. Neuropathic pain is likely due to long-term plastic changes along the nociceptive pathway.[2,21] Therefore, we review NMDA receptor-dependent synaptic plasticity in pain pathways, with particular emphasis on LTP.

In the dorsal horn of spinal cord, NMDA receptors are well known to be required for triggering central sensitization and wind-up under chronic pain conditions. Sustained noxious stimuli that are associated with tissue injury in neuropathic pain result in a temporal summation of postsynaptic depolarization, which could remove the Mg^{2+} blockade of NMDA receptor. The activation of NMDA receptor would allow Ca^{2+} influx, which in turn will activate calcium-sensitive intracellular signal cascades that lead to the phosphorylation of the NMDA and other receptor–ion channels, initiating prolonged increases in the excitability of spinal cord neurons, a process described as central sensitization.[22] The mechanism underlying wind-up also involves the activation of NMDA receptor and ketamine, a NMDA receptor antagonist, can block wind-up.[22]

Studies of LTP in spinal dorsal horn neurons draw much attention because it is believed that the potentiation of sensory responses after injury may explain chronic pain.[23] In spinal slices *in vitro*, LTP in the spinal dorsal horn neurons could be induced by several different protocols, including high-frequency stimulation, low-frequency stimulation, and pairing protocol. The mechanism of LTP induction involves the activation of NMDA receptors, neurokinin 1 receptors, and the downstream mitogen-activated protein kinase (MAPK) pathway.[24,25] In addition, *in vivo* LTP of C-fiber-evoked responses could also be induced by low-frequency or high-frequency stimulation of sciatic nerve fibers.[24] The LTP induction is also dependent on NMDA receptor activation. In particular, recent studies have shown that NR2B-containing NMDA receptor is required for spinal LTP induction.[26,27] More importantly, in animals with spinal cord and descending pathways intact, intraplantar injections of formalin or sciatic nerve jury induced LTP in the dorsal horn, and this was dependent on NMDA receptor activation.[28,29]

The thalamus plays an essential role in processing and relaying nociceptive information to the pain-related cortex. Stimulation in the human somatosensory thalamus can reproduce both the affective and sensory dimensions of previously experienced pain.[30] In addition, the thalamus may play an important role in pathological pain, because plastic changes also occur in the thalamus after neuropathic pain. For instance, long-term rearrangement or reorganization within the thalamus occurs after peripheral nerve injury.[31] Recent studies using dual whole-cell recording found that activation of metabotropic glutamate receptors causes long-term reduction of electrical synapse strength between the inhibitory neurons of the rat thalamic reticular nucleus.[32] However, there is no report on NMDA receptor-dependent synaptic plasticity in thalamus.

The somatosensory cortex, which is important for determining the location and quality of noxious stimulation, is under the reorganization of cortical representational maps after peripheral denervation, such as amputation.[33] In a study using *in vitro* slice recordings, our research group demonstrated that the somatosensory cortex can exhibit LTP induced by theta-burst stimulation.[34] The LTP is dependent on CaMKIV; however, it remains unsolved whether NMDA receptor is required. Thalamocortical synapses can undergo LTP by pairing protocol. The LTP is dependent on NMDA receptor activation.[35]

Human imaging and clinical studies have documented that the insular cortex is involved in pain, particularly related to emotional part of pain perception.[36] *In vitro* electrophysiological studies in this brain area, however, are rare. Our studies showed that forebrain NR2B overexpression increased NMDA receptor excitatory postsynaptic currents in the insular cortex and behavior sensitization,[37] and field recordings indicated that theta-burst stimulation induced LTP in insular slices.[34] The LTP is diminished in CaMKIV knockout mice, suggesting the involvement of the CaMKIV pathway in insular LTP.[34] Consistently, *in vivo* LTP in the insular cortex has been reported, and it is dependent on NMDA receptor activation.[38]

