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Targeting *N*-methyl-D-aspartate receptors for treatment of neuropathic pain

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Abstract

Neuropathic pain remains a major clinical problem and a therapeutic challenge because existing analgesics are often ineffective and can cause serious side effects. Increased *N*-methyl-D-aspartate receptor (NMDAR) activity contributes to central sensitization in certain types of neuropathic pain. NMDAR antagonists can reduce hyperalgesia and allodynia in animal models of neuropathic pain induced by nerve injury and diabetic neuropathy. Clinically used NMDAR antagonists, such as ketamine and dextromethorphan, are generally effective in patients with neuropathic pain, such as complex regional pain syndrome and painful diabetic neuropathy. However, patients with postherpetic neuralgia respond poorly to NMDAR antagonists. Recent studies on identifying NMDAR-interacting proteins and molecular mechanisms of increased NMDAR activity in neuropathic pain could facilitate the development of new drugs to attenuate abnormal NMDAR activity with minimal impairment of the physiological function of NMDARs. Combining NMDAR antagonists with other analgesics could also lead to better management of neuropathic pain without causing serious side effects.

Keywords

complex regional pain syndrome; diabetic neuropathy; neuropathic pain; NMDA receptors; spinal cord; synaptic plasticity; synaptic transmission

Chronic pain, such as neuropathic pain caused by peripheral nerve injury and diabetic neuropathy, can result in prolonged suffering and reduced quality of life. Pain can occur spontaneously or as a result of exposure to mildly painful stimuli (hyperalgesia) or stimuli not normally perceived as painful (allodynia). However, the treatment of chronic pain remains a major challenge, with only approximately 50% of patients receiving adequate pain relief [1].

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The glutamate *N*-methyl-D-aspartate receptors (NMDARs), especially those located in the dorsal horn of the spinal cord, are critically involved in nociceptive transmission and synaptic plasticity and have long been considered a target for the treatment of neuropathic pain. However, despite numerous preclinical studies demonstrating the efficacy of NMDAR antagonists in treating neuropathic pain, clinical studies suggest that NMDAR antagonists have limited effects in a subpopulation of patients with chronic neuropathic pain. Here, we review the current literature about the function and regulation of NMDARs and the therapeutic effects of NMDAR antagonists on chronic neuropathic pain.

Structure, function & distribution of NMDARs

N-methyl-D-aspartate receptors are heteromeric protein complexes, and three families of NMDAR subunits have been identified: NR1, NR2 and NR3. There are eight different NR1 (*GluN1*) subunits, four different NR2 subunits (NR2A-2D and *GluN2A-2D*) and two NR3 subunits (NR3A and NR3B, and *GluN3A* and *GluN3B*). While the eight different NR1 subunits are generated by alternative splicing from a single gene [2], the NR2 and NR3 subunits are encoded by six separate genes [3–7]. NMDARs are tetramers that typically incorporate two NR1 subunits plus two NR2 subunits and, in some cases, include an NR3 subunit [8]. The subunit composition determines the pharmacological and physiological properties of the NMDARs [4,9]. NR1/NR2A channels display much faster inactivation than do NR1/NR2B, NR1/NR2C and NR1/NR2D subunits [9].

The NR1 subunits are ubiquitously distributed in all neurons and at all developmental stages in the CNS [10]. NR1/NR2A subunits become predominant as synapses stabilize and mature [11]. NR2A is gradually increased in the adult CNS but is absent in the embryonic CNS [10,12]. In early developmental stages, NR2B is abundant, but it declines with age and becomes more restricted to the forebrain, including the cortex, hippocampus, striatum, thalamus and olfactory bulb [10,12]. Electrophysiological data suggest that NR2A-containing NMDARs tend to be localized synaptically, whereas NR2B-containing receptors tend to be localized perisynaptically or extrasynaptically [13–15]. In the spinal cord, the predominant subunits of NMDARs include NR2A at synaptic sites and NR2B at extrasynaptic sites [16]. NR2C is absent at early developmental stages but becomes progressively predominant in the cerebellum. NR2D is mainly found in the thalamus, hippocampus and brainstem [12,17]. Of note, NR1, NR2A, NR2B and NR2D have been detected in the superficial dorsal horn of the spinal cord [16,18–20]. NR1 subunits are ubiquitously distributed in all laminae of the spinal cord, but the distribution of NR2B subunits appears to be restricted to the superficial dorsal horn of the spinal cord [19]. In addition, the NR1 subunits are present in the cell bodies and central terminals of both small- and large-diameter dorsal root ganglia neurons [21–23]. Dorsal root ganglia neurons also contain NR2B, NR2C and NR2D, but not NR2A [23,24].

