

NR2 NMDAR channels, such as sensitivity to magnesium block, kinetics of desensitization and offset decay, susceptibility to modulation by glycine, reducing agents, polyamines and phosphorylation, and affinity for agonists and antagonists, depend on the type of NR2 subunit included in a heteromeric complex (6,7). For example, a brief application of glutamate onto NR1/NR2A assemblies generates a macroscopic current with a deactivation time constant of tens of milliseconds, compared with several seconds for NR1/NR2D receptors. In addition, NR1/NR2A assemblies are less sensitive to glutamate than other heteromeric channels. Aside from these kinetic differences, the most obvious subunit-dependent properties of NMDARs are their single-channel conductance and sensitivity to magnesium block. For example, the NR2A or NR2B subunits-containing NMDARs generate high-conductance channel openings with a high sensitivity for blocking by magnesium, whereas NR2C- or NR2D-containing receptors give rise to low-conductance openings with a lower sensitivity to magnesium. A number of pharmacological agents have been shown to distinguish between certain NMDAR subtypes. The best characterized of these compounds is ifenprodil, which inhibits NR2B-containing receptors at concentrations 400-fold lower than those required for inhibition of other NR2-containing receptors (8). Thus, ifenprodil and several structurally related compounds (eliprodil, CP-101,606, Ro25-6189, and CI-1041) are highly selective for NMDARs containing NR2B subunits. Importantly, they are better tolerated than other classes of NMDAR antagonists (9).

Similar to NR2, NR3 subunits do not by themselves generate agonist-activated currents; however, when co-expressed with NR1 and NR2, NR3A/3B subunits act in a dominant-negative manner against NMDARs to reduce such currents (10–12). Surprisingly, in contrast to conventional NR1/NR2 receptors, co-assembly of NR1 with NR3A or -3B subunits forms excitatory glycine receptors that are unaffected by glutamate or NMDA, are impermeable to calcium, and are resistant to magnesium block and NMDAR antagonists (13).

No experimental studies have been performed related to the role of NR3 subunits in pain mechanisms. Therefore, when addressing the role of NMDAR in pain, this article will emphasize the contribution of NR2 subunits, particularly NR2B, in nociception.

NMDA Receptor Activation and Persistent Pain

There is considerable evidence that pain associated with peripheral tissue or nerve injury involves NMDAR

activation (14). Consistent with this, NMDAR antagonists have been shown to effectively alleviate pain-related behavior in animal models as well as in clinical situations (15,16). However, NMDARs are important for normal CNS functions, and the use of NMDAR antagonists can often be limited by serious side effects, such as memory impairment, psychotomimetic effects, ataxia, and motor incoordination. In this regard, a reduced side effect profile and an improved efficacy of NR2B-selective antagonists in animal pain models bring optimism for this class of compounds becoming useful for the treatment of pain in humans (9).

Although central NMDARs, especially ones located in the spinal cord, still receive a great deal of attention, evidence is accumulating that suggests that NMDARs located in peripheral somatic tissues and visceral pain pathways play an important role in nociception. Thus, in chronic pain settings, NMDAR activation occurs at each level of the neural axis, and therefore, each level should be considered as a potential target for therapeutic intervention. The contribution of peripheral NMDARs to the generation of pain is discussed first.

Peripheral NMDA Receptors

Morphologic studies in animals have identified NMDARs on unmyelinated and myelinated axons in peripheral somatic tissues (17,18). NMDARs are expressed on nerves in human tendons and, together with increased concentrations of glutamate, have been implicated in the pathogenesis of chronic pain from tendons (19).