Recent studies from both human and animal tests consistently suggest that the ACC and its related areas are important for processing pain perception. Neurons in the ACC respond to nociceptive stimuli, and activity within the ACC is related to the unpleasantness or discomfort of somatosensory stimuli.[39] Electric stim-

ulation or chemical activation of the ACC induced pain and fear in animals,[40,41] whereas blocking excitatory transmission or downstream cAMP and adenylyl cyclase 1 and 8 (AC1 and AC8) pathway inhibited behavioral sensitization.[42]

Both NR2A and NR2B NMDA receptors are required for LTP induction in the ACC. The downstream multiple signaling pathways, such as calmodulin (CaM), calcium-stimulated AC1 and AC8, CaMKIV, and MAPK, are involved in LTP in the ACC.[34,43–45] In anesthetized rat, digit amputation induced LTP-like response *in vivo*. After amputation of a central digit of the hindpaw, a rapid enhancement of sensory responses to peripheral electrical shocks delivered to the normal hindpaw was observed. The potentiation was long-lasting: evoked responses remained enhanced for at least 120 min.[46]

A proposed model for the molecular mechanism of LTP in the ACC is given in FIG. 1, based on studies by our research group.[2,21,47] Neural activity triggered by injuries releases the excitatory neurotransmitter glutamate in the ACC synapse. The activation of glutamate NMDA receptors leads to an increase in postsynaptic calcium in dendritic spines. Calcium binds to CaM, leading to the activation of calcium-stimulated signaling pathways. Among these are calcium–CaM-stimulated ACs, including AC1 and AC8, and the calcium–CaM-dependent protein kinases PKC, CaMKII and CaMKIV. The calcium–CaM-dependent protein kinases phosphorylate glutamate AMPA receptors, thereby increasing their sensitivity to glutamate or the number of synaptic receptors. Activation of CaMKIV, a kinase predominantly expressed in the nucleus, triggers CaMKIV-dependent cAMP response element binding protein (CREB). In addition, activation of AC1 and AC8 leads to the activation of PKA, as well as CREB. In turn, CREB, as well as other immediate early gene proteins, activate targets that are thought to lead to long-lasting synaptic functional or structural changes (FIG. 1).