In the three families of NMDAR subunits, NR1 binds to glycine, NR2 binds to glutamate and NR3 is found to bind to glycine. Thus, NMDARs composed of NR1 and NR3 subunits only require glycine for activation. The recent discovery of the functionally different NR3 subunits has caused many of NMDARs' known features to be reassessed [6,8,25,26]. NR3A can coassemble with NR1 and NR2A to form a receptor complex with distinct single-channel properties and reduced relative calcium permeability [26]. Interestingly, cotransfection of NR1, NR2A and NR3B into HEK293 cells results in significantly lower amplitude and calcium-permeability of the whole-cell currents than that permitted by NR1/NR2A receptors [7,25]. In addition, increasing the proportion of NR3B cDNA relative to NR1 and NR2A cDNA significantly reduces the NMDAR current amplitude [7]. A reduced shift in reversal potential, induced by switching the extracellular Ca^{2+} concentration from 1 to 20 mM, can be detected in NR3B-overexpressed hippocampal neurons (16.1 mV)

compared with native neurons (27.2 mV) [27]. Thus, NR3 subunits seem to negatively interfere with NMDAR activity. NR3A expression is relatively high at younger ages and then decreases with age [28], whereas NR3B expression gradually increases with age [29]. High levels of NR3A are present in the spinal cord, thalamus, hypothalamus, brainstem, CA1 of the hippocampus, amygdala and certain parts of the cortex [28]. NR3B is distributed in the forebrain, cerebellum and lumbar spinal cord [30]. Owing to the lack of selective agonists and antagonists, the function of NR3-containing NMDARs in synaptic transmission is not well understood.

Regulation of NMDAR function by subunit composition & phosphorylation

The unique feature of the NMDAR is its voltage and ligand dependence [31,32]. NR3A subunits have a larger influence on the voltage dependence of NMDARs [33]. The NMDAR is permeable to monovalent cations, including Na^+ and K^+ , and divalent cations, most notably Ca^{2+} . However, the NMDARs containing NR2C and NR2D are less sensitive to Mg^{2+} blocking than are NMDARs containing NR2A or NR2B [34]. The NMDAR's voltage dependence follows directly from channel blocking by submillimolar concentrations of extracellular Mg^{2+} , rather than from the voltage dependence of conformational changes [31,32]. At the resting membrane potential, Mg^{2+} largely blocks ion flow through the NMDAR channel. When the membrane is depolarized, Mg^{2+} is expelled from the channel, allowing for greatly enhanced passage of ions. The current–voltage relationship of typical NMDAR currents has a negative slope conductance [31,32]. In spinal dorsal horn neurons, the current–voltage curve shows the typical J shape of NMDAR-mediated currents [35]. Therefore, both depolarization of the postsynaptic neuron and presynaptic release of glutamate and glycine are required for maximum current flow through the NMDAR channel. Although glycine is considered a co-agonist for NMDARs, increased glycine release does not potentiate NMDAR currents in dorsal horn neurons [36]. Thus, an increase in synaptic glycine release probably activates inhibitory glycine receptors, rather than NMDARs, in the spinal dorsal horn.

