Presynaptic NMDA Receptors Modulate Glutamate Release from Primary Sensory Neurons in Rat Spinal Cord Dorsal Horn

Rita Bardoni, 1 Carole Torsney, 2 Chi-Kun Tong, 2 Massimiliano Prandini, 1 and Amy B. MacDermott 1

1 Department of Biomedical Sciences, University of Modena and Reggio Emilia, 41100 Modena, Italy, and 2 Department of Physiology and Cellular Biophysics and 3 The Center for Neurobiology and Behavior, Columbia University, New York, New York 10032

NMDA receptors have the potential to produce complex activity-dependent regulation of transmitter release when localized presynaptically. In the somatosensory system, NMDA receptors have been immunocytochemically detected on presynaptic terminals of primary afferents, and these have been proposed to drive release of substance P from central terminals of a subset of nociceptors in the spinal cord dorsal horn. Here we report that functional NMDA receptors are indeed present at or near the central terminals of primary afferent fibers. Furthermore, we show that activation of these presynaptic receptors results in an inhibition of glutamate release from the terminals. Some of these NMDA receptors may be expressed in the preterminal axon and regulate the extent to which action potentials invade the extensive central arborizations of primary sensory neurons.

Key words: glutamate; spinal cord; pain; presynaptic modulation; patch clamp; primary afferent depolarization

Introduction

The transfer of sensory input from the periphery to the CNS is critically dependent on the release of glutamate from central terminals of primary afferent fibers within the spinal cord dorsal horn. Regulation of this glutamate release by GABA was the first demonstration of presynaptic inhibition by an endogenous mediator, and it was shown to be attributable to local depolarization at the terminals, or primary afferent depolarization (PAD) (Frank and Fuortes, 1957; Eccles et al., 1961). More recently, modulation of primary afferent glutamate release was demonstrated by exogenous activation of presynaptic AMPA and kainate receptors, likely attributable to the induction of PAD (Kernrner et al., 2001; Lee et al., 2002).

Immunocytochemical studies demonstrate NMDA receptor expression in the cell bodies of both small- and large-diameter sensory neurons (Sato et al., 1993; Liu et al., 1994; Marvizon et al., 2002). In addition, NMDA receptors have been specifically detected within the presynaptic terminals of primary afferent fibers at the electron microscope level (Liu et al., 1994; Lu et al., 2003). Functional NMDA receptor expression on the cell bodies of dorsal root ganglion (DRG) neurons has only been reported rarely (Lovingier and Weight, 1988), despite efforts by others to detect functional receptors at the level of the soma and central axons (Agrawal and Evans, 1986; Huettner, 1990).

Activation of presynaptic NMDA receptors has, however, been implicated in the release of substance P from the central terminals of a subset of nociceptors in the rat dorsal horn. This hypothesis was proposed to account for pain behavior and strong internalization of neurokinin 1 (NK1) receptors in a subpopulation of dorsal horn neurons after intrathecal injection of NMDA in rats (Liu et al., 1997) and for the activity-dependent release of substance P, induced by high-frequency dorsal root stimulation in spinal slices (Marvizon et al., 1997). In other regions of the CNS, such as hippocampus, cerebellum, and enthorinal cortex, immunocytochemical and functional studies have documented presynaptic NMDA receptor expression and their involvement in modulating transmitter release (Pittaluga and Raiteri, 1990; Berretta and Jones, 1996; Cochilla and Alford, 1999; Glitsch and Marty, 1999; Casado et al., 2000, 2002). Although the release of substance P from a subset of nociceptors is proposed to be regulated by presynaptic NMDA receptors, it is not known whether the release of glutamate, which is thought to be used as a transmitter by all primary afferents (De Biasi and Rustioni, 1988), is regulated by presynaptic NMDA receptor activation.

