Intracisternal Administration of NR2 Antagonists Attenuates Facial Formalin-induced Nociceptive Behavior in Rats

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Aims: *To examine the antinociceptive effects of N-Methyl-D-aspartate (NMDA) receptor NR2 subunit antagonists in a rat model of the facial formalin test.* ***Methods:*** *Experiments were carried out on adult male Sprague-Dawley rats weighing 220 to 280 g. Anesthetized rats were individually mounted on a stereotaxic frame and a polyethylene tube was implanted for intracisternal injection and, 72 hours later, formalin tests were performed. NMDA receptor antagonists were administered intracisternally 10 minutes prior to subcutaneous injection of 5% formalin (50 μL) into the vibrissal pad.* ***Results:*** *The intracisternal administration of 25, 50, or 100 μg of memantine, an antagonist that acts at the NMDA ion channel site, significantly suppressed the number of scratches in the second phase of the behavioral responses to formalin. Intracisternal administration of a range of doses of 5,7-dichlorokynurenic acid, a glycine site antagonist, or DL-2-amino-5-phosphonopentanoate (AP-5), a nonselective NMDA site antagonist, produced significant antinociceptive effects in the second phase. Intracisternal administration of 1, 2.5, or 5 μg of (2R,4S)-4-(3 Phosphonopropyl)-2-piperidinecarboxylic acid (PPPA), a competitive NR2A antagonist, significantly suppressed the number of scratches in the second phase, while only the highest dose of PPPA (5 μg) significantly suppressed the number of scratches in the first phase. The antinociceptive effects of intracisternal injection of (αR,βS)-α-(4Hydroxyphenyl)-β methyl-4-(phenylmethyl)-1-Piperidinepropanol maleate (Ro 25-6981), a selective NR2B antagonist, were similar to those of PPPA. Injection of memantine, AP-5, Ro 25-6981, or vehicle did not result in any motor dysfunction. A low dose of PPPA (1 μg) or 5,7-dichlorokynurenic acid (2.5 μg) did not affect motor function. However, higher doses of PPPA and 5,7-dichlorokynurenic acid produced motor dysfunction.* ***Conclusion:*** *The present results suggest that central NR2 subunits play an important role in orofacial nociceptive transmission. Moreover, this data also indicate that targeted inhibition of the NMDA receptor NR2 subunit is a potentially important new treatment approach for inflammatory pain originating in the orofacial area.* J OROFAC PAIN 2010;24:203–211

Key words: antinociception, formalin, NMDA, NR2 receptor, orofacial pain

N-Methyl-D-aspartate (NMDA) receptors are major mediators of fast excitatory neurotransmission in the central nervous system (CNS). These multifunctional receptors have important roles in long-term potentiation, depression, synaptogenesis during neuronal development, synaptic plasticity under physiological conditions, and neuronal death under excitotoxic or

pathological conditions in the CNS.[1,2] A number of distinct NMDA receptor subtypes have been identified which differ in their sensitivity to a variety of ligands, kinetic properties, and interactions with intracellular proteins.[3] The NMDA receptor is composed of seven subunits, NR1, NR2A–D, and NR3A and B, which are all products of separate genes.[4] Expression of functional recombinant NMDA receptors in mammalian cells requires the coexpression of at least one NR1 subtype, an essential channel-forming subunit, and one NR2 subtype.[1,2,5,6] Receptor affinity for agonists and antagonists is dependent upon the type of NR2 subunit.[7,8]

Subcutaneous injection of formalin into the hind paw of rats has been reported to induce expression of NR2A, NR2B, NR2C, and NR2D subunits in the spinal cord.[9] In support of this data, spinal administration of Conantokin G, which selectively inhibits the NR2B subunit, may produce antinociception in formalin tests and in models of peripheral nerve injury and inflammation.[10] Consistent with an increasing number of reports implicating the NR2 subunit as an important contributor to pain mechanisms, several experimental studies have demonstrated the antinociceptive efficacy of NR2B-selective NMDA receptor antagonists.[11–13] These results show that NR2-containing NMDA receptors may play an important role in nociceptive transmission and suggest that they may be promising new therapeutic targets for the control of pain. Although chronic pain depends on the NMDA receptor, clinical use of competitive and noncompetitive NMDA receptor antagonists is limited by antagonist-induced side effects resulting from the suppression of normal physiological functions and by very narrow therapeutic indices.[14] Although differential modulation of NR1 or NR2 subtypes in the trigeminal ganglion has been demonstrated in rats with craniofacial muscle inflammatory pain,[15,16] there is little behavioral evidence for the role of NR2-containing NMDA receptors in orofacial nociceptive transmission.

The aim of the present study was to examine the antinociceptive effects of NR2 antagonists in a rat model of the facial formalin test. For this purpose, the study examined changes in nociceptive scratching behavior produced by formalin injection into the vibrissa pad after intracisternal administration of DL-2-amino-5-phosphonopentanoate (AP-5), a nonselective NMDA site antagonist; (2R,4S)-4-(3 Phosphonopropyl)-2-piperidinecarboxylic acid (PPPA), a competitive NR2A antagonist; and (αR,βS)-α-(4Hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-Piperidinepropanol maleate (Ro 25-6981), a selective NR2B antagonist. The effects of intracisternal injection of memantine, an antagonist that acts at the NMDA ion channel site, and 5,7-dichlorokynurenic acid, a glycine site antagonist, were also investigated.