Consistent with the presence of NMDARs in the periphery, local injections of glutamate or NMDA result in nociceptive behaviors that can be attenuated by peripheral administration of NMDAR antagonists (20–22). In addition, peripheral administration of MK-801, a noncompetitive NMDAR antagonist, produces local anesthetic-like effects (23). Furthermore, hyperalgesia and spontaneous pain behavior observed in a formalin test or after inflammation can be effectively inhibited by peripheral administration of NMDAR antagonists, including those clinically available, and by NR2B-selective compounds (20,24–27). The number of NMDARs on peripheral nerve fibers increases during inflammation, and this may contribute to peripheral sensitization in inflammation (28). The nociceptive responses induced by injection of glutamate into the mouse paw appear to involve not only peripheral but also spinal and supraspinal NMDARs and are largely mediated by release of nitric oxide (NO) (29). Unlike inflammatory pain, which can be attenuated by peripheral administration of NMDAR antagonists, intradermal MK-801 failed to affect the established hyperalgesia in four models of metabolic/toxic and trauma-induced neuropathic pain, suggesting that NMDARs

do not contribute to peripheral sensitization of nociceptors in this setting (30).

In humans, the peripheral administration of ketamine enhanced the local anesthetic and analgesic actions of bupivacaine used for infiltration anesthesia (31) and inhibited the development of primary and secondary hyperalgesia after an experimental burn injury (32). Another study demonstrated a dose-dependent antihyperalgesic effect for IV ketamine in patients with neuropathic pain (33). Because only minimal CNS side effects were encountered, it was suggested that these effects of ketamine were mediated, at least in part, by peripheral neurons. Finally, topical application of ketamine ointment has been recently reported to reduce pain intensity and to attenuate allodynia in patients with an acute early dystrophic stage of complex regional pain syndrome type I (34). Ketamine interacts with many receptors related to nociceptive transmission (35), and although further studies using pure NMDAR antagonists are needed to clarify the exact mechanism of ketamine's action, local administration of ketamine appears to be beneficial for some patients with chronic pain.

Recently, it has been shown that peripheral nociceptive fibers express NR2B and NR2D subunits, whereas NR2A subunits appear to be absent from the peripheral terminals of primary afferents (Fig. 1A) (36,37). Because NR2B-selective antagonists potentiate NMDAR inhibition by endogenous protons (38), this mode of action may be beneficial under conditions of tissue injury, ischemia, or inflammation (presumably accompanied by acidosis) when a greater degree of inhibition of NMDARs can be expected in the affected tissues than normal (9). This could also explain the improved efficacy of NR2B-selective antagonists under these conditions.

Central NMDA Receptors

Spinal NMDA Receptors. Changes in the periphery after trauma lead to the phenomenon of peripheral sensitization and primary hyperalgesia. The sensitization that occurs, however, can only be partly explained by the changes in the periphery, indicating that the hyperalgesia and allodynia after injury has a central component. This is the phenomenon of central sensitization (14). Central sensitization is the state where dorsal horn excitability is increased and, as a consequence, its response to sensory input is facilitated. A low-intensity stimulus acting via low-threshold afferents then generates pain (the phenomenon of allodynia) and noxious inputs result in a pain response that is augmented in amplitude and duration, so-called "hyperalgesia." Convincing evidence has demonstrated that the development of spinal hyperexcitability and persistent pain involves activation of NMDARs (Fig. 2). The increased NMDAR function