Here we tested the prediction that primary afferent fibers express functional NMDA receptors. We found that, although functional receptors do not appear to be detectable along the central axon, they are strongly expressed near the central terminals. Moreover, we showed that exogenous activation of these receptors inhibits and slows the synaptic release of glutamate without affecting postsynaptic AMPA receptors. Our data strongly suggests that these presynaptic NMDA receptors have the potential to powerfully influence the transmission of sensory input to the spinal cord dorsal horn.
Materials and Methods

Recording from dorsal root and primary afferent terminals. Lumbar and sacral spinal cords with attached dorsal roots were obtained from rats of postnatal day 6 (P6) to P12. The spinal cords were excised and placed in ice-cold oxygenated Krebs’ solution (95% O2–5% CO2, saturated Krebs’ solution (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 2.5 glucose, 1 MgCl2, and 2 CaCl2, pH 7.4 (320 mOsm)). After removal of the dura mater, all ventral roots were cut close to the cord. Several parallel cuts were made through the spinal cord between L1 and S4 roots, and then the spinal cord sections were hemisected sagittally using a razor blade. After recovery in oxygenated Krebs’ solution for at least 1 hr at room temperature, the tissues or isolated roots were placed in a submersion-type recording chamber and superfused continuously with the Krebs’ solution at 23–25°C.

For compound action potential recording, dorsal roots of S1–S4 with DRG attached were transferred to a recording chamber. Two suction electrodes were used: one for electrical stimulation of the root and the other for recording compound action potentials. Electrical stimulation was performed at 0.1–0.2 Hz for 100 μsec using an ISO-Flex Stimulus Isolator (A.M.P.I., Jerusalem, Israel). Experiments were performed at room temperature (23–25°C) in oxygenated Krebs’ solution. To rule out the possibility that the drug had some effect on the DRG itself, a Vaseline barrier was used to separate the compartments of the chamber to isolate the DRG from any contact with drug. Compound action potentials were filtered at 10 kHz and sampled at 20 kHz.

The procedure for spinal cord–dorsal root DC depolarization (PAD) recording has been described previously (Lee et al., 2002). Briefly, the tissue was placed across a Vaseline seal to separate the hemisected cord and the dorsal root (see Fig. 1). The Vaseline seal made the drug containing Krebs’ solution flow exclusively through the cord without contacting the DRG and increased the output resistance of the dorsal root to magnify the amplitude of the voltage signals. A suction recording electrode was used with an indifferent electrode made of 3 M NaCl–3% agarose as the ground in the same chamber as the cord. DC potential was measured between the indifferent electrode and the root recording electrode. All experiments were performed in low calcium (20 μM) and zero magnesium Krebs’ solution. Potentials were filtered with a low-pass 10 kHz filter and sampled at 160 Hz. All of the extracellular voltage signals were recorded by a Cygnus ER1 differential amplifier (Cygnus Technology, Delaware Water Gap, PA) or Dagan EX1 differential amplifier (Dagan Corporation, Minneapolis, MI) with a 4001 head stage and stored with the pClamp 6 data acquisition system (Axon Instruments, Union City, CA). Reported peak amplitudes are averages calculated over an 0.6 sec duration. Statistical analysis was performed using repeated measures ANOVA, followed by Newman-Keuls post hoc tests.

Spinal cord slice preparation and stimulation of dorsal roots. Recordings of EPSCs were performed on lamina II neurons in spinal cord slices from postnatal rats (P3–P15). Postnatal rats were anesthetized with halothane and decapitated, and the lumbar region of the spinal cord was removed. The spinal cord was laid down on an agarose block, and transverse slices (500–600 μm) with attached dorsal roots were obtained. Slices were incubated in oxygenated Krebs’ solution at 35°C for 1 hr and used for recording. All experiments were performed in low calcium (95% O2–5% CO2, saturated Krebs’ solution) with the following composition (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 2.5 glucose, 1 MgCl2, and 2 CaCl2, pH 7.4 (320 mOsm). Dorsal roots were stimulated using a glass suction electrode. The stimulus had intensities ranging from 20 μA to 10 mA (constant current stimulus isolation unit output) and durations of 0.05–0.5 msec at a frequency of 0.03–0.05 Hz. Threshold was found by adjusting the intensity from low to high. Once the threshold was verified, the stimulation intensity was adjusted slightly higher than threshold, to avoid synaptic failures in control conditions. Thus, the majority were evoked by low-stimulus intensity and likely to be A fiber, but some (20%) were of a higher intensity (>1 mA), likely to be C fiber. Monosynaptic responses were selected on the basis of the low variability of latency (SD, typically 0.1–0.2 msec). Polysynaptic responses were identified as responses with more variable latency (SD, 0.7–1.9 msec). The reliability of this classification criterion was verified in some cells by stimulating the root at higher frequency (1 Hz). At this frequency, polysynaptic responses tended to fail and markedly change their latency from one response to the following one.