Materials and Methods

Animals

Experiments were carried out on adult male Sprague-Dawley rats weighing 220 to 280 g. The animals were maintained in a temperature-controlled room (23 ± 1°C) with a 12/12 hour light-dark cycle (light on at 7:00 AM). All procedures involving the use of the animals were approved by the Institutional Animal Care and Use Committee of the School of Dentistry, Kyungpook National University, and were carried out in accordance with the ethical guidelines for the investigation of experimental pain in conscious animals proposed by the International Association for the Study of Pain. All treatments were randomized during experiments. All behavioral responses were measured by an experimenter who was blind to the treatment group in each experiment and the data were analyzed before the blind code was removed.

Intracisternal Cannulae Implantation

Anesthetized rats (pentobarbital sodium, 40 mg/kg ip) were individually mounted on a stereotaxic frame (model 1404, David Kopf Instruments) and a polyethylene tube (PE10, Caly Adams) was implanted for intracisternal injection.[17–19] The polyethylene tube was inserted through a small hole that was made in the atlantooccipital membrane and dura by the use of a 27-gauge syringe needle. The tip of the cannula was placed at the level of the obex. The polyethylene tube was then subcutaneously guided to the top of the skull and secured in place by a stainless-steel screw and dental acrylic resin. Facial formalin tests were initiated 72 hours after the surgery because previous studies have demonstrated that rats were fully recovered in 72 hours.[19,20] Because intracisternal catheterization may produce motor dysfunction, animals that showed motor dysfunction or malposition of the catheter after intracisternal catheterization were excluded from the analysis. For confirmation of the placement of the intracisternal cannula and the extent of drug dissemination from the cannula, pontamine sky blue dye was injected through the cannula at the end of the tests.

Facial Formalin Test

Facial formalin tests were performed as previously described.[21–25] In brief, 50 μL of 5% formalin was applied to the vibrissal pad subcutaneously. For each animal, the number of nociceptive behavioral responses, including rubbing or scratching the facial region proximal to the injection site, was recorded for nine sequential 5-minute intervals. The formalin-induced responses showed two distinct phases consisting of an early short-lasting response (0 to 10 minutes, first phase) and a continuous prolonged response (11 to 45 minutes, second phase) separated by an interval of relative inactivity.

Administration of Chemicals

Intracisternal injection of AP-5 (0.02, 0.2, 2 μg), a nonselective NMDA site antagonist, PPPA (1, 2.5, 5 μg), a competitive NR2A antagonist, or Ro 25-6981 (12.5, 25, 50 μg), a selective NR2B antagonist, was performed 10 minutes before formalin injection. This concentration of PPPA has been shown to attenuate the glutamatergic NMDA-dependent potentiation of spinal reflex activity in rats.[26] The intrathecal pretreatment with Ro 25-6981 has been shown to dose-dependently inhibit the hyperalgesia induced by mGluR1/5 receptor agonist in mice.[27] Changes in the number of noxious scratching responses were recorded for 45 minutes after formalin injection. This study also investigated the antinociceptive effects of memantine, an antagonist acting on the NMDA ion channel site, and 5,7-dichlorokynurenic acid, a glycine site antagonist.[28,29] All animals were used only for one treatment. The number of animals in each treatment was eight. All NMDA receptor-related chemicals were purchased from Tocris Cookson Inc, Ellisville, MO, and dissolved in normal saline, with the exception of 5,7-dichlorokynurenic acid, which was dissolved in 70% dimethyl sulfoxide (DMSO).

In the control group, 10 ml of saline was administered intracisternally as a vehicle of AP-5, PPPA, Ro 25-6981 or memantine and 10 ml of 70% of DMSO/saline was administered intracisternally as a vehicle of 5,7-dichlorokynurenic acid.

Rotarod Test

Changes in motor performance after the intracisternal administration of AP-5, PPPA, Ro 25-6981, memantine, or 5,7-dichlorokynurenic acid were measured using a rotarod (Ugo Basil, Comerio) as described previously.[19,30] The rotarod speed increased to 16 rpm, with the maximum time spent on the rod set at 180 seconds. For acclimatization, naïve rats received two or three training trials on two separate days prior to testing. On the day of the experiment, the time course of motor performance was examined before and after intracisternal administration of NMDA receptor antagonists.

Statistical Analyses

For the behavioral analyses, the number of scratches in the first and second phases was measured, respectively, after intracisternal administration of NMDA receptor-related chemicals. Effects of NMDA receptor-related chemicals on behavioral responses were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett post-hoc analysis. In all statistical comparisons, $P < .05$ was used as the criteria for statistical significance. All data are presented as the mean ± standard error (SE).

Results

Subcutaneous injection of 50 μL of 5% formalin into the vibrissal pad of rats increased the frequency of noxious scratching behavior in the orofacial area. The formalin-induced responses showed two distinct phases of an early short-lasting response (0 to 10 minutes, first phase) and a continuous prolonged response (11 to 45 minutes, second phase) separated by a time of relative inactivity. For confirmation of the extent of drug dissemination from the cannula, pontamine sky blue dye was injected at the end of the tests. Pontamine sky blue was intensively observed at the obex level.

