ELSEVIER

Neuroscience Letters 204 (1996) 17–20

NEUROSCIENCE LETTERS

NMDA receptor-mediated calcium entry in the absence of AMPA receptor activation in rat dorsal horn neurons

David B. Reichling[a,b,1], Amy B. MacDermott[a,b,*]

[a]*Department of Physiology and Cellular Biophysics, Columbia University, College of Physicians and Surgeons, 630 West 168 Street, New York, NY 10032, USA*

[b]*Center for Neurobiology and Behavior, Columbia University, College of Physicians and Surgeons, 630 West 168 Street, New York, NY 10032, USA*

Received 16 October 1995; revised version received 15 December 1995; accepted 15 December 1995

Abstract

This study tested quantitatively the degree to which selective activation of NMDA receptors increases the intracellular concentration of free Ca^{2+} ions ($[Ca^{2+}]_i$) in neurons isolated from the rat spinal cord dorsal horn and grown in culture. The results show that activation of NMDA receptors, without additional depolarizing stimuli, produce $[Ca^{2+}]_i$ increases in the neuronal cell bodies in the presence of physiological concentrations of Mg^{2+}. Furthermore, this phenomenon occurs during synaptic activation of NMDA receptors. Thus, it cannot be assumed that in physiological extracellular $[Mg^{2+}]$, there is an absolute requirement for additional membrane depolarization before NMDA receptor activation stimulates significant increases in $[Ca^{2+}]_i$.

Keywords: Magnesium; Spinal cord; Indo-1; Glutamate; Excitatory amino acid; Pain

NMDA receptors have been implicated in a number of physiological and pathological phenomena in the spinal cord dorsal horn, including activity-dependent synaptic plasticity [2,3], central sensitization associated with the development of hyperalgesia [8] and neurotoxicity associated with trauma and ischemia [4,16]. Specifically, Ca^{2+} entry via NMDA receptors plays an important role in NMDA receptor-mediated plasticity and neurotoxicity [8,16]. Because NMDA-gated channels are both Ca^{2+}-permeable [5,6], and subject to voltage-dependent block by Mg^{2+} [6,7,11], it is often assumed that NMDA receptors only stimulate significant increases in $[Ca^{2+}]_i$ in the presence of some additional, depolarizing stimulus (e.g. AMPA receptor activation) [1,9]. To test this assumption, we have measured increases in $[Ca^{2+}]_i$ in dorsal horn neurons evoked by selective activation of NMDA receptors alone, in physiological $[Mg^{2+}]$.

Dorsal horn neurons were prepared from embryonic rats (E15), as described previously [13]. After 7–21 days in culture, the neurons were loaded with 10 μM indo 1 ester (with 0.025% pluronic acid; Molecular Probes) for 30–45 min at room temperature. The coverslip was then mounted in a chamber with continuously flowing room-temperature bath composed of (in mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 10 HEPES, 5×10^{-3} glycine and 0.1% glucose, with osmolarity adjusted to 325 mOsM with sucrose. The bath also contained 0.5 μM tetrodotoxin, except when synaptic activation was being studied. Drugs were applied via a large Y-tube that rapidly delivered drug to an entire neuron [13,14]. Indo-1 fluorescence was measured with photomultiplier tubes and the data were digitized at a rate of 20 Hz. Calculated $[Ca^{2+}]_i$ was further filtered at 4 Hz [14].

For the synaptic experiments, dorsal root ganglion explants were co-cultured with dorsal horn neurons. Dorsal root ganglia were removed from the same 15-day-old embryo from which spinal cord neurons were obtained. The ganglia were cut in half and each half was placed on a coverslip with dissociated dorsal horn neurons. The medium was supplemented with 100 ng/ml nerve growth factor (2.5 S fragment, Boehringer Mannheim). To elec-

* Corresponding author. Department of Physiology and Cellular Biophysics, Columbia University, 630 W. 168th Street, 1109 BB, New York, NY 10032, USA. Tel.: +1 212 3053889; fax: +1 212 3055775; e-mail: abm1@columbia.edu

[1] Present address: Department of Anatomy, Box 0452A, University of California, San Francisco, San Francisco, CA 94143, USA.