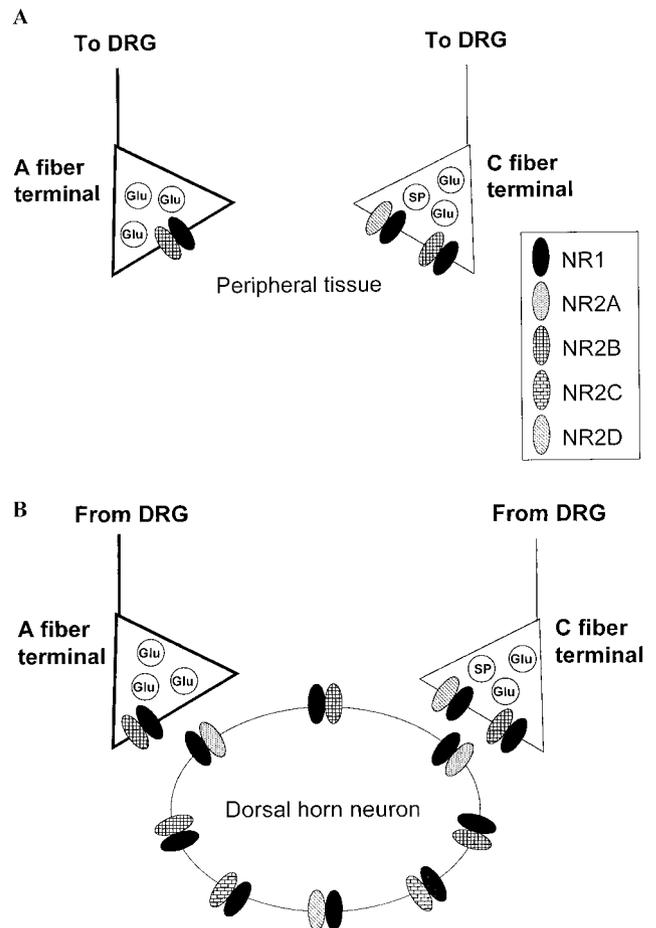


Figure 1. Schematic representation of possible subunit composition of peripheral (A) and spinal (B) NMDARs. Based on References 17, 18, 36, 37, 39, 40, and 55. DRG = dorsal root ganglion; Glu = glutamate; SP = substance P.

is expressed as an increase in channel openings and may involve transcriptional, translational, and post-translational modulation.

Recent data suggest that the mRNA expression pattern of NMDAR subunits is altered as a consequence of peripheral tissue or nerve injury. For example, one study using reverse transcription polymerase chain reaction (RT-PCR) investigated the effect of formalin injection into the hind paw on NR subunit expression in rat lumbar spinal cord (39). In control animals, NR2B exhibited the largest expression among NR2 subunits, followed by decreasing proportions of NR2C, NR2A, and NR2D (Fig. 1B). Formalin treatment did not affect the expression of NR1 splice variants or NR2B, but it significantly increased and decreased the proportion of NR2A and NR2C mRNA, respectively. Of note, NR1/NR2C heteromers have a much longer deactivation time, lower conductance, higher affinity for glutamate, and weaker magnesium block than NR1/NR2A receptors, but they have comparable calcium permeability (7). Speculatively, the