Figure 1 illustrates changes in the number of scratches produced by formalin after intracisternal administration of memantine (a NMDA ion channel site antagonist) or 5,7-dichlorokynurenic acid (a glycine site antagonist). Intracisternal administration of 100 μg but not 25 or 50 μg of memantine attenuated the scratching behavior in the first phase ($P < .05$). In the prolonged response, 25, 50, and 100 μg of memantine significantly suppressed the number of scratches produced by formalin injection as compared with vehicle treatment ($P < .05$). In addition, only 25 μg of 5,7-dichlorokynurenic acid decreased the number of formalin-induced scratches in the first phase ($P < .05$). However, in the second phase, intracisternal administration of 0.25, 2.5, or 25 μg of 5,7-dichlorokynurenic acid significantly decreased the number of scratches produced by formalin as compared with vehicle treatment ($P < .05$).

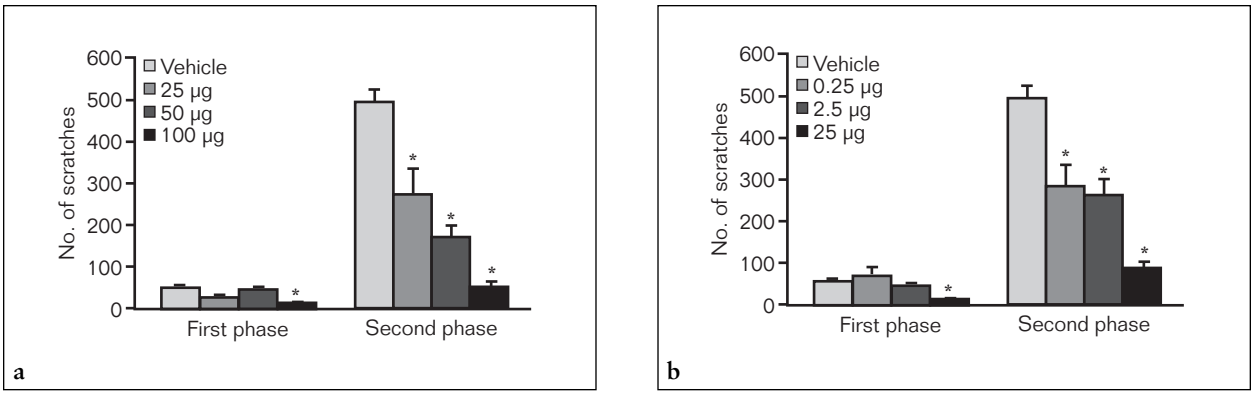

Fig 1 Effect of intracisternal injection of *(a)* memantine, a NMDA antagonist that acts at the ion channel site, or *(b)* 5,7-dichlorokynurenic acid, a glycine site antagonist, on the number of scratching responses produced by facial formalin injection. n = 8 rats per each group. An asterisk indicates significant decrease as compared with vehicle injection (*$P < .05$).