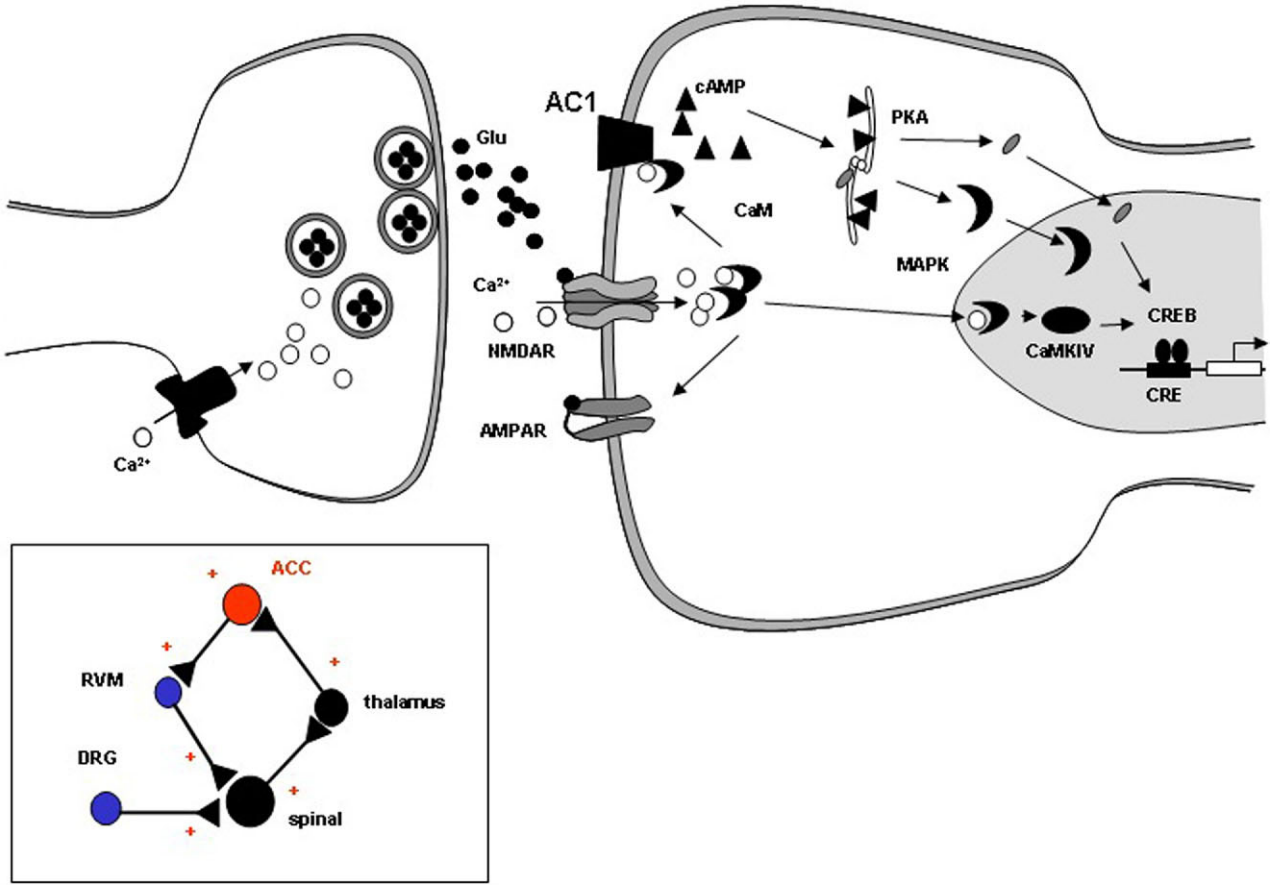

FIG. 1. Signaling pathways for NMDA receptor-dependent long-term potentiation in the anterior cingulate cortex (ACC). Neural activity triggered by injury releases glutamate in the ACC synapses. AMPA receptors (AMPAR) mediate the primary excitatory transmission and induce postsynaptic depolarization. Subsequent to activation of glutamate NMDA receptors (NMDAR), Ca^{2+} binds to calmodulin (CaM) and leads to activation of calcium-stimulated adenylyl cyclases (ACs), including AC1 and AC8, and Ca^{2+}–CaM-dependent protein kinases (PKC, CaMKII, and CaMKIV). Activation of CaMKIV, a kinase predominantly expressed in the nucleus, triggers CaMKIV-dependent cAMP response element binding protein (CREB). In addition, activation of AC1 and AC8 leads to activation of PKA, and subsequently CREB. CREB family and other immediate early gene proteins (e.g., EGR1) in turn activate targets that are thought to lead to more permanent structural changes. Inset: The pain transmission pathway, from dorsal root ganglia (DRG).

Pharmacology: NR2A *vs* NR2B receptor antagonist

Selective broad-spectrum competitive NMDA receptor antagonists (e.g., AP5, D-CPP, and channel blockers such as MK-801, ketamine, or memantine) have been developed since the NMDA receptor was discovered in the early 1980s.[5,48] The cloning of NMDA receptor subunits triggered a subsequent campaign to identify receptor subtype-selective compounds. The dissection of subunit-selective NMDA receptor functions will promote understanding of the molecular mechanisms underlying physiological and pathological processes, and the development of NMDA receptor subunit-selective antagonists has been the subject of intense research in recent years. However, subtype-selective antagonists for NMDA receptors are still limited.[49]

The most selective antagonist is ifenprodil, which has >200-fold greater preference for NR1/NR2B than for NR1/NR2A.[50,51] The ifenprodil derivative Ro 25-6981 has >3000-fold greater selectivity for NR1/NR2B than for NR1/NR2A. Zinc, an ion naturally occurring in the brain, has also been shown to be a selective antagonist of NR2A-containing receptors at nanomolar concentrations. Its selectivity for NR1/NR2A has >100-fold preference for NR1/NR2B. A recently developed and relatively selective NR1/NR2A antagonist, NVP-AAM077, has been found to have >100-fold preferential blockade of NR1/NR2A, compared with NR1/NR2B.[52,53]