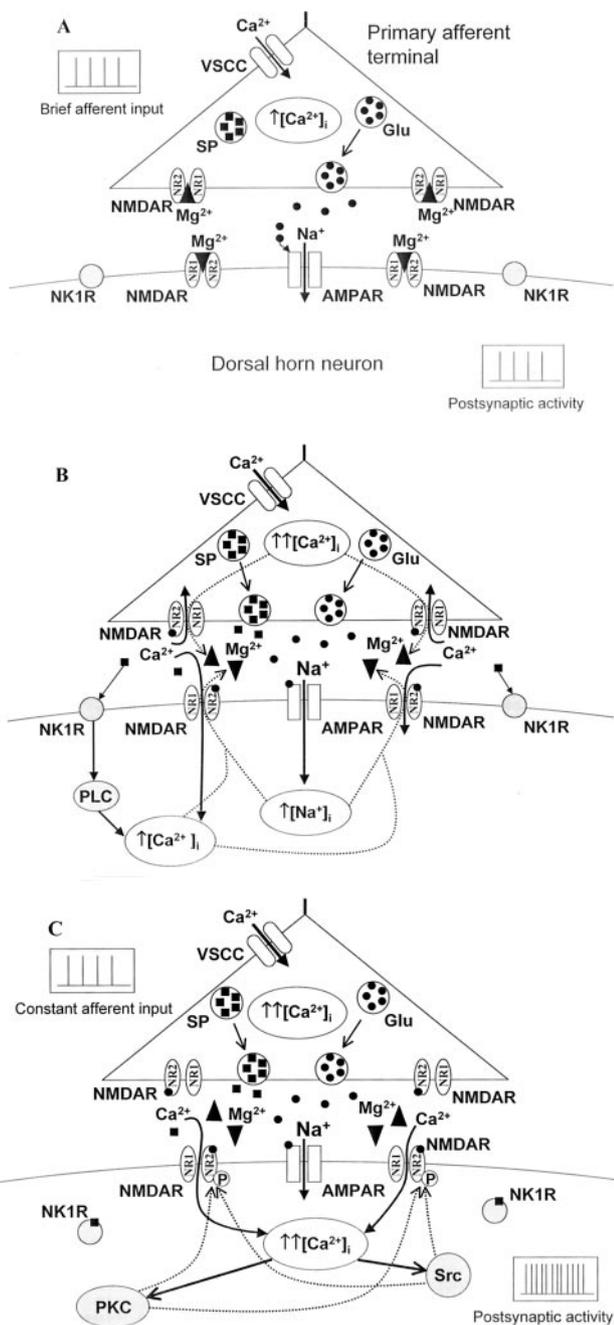


Figure 2. Schematic illustration of the role of NMDARs in central sensitization. *A:* Normal synaptic transmission. NMDARs do not participate in normal synaptic transmission because of their voltage-dependent block by extracellular magnesium. *B:* Postsynaptic depolarization and removal of the magnesium block of NMDARs. A constant drive of noxious afferent input after tissue damage depolarizes membrane strong enough to permit participation of NMDARs in synaptic transmission. Nociceptive input to the dorsal horn is further increased via positive feedback through presynaptic NMDARs. *C:* Posttranslational changes of NMDARs. Calcium entry causes activation of protein kinases and results in phosphorylation of NMDARs. As a consequence, the magnesium block at resting membrane potentials is decreased and channel opening time is prolonged. AMPAR = α -amino-3-hydroxy-5-methyl-4-isoxasolepropionic acid receptor; NK1R = neurokinin 1 receptor; NMDAR = *N*-methyl-D-aspartate receptor; VSCC = voltage-sensitive calcium channel; Glu = glutamate; SP = substance P; PLC = phospholipase C; PKC = protein kinase C; Src = protein tyrosine kinase; P = phosphate group.

observed changes in the NR subunit expression may represent an adaptive response aimed to reduce excessive neuronal excitability resulting from tissue injury.

As demonstrated by single-cell PCR, fewer rat dorsal horn neurons expressed NR2A mRNA compared with controls 1–2 wk after L5 spinal nerve transection (40). As NR2B was the subunit most commonly expressed in rat dorsal horn, the reduction of NR2A expression after a peripheral nerve lesion could make the contribution of the former subunit proportionally larger. Indeed, the observed changes in the dose-response curve of NMDAR currents were consistent with a relative increase in NR2B expression. The authors suggested the importance of the NR2B but not the NR2A subunit in neuropathic pain conditions. Also, the relative increase in NR2B, NR2C, and NR2D populations could well be part of an adaptive or “fail safe” response that would serve to enhance NMDAR-mediated currents, thereby ensuring the adequacy of neurotransmission compromised by nerve damage (41).

Peripheral inflammation may alter the properties of NMDARs in the spinal dorsal horn (42). After complete Freund’s adjuvant (CFA) treatment, the magnesium blockade of NMDA responses was reduced and the current-voltage relationship of NMDAR channels was shifted in the hyperpolarized direction. These changes, which were mediated by protein kinase C (PKC) and resulted in enhanced NMDA responses at negative potentials, could lead to an increase in synaptic transmission in the dorsal horn and contribute to the development of pathological nociceptive responses associated with tissue injury.

Protein phosphorylation is a major mechanism for the regulation of NMDAR function (Fig. 2C). Indeed, direct phosphorylation may be one mechanism by which PKC regulates the function of NMDARs (43). Also, PKC potentiates NMDA responses indirectly by activation of the tyrosine kinase (Src) signaling cascade (44). In addition, increasing evidence also suggests that PKC modulates the function of NMDARs by participating in their interactions with postsynaptic density and cytoskeletal proteins (45).