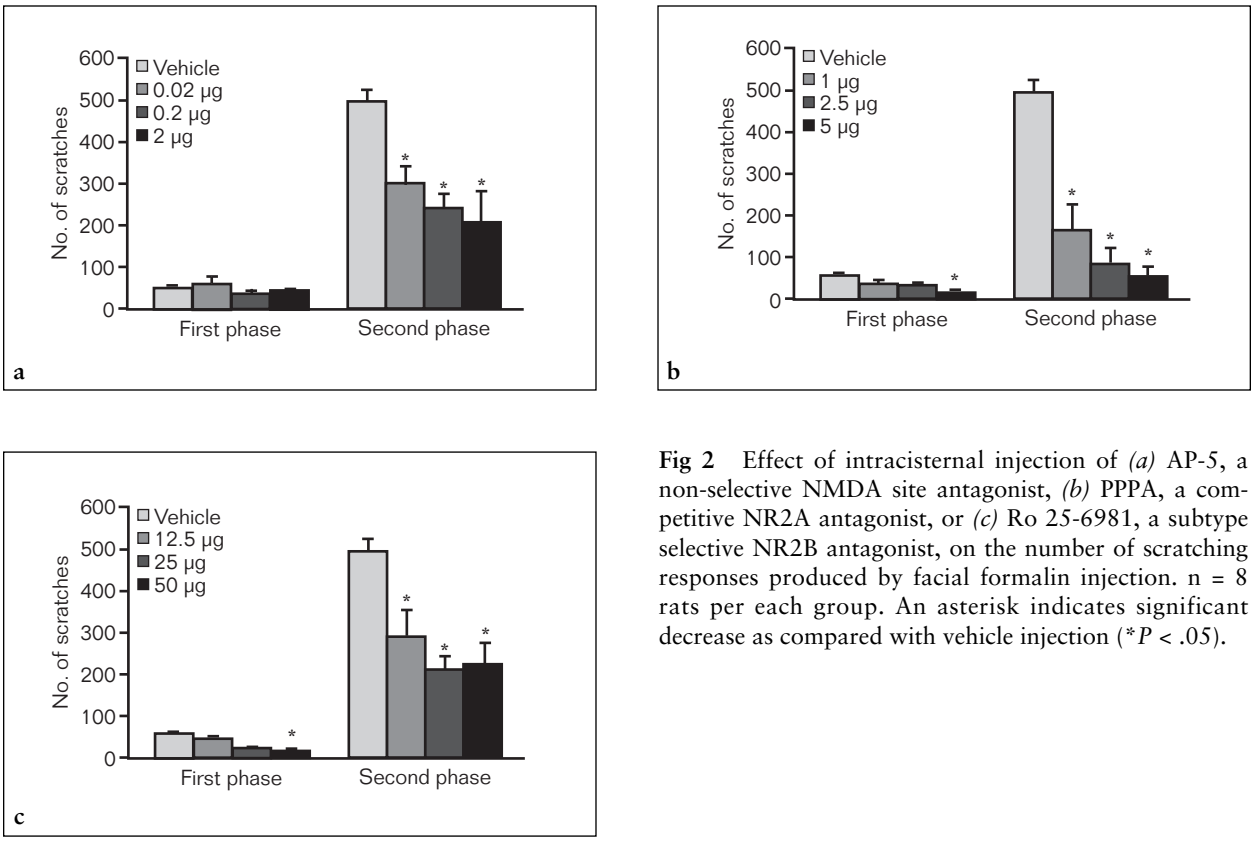

Fig 2 Effect of intracisternal injection of *(a)* AP-5, a non-selective NMDA site antagonist, *(b)* PPPA, a competitive NR2A antagonist, or *(c)* Ro 25-6981, a subtype selective NR2B antagonist, on the number of scratching responses produced by facial formalin injection. n = 8 rats per each group. An asterisk indicates significant decrease as compared with vehicle injection (*$P < .05$).

Figure 2a illustrates the changes in the number of scratches after intracisternal administration of AP-5 (a nonselective NMDA site antagonist). Although AP-5 did not inhibit the number of scratches in the first phase, 0.02, 0.2, or 2 μg of AP-5 produced significant antinociceptive effects in the second phase as compared with the vehicle treatment ($P < .05$). Figures 2b and 2c illustrate the effects of intracisternal injection of NR2A and NR2B antagonists, respectively, on the scratching behavior produced by formalin injection. Only intracisternal administration of high doses of PPPA (5 μg) (a competitive NR2A antagonist) significantly decreased the number of scratches in the first phase as compared with the vehicle treatment, while 1, 2.5, or 5 μg of PPPA significantly decreased the number of scratches in

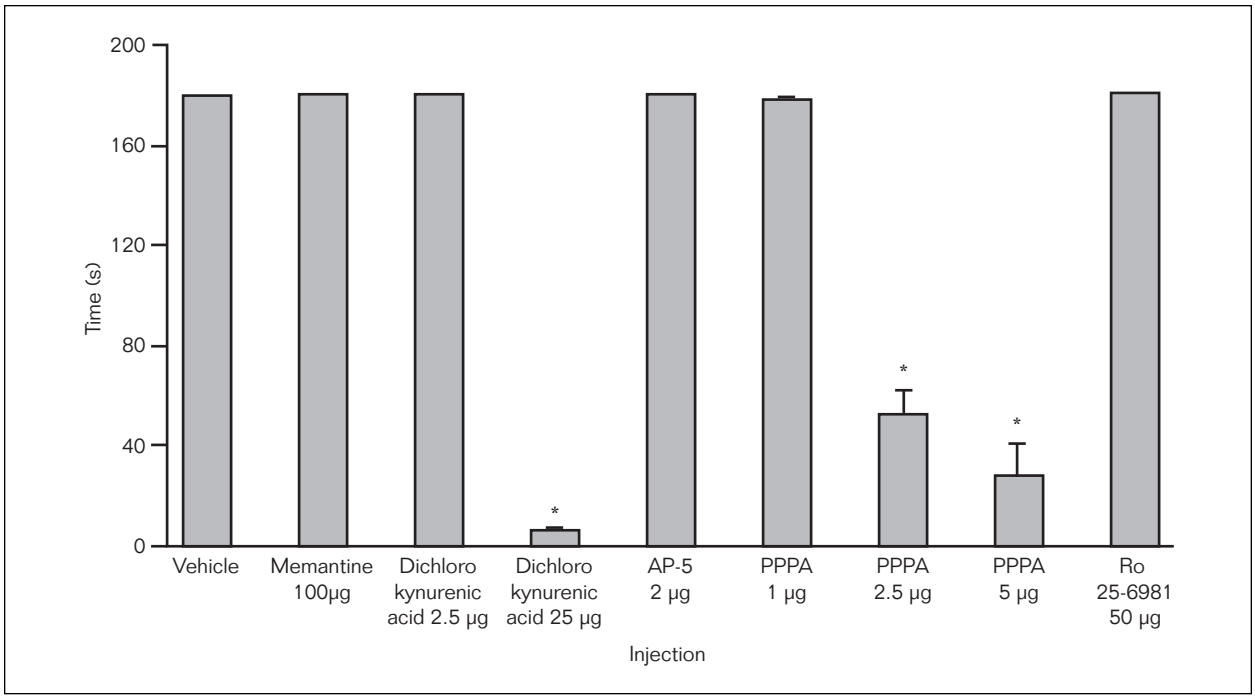

Fig 3 Effects of intracisternal administration of NMDA receptor antagonists on motor functions. Motor functions were evaluated using a rotarod test following NMDA receptor antagonist injection. n = 8 rats per group. An asterisk indicates significant inhibition as compared with the vehicle-treated group (*$P < .05$).

the second phase ($P < .05$, Fig 2b). Intracisternal administration of Ro 25-6981 (a selective NR2B antagonist) elicited a response which was similar to that of the competitive NR2A antagonist. Only intracisternal administration of high doses of Ro 25-6981 (50 μg) produced antinociceptive effects in the first phase, while 12.5, 25, and 50 μg of Ro 25-6981 produced an antinociceptive action in the second phase as compared with vehicle treatment ($P < .05$, Fig 2c).