Patch-clamp recording and analysis. Patch-clamp recording in whole-cell configuration was performed on visually identified lamina II neurons at room temperature. Intracellular solution had the following composition (in mM): 130 Cs-gluconate, 10 CsCl, 11 EGTA, 1 CaCl2, 10 HEPEs, and 2 Mg2+–ATP, pH adjusted to 7.2 with NaOH (osmolality adjusted to 305 with sucrose). Junction potential was corrected before recording.

Data were recorded and acquired using an Axopatch 1-D amplifier and pClamp 6 software (Axon Instruments). Sampling rate was 10 kHz, and data were filtered at 2 kHz. The evoked EPSC amplitudes and latencies were detected with Clampfit 6 (Axon Instruments). When the evoked EPSCs presented multiple peaks, the first peak was analyzed because it was in the minimal value within a fixed temporal range (~2 msec). Evoked EPSC latency was measured as the time interval between the stimulus artifact and the point at which the trace slope started changing significantly (visually determined). Percentage of depression of EPSC peak in NMDA was determined using average traces and was defined as the following: % depression = (EPSC peakcontrol – EPSC peakNMDA) × 100/EPSC peakcontrol.

Significance of evoked EPSC amplitude depression or latency change was determined using an unpaired t test with a two-tailed p value (p < 0.05 was considered significant). The t test was used to compare peak amplitudes or latency changes between samples of 10 traces, obtained in control and in NMDA for each cell individually. The effect of NMDA on peak amplitude and latency change was considered significant in those cells in which the t test produced a significant p value.

Miniature EPSC (mEPSC) amplitudes and frequencies were determined using Minianalysis software (Synaptosoft, Decatur, GA). Detection threshold for mEPSCs was set at 7–10 pA, depending on the amplitude of the membrane noise observed during NMDA application. Significance of miniature EPSC amplitude and frequency changes in NMDA was evaluated using a t test (again, p < 0.05 was considered significant). When a sufficient number of events was recorded in NMDA, the Kolmogorov–Smirnov test was also applied. The absence of correlation between miniature EPSC frequency or amplitude change in NMDA and the NMDA-induced postsynaptic current (see Fig. 4C,D) was determined by assessing, using a t test, that the parameter r2 was not significantly different from zero.

All of the averaged data are expressed as mean ± SE. Average EPSC traces were obtained from five single traces.

Potassium tail currents were recorded using intracellular solution with the following composition (in mM): 130 K+–gluconate, 10 KCl, 0.1 CaCl2, 1 EGTA, 10 HEPEs, and 2 Mg2+–ATP, pH adjusted to 7.2 with NaOH (osmolality adjusted to 305 with sucrose). Tail currents were evoked from a holding potential of ~85 mV, after a 20 msec depolarizing step to +10 mV. Leak currents were subtracted using the P4 procedure (Armstrong and Bezanilla, 1974; Bardoni and Belluzzi, 1993). Voltage-dependent Na+ and Ca2+ currents were blocked by adding 5 mM QX-314 to the intracellular solution and 0.5 mM CdCl2 to the extra- cellular solution, respectively. The tail current amplitude was measured after ~2 msec from the beginning of the hyperpolarizing step.

Materials. Bicuculline, d-APV, SCH50911, PPADS, NMDA, LY341495, QX-314, and MPP+ were purchased from Tocris Cookson (Bristol, UK). TTX was purchased from Alomone Labs (Jerusalem, Israel). Other drugs were obtained from Sigma (St. Louis, MO).

Results

Functional expression of NMDA receptors by sensory afferents

Strong depolarization of axons blocks propagation of action potentials. In a study by Agrawal and Evans (1986), kainate depolarized primary afferent axons in dorsal roots and caused depression of C fiber conduction. In their study, application of 10 μM NMDA did not evoke any fiber depolarization, suggesting that no functional NMDA receptors are expressed along primary afferent central axons. However, these experiments did not exclude the possibility that some functional NMDA receptors are located...
near the central terminals of primary afferent fibers, within the dorsal horn, as suggested by the role of NMDA receptors in mediating substance P release (Liu et al., 1994; Marvizon et al., 1997). By recording from dorsal roots, isolated or attached to the spinal cord, we further investigated possible sites of expression of functional NMDA receptors.