Although PKC enhances NMDA-evoked currents in most preparations, its effects on the magnesium blockade of NMDA currents vary. One obvious factor that can contribute to the dissimilar PKC effects found among the various studies is the differential NMDAR subunit expression in the CNS. Compared with NR1/NR2C and NR1/NR2D receptors, NR1/NR2A and NR1/NR2B receptors are more sensitive to magnesium block and PKC potentiation. Thus, consistent with the larger expressions of NR2B and NR2A subunits in the superficial dorsal horn, PKC resulted in magnesium-dependent potentiation of NMDAR-mediated responses in rat dorsal horn neurons that

receive sensory inputs from an inflamed hind paw (42).

As noted above, protein phosphorylation is important for the up-regulation of NMDAR function. Although both NR2A and NR2B subunits can be tyrosine phosphorylated *in vitro*, tyrosine phosphorylation of the NR2B, but not the NR2A, is associated with the development of persistent pain after inflammation (46). The increase in NR2B tyrosine phosphorylation is dependent on primary afferent drive from the inflamed hind paw and correlates with the development and maintenance of inflammatory hyperalgesia. Signal transduction upstream to NR2B tyrosine phosphorylation involves G-protein-coupled receptors, PKC and the Src family protein tyrosine kinases. Thus, the enhanced tyrosine phosphorylation of the NR2B subunit of NMDAR may contribute to the development of nociceptor activity-dependent changes in the spinal cord.

NMDARs are anchored in the postsynaptic membrane by interactions between the cytoplasmic C-terminal tails of their NR2 subunits and the PDZ domains of PSD-95/SAP90, an abundant scaffold protein that assembles a specific set of signaling proteins around the NMDAR. These proteins, such as neuronal NO synthase, SynGAP, and SPAR, may participate in downstream signaling by NMDARs (47). Not surprisingly, the NMDAR-PSD-95/SAP90 interaction has been recently implicated in the processing of spinal nociceptive information. PSD-95/SAP90 mRNA and protein are enriched in the spinal cord and are selectively distributed in the superficial dorsal horn neurons, where PSD-95/SAP90 interacts with NR2A/2B subunits (48). Additionally, PSD-95/SAP90 is required for NMDAR-mediated thermal hyperalgesia. Furthermore, PSD-95/SAP90 knockdown can delay the onset of mechanical and thermal hyperalgesia in the chronic neuropathic pain model (49).

Another protein that is likely to mediate many aspects of postsynaptic signaling by NMDAR is calcium-calmodulin-dependent protein kinase II (CaMKII). It has received much attention because it is persistently activated after NMDAR stimulation. The activation of CaMKII stimulates its binding to the cytoplasmic domain of the NMDAR subunit NR2B. By interfering with autoinhibitory interactions within CaMKII, binding to NR2B locks CaMKII in an activated state that cannot be reversed by phosphatases (50). In addition, the CaMKII-NR2B interaction leads to the trapping of CaM that may reduce down-regulation of NMDA receptor activity. It is interesting that although CaMKII binds to the NR2B subunit of NMDAR in more than one mode, its affinity to the closely related NR2A subunit is decreased (51).

Recently, evidence has pointed to a key role for CaMKII in nociceptive transmission. In fact, CaMKII α , which is a major CaMKII isoform expressed in the

brain, is preferentially localized in pain-processing regions in the CNS such as lamina II of the spinal cord dorsal horn and the dorsal root ganglion (DRGs) (52). Also, CaMKII is up-regulated in the superficial laminae of the dorsal horn and DRG cells after inflammation or injuries to peripheral tissues (53,54). Thus, the ability of CaMKII to specifically interact with NR2B, together with their co-localization in the superficial dorsal horn (a region strongly involved in nociception), indicates the particular importance of the NR2B-CaMKII interaction in the development and maintenance of nociceptive hypersensitivity and provides a novel target for treatment of chronic pain.