In order to evaluate whether an antinociceptive dose of a NMDA receptor antagonist was also associated with motor dysfunction, rotarod tests were performed after intracisternal administration of a high dose of memantine (100 μg), 5,7-dichloro - kynurenic acid (2.5 or 25 μg), AP-5 (2 μg), PPPA (1, 2.5, or 5 μg), or Ro 25-6981 (50 μg). Injection of memantine, AP-5, Ro 25-6981, or vehicle did not result in any motor dysfunction. A low dose of PPPA (1 μg) or 5,7-dichlorokynurenic acid (2.5 μg) did not affect motor function. However, as Fig 3 illustrates, a high dose of either PPPA (2.5 or 5 μg) or 5,7-dichlorokynurenic acid (25 μg) significantly reduced the time course of motor performance to 50 ± 11 seconds and 4 ± 1 seconds ($P < .05$), respectively, as compared with the vehicle-treated group (180 second cut-off time).

Discussion

The present study has demonstrated that intracisternal administration of NR2 subunit antagonists produces antinociceptive effects in rats undergoing a facial formalin test. The antinociceptive actions of NR2 subunit antagonists are comparable to those of other NMDA receptor antagonists, such as ion channel blockers or glycine site antagonists.

Glutamate, the major excitatory neurotransmitter in the brain and spinal cord, exerts its postsynaptic effects via interaction with diverse membrane receptors. Ionotropic receptors are divided into three major subclasses: AMPA, kainate, and NMDA receptors. Of these subclasses, NMDA receptors have received particular attention due to their crucial roles in excitatory synaptic transmission, neuronal plasticity, and neurodegeneration in the CNS.[31] Since the spinal delivery of NMDA receptor antagonists may result in inhibition of the hyperexcitability of nociceptive neurons induced by C-fiber stimulation in the spinal cord,[32,33] several reports have demonstrated that activation of NMDA receptors facilitates nociceptive processing in the spinal cord during tissue injury and inflammation. Consistent with these findings, NMDA receptor antagonists have

been found to effectively alleviate pain-related behavior both in animal models as well as in human clinical investigations.[34,35] Although NMDA receptors are important for nociceptive transmission at the spinal cord level, the use of NMDA receptor antagonists is often limited by deleterious side effects such as memory impairment, psychotomimetic effects, ataxia, and impaired motor coordination.

Participation of central NMDA receptors in nociceptive transmission in the trigeminal system is well known. Central NMDA receptor mechanisms have mediated neuroplastic changes of nociceptive neurons in the subnuclei oralis and caudalis[36] that were produced by mustard oil application to the exposed pulp in rats.[37,38] Application of NMDA or non-NMDA receptor antagonists to the surface of the caudal brainstem overlying the subnucleus caudalis significantly suppressed temporomandibular joint-evoked jaw muscle activity, suggesting that the possible involvement of brainstem excitatory amino acid receptor mechanisms in the trigeminal subnucleus caudalis in rats with temporomandibular joint pain.[39] Although several studies have demonstrated central mechanisms of NMDA receptor in the trigeminal system, there is little direct behavioral evidence for the role of NR2-containing NMDA receptors in orofacial pain transmission.

NMDA receptors are a family of multiple receptor subtypes with distinct pharmacological and biophysical properties that are largely determined by the type of NR2 subunit,[40] since receptor affinity for agonists and antagonists may be dictated by the specific NR2 subunit incorporated into the multiprotein receptor complex. Therefore, the present study evaluated the role of NR2 receptors in the modulation of nociceptive transmission. The present study demonstrated that intracisternal administration of AP-5, a nonselective NMDA site antagonist, produced significant antinociceptive effects in rat facial formalin experiments. Moreover, intracisternal administration of PPPA, a competitive NR2A antagonist, and Ro 25-6981, a selective NR2B antagonist, produced antinociceptive effects when these chemicals were administered intracisternally 10 minutes prior to formalin injection. These results suggest that specific NR2 receptors participate in orofacial nociceptive transmission. Thus, targeted blockade of specific NR2 receptors may represent an important new therapeutic approach to the treatment of orofacial pain.