0304-3940/96/$12.00
PII: S0304-3940(96)12305-1

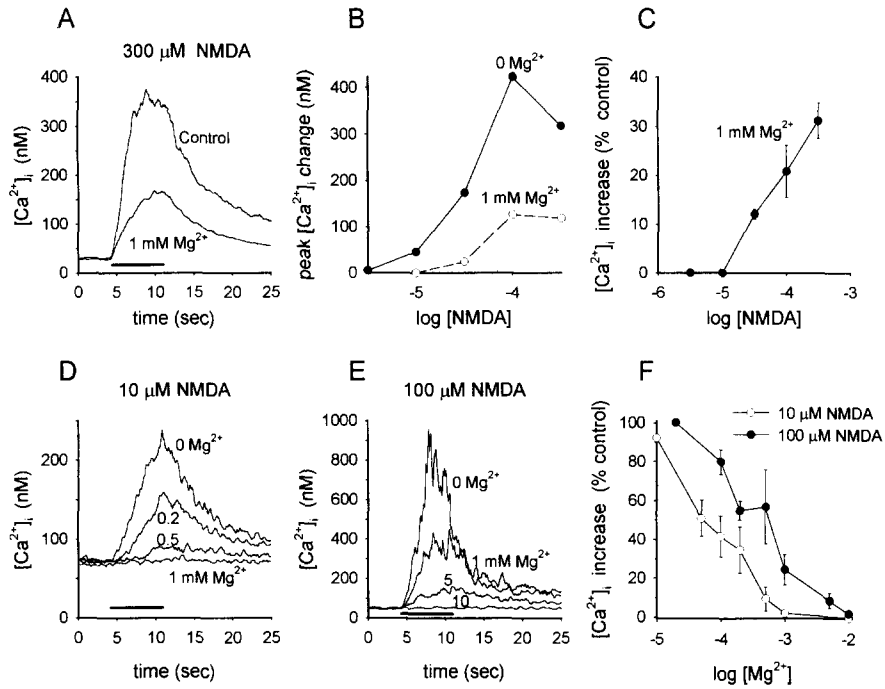

Fig. 1. (A) The change in $[Ca^{2+}]_i$ evoked by 300 μM NMDA in the presence and absence of 1 mM Mg^{2+}. (B) The complete dose-response data for the cell in (A). (C) The same data as in (B), with Ca^{2+} elevation in 1 mM Mg^{2+} expressed as a percent of that in 0 Mg^{2+}. (D) Ca^{2+} responses to 10 μM NMDA in the presence of 0.001–1 mM Mg^{2+}. (E) The Ca^{2+} responses to 100 μM NMDA in 0–10 mM Mg^{2+} in a different cell than (D). (F) The population data for Mg^{2+} inhibition of 10 and 100 μM NMDA evoked Ca^{2+} transients.

trically evoke synaptic activity in the culture, a bipolar tungsten electrode was inserted into the center of the DRG explant and was stimulated with single, 10 ms voltage pulse.

Physiological concentrations of Mg^{2+} only partially blocked $[Ca^{2+}]_i$ transients in rat dorsal horn neurons. For example, as shown in Fig. 1A, 300 μM NMDA caused $[Ca^{2+}]_i$ to increase in a cultured dorsal horn neuron from a resting value of approximately 30 nM to a peak of approximately 350 nM. This increase in $[Ca^{2+}]_i$ was presumably due to Ca^{2+} entry through the NMDA receptors as well as Ca^{2+} entry through voltage-gated Ca^{2+} channels. When the same concentration of NMDA was applied in the presence of 1 mM Mg^{2+}, the $[Ca^{2+}]_i$ transient, although attenuated, reached a peak amplitude of approximately 150 nM.

The ability of NMDA receptors to mediate $[Ca^{2+}]_i$ elevation in the presence of Mg^{2+} should depend on the number of receptors activated and therefore the concentration of agonist applied. This was shown by determining the dose dependence of NMDA-evoked $[Ca^{2+}]_i$ transients in the presence of Mg^{2+} (Fig. 1B,C). The only doses of NMDA tested at which 1 mM Mg^{2+} was able to completely block the agonist-evoked $[Ca^{2+}]_i$ transient were equal to or less than 10 μM, well below the EC_{50} for NMDA [13]. Using concentrations as low as 30 μM, NMDA-mediated activation of these cells was sufficient to produce significant increases in $[Ca^{2+}]_i$. Mg^{2+} block was progressively overcome as the concentration of NMDA was increased above 10 μM.

We also assessed the degree to which NMDA receptors mediating a $[Ca^{2+}]_i$ transient depends on the concentration of Mg^{2+}. As shown in Fig. 1D–F, Mg^{2+} block of NMDA-evoked $[Ca^{2+}]_i$ transients became progressively more effective as $[Mg^{2+}]$ increased between 0.01 and 10 mM. NMDA (100 μM) was more potently able to elevate $[Ca^{2+}]_i$ at all Mg^{2+} concentrations tested compared to 10 μM NMDA (Fig. 1D–F).