Recent studies using these antagonists have shown that NR2A-containing NMDA receptors are required for LTP, whereas NR2B receptors are required for LTD.[53,54] However, the concept of subtype-dependent LTP and LTD was put in question by studies reporting the lack of NMDA subtype receptor selectivity for bidirectional synaptic plasticity.[45,55,56] Moreover, other researchers have argued that NVP-AAM077 is not sufficient to discriminate between NR2A- and NR2B-containing NMDA receptors with less than 10-fold selectivity.[57,58] In the ACC, our research group found that NVP-AAM007 shows great preference for the NR2A subunit and could be used as a selective antagonist for NR2A-containing NMDA receptors.[45,59] Although pharmacological agents that selectively block NR2C- or NR2D-containing receptors have not been discovered, (±)-*cis*-1-(phenanthrene-2-carbonyl) piperazine-2,3-dicarboxylic acid (PPDA) has been suggested to preferentially block NR2C- and NR2D-containing NMDA receptors.[60]

Function in chronic pain

As already noted, NMDA receptors are located in the DRG, the spinal cord, and, widely distributed, in the brain. Activation of NMDA receptors expressed on the peripheral terminals of primary sensory afferents by endogenously released glutamate during injury or inflammation causes pain-related behavior.[61] Peripheral administration of MK801, a noncompetitive NMDA receptor antagonist, produces local anesthetic-like effects and inhibits formalin-induced inflammatory pain.[62] The central sensitization that occurs in the spinal dorsal horn is widely held to be an important event in the pathway leading to neuropathic pain. In a rat neuropathic pain model of L5 spinal nerve transection, there was reduced expression of NR2A,[15] whereas in a model of partial chronic constriction injury to the sciatic nerve there was increased expression of NR2B (but not NR2A) and reduced NR1 in the superficial dorsal horn.[63]

In animal neuropathic pain models, NMDA receptor channel blockers such as memantine, MK801, and ketamine attenuate thermal and mechanical hyperalgesia in rats.[64] In a mouse model, conditional deletion of the NR1 subunit in spinal cord resulted in reductions in NMDA-mediated current and inflammatory pain,[65] but that study did not address neuropathic pain. Notably, NR2A knockout mice showed unaltered pain phenotype in both acute and neuropathic pain models,[66] which may suggest the potential role of NR2B-containing NMDA receptors in neuropathic pain. In support of this notion, spinal administration of NR2B antagonist ifenprodil or Ro 25-6981 produced potent antinociception and inhibited LTP in spinal dorsal horn in neuropathic pain model.[26,67]

Although spinal NMDA receptors have received a great deal of attention, evidence is accumulating that NMDA receptors located in supraspinal structures, such as brain stem, play an important role in chronic pain. Upregulation of mRNAs encoding NMDA receptor subunits, such as NR1, NR2A, and NR2B, has been observed in the RVM after inflammation.[18] Electrophysiological data, however, have been lacking for the functional upregulation of NMDA receptor-mediated responses in these studies. Microinjection of selective NMDA receptor antagonists prevented the inflammation-induced increase in RVM excitability.[68] Thus, the enhanced descending modulation appears to be mediated by changes in the activation of the NMDA receptor in the RVM.

Recent studies from our research group demonstrate the important role of cortical NMDA receptors, particularly NR2B-containing receptors, in chronic pain.[2,21] Evidence for the involvement of cortical NR2B receptor in pathological pain comes first from the so-called Doogie smart mice.[69] In this transgenic mouse with selective forebrain NMDA NR2B overexpression, electrophysiological and behavioral studies found that the NR2B transgenic mice are superior performers in memory tests, with significant enhanced hippocampal CA1 LTP.[69] In terms of tissue injury and inflammation, both inflammatory pain and allodynia were significantly enhanced without any effect on acute pain,[37] providing the first genetic evidence that NR2B-containing NMDA receptor in forebrain neurons may encode information related to pathological pain. In accord with these findings, a recent study using cortex-specific NR1 knockout mice showed reduced formalin-induced inflammatory pain.[70]