The response of ionotropic glutamate receptors to agonists is usually potentiated after phosphorylation. NMDARs can be phosphorylated by protein kinase A (PKA) [37–39], PKC [38–40], casein kinase 2 (CK2) [41,42], Ca^{2+} /calmodulin-dependent protein kinase II (CAMKII) [43,44] and tyrosine kinases Src [45] and Fyn [46–48]. Phosphorylation by PKC increases the opening probability and decreases the affinity of NMDARs for extracellular Mg^{2+} [40]. Activation of PKC, possibly via upstream activation of group I metabotropic glutamate receptors, facilitates upregulation of NMDAR activity and enhances long-term potentiation (LTP) [49]. Ser-890, Ser-896 and Thr-879 in the C1 cassette of the NR1 subunit have been identified as PKC phosphorylation sites [38]. However, PKC-induced phosphorylation of NR1 at Ser-890 can inhibit clustering of NR1 [38], which might explain why stimulation of PKC suppresses NMDAR currents [50]. Although PKC directly phosphorylates the NR1 subunit [38,39,51], the PKC–cell adhesion kinase β /proline-rich tyrosine kinase 2–Src signaling cascade can indirectly upregulate NMDAR function [52]. In addition, PKC can phosphorylate Ser-1291 and Ser-1312 of NR2A [53] and Ser-1303 and Ser-1323 of NR2B [54] to potentiate currents through NR2A- or NR2B-containing NMDA channels.

Protein kinase A phosphorylates NR1 at Ser-879 in the C1 cassette of NMDARs [38]. In hippocampal neurons, PKA activation potentiates NMDAR activity indirectly by inhibiting calcineurin [37]. Activation of PKA is sufficient to induce synaptic clustering of NR1 [55]. By contrast, phosphorylation of NR1 by PKA can antagonize its interaction with spectrin and may initiate the morphological changes in dendrites associated with synaptic activity and plasticity [47]. CaMKII binds directly to NR1 and NR2B subunits, and activation of

CaMKII by stimulation of NMDARs in the hippocampal slices increases their association [43]. Ser-1303 of NR2B and perhaps the homologous serine in NR2A are phosphorylation sites for CAMKII in hippocampal neurons [56]. Switching from NR2B-containing NMDARs (with high affinity for CaMKII) to NR2A-containing NMDARs (with low affinity for CaMKII) dramatically reduces LTP [44].

Activation of tyrosine kinases increases NMDAR-mediated responses in neurons [57–59]. Approximately 2% of NR2A and 4% of NR2B, but not NR1, subunits are phosphorylated by tyrosine in synaptic plasma membranes [60]. Within the NMDAR complex, the nonreceptor tyrosine kinase Src is involved in the control of NMDAR activity through a cascade of intracellular signaling [45]. Thus, Src is considered to be an endogenous kinase that regulates NMDARs [45]. Tyr-1292, Tyr-Y1325 and Tyr-Y1387 of NR2A have been identified as the major Src-mediated phosphorylation sites [61]. NR2B is another major tyrosine-phosphorylated NMDAR subunit in the postsynaptic density [62], and its phosphorylation level is enhanced after LTP and by brain-derived neurotrophic factor treatments [63,64]. Seven specific tyrosine residues in the C-terminal cytoplasmic region of the NR2B subunit are phosphorylated by Fyn *in vitro* [65]. Of these seven residuals, three tyrosine phosphorylation sites (Tyr-1252, Tyr-1336 and Tyr-1472) are phosphorylated when active Fyn is coexpressed in the cell line [65]. Phosphorylation of NR2A by Src and Fyn kinases can potentiate NMDAR function [46]. Because Src and Fyn kinases have no effect on a receptor consisting of NR1 and a C-terminal truncation mutant of NR2A, the tyrosine phosphorylation sites may be targeted in the C-terminal domain of NR2A [46]. The potentiation of NR1/NR2B and NR1/NR2D by Src is much less than that of NR1/NR2A receptors [66].