To determine if the endogenous excitatory amino acid transmitter, glutamate, also activates NMDA receptors

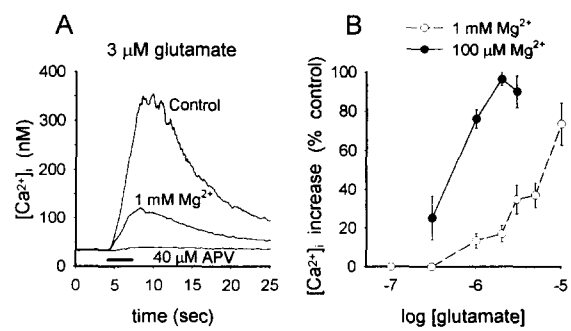

Fig. 2. (A) Glutamate (3 μM) activates a large Ca^{2+} transient both in the absence and presence of 1 mM Mg^{2+}. (B) The dose-dependence of the $[Ca^{2+}]_i$ response to L-glutamate in the presence of 100 μM and 1 mM Mg^{2+}.

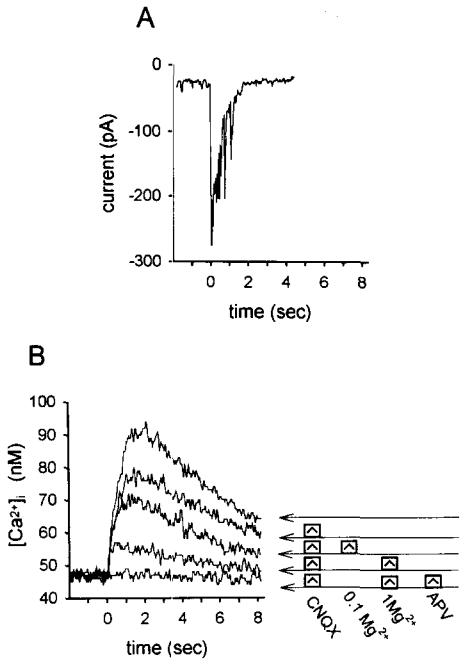

Fig. 3. Synaptic activity in dorsal horn neurons following stimulation of DRG explant. (A) Current evoked in dorsal horn neuron by 10 ms stimulation of explant. Response appears to be a large burst of synaptic activity. (B) With a comparable stimulation, Ca^{2+} transients are evoked in a different neuron. (Recording was focused on the soma.) Each trace is an average of multiple sweeps in each condition: 8 sweeps for control, 7 sweeps for 50 μM CNQX, 5 sweeps for 100 μM Mg^{2+} plus 50 μM CNQX, 7 sweeps for 1 mM Mg^{2+} plus 50 μM CNQX, and 8 sweeps for 30 μM APV, 1 mM Mg^{2+}, and 50 μM CNQX.

sufficiently to result in $[Ca^{2+}]_i$ increases, we measured the effect of l-glutamate at concentrations low enough to selectively activate the NMDA receptor [12]. As shown in Fig. 2A, 1 mM Mg^{2+} was unable to completely block the $[Ca^{2+}]_i$ transient evoked by 3 μM glutamate. At higher concentrations of agonist, there was a dose-dependent $[Ca^{2+}]_i$ elevation (Fig. 2B). For example, 3 μM glutamate, near the EC_{50} for NMDA receptors [12], evoked a $[Ca^{2+}]_i$ transient in the presence of 1 mM Mg^{2+} of approximately 100 nM $[Ca^{2+}]_i$ above baseline (Fig. 2B). The selectivity of 3 μM glutamate for NMDA receptor activation in these cells was tested by blocking the $[Ca^{2+}]_i$ response with 40 μM APV (Fig. 2A).

Even knowing that modest glutamate concentrations are able to elevate $[Ca^{2+}]_i$ in the presence of 1 mM Mg^{2+}, it is not possible to predict whether synaptic activation of NMDA receptors alone will act similarly. Previous investigation of A-δ fiber input onto adult dorsal horn neurons suggested that NMDA receptors in these neurons are effectively blocked in the presence of 1 mM Mg^{2+} at resting membrane potentials [18]. We assessed whether synaptically released glutamate acting on NMDA receptors alone elevates $[Ca^{2+}]_i$ in the presence of 1 mM Mg^{2+} in cultured dorsal horn neurons. Electrical stimulation of the DRG explant with a bipolar metal electrode caused polysynaptic excitation of the dorsal horn neurons (Fig. 3A). Elimination of any contribution of synaptically activated AMPA receptors by adding 50 μM CNQX to the bath showed that, in this example, the synaptically evoked $[Ca^{2+}]_i$ transient was largely a consequence of NMDA receptor activation when the bath contained 0 Mg^{2+}. The NMDA receptor-mediated $[Ca^{2+}]_i$ transient was only partly blocked with addition of 0.1 mM Mg^{2+} to the bath, and the $[Ca^{2+}]_i$ increase was still detectable when $[Mg^{2+}]$ was increased to 1 mM (Fig. 3B). The remaining Ca^{2+} transient was completely blocked by 30 μM APV.