Functional NMDA receptor expression at or near primary afferent central terminals was tested directly by recording PAD from a dorsal root while applying NMDA (50 \mu M) to the attached hemisected spinal cord isolated from the root by a Vaseline gap (Fig. 1A). The hemisected spinal cord was perfused with Krebs’ solution containing a low concentration (20 \mu M) of CaCl2 to block any Ca2+–dependent release of transmitters. This bath was sufficient to completely block dorsal root-evoked dorsal root potentials within 5 min. NMDA (50 \mu M) routinely evoked a depolarization of the afferent fibers or PAD (1.1 \pm 0.27 mV; n = 4). This depolarization was mediated by NMDA receptors because it was significantly blocked by 50 \mu M d-APV (Fig. 1B,C) (92% depression; n = 4; p < 0.05). This observation suggests that functional NMDA receptors are indeed expressed at or near the presynaptic terminals of primary afferent fibers.

NMDA was applied to isolated dorsal roots from postnatal rats while compound action potentials were stimulated and recorded. Application of 100 \mu M NMDA failed to cause any change in the afferent volley evoked by root stimulation (data not shown; n = 6). In similar experiments, kainate and capsaicin selectively inhibited the slow volley (Agrawal and Evans, 1986; Labrakakis et al., 2003). Neither fast- nor slow-conducting fiber volleys showed NMDA sensitivity, demonstrating that central primary afferent axons lack functional NMDA receptor expression or that axonal NMDA receptors do not have good access to applied NMDA.

Interestingly, NMDA-induced PAD was still observed, although significantly reduced, when 1 mM Mg2+ was included in the extracellular solution (57% depression; n = 5) (Fig. 1D). This suggests that NMDA receptors expressed on primary afferent terminals are not completely blocked by magnesium ions at the resting membrane potential. Almost complete inhibition of NMDA-induced PAD was obtained in the presence of 10 mM extracellular Mg2+ (96% depression; n = 9) (Fig. 1E).

## NMDA receptor-mediated modulation of glutamate release

The PAD evoked by NMDA suggests that NMDA receptors are near primary afferent central terminals. These receptors are well located to modulate primary afferent fiber excitability and regulate release of glutamate at synapses with dorsal horn neurons (Liu et al., 1994; Lu et al., 2003). We therefore investigated the modulatory effect of NMDA application on AMPA receptor-mediated EPSCs, recorded from spinal cord lamina II neurons from postnatal rats (P6–P15) (Fig. 2A,B). Monosynaptic EPSCs were evoked by dorsal root stimulation and recorded at a frequency of 0.03–0.05 Hz. Membrane potential was held at −85 mV under voltage clamp to minimize current flow through activated NMDA receptors on the postsynaptic neuron. All of the experiments on spinal cord slices were performed in 10 \mu M bicuculline and 5 \mu M strychnine to block the inhibitory inputs mediated by GABA\(_{\text{A}}\) and glycine receptors and to isolate AMPA receptor-mediated EPSCs.

Bath application of NMDA (10, 25, and 50 \mu M) rapidly caused an increase in spontaneous synaptic activity and a depression of AMPA EPSC peak amplitude. Both of these effects were reversible after wash. In most neurons tested, NMDA caused a significant decrease of peak EPSC amplitude (p < 0.05); the mean percentage depression within this group of neurons was 47.6 ± 15.6% in 10 \mu M NMDA (n = 4 of 5), 45.8 ± 8.5% in 25 \mu M NMDA (n = 3 of 4), and 76 ± 7.3% in 50 \mu M NMDA (n = 5 of 5) (Fig. 2C). The application of NMDA at different concentrations also resulted in synaptic failures in the majority of lamina II neurons tested (n = 9 of 14) (Fig. 2D). The inhibition of AMPA EPSC amplitude induced by NMDA was significantly decreased when 50 \mu M d-APV was added to 50 \mu M NMDA (n = 3; p < 0.01), confirming that depression of AMPA EPSC peak was mediated by NMDA receptors (Fig. 2C). We observed a significant NMDA-induced depression of AMPA EPSC amplitudes evoked by both low stimulus intensities (in the A fiber range) and high intensities (in the C fiber range). There was no correlation between the magnitude of depression and the stimulus intensity, consistent with immunohistochemical observations of NMDA receptor subunit expression in large numbers of both A- and C-type neurons in dorsal root ganglia (Marvizon et al., 2002). Although membrane potential was held negative to resting...
potential \((-85\,\text{mV})\), which should be sufficient to maintain the Mg\(^{2+}\) blockade of NMDA receptors, the application of NMDA produced a constant increase of the negative holding current in all tested cells \((n = 40)\) (Fig. 3B). The mean amplitude of this inward current in the postsynaptic neuron was dose dependent and significantly reduced in the presence of 50 \(\mu\text{M}\) NMDA \((p < 0.01)\). Numbers above the bars represent the number of cells in which synaptic failures were observed over the total number of tested cells.