In the hippocampus, the protein kinase CK2 seems to selectively potentiate the function of NMDARs but not that of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors [41]. High-frequency synaptic activity induces a transient increase in CK2 activity during induction of LTP in an NMDAR-dependent manner [67]. CK2 selectively enhances synaptic NMDARs and induces LTP in the hippocampus [68]. It has been suggested that CK2 may increase NMDAR function through indirect mechanisms, possibly involving postsynaptic density (PSD)-95/synapse-associated protein (SAP)90, because the NR2A/B and NR1 subunits are not CK2 substrates [69]. However, others have demonstrated that CK2 phosphorylates Ser-1480 of the NR2B subunit within its PDZ binding domain [42]. Phosphorylation of Ser-1480 disrupts the interaction of NR2B with the PDZ domains of PSD-95 and SAP102 and decreases surface NR2B expression in cortical neurons [42]. Results from a recent study suggest that during neuronal development, increased CK2 activity phosphorylates NR2B Ser-1480 to drive NR2B endocytosis and remove NR2B from synapses, causing an increase in synaptic NR2A expression in cortical neurons [70]. Thus, CK2 may be important in determining the NR2 subunit composition of synaptic NMDARs.

Conversely, NMDAR activity can be inhibited by serine and threonine protein phosphatases. Phosphatases 1 and 2A reduce the opening probability of NMDARs in cultured hippocampal neurons [71]. Calcineurin also shortens the opening time of NMDARs in acutely dissociated dentate gyrus granule cells [72]. CK2 and calcineurin appear to have opposite effects on NMDAR channel gating [41]. Endogenous tyrosine phosphatases may also regulate the probability of NMDAR opening in spinal dorsal horn neurons [73].

Role of NMDARs in nociceptive transmission in neuropathic pain

Neuropathic pain caused by diabetic neuropathy and nerve injury is associated with increased glutamate release from primary afferent terminals and stimulation of AMPA receptors and metabotropic glutamate receptors, especially mGluR5, in the spinal cord [74–

77]. Although glutamate mainly acts on postsynaptic receptors to mediate excitatory neurotransmission, presynaptic NMDARs can increase the release of neurotransmitters such as substance P from the primary afferent terminals in the spinal dorsal horn [78]. Furthermore, presynaptic NMDARs mediate increased glutamate release from the primary afferent terminals and LTP triggered by μ -opioid receptor stimulation [79]. On the other hand, it has been reported that activation of presynaptic NMDARs inhibits electrically evoked glutamate release from the primary afferent terminals [80]. It is possible that stimulation of presynaptic NMDARs can indirectly reduce neurotransmitter release through the inhibition of voltage-activated Ca^{2+} channels because of Ca^{2+} influx and calcineurin stimulation [81].

The pathological role of NMDARs and the therapeutic effects of NMDAR antagonists on neuropathic pain have been studied primarily using animal models of peripheral nerve injury. Bath application of NMDA induces a greater increase in the whole-cell currents and calcium influx in spinal lamina II neurons in nerve-ligated rats than in control rats [82]. However, an increase in NMDAR activity in the spinal cord in diabetic neuropathy and postherpetic neuralgia has not yet been specifically documented. By directly recording NMDAR currents of dorsal horn neurons triggered by primary afferent stimulation, our preliminary data suggest that the activity of NMDARs in the spinal cord is increased in rats subjected to peripheral nerve ligation injury but not in the rat models of painful diabetic neuropathy and postherpetic neuralgia [83]. Partial ligation of the sciatic nerve significantly increases the phosphorylated proportion of the NR1 subunit of NMDARs in the dorsal horn [84]. Furthermore, PKA may increase NR1 subunit phosphorylation in the spinal cord in rats subjected to ligation of the L5 spinal nerve [85]. However, the total protein levels of NR1 and NR2A–2D subunits in the spinal cord remain unchanged after nerve injury [84,85]. Thus, increased NMDAR phosphorylation may be critical for central sensitization induced by nerve injury. In neuropathic pain induced by transection of the L5 spinal nerve, Fyn kinase phosphorylates the NR2B subunit at Tyr1472, and neuropathic pain is reduced in Fyn-knockout mice [48]. Furthermore, peripheral nerve injury can increase tyrosine phosphorylation of NR2B in the spinal cord, with no effect on the total NR2B protein level [86].