Our observations are consistent with molecular biological evidence concerning the subunit composition of NMDA receptors in the spinal cord. Embryonic spinal cord neurons express low levels of NR2B and higher levels of NR2D form of the NR2 subunit [10]. The NR2D subunit is associated with lower sensitivity to voltage-dependent Mg^{2+} blockade [10]. In contrast with many other areas of the central nervous system in which, during maturation, NR2A and NR2C become the major forms of NR2, dorsal horn neurons in adult rat continue to predominantly express NR2D [15]. Thus, due to their subunit composition, NMDA receptors in dorsal horn neurons of both embryonic and adult rat may be particularly resistant to Mg^{2+} blockade.

In recent years, the potential role of NMDA and AMPA receptors in dorsal horn neuronal plasticity, hyperalgesia, and pain sensation has become the subject of intense research [2,3,8,9,17]. In this regard it is often assumed that NMDA receptor activation must coincide with other depolarizing stimuli in order for NMDA receptors to overcome voltage-dependent Mg^{2+} block. Contrary to this assumption, our results indicate that, in physiological $[Mg^{2+}]$, and at concentrations of transmitter that are achieved by synaptic release, activation of NMDA receptors alone can stimulate significant increases in $[Ca^{2+}]_i$.

We would like to thank Jianguo Gu for thoughtful comments on the manuscript and Bernadette Rodriguez for technical assistance. This work was supported by NIH GM32099 and APA MB1-9102

[1] Daw, N.W., Stein, P.S. and Fox, K., The role of NMDA receptors in information processing, Annu. Rev. Neurosci., 16 (1993) 207–222.

[2] Dickenson, A.H., A cure for wind up: NMDA receptor antagonists as potential analgesics, Trends Pharmacol. Sci., 11 (1990) 307–309.

[3] Dubner, R. and Ruda, M.A., Activity-dependent neuronal plasticity following tissue injury and inflammation, Trends Neurosci., 15 (1992) 96–103.

[4 Faden, A.I. and Simon, R.P. A potential role for excitotoxins in the pathophysiology of spinal cord injury, Ann. Neurol., 23 (1988) 623–626.

[5] MacDermott, A.B., Mayer, M.L., Westbrook, G.L., Smith, S.J. and Barker, J.L., NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones, Nature, 321 (1986) 519–522.

[6] Mayer, M.L. and Westbrook, G.L., Permeation and block of N-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones, J. Physiol., 394 (1987) 501–527.

[7] Mayer, M.L., Westbrook, G.L. and Guthrie, P.B., Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones, Nature, 309 (1984) 261–263.

[8] McMahon, S.B., Lewin, G.R. and Wall, P.D., Central hyperexcitability triggered by noxious inputs, Curr. Opin. Neurobiol., 3 (1993) 602–610.

[9] Meller, S.T., Thermal and mechanical hyperalgesia A distinct role for different excitatory amino acid receptors and signal transduction pathways? Am. Pain Soc. J. (Pain Forum), 3 (1994) 215–231.

[10] Monyer, H., Burnashev, N., Laurie, D.J., Sakmann, B. and Seeburg, P.H., Developmental and regional expression in the rat brain and functional properties of four NMDA receptors, Neuron, 12 (1994) 529–540.

[11] Nowak, L., Bregestovski, P., Ascher, P., Herbet, A. and Prochiantz, A., Magnesium gates glutamate-activated channels in mouse central neurones, Nature, 307 (1984) 462–465.

[12] Patneau, D.K. and Mayer, M.L., Structure-activity relationships for amino acid transmitter candidates acting at N-methyl-D-aspartate and quisqualate receptors, J. Neurosci., 10 (1990) 2385–2399.

[13] Reichling, D.B. and MacDermott, A.B., Lanthanum actions on excitatory amino acid-gated currents and voltage-gated calcium currents in rat dorsal horn neurons, J. Physiol., 441 (1991) 199–218.

[14] Reichling, D.B. and MacDermott, A.B. Brief calcium transients evoked by glutamate receptor agonists in rat dorsal horn neurons: fast kinetics and mechanisms, J. Physiol., 469 (1993) 67–88.

[15] Tölle, T.R., Berthele, A., Zieglgänsberger, W., Seeburg, P.H. and Wisden, W., The differential expression of 16 NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray, J. Neurosci., 13 (1993) 5009–5028.

[16] Tymianski, M., Charlton, M.P., Carlen, P.L. and Tator, C.H., Source specificity of early calcium neurotoxicity in cultured embryonic spinal neurons, J. Neurosci., 13 (1993) 2085–2