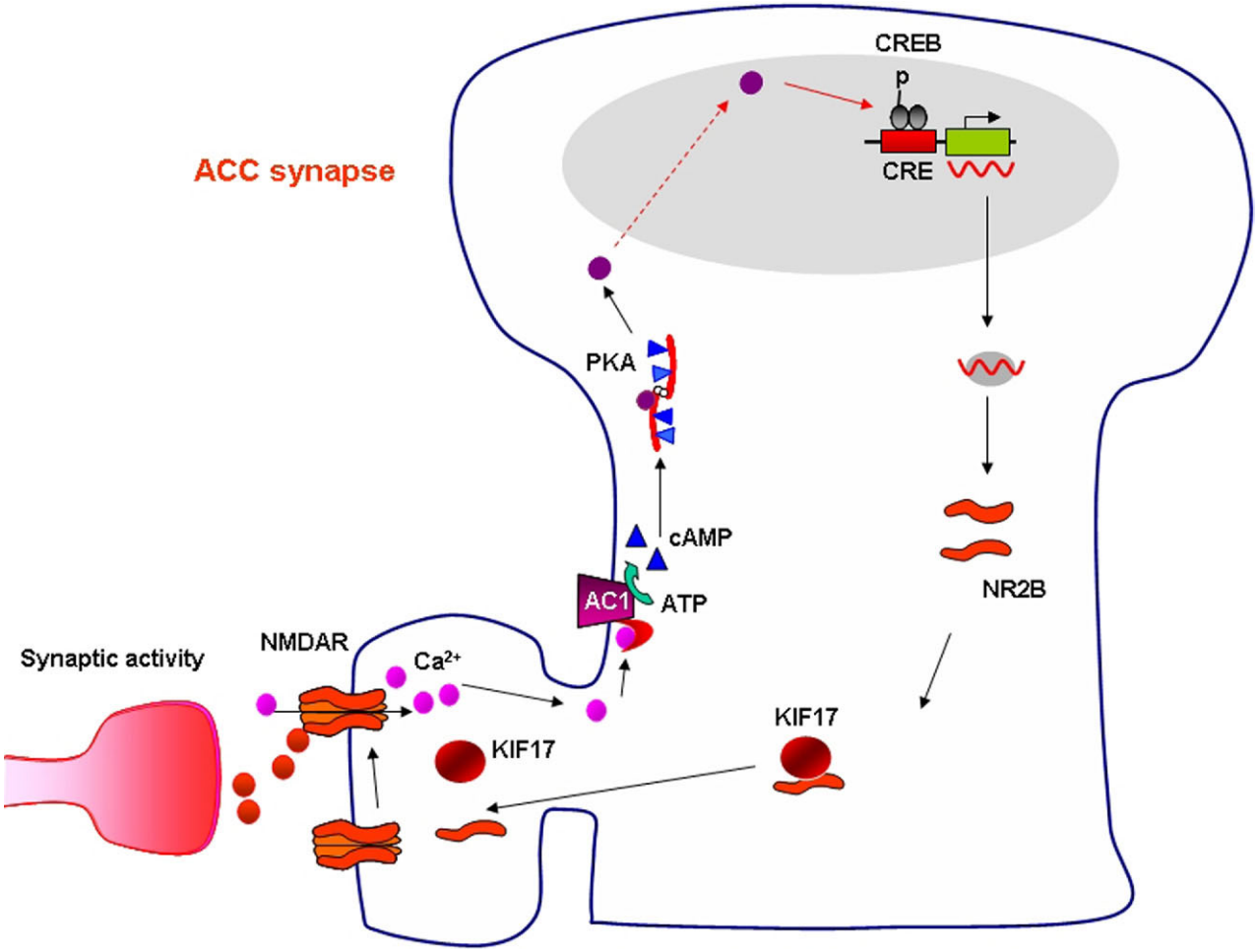

FIG. 2. Model for NR2B upregulation in chronic pain. Peripheral injuries trigger a burst of abnormal activity in the anterior cingulate cortex (ACC) circuits, and subsequently activate postsynaptic NMDA receptors (NMDAR) on cingulate pyramidal cells located in layer II–III. Activation of NMDAR triggers calcium influx. Postsynaptic increases in Ca^{2+} leads to activation of Ca^{2+}–calmodulin (CaM)-dependent pathways. Among them, Ca^{2+} and CaM stimulated AC1 is activated, and this activation leads to the generation of the key second messenger, cAMP. Subsequently, cAMP activates PKA. The PKA catalytic subunit then translocates to the nucleus and phosphorylates CREB. NR2B contains a CREB binding domain, which may couple increases in intracellular calcium with the increase in NR2B expression. Subsequently, postsynaptic synthesis of NMDA NR2B is increased; together with endogenous motor protein KIF17, these new NR2B subunits are added to postsynaptic NMDA receptors. Such positive feedback control may further enhance neuronal excitability within the ACC and so contribute to chronic pain.

In a recent study examining potential changes in NR2B receptors in the cortex of injured mice, peripheral inflammation increased the expression of NMDA NR2B receptors within the ACC, as predicted.[71] The changes in NMDA receptor protein expression were subtype selective, because other NMDA receptor subunits such as NR1 and NR2A did not show any significant change.[71] The increased NMDA NR2B receptors are likely to be within synapses, and single-shock focal stimulation-induced NMDA NR2B receptor-mediated synaptic currents were also enhanced in the ACC pyramidal neurons.[71] The mechanism of the NR2B upregulation may involve the AC1/AC8-PKA-CREB pathway (L.J. Wu et al., unpublished data), and new NR2B subunits may be added to postsynaptic NMDA receptors through motor protein KIF17 (FIG. 2).

The upregulation of NMDA NR2B receptors in the ACC can also be detected in freely moving animals.[71] In addition, local ACC injection or intraperitoneal injection of NR2B selective antagonist, Ro 25-6981, reduced the mechanical allodynia after inflammation.[71] In support of the cortical role of NR2B in chronic pain, recent studies showed that the environmental enrichment paradigm commonly used to enhance learning and memory can also enhance chronic pain, by increasing the function of NR2B-containing NMDA receptors in the ACC neurons.[72] In a nerve-ligation-induced neuropathic pain model, our research group found Ca^{2+}-activated AC1- and AC8-mediated enhancement of presynaptic glutamate release and postsynaptic AMPA receptor function.[73] It is conceivable that the NMDA receptor may act as the upstream of AC1 and AC8 activation after neuropathic pain.