Wind-up is a frequency-dependent increase in the excitability of spinal cord neurons evoked by electrical stimulation of C-fiber primary afferent nerves. Wind-up has been interpreted as a system for the amplification in the spinal cord of the nociceptive message that arrives from peripheral nociceptors [87]. Blocking NMDARs with ketamine or (2R)-amino-5-phosphonopentanoate can largely inhibit the wind-up phenomenon of spinal dorsal horn neurons [88,89]. Blocking NMDARs reduces the hypersensitivity of spinal dorsal horn neurons in neuropathic pain models. The NMDAR antagonists ketamine, memantine and MK-801 potently reduce evoked responses of dorsal horn neurons in spinal nerve-ligated rats [90]. In addition, the NR2B subunit-specific antagonist ifenprodil reduces the amplitude of NMDAR currents in nerve-ligated mice [91]. Administration of Ro 25-6981, another selective NR2B antagonist, to the spinal cord not only decreases the C-fiber responses of dorsal horn neurons in both normal and spinal nerve-injured rats but also significantly blocks the LTP induced by high-frequency stimulation [92]. It has been reported that nerve injury decreases calcineurin levels in the spinal cord [93], suggesting that decreased calcineurin levels may contribute to the increase in NMDAR phosphorylation that accompanies neuropathic pain. In addition, nerve injury-induced increases in NMDAR activity in the spinal cord could result from the subunit switch from NR2B to NR2A, but this possibility has not yet been carefully investigated.

Effects of NMDAR antagonists in animal models of neuropathic pain

Animal studies of the antinociceptive effects of NMDAR antagonists have been conducted almost exclusively using rodents subjected to different types of peripheral nerve injuries. Various noncompetitive NMDAR antagonists (i.e., MK-801, ketamine, memantine and dextrorphan) decrease the development of allodynia and hyperalgesia following constrictive injury of the sciatic nerve [94–97] and spinal nerve ligation [90,98,99]. Moreover, intrathecal application of amino-5-phosphonopentanoate, a competitive NMDAR antagonist, reduces mechanical allodynia caused by spinal cord injury [100]. Parenteral and oral administration of norketamine, a primary metabolite of ketamine, has been reported to alleviate mechanical and thermal hyperalgesia, but not tactile allodynia, in the sciatic nerve injury model without significant side effects [101]. Compared with ketamine and MK-801, the low-affinity NMDAR blockers memantine and neramexane display faster unblocking kinetics and greater voltage dependency. Thus, these agents may be able to inhibit the ongoing NMDAR activation presumed to occur in neuropathic pain with a lower incidence of side effects. Chronic administration of the noncompetitive NMDAR antagonists neramexane and memantine for 2 weeks produces persistent antinociceptive effects on mechanical hyperalgesia and allodynia in a rat model of diabetic neuropathic pain [102].

Several studies have demonstrated that selective glycine-site NMDAR antagonists such as L-701,324, MRZ2/576 and 5,7-dichlorokinurenic acid (5,7-DCK) reverse allodynia induced by sciatic and spinal nerve injuries [103,104]. Another glycine-site NMDAR antagonist, GV196771, also appears to be effective in the animal model of sciatic nerve injury [105,106]. Pyridazinoquinolinetriones are new glycine-site NMDAR antagonists with better aqueous solubility and oral bioavailability, and they can reduce neuropathic pain induced by sciatic nerve injury in rats [107].