The importance of the NR2 receptor subunit in nociceptive mechanisms has been demonstrated in several previous studies.[11–13] Interestingly, the NMDA receptor subunits, NR2A and NR2B, are differentially expressed in the spinal cord at the cellular level.[41] NR2A subunits predominate at synapses, whereas NR2B subunits are present extrasynaptically and do not participate in synaptic transmission in adult rat lamina II neurons. These results suggest that an accumulation of extracellular glutamate can potentially activate extrasynaptic NR2B during pain states.[41,42] Moreover, a deficiency of postsynaptic density (PSD)-93, a neuronal scaffolding protein, significantly decreases the amount of both NR2A and NR2B in the synaptosomal membrane fractions derived from the spinal cord dorsal horn in mice.[43] These results, taken together with the present data, suggest that central expression of the NR2 receptor subunit plays an important role in orofacial nociceptive transmission. Conversely, phosphorylation of the NR1 subunit of the NMDA receptor, but not the NR2A, NR2B, NR2C, or the NR2D subunits, significantly increases in the ipsilateral dorsal horn of nerve-injured rats.[44] These data suggest that phosphorylation of NR1 but not NR2 subunits may be correlated with the presence of signs of neuropathy and possibly also with persistent pain following nerve injury. In addition, mice deficient in the NR2 subunit gene exhibit nociceptive responses similar to wild-type control mice.[44] These results suggest that NR2 subunit disruption does not result in altered nociceptive behavior in mice.[45] Although the present study demonstrated that intracisternal administration of NR2 antagonists produces antinociception in rats undergoing a formalin test, central expression of the NR2 receptor subunit may have a different role under different pain conditions.

The cellular mechanisms involving NR2-dependent nociceptive transmission may be limited. Small-diameter primary afferent fibers terminating in the dorsal horn express NMDA receptors and the activation of presynaptic NMDA receptors causes the release of substance P from primary afferent fibers.[46] Furthermore, NR2B subunits are predominantly expressed on small-diameter primary afferent fibers.[47] These expression patterns suggest that presynaptic NR2B-containing NMDA receptors can facilitate and prolong the transmission of nociceptive messages through the release of neurotransmitters including substance P, calcitonin gene-related peptide, or glutamate at small-diameter primary afferent terminals.[46,47] On the other hand, NR2A subunits predominate at synapses, which are involved in the synaptic transmission of nociceptive signals.[41]

Clinical use of NMDA receptor inhibitors is very limited, since preclinical and clinical studies with competitive and noncompetitive NMDA receptor antagonists have demonstrated significant side effects resulting from the suppression of the physiological functions of these receptors.[14] In this regard, a reduction in side effects and improved efficacy of NR2 selective antagonists are important for the development of new pain treatment modalities in humans.[48] Encouragingly, human administration of neuroprotective doses of selective NR2B antagonists does not induce the side effects that are typically seen with nonselective NMDA receptor antagonists.[49,50] Spinal administration of Conantokin G, a selective inhibitor of the NR2B subunit, produces potent antinociception in formalin tests and in models of peripheral nerve injury and inflammation, at doses approximately 20 times smaller than those required to impair motor function.[10] Although a high dose of the NR2A antagonist PPPA (2.5, 5 μg) reduced the time course of motor performance in the present study, a low dose of PPPA did not cause any motor dysfunction in the present study. These results suggest that unwanted side effects of NR2A receptor antagonists may limit their clinical use.

NMDA receptors display a number of unique properties that distinguish them from other ligand-gated ion channels. First, the receptor controls a cation channel that is highly permeable to monovalent ions and calcium. Second, the simultaneous binding of glutamate and glycine is required for efficient activation of the NMDA receptor. Since glycine is a required co-agonist in conjunction with glutamate,[51] a number of glycine-binding site modulators have recently been characterized as potential drugs.[3,52] Systemic administration of GV196771A, a NMDA receptor/glycine antagonist, has been reported to inhibit established mechanical allodynia or thermal hyperalgesia in rats with chronic constriction of the sciatic nerve[53] and reduce the duration of licking time in the late response phase of mice receiving orofacial formalin.[54] The present study has demonstrated that intracisternal administration of memantine, an NMDA antagonist that acts at the ion channel site, and 5,7-dichlorokynurenic acid, a glycine site antagonist, significantly decreased the number of scratches in rats subjected to facial formalin. However, 25 μg of 5,7-dichlorokynurenic acid produced severe motor dysfunction, which may make clinical use of this antagonist difficult. The magnitude of the NR2 antagonist antinociceptive effects were comparable to those of memantine or 5,7-dichlorokynurenic acid, which represent well-known categories of antinociceptive chemicals. These results also suggest that central NR2 subunits play an important role in nociceptive transmission originating in the orofacial area.

In summary, the present study has demonstrated that intracisternal administration of NR2 antagonists produces significant antinociceptive effects in rats subjected to facial formalin. NR2 antagonist-induced antinociceptive effects were comparable to responses to memantine, a NMDA antagonist that acts at the ion channel site, and to 5,7-dichlorokynurenic acid, a glycine site antagonist. These results suggest that central NR2 subunits play an important role in orofacial nociceptive transmission. Moreover, the present results indicate that targeted blockade of NR2 receptor subunits is a potentially important new treatment approach for inflammatory pain originating in the orofacial area.