PRECLINICAL AND CLINICAL STUDIES OF NMDA RECEPTOR ANTAGONISTS

As noted earlier, action at the NMDA receptors plays a critical role in chronic pain in animals. Among clinically assessed NMDA antagonists, however, the narrow separation between effectiveness and liabilities such as sedation, memory impairments, motor incoordination, and psychotomimetic effects has severely hampered their utility for the treatment of neuropathic pain. For example, intrathecal administration of CPP, a competitive NMDA antagonist, prevented radiation of the pain outside the territory of the injured nerve in a patient suffering from surgery-induced nerve injury,[74] but psychotomimetic adverse effects of the drug resulted in the termination of the study.

Preclinical data also showed perzinfotel (a potent, competitive NMDA receptor antagonist) to be efficacious in both inflammatory and neuropathic pain models.[75] Perzinfotel, however, shows low bioavailability after oral administration. Recently, oxymethylene-spaced prodrugs of perzinfotel were developed that exhibited improved oral bioavailability and were significantly more potent than perzinfotel after oral administration in a rodent model of inflammatory pain.[76] No data were available, however, for the analgesic effect of the compounds in neuropathic pain models.

Noncompetitive NMDA receptor channel blockers have shown positive activity in a number of preclinical and clinical neuropathic pain models.[77] For example, in clinical studies ketamine was effective in relieving pain intensity, wind-up, and allodynia in a number of pathological pain syndromes, including postherpetic neuralgia, spinal cord injury–induced central neuropathic pain, and peripheral neuropathy.[78,79] High doses of dextromethorphan showed statistically significant effects in diabetic neuropathy patients (although the reported effects may be underestimated, given the log-linear nature of the Gracely pain intensity scale), but had no effect in postherpetic neuralgia.[80,81] Amantadine relieved the intensity of ongoing postsurgical neuropathic pain in cancer patients by 31%, compared with placebo.[82] Unfortunately, the utility of these compounds was also limited by unacceptable psychotomimetic behavioral adverse effects.