Intrathecal application of the NR2B antagonists ifenprodil and Ro 25-6981 has been reported to dose-dependently reduce allodynia caused by spinal nerve ligation [92]. Furthermore, intrathecal administration of ifenprodil attenuates thermal hyperalgesia and mechanical allodynia induced by chronic compression of the dorsal root ganglia and in a model of bone cancer pain [108,109]. However, we found that intrathecal injection of ifenprodil produced no effect on tactile allodynia induced by spinal nerve ligation in rats [Chen S-R *et al.*, Unpublished Data]. Systematic administration of the NR2B antagonist CP-101,606 or Ro 25-6981 decreases neuropathic pain in the sciatic nerve injury model with fewer side effects than existing NMDAR antagonists [19]. Because intrathecal injection of CP-101,606 does not inhibit allodynia caused by sciatic nerve injury [110], CP-101,606 may exert its antinociceptive effect at the supraspinal level. Thus, NR2B at the spinal level does not appear to be important for the maintenance of neuropathic pain induced by nerve injury.

Because of the essential role of NMDARs in many physiological functions, the ideal approach for treating chronic pain would be to preferentially inhibit enhanced NMDARs but not alter basal NMDAR channel activity. Src is anchored within the NMDAR complex through NADH dehydrogenase subunit 2, an adaptor protein [111]. Thus, blocking the interaction between the Src unique domain and NADH dehydrogenase subunit 2 can release Src from the NMDAR complex, separating the enzyme and substrate, thereby inhibiting Src-mediated upregulation of NMDAR activity [111]. Systemic or intrathecal injection of a synthetic peptide consisting of amino acids 40–49 of Src fused to the protein transduction domain of the HIV Tat protein can reverse tactile and cold hypersensitivity induced by sciatic nerve injury in rats [86]. In our preliminary study, we found that inhibition of CK2 can also normalize NMDAR activity in the spinal cord and attenuate tactile allodynia induced by spinal nerve ligation without altering NMDAR currents and nociception in normal control rats [83].

Clinical studies on the efficacy of NMDAR antagonists in neuropathic pain

Intravenous infusion of ketamine at anesthetic and subanesthetic doses produces significant pain relief in patients suffering from refractory complex regional pain syndrome [112,113]. However, ketamine infusion does not prevent chronic neuropathic pain caused by thoracotomy in patients [114]. Intranasal administration of low-dose ketamine also decreases pain scores in patients with neuropathic pain of various origins [115]. In addition, high doses of dextromethorphan show modest effects on diabetic neuropathic pain but have no effect on postherpetic neuralgia [116]. Intravenous infusion of another NMDAR antagonist, amantadine, reduces the intensity of ongoing postsurgical neuropathic pain in cancer patients [117]. However, oral amantadine is not clinically useful because of frequent intolerable side effects and insufficient reduction of neuropathic pain [118]. Systemic administration of high-affinity NMDAR antagonists, such as ketamine, can produce various adverse effects in patients, such as hallucinations, drowsiness, restlessness, dissociation, vivid dreams, and memory and motor deficits. However, when ketamine infusion is carefully controlled to target certain plasma levels, it can be a safe and effective treatment for patients with neuropathic pain. Several clinical studies have demonstrated that intravenous ketamine infusion effectively reduces pain scores in patients with complex regional pain syndrome [112,119–121]. Oral administration of dextromethorphan also can reduce pain scores in patients with painful diabetic neuropathy, but it is not effective in patients with postherpetic neuralgia [116].

Although some new NMDAR antagonists have been tested clinically, they are no more effective than ketamine in reducing neuropathic pain. Memantine generally has a low incidence of CNS side effects because it exhibits fast blocking/unblocking kinetics and relatively strong voltage dependence at the NMDARs. Treatment with memantine significantly reduces pain scores in patients with complex regional pain syndrome caused by a traumatic injury to an upper extremity [122]. However, other studies have shown that despite its intriguing efficacy in animal models, memantine seems to have little effects on patients with painful diabetic neuropathy and postherpetic neuralgia [123], chronic limb pain [124] and sensory neuropathy associated with immunodeficiency virus infection [125].