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References

1. Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. Pharmacol Rev 1999;51:7–61.
2. Paoletti P, Neyton J. NMDA receptor subunits: Function and pharmacology. Curr Opin Pharmacol 2007;7:39–47.
3. Childers WE Jr, Baudy RB. N-methyl-D-aspartate antagonists and neuropathic pain: The search for relief. J Med Chem 2007;50:2557–2562.
4. McBain CJ, Mayer ML. N-methyl-D-aspartic acid receptor structure and function. Physiol Rev 1994;74:723–760.
5. Mori H, Mishina M. Structure and function of the NMDA receptor channel. Neuropharmacology 1995;34:1219–1237.
6. Kew JN, Kemp JA. Ionotropic and metabotropic glutamate receptor structure and pharmacology. Psychopharmacology (Berl) 2005;179:4–129.
7. Yamakura T, Shimoji K. Subunit- and site-specific pharmacology of the NMDA receptor channel. Prog Neurobiol 1999;59:279–298.
8. Cull-Candy S, Brickley S, Farrant M. NMDA receptor subunits: Diversity, development and disease. Curr Opin Neurobiol 2001;11:327–335.
9. Gaunitz C, Schüttler A, Gillen C, Allgaier C. Formalin-induced changes of NMDA receptor subunit expression in the spinal cord of the rat. Amino Acids 2002;23:177–182.
10. Malmberg AB, Gilbert H, McCabe RT, Basbaum AI. Powerful antinociceptive effects of the cone snail venom-derived subtype-selective NMDA receptor antagonists conantokins G and T. Pain 2003;101:109–116.

11. Bernardi M, Bertolini A, Szczawinska K, Genedani S. Blockade of the polyamine site of NMDA receptors produces antinociception and enhances the effect of morphine, in mice. Eur J Pharmacol 1996;298:51–55.
12. Taniguchi K, Shinjo K, Mizutani M, et al. Antinociceptive activity of CP-101, 606, an NMDA receptor NR2B subunit antagonist. Br J Pharmacol 1997;122:809–812.
13. Boyce S, Wyatt A, Webb JK, et al. Selective NMDA NR2B antagonists induce antinociception without motor dysfunction: Correlation with restricted localisation of NR2B subunit in dorsal horn. Neuropharmacology 1999;38:611–623.
14. Liu XJ, Gingrich JR, Vargas-Caballero M, et al. Treatment of inflammatory and neuropathic pain by uncoupling Src from the NMDA receptor complex. Nat Med 2008;14:1325–1332.
15. Lee J, Ro JY. Differential regulation of glutamate receptors in trigeminal ganglia following masseter inflammation. Neurosci Lett 2007;421:91–95.
16. Dong XD, Mann MK, Kumar U, et al. Sex-related differences in NMDA-evoked rat masseter muscle afferent discharge result from estrogen-mediated modulation of peripheral NMDA receptor activity. Neuroscience 2007; 146:822–832.
17. Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–1036.
18. Wang XM, Zhang ZJ, Bains R, Mokha SS. Effect of antisense knock-down of alpha(2a)- and alpha(2c)-adrenoceptors on the antinociceptive action of clonidine on trigeminal nociception in the rat. Pain 2002;98:27–35.
19. Ahn DK, Choi HS, Yeo SP, et al. Blockade of central cyclooxygenase (COX) pathways enhances the cannabinoid-induced antinociceptive effects on inflammatory temporomandibular joint (TMJ) nociception. Pain 2007;132: 23–32.
20. Lee MK, Choi BY, Yang GY, et al. Low doses of cannabinoids enhance the antinociceptive effects of intracisternally administered mGluRs groups II and III agonists in formalin-induced TMJ nociception in rats. Pain 2008;139: 367–375.
21. Clavelou P, Pajot J, Dallel R, Raboisson P. Application of the formalin test to the study of orofacial pain in the rat. Neurosci Lett 1989;103:349–353.
22. Gilbert SD, Clark TM, Flores CM. Antihyperalgesic activity of epibatidine in the formalin model of facial pain. Pain 2001;89:159–165.
23. Choi HS, Ju JS, Lee HJ, et al. Effects of TNF-alpha injected intracisternally on the nociceptive jaw-opening reflex and orofacial formalin test in freely moving rats. Prog Neuropsychopharmacol Biol Psychiatry 2003;27: 613–618.
24. Choi HS, Ju JS, Lee HJ, Kim BC, Park JS, Ahn DK. Effects of intracisternal injection of interleukin-6 on nociceptive jaw opening reflex and orofacial formalin test in freely moving rats. Brain Res Bull 2003;59:365–370.
25. Lee HJ, Choi HS, Jung CY, et al. Intracisternal NMDA produces analgesia in the orofacial formalin test of freely moving rats. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:497–503.
26. Peng HY, Chang HM, Chang SY, et al. Orexin-A modulates glutamatergic NMDA-dependent spinal reflex potentiation via inhibition of NR2B subunit. Am J Physiol Endocrinol Metab 2008;295:117–129.
27. Gabra BH, Kessler FK, Ritter JK, Dewey WL, Smith FL. Decrease in N-methyl-D-aspartic acid receptor-NR2B subunit levels by intrathecal short-hairpin RNA blocks group I metabotropic glutamate receptor-mediated hyperalgesia. J Pharmacol Exp Ther 2007;322:186–194.
28. Christoph T, Reissmüller E, Schiene K, Englberger W, Chizh BA. Antiallodynic effects of NMDA glycine(B) antagonists in neuropathic pain: Possible peripheral mechanisms. Brain Red 2005;1048:218–227.
29. Berrino L, Oliva P, Massimo F, et al. Antinociceptive effect in mice of intraperitoneal N-methyl-D-aspartate receptor antagonists in the formalin test. Eur J Pain 2003; 7:131–137.
30. Ahn DK, Lim EJ, Kim BC, et al. Compression of the trigeminal ganglion produces prolonged nociceptive behavior in rats. Eur J Pain 2009;13:568–575.
31. Petrenko AB, Yamakura T, Baba H, Shimoji K. The role of N-methyl-D-aspartate (NMDA) receptors in pain: A review. Anesth Analg 2003;97:1108–1116.
32. Davies SN, Lodge D. Evidence for involvement of N-methylaspartate receptors in "wind-up" of class 2 neurones in the dorsal horn of the rat. Brain Res 1987;424: 402–406.
33. Dickenson AH, Sullivan AF. Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. Neuropharmacology 1987;26:1235–1238.
34. Fisher K, Coderre TJ, Hagen NA. Targeting N-methyl-D-aspartate receptor for chronic pain management. Preclinical animal studies, recent clinical experience and future research directions. J Pain Symptom Manage 2000; 20:358–373.
35. Hewitt DJ. The use of NMDA-receptor antagonists in the treatment of chronic pain. Clin J Pain 2000;16:S73–S79.
36. Chiang CY, Park SJ, Kwan CL, Hu JW, Sessle BJ. NMDA receptor mechanisms contribute to neuroplasticity induced in caudalis nociceptive neurons by tooth pulp stimulation. J Neurophysiol 1998;80:2621–2631.
37. Luccarini P, Sessle BJ, Woda A. Superficial and deep convergent nociceptive neurons are differentially affected by N-methyl-D-aspartate applied on the brainstem surface of the rat medullary dorsal horn. Neuroscience 2001;107: 311–316.
38. Park SJ, Chiang CY, Hu JW, Sessle BJ. Neuroplasticity induced by tooth pulp stimulation in trigeminal subnucleus oralis involves NMDA receptor mechanisms. J Neurophysiol 2001;85:1836–1846.
39. Cairns BE, Sessle BJ, Hu JW. Temporomandibular-evoked jaw muscle reflex: Role of brain stem NMDA and non-NMDA receptors. Neuroreport 2001;12:1875–1878.
40. Gielen M, Siegler Retchless B, Mony L, Johnson JW, Paoletti P. Mechanism of differential control of NMDA receptor activity by NR2 subunits. Nature 2009;459: 703–707.
41. Momiyama A. Distinct synaptic and extrasynaptic NMDA receptors identified in dorsal horn neurones of the adult rat spinal cord. J Physiol 2000;523:621–628.
42. Karlsson U, Sjödin J, Angeby Möller K, Johansson S, Wikström L, Nasström J. Glutamate-induced currents reveal three functionally distinct NMDA receptor populations in rat dorsal horn: Effects of peripheral nerve lesion and inflammation. Neuroscience 2002;112:861–868.