Application of 50 \(\mu\text{M}\) NMDA caused a significant decrease of mEPSC frequency in most cells \((5\) of 7; data not shown). At this concentration, however, we were able to obtain full recovery only in one neuron. The application of a lower concentration of NMDA \((25\,\mu\text{M})\) still produced a significant decrease of mEPSC frequency in all tested neurons, followed by a complete recovery.
Presynaptic NMDA Receptors in Rat Spinal Cord

Because NMDA application in the presence of TTX produces a depression of mEPSC frequency caused by 25 μM NMDA was not accompanied by any significant change of mEPSC amplitude (n = 4) (Fig. 4B), and there was also no significant correlation between depression of mEPSC frequency and the amplitude of NMDA-induced holding current increase (r² = 0.0003; p > 0.05; n = 5) (Fig. 4C). A decrease in mEPSC frequency with no change in amplitude points to a likely presynaptic site of action of NMDA.

Because NMDA application in the presence of TTX produces a depression of mEPSC frequency, the increase in spontaneous activity, observed when TTX is absent, may be attributable to action potential firing of dorsal horn neurons or primary afferent terminals, after depolarization induced by NMDA. This hypothesis is supported by the presence of the NMDA-induced inward current in postsynaptic neurons, even at very negative membrane potentials. This also raises the possibility that NMDA receptor-mediated depolarization of dorsal horn neurons might induce the release of neurotransmitters capable of inhibiting glutamate release from primary afferent terminals. To probe the potential involvement of intraspinal circuits in the AMPA EPSC depression, we applied NMDA in the presence of a mixture of antagonists to receptors potentially present on primary afferent terminals. These include the following (in μM): 10 DPCPX, 20 SCH50911, 50 PPADS, 25 LY341495, and 100 MPPG (bicuculline and strychnine are also included). The application of 50 μM NMDA was still able to induce a significant depression of AMPA EPSC amplitude. The histogram compares the mean percentage of AMPA EPSC depression in 50 μM NMDA alone with the depression in 50 μM NMDA plus mixture. The amount of depression obtained in NMDA plus mixture is not significantly different from the inhibition obtained in NMDA alone (unpaired t test; p > 0.05). Numbers above each bar represent the number of tested cells.

Tonic activation of dorsal horn neurons by applied NMDA is likely to cause accumulation of K⁺ in the tissue. Potassium ions enhance or depress transmitter release in some preparations, depending on the concentration (Schmitz et al., 2001). Therefore, it is possible that NMDA-induced K⁺ accumulation is responsible for the depression of the evoked EPSC amplitude by NMDA. To address this possibility, we estimated the increase of extracellular [K⁺]ᵢ induced by 50 μM NMDA using the equilibrium potential of potassium tail currents recorded from lamina II neurons, evoked by a test pulse to +10 mV. NMDA application caused a decrease of the potassium equilibrium potential by 9.3 ± 2.7 mV, corresponding to an average [K⁺]ᵢ increase of 1.2 ± 0.3 mM (n = 3; data...
to 5.5 mM does not significantly depress dorsal root potential and NMDA application causes a reversible increase in latency of sciatic axon. This hypothesis is supported by the observation that depress action potential-evoked glutamate release by interfering nerve terminals by presynaptic NMDA receptor activation could be involved in the mechanisms. Depolarization of primary afferent fibers near the presynaptic terminals can interfere with the transmission of action potentials along primary afferent axons.

Discussion

We showed that NMDA receptors are functionally expressed near central terminals of primary afferent fibers in postnatal rat spinal cord dorsal horn. Their activation produces a depolarization of primary afferent fibers, even in the presence of 1 mM Mg\(^{2+}\). Activation of these NMDA receptors causes depression of action potential-evoked glutamate release accompanied by an increase in failure of transmitter release from primary afferent fibers onto lamina II neurons and an increase in synaptic latency, suggesting that at least part of the inhibition is attributable to slowing and suppression of axonal action potentials in the preterminal membrane. However, NMDA receptors are both depolarizing and permeable to Ca\(^{2+}\). Thus, modulation of the release machinery by presynaptic NMDA receptors could also be involved in the synaptic depression.