Oral administration of a glycine-site NMDAR antagonist, GV196771, is not effective in reducing the intensity of evoked pain in patients with neuropathic pain (including diabetic neuropathy, postherpetic neuralgia and complex regional pain syndrome), although GV196771 does reduce the areas of static and dynamic mechanical allodynia [126]. The specificity and clinical efficacy of glycine-site NMDAR antagonists in neuropathic pain remain to be determined. In addition, a recent Phase II randomized, double-blind, placebo-controlled trial showed that an NR2B antagonist, radiprodil (RGH-896), has no significant effect on the mean daily pain scores of patients with diabetic neuropathy.

When combined with other classes of analgesics, NMDAR antagonists could have the potential for increased efficacy and reduced adverse effects. A double-blind study suggests that intravenous infusion of a low dose of ketamine plus oral gabapentin is safe and efficacious in reducing pain scores in patients with neuropathic pain secondary to spinal cord injury [127]. Combined treatments with intravenous ketamine and opioids also show improved efficacy in neuropathic pain in cancer patients [128]. In a Phase III clinical trial, zenvia, a combination of dextromethorphan and quinidine (a clinically used drug that can block Na⁺ and K⁺ channels and slow down the metabolism of dextromethorphan), lowers pain ratings more than placebo does in patients with diabetic neuropathic pain.

Expert commentary

N-methyl-D-aspartate receptors have been an attractive target for treatment of chronic neuropathic pain for two decades. Ketamine and dextromethorphan remain the most studied NMDAR antagonists in the clinical setting for the treatment of patients with neuropathic pain. NMDAR antagonists are very effective in reducing hyperalgesia and allodynia caused by nerve injury in animal models. However, clinical studies suggest that the therapeutic effects of this class of drugs are mostly limited to patients with complex regional pain syndrome and painful diabetic neuropathy. Further studies are warranted to determine whether increased NMDAR activity in the spinal cord after nerve injury results from direct phosphorylation of NR1 or a switch from NR2B to NR2A on the plasma membrane. On the basis of our current knowledge, targeting NR2B for neuropathic pain treatment does not seem to be effective or well justified. It would be interesting to determine whether overexpressing NR3A and/or NR3B at the spinal level could impair NMDAR function and reduce neuropathic pain.

It should be recognized that neuropathic pain is not a homogeneous disease condition, and different mechanisms may be involved in the neuropathic pain caused by traumatic nerve injury, diabetic neuropathy and viral infections. It is striking that most preclinical studies showing the importance of NMDARs in central sensitization, and the effects of NMDAR antagonists on neuropathic pain are conducted almost exclusively using ligation or transection of peripheral nerves in animal models. Increased NMDAR activity in the spinal cord has only been shown in traumatic nerve injury models, but this increased NMDAR activity has not been demonstrated directly in other neuropathic pain conditions such as painful diabetic neuropathy and postherpetic neuralgia. Furthermore, while spontaneous pain (i.e., pain scores reported by patients) is evaluated in most clinical studies, evoked pain or pain hypersensitivity (e.g., hyperalgesia and allodynia) is only measured in preclinical studies. These differences in pain assessments may account for the differences in the efficacy of NMDAR antagonists in neuropathic pain between clinical and animal studies. The possibility of a placebo effect should also be taken into account when interpreting the observed effects in patients.

N-methyl-D-aspartate receptor channel blockers, such as ketamine often produce several adverse effects due to their interference with normal NMDAR function in the CNS. An ideal treatment for neuropathic pain would be to reduce the increased activity of NMDARs while maintaining their physiological function. Identifying additional mechanisms of increased NMDAR activity after nerve injury could lead to the development of improved treatments for neuropathic pain. For example, inhibiting the NMDAR phosphorylation sites without blocking the channels themselves could improve the therapeutic window. In addition, targeting the interacting proteins and kinases that regulate NMDAR function at the spinal level, such as disrupting the Src-NADH dehydrogenase subunit 2 interaction and inhibiting CK2 activity, could selectively attenuate the increased NMDAR activity and neuropathic pain caused by nerve injury.