Interestingly, NR2A and NR2B, the prevailing NR2 subunits in the spinal cord, have different locations at the cellular level (Fig. 1B) (55). In adult rat lamina II neurons, identified on the basis of patch-clamp recordings, NR2A subunits predominate at synapses, whereas NR2B subunits are present extrasynaptically and do not participate in synaptic transmission. However, during pain states, the building-up of extracellular glutamate can potentially activate extrasynaptic NMDARs, thus suggesting a possible role of NR2B subunits in this situation.

It has been assumed that central sensitization in the spinal cord dorsal horn is mediated by activation of postsynaptic NMDARs. However, one of the features unique to the spinal cord is the presence of presynaptic NMDARs (Figs. 1B, 2B). In fact, many small-diameter primary afferent fibers terminating in the dorsal horn express NMDARs, and activation of presynaptic NMDARs causes the release of substance P (SP) from primary afferents (56). In addition, glutamate released from the presynaptic terminal can potentially enhance its own release in a feed-forward manner in response to subsequent stimuli. Rat DRG neurons contain NR1, NR2B, NR2C, and NR2D, but not NR2A, subunits (37). Furthermore, NR2B subunits are predominantly expressed on small-diameter primary afferents (36). Therefore, because SP, calcitonin gene-related peptide and glutamate co-occur in small-diameter primary afferent terminals, presynaptic NR2B-containing NMDARs can facilitate and prolong the transmission of nociceptive messages through the release of these neurotransmitters.

The particular importance of NR2B subunits in mediating pain is further substantiated by the fact that changes in pain-related behaviors in NR2A subunit knockout mice have not been demonstrated compared with wild controls in several acute and chronic pain models (57).

Consistent with an increasing number of reports implicating the importance of the NR2B subunit in pain mechanisms, several experimental studies have demonstrated the efficacy of NR2B-selective NMDAR

antagonists (24,58,59). In support of these data, spinal administration of Conantokin G, a 17 amino acid peptide isolated from the cone snail that selectively inhibits the NR2B subunit of the NMDAR, produced 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 (60). Although some data suggest that the spinal cord is not the primary site of action of NR2B-selective compounds (61), this study argues for an action at a spinal NR2B site and further supports the notion that drugs directed against NR2B-containing NMDARs hold promise as novel therapeutics for the control of pain.

Supraspinal NMDA Receptors. There is evidence to suggest a role for NMDARs in mediating supraspinal sensitization as well. Indeed, increased NMDAR activation underlies inflammation-induced neuronal hyperexcitability of brainstem circuitry (62). Furthermore, as indicated by RT-PCR analysis, there is an up-regulation of NR1, NR2A, and NR2B mRNA subunits gene expression in the brainstem after inflammation (63).

Transgenic mice overexpressing NR2B subunits in the forebrain exhibited enhanced responsiveness to the peripheral injection of two inflammatory agents, formalin and CFA (64). Neuronal c-Fos expression was most prominent, and significantly different from wild type controls, in the anterior cingulate (ACC) and insular cortices. Interestingly, the ACC is tonically activated in chronic pain states (65), and its activity is correlated with the suffering component of pain (66).

Blockade of Inhibitory Mechanisms Leads to NMDA Receptor Activation

Although many reports have demonstrated a pivotal role for increased excitation in facilitating the processing of afferent input in the dorsal horn after tissue or nerve injury, similar changes may potentially occur because of the blockade of the inhibitory mechanisms (disinhibition). Disinhibition may be caused by a number of factors, such as a reduction in inhibitory transmitters, for example γ -aminobutyric acid (GABA) or its receptors (67), or losses of inhibitory neurons (68). However, irrespective of the cause of disinhibition, the unopposed activation of NMDARs is the underlying mechanism. In a normal state (Fig. 2A), the participation of NMDARs in synaptic transmission is prevented by GABA_A receptor-mediated currents (69), which restore the depolarized membrane potential to a resting level, thus preventing the relief of the magnesium block of NMDAR, and only C-fiber input can reliably trigger central sensitization (70). However, the

situation changes after the removal of GABAergic inhibition, and low-intensity stimulation begins to induce central sensitization, which never occurs under a physiological state.