NR2B subunit-containing NMDA receptors are localized predominantly in pain-relevant structures, such as in DRG cells, superficial layers of the dorsal spinal horn, thalamus, hippocampus, and cortex. In particular, NR2B has very low expression in the cerebellum.[17,20] The restricted distribution of NR2B receptors makes them promising candidates as targets of adverse effect-free analgesic drugs.[83] Indeed, NR2B antagonists, such as ifenprodil and related compounds, are effective in neuropathic pain in animals and in patients, and show better separation between efficacy and adverse effects than nonselective NMDA receptor blockers.[5,49] For example, antinociceptive doses of ifenprodil and eliprodil appear to be devoid of psychotomimetic effects, as well as of motor deficits. Importantly, these drugs do not lead to abuse and may otherwise have effect on the development of morphine-induced conditioned place preference.[84,85]

After the identification of ifenprodil, a second generation of compounds was developed, such as Ro 25-6981 and CI-1041, which have demonstrated efficacy in a number of animal pain models, apparently with superior adverse effect profiles, relative to earlier analogues.[5,77] One compound, traxoprodil (CP-101,606) has progressed to phase II clinical trials. A preliminary report indicates that intravenous administration of the compound is effective in patients suffering from central pain such as spinal cord injury, but show no typical psychotomimetic effects.[5] Therefore, although there is debate on spinal *versus* brain NR2B in mediating antinociceptive effects, NR2B-containing NMDA receptor is one of the best potential targets for neuropathic pain.[3,49,86]

FUTURE DIRECTIONS

After two decades of searching, there is still a lack of effective clinical treatments for neuropathic pain. Improved understanding of the molecular and cellular mechanisms of NMDA receptors in neuropathic pain offers hope for developing painkillers targeting at NMDA receptors. There is cumulative evidence that NMDA NR2B receptors play important roles in various cognitive functions, so one obvious challenge for targeting the NMDA NR2B receptor for pain treatment is adverse effects on cognitive functions, The use of such drugs in patients with chronic pain is likely to rest on the balance of gain and loss of brain functions.

Future research is needed to focus on the following routes to develop painkillers with improved therapeutic index.

1) Targeting NR2B-selective NMDA receptors. Over the past decade, accumulating evidence has suggested that the NR2B is effective in neuropathic pain treatment with fewer adverse effects. More effort should be devoted to developing selective NR2B antagonists and to testing them in preclinical and clinical trials.
2) Targeting forebrain NMDA receptors. Recent studies from both human and animal tests consistently suggest the forebrain areas are important for processing pain perception and unpleasantness of somatosensory stimuli.[87] Therefore, selectively targeting to NMDA receptor in forebrain can be expected to provide new ideas and insights into controlling neuropathic pain.[3]

3) Targeting extrasynaptic NMDA NR2B receptor. Studies have shown that NR2B receptors located at extrasynaptic sites may act differently than those within the synapses.[88] Investigation of the roles of extrasynaptic NMDA NR2B receptors, and the development of selective drugs targeted at these sites, may help develop treatment of neuropathic pain with reduced adverse effects.

4) Targeting at downstream signaling pathways of NMDA receptors. In addition to NMDA receptor, its downstream molecules, such as AC1, have also been shown to be involved in synaptic potentiation and central sensitization. Indeed, AC1 is reported to facilitate glutamatergic neurotransmission after neuropathic pain, and AC1 knockout mice have a phenotype of less mechanical allodynia in models of neuropathic pain.[42,73] CaMKIV can be another target for consideration as well.

In summary, more basic research is clearly needed to advance our understanding of neuropathic pain at different system levels. These include molecular, cellular, systemic, and behavioral levels. It is risky to develop any new drug without a basic understanding of its involvement, location, and mechanisms of action.

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