Subcellular localization of NMDA receptors on sensory neurons

Several immunohistochemical and molecular studies have shown NMDA receptor expression at the level of DRG somata. A high percentage of DRG neurons have been reported to express the NR1 subunit (Sato et al., 1993; Liu et al., 1994; Coggeshall and Carlson, 1998; Marvizon et al., 2002). NR2 subunits have also been detected, particularly NR2B and NR2D (Ma and Hargreaves, 2000; Marvizon et al., 2002). We observed significant NMDA-induced depression of AMPA EPSCs in most lamina II neurons tested, consistent with the widespread expression pattern of presynaptic NMDA receptors on DRG neurons. NMDA receptors are present on both A- and C-type primary afferents (Marvizon et al., 2002), although the presynaptic NR1 subunit seems to be mainly expressed on myelinated, mechanosensitive fibers (Lu et al., 2003). In our experiments on spinal cord slices, most AMPA EPSCs were evoked by low-intensity stimulation and were probably mediated by myelinated A-type fibers. However, in a small percentage of neurons (~20%), stimulation thresholds were quite high (>1 mA), suggesting that C fibers could be involved. NMDA-induced depression of EPSC amplitude, however, did not correlate with stimulus intensity.

The subcellular localization of functional NMDA receptors on DRG neurons has been a matter of debate for some time. Only one electrophysiological study has described NMDA receptor-mediated current in a substantial proportion of DRG neurons held at resting potential (Lovinger and Weight, 1988) (but see Huettner, 1990). Extracellular recordings from the isolated dorsal roots failed to show any depolarization in response to NMDA (Agrawal and Evans, 1986). In our experiments, NMDA did not affect the propagation of the afferent volley along primary afferent central axons in isolated dorsal root, whereas application of NMDA to hemisected spinal cords, recorded in low Ca\(^{2+}\) bath to block internal synaptic circuits, resulted in depolarization of the primary afferent fibers. This indicates that NMDA receptors are expressed on or near the central terminals of the afferents. In agreement with this observation, electron microscopy has revealed that a high percentage of glutamatergic synapses in superficial dorsal horn express NMDA receptors at presynaptic terminals (Liu et al., 1994; Lu et al., 2003).
NMDA receptor activation in the presence of Mg\textsuperscript{2+}

We observed depolarization of primary afferent axon terminals and depression of EPSC amplitude in the presence of 1 mM extracellular Mg\textsuperscript{2+}. At the DRG neuron resting potential of approximately −60 mV (Waddell and Lawson, 1990), NMDA receptors composed of NR1 and NR2A and/or NR2B are more strongly blocked by Mg\textsuperscript{2+} than receptors expressing the NR2D subunit (Monyer et al., 1994). Thus, our data indicate that the NR2D subunit may be an important constituent of these presynaptic NMDA receptors, as shown for presynaptic NMDA receptors in hippocampus (Thompson et al., 2002). However, it may be that, at −60 mV, there would be sufficient remaining current through NR1 and NR2A/B to relieve the Mg\textsuperscript{2+} block, resulting in membrane depolarization and further unblocking of other NMDA receptors. NMDA also induced an increase in holding current in lamina II dorsal horn neurons in the presence of 1 mM Mg\textsuperscript{2+}. The current–voltage relationship of this postsynaptic current had a negative slope conductance as is typical of NMDA receptors. However, the current amplitude, dependent on NMDA concentration, was substantial even at the holding potential of −85 mV, implying that it is sustained by NMDA receptors having a low sensitivity to Mg\textsuperscript{2+}. Extrasynaptic NMDA receptors containing the NR2D subunit, reported in dorsal horn neurons by Momiyama (2000), could be involved in the generation of the postsynaptic current observed during NMDA application.