43. Liaw WJ, Zhu XG, Yaster M, Johns RA, Gauda EB, Tao YX. Distinct expression of synaptic NR2A and NR2B in the central nervous system and impaired morphine tolerance and physical dependence in mice deficient in postsynaptic density-93 protein. Mol Pain 2008;4:45.
44. Ultenius C, Linderoth B, Meyerson BA, Wallin J. Spinal NMDA receptor phosphorylation correlates with the presence of neuropathic signs following peripheral nerve injury in the rat. Neurosci Lett 2006;399:85–90.
45. Petrenko AB, Yamakura T, Baba H, Sakimura K. Unaltered pain-related behavior in mice lacking NMDA receptor GluR epsilon 1 subunit. Neurosci Res 2003;46:199–204.
46. Liu H, Mantyh PW, Basbaum AI. NMDA-receptor regulation of substance P release from primary afferent nociceptors. Nature 1997;386:721–724.
47. Ma QP, Hargreaves RJ. Localization of N-methyl-D-aspartate NR2B subunits on primary sensory neurons that give rise to small-caliber sciatic nerve fibers in rats. Neuroscience 2000;101:699–707.
48. Chizh BA, Headley PM, Tzschentke TM. NMDA receptor antagonists as analgesics: Focus on the NR2B subtype. Trends Pharmacol Sci 2001;22:636–642.
49. Kemp JA, McKernan RM. NMDA receptor pathways as drug targets. Nat Neurosci 2002;5:1039–1042.
50. Chizh BA, Headley PM. NMDA antagonists and neuropathic pain: Multiple drug targets and multiple uses. Curr Pharm Des 2005;11:2977–2994.
51. Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 1987; 325:529–531.
52. Cai SX. Glycine/NMDA receptor antagonists as potential CNS therapeutic agents: ACEA-1021 and related compounds. Curr Top Med Chem 2006;6:651–662.
53. Quartaroli M, Fasdelli N, Bettelini L, Maraia G, Corsi M. GV196771A, an NMDA receptor/glycine site antagonist, attenuates mechanical allodynia in neuropathic rats and reduces tolerance induced by morphine in mice. Eur J Pharmacol 2001;430:219–227.
54. Quartaroli M, Carignani C, Dal Forno G, et al. Potent antihyperalgesic activity without tolerance produced by glycine site antagonist of N-methyl-D-aspartate receptor GV196771A. J Pharmacol Exp Ther 1999;290:158–169.