It is noteworthy that although the enhancement of NMDAR function is essential for persistent pain, a drug that potentiates NMDA responses is not necessarily pain promoting because NMDARs are located on both excitatory and inhibitory neurons. Indeed, in rats with inflammation, gabapentin, a widely prescribed analgesic, may exert its antinociceptive effects by increasing the activity of inhibitory neurons in the dorsal horn by NMDAR activation (71). Interestingly, the activation of extrasynaptic NMDARs in the spinal dorsal horn (presumably containing NR2B subunits) may contribute to the antinociceptive action of gabapentin (72).

NMDA Receptors and Visceral Pain

Despite extensive experimental and clinical literature related to somatic nociception, relatively few studies have been performed in models of visceral pain. However, accumulating evidence indicates a possible role for NMDARs in mediating pain from internal organs. In fact, IV ketamine dose-dependently attenuated increases in blood pressure evoked by graded distentions of the ureter in rats (73). Intrathecally administered NMDA facilitated dorsal horn neuronal and behavioral responses to noxious colorectal distention (CRD) (74). NMDARs have been found on peripheral terminals of primary afferent nerves innervating the colon (75). That study also demonstrated that systemically, but not intrathecally, administered NMDAR antagonists attenuate the reflex responses to noxious CRD, suggesting that peripherally located NMDARs mediated the response. However, the mechanism and site of action of such an effect remains controversial. Indeed, the effect of ketamine on pain originating in the urinary bladder has recently been characterized in intact, halothane-anesthetized rats (76). A direct inhibitory effect on cardiovascular and visceromotor responses (VMR) to urinary bladder distention (UBD) was observed when ketamine was administered IV and intrathecally, with a similar effect from two other clinically available NMDAR antagonists, dextromethorphan and memantine. A spinally mediated effect was thereby suggested. A related study examining spinal cord dorsal horn neuronal responses to UBD in acutely spinalized decerebrate rat confirmed that a site of action of ketamine appears to be localized in the spinal cord (77).

Another study has examined the role of NMDARs in processing of visceral stimuli of graded intensities (78). The NMDAR channel blocker MK-801 attenuated c-Fos expression, widely used as a correlative indicator for neuronal activity, in the lumbosacral spinal

cord induced by noxious, as well as innocuous, CRD. Additionally, in awake rats, intrathecally administered competitive NMDAR antagonist 2-amino-5-phosphonovalerate (APV) dose-dependently attenuated VMRs to both stimulus paradigms. In contrast to a previous report (75), these data also suggest that spinally located NMDARs mediated the response to colorectal stimulation. Interestingly, unlike somatic sensory system, an innocuous visceral stimulus appears to be susceptible to NMDAR antagonism.

In visceral pain pathways, NMDARs may also be involved under inflammatory conditions. For, example, systemically administered NMDAR antagonists can attenuate visceral pain because of cyclophosphamide-induced cystitis in rats (79). The stronger antinociceptive effect produced by intrathecal MK-801 suggests the spinal localization of NMDARs involved in vesical pain. Another possible site of action for the effects of NMDAR antagonists, that is peripheral afferents, was not eliminated in that study. Although in a previous report the inhibition of responses to UBD was not observed after intravesical application of ketamine (76), the situation may be different in the chemically damaged bladder when better delivery of the drug to nociceptors can be expected.

Thus, experimental evidence currently available suggests that NMDAR antagonists may be useful analgesics for the treatment of visceral pain, (e.g, irritable bowel syndrome).

Conclusion

It is clear that NMDARs are critically involved in the induction and maintenance of neuronal hyperexcitability after noxious events. Until recently, only central NMDARs were a primary focus of investigations. With the recognition of peripheral somatic and visceral NMDARs, it is now apparent that the role of NMDARs in pain is much greater than thought previously.

Over the past decade, accumulating evidence has suggested that the NR2B subunit of NMDAR is particularly important for pain perception. Given the small side effect profile and good efficacy of NR2B-selective compounds, it is quite likely that NR2B-selective blockade will emerge as a viable strategy for pharmacological treatment of pain.

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