Five-year view

Using NMDAR antagonists alone may not produce adequate pain relief in the majority of patients. Moreover, NMDAR antagonists used in the clinical setting have a narrow therapeutic window, which could be improved by their combined use with other analgesic drugs. We predict that more studies will seek optimal combinations of NMDAR antagonists with other drugs such as opioids, α 2-adrenoceptor agonists and anticonvulsants.

Recent understanding of the mechanisms involved in regulating NMDAR function provides new opportunities to selectively reduce the NMDAR activity associated with neuropathic

pain. In the near future, more will be learned about changes in NMDAR activity in the spinal cord that are directly related to specific etiologies of neuropathic pain (e.g., traumatic nerve injury, diabetic neuropathy and postherpetic neuralgia). The only subtype-selective agents that have been developed are antagonists (e.g., ifenprodil) that selectively block NR1/NR2B receptors. Using a high-throughput screening utilizing cell lines expressing NR1/NR2A subunits and a fluorometric imaging plate reader/ Ca^{2+} assay, several sulfonamide derivatives have been identified, which display submicromolar and micromolar potency at NR1/NR2A receptors [129]. These recent efforts to develop selective NR2A antagonists [129] could prompt more studies focusing on targeting NR2A-containing NMDARs as a treatment option for neuropathic pain. Still, the drugs targeting NR2C and NR2D subunits may also have some therapeutic potential for treatment of neuropathic pain.

In addition, several subtype-selective positive and negative allosteric modulators of NMDARs have been developed recently [130], and these new NMDAR modulators may be promising in treatments for neuropathic pain without causing severe side effects. This includes a series of naphthalene and phenanthrene derivatives that display inhibitory and/or potentiating activity with remarkably different patterns of selectivity at NMDARs containing different NR2 subunits. In brief, several compounds have been shown to selectively potentiate responses at NR1/NR2A (UBP512), NR1/NR2A and NR1/NR2B (UBP710), NR1/NR2D (UBP551) or NR1/NR2C and NR1/NR2D receptors (NSC339614) and have no effect or inhibit responses at the other NMDARs [130]. Furthermore, UB512 only inhibits NR1/NR2C and NR1/NR2D receptors, whereas UB608 inhibits NR1/NR2A receptors with a 23-fold selectivity compared with NR1/NR2D receptors [130]. These agents represent a novel class of NMDAR allosteric modulator drugs that do not act at the glutamate- or glycine-binding sites, the ion channel, or the *N*-terminal regulatory domain, but appear to act at the dimer interface between individual subunit ligand-binding domains. Therefore, these new compounds could become valuable tools in identifying the physiological roles of distinct NMDAR subtypes.

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Key issues

- Increased *N*-methyl-D-aspartate receptor (NMDAR) activity at the spinal level plays an important role in the development of neuropathic pain caused by peripheral nerve injury. However, increased NMDAR activity has not been demonstrated specifically in other types of neuropathic pain, such as diabetic neuropathy and postherpetic neuralgia.
- Increased NMDAR activity in the dorsal horn of the spinal cord after a nerve injury probably results from increased phosphorylation of NMDARs. However, the possibility of a NMDAR subunit switch from NR2B to NR2A should be investigated further in neuropathic pain conditions.
- Disrupting specific NMDAR phosphorylation sites or inhibiting Src and CK2 may inhibit pathological NMDAR activity without blocking the physiological function of NMDARs.
- Patients with neuropathic pain are not a homogeneous population, and treatments should be tailored to the specific etiologies and underlying mechanisms of their neuropathic pain. For example, NMDAR antagonists such as ketamine and dextromethorphan would be used to treat complex regional pain syndrome but not postherpetic neuralgia.
- Because of the complex mechanisms involved in neuropathic pain, optimal combinations of NMDAR antagonists with other available analgesics could improve the relief of neuropathic pain and minimize adverse effects.