Mechanism of NMDA receptor modulation of glutamatergic transmission

NMDA caused a depression of peak amplitude and an increase in synaptic latency and failures of evoked monosynaptic AMPA EPSCs in most lamina II neurons. Our data suggest that this synaptic inhibition is attributable to a presynaptic mechanism, directly mediated by NMDA receptors. Presynaptic NMDA receptors could depress evoked release by decreasing the input resistance of the afferent terminal, shunting the propagation of incoming action potentials (Segev, 1990). NMDA receptors could also substantially depolarize the afferent axons to inactivate Na\textsuperscript{+} channels (Graham and Redman, 1994). Both mechanisms would produce either a depression of action potential amplitude, causing a decrease of glutamate release, or a complete block of action potential propagation, causing the appearance of synaptic failures. Na\textsuperscript{+} channel inactivation could also be responsible for the slowing of action potential propagation, resulting in an increase of synaptic latency. Finally, the activation of presynaptic NMDA receptors could inhibit evoked synaptic transmission by causing the inactivation of voltage-dependent Ca\textsuperscript{2+} channels; this would affect the level of Ca\textsuperscript{2+} inside the terminal and, indirectly, interfere with the release machinery.

NMDA receptors are permeable to Ca\textsuperscript{2+}. This property underlies the facilitating effect that NMDA receptor activation has on glutamate release as a result of Ca\textsuperscript{2+} entry through presynaptic NMDA receptors (Cochilla and Alford, 1999; Glitsch and Marty, 1999). However, rather than an increase in mEPSC frequency being induced by 25 μM NMDA in our experiments, a decrease was observed. If the NMDA receptors are predominantly localized in the preterminal membrane, the Ca\textsuperscript{2+} entering the cells through the receptors might not reach into the terminals. Presynaptic NMDA receptors might activate a second-messenger pathway, as shown in the cerebellum (Casado et al., 2000), leading to the modulation of voltage-dependent channels or of other components involved in synaptic release. However, the decrease in mEPSC frequency may not be associated with the same terminals stimulated in the evoked release studies, the primary afferent terminals, but instead could be associated with other glutamatergic terminals forming synapses on the neurons under study.

The effect of presynaptic NMDA receptors on glutamate release in lamina II is paradoxically opposite to the facilitating action of NMDA on substance P release (Marvizon et al., 1997). In this study of substance P release, 100 μM NMDA was used, a concentration that in our preparation caused such strong activation of NMDA receptors on dorsal horn neurons that it was difficult to observe any presynaptic effects. It is possible that the higher concentration of NMDA used in that study produces a strong depolarization of the terminal, unblocking other NMDA receptors and causing substantial Ca\textsuperscript{2+} entry into the terminal with subsequent substance P release. Substance P may also be released under our experimental conditions. However, the depressant effect of NMDA on evoked EPSC amplitude is unlikely to be directly attributable to the action of substance P because NK1 receptor activation potentiates evoked EPSC amplitude instead of depressing it (Ikeda et al., 2003).

Physiological role of NMDA receptor-mediated regulation of glutamate

Although many of the presynaptic terminals contacting primary afferents in the dorsal horn seem to be GABAergic (Alvarez, 1998), a recent study reports the presence of glutamatergic synaptic contacts on primary afferents in frog spinal cord, likely formed by glutamatergic interneurons (Vesselinik et al., 2003). Furthermore, NMDA receptor subunit NRI has been detected on several primary afferent terminals at the center of glomerular structures in inner lamina II (Lu et al., 2003). Given that glomeruli are the proposed morphological basis for modulation of primary afferent input (Willis and Coggleshall, 1991), this suggests that these presynaptic NMDA receptors could be endogenously activated synaptically and modulate sensory throughput.

Endogenous activation of presynaptic NMDA receptors could also occur through glutamate spillover. Russo et al. (2000), working in the turtle spinal cord, reported dorsal root potentials mediated by GABA\textsubscript{A}, non-NMDA, and NMDA receptors that are reduced, but not blocked, in the presence of tetrodotoxin, indicating that TTX-insensitive primary afferents may drive release of transmitters onto other primary afferent terminals. Glutamate released by primary afferent fibers could activate NMDA receptors expressed on the same or on nearby terminals, depolarize them, and control the subsequent release of glutamate during repetitive stimulation. Kainate receptors have been proposed to have this type of autoreceptor function on mossy fiber terminals in the hippocampus (Kamiya et al., 2002). The high affinity of NMDA receptors for glutamate and their ability to depolarize the terminals in the presence of physiological concentrations of Mg\textsuperscript{2+} suggests that presynaptic NMDA receptors could act as sensors and fine modulators of primary afferent fiber activity. The role of second-messenger pathways in NMDA receptor-mediated modulation of glutamate release by primary afferent fibers remains to be investigated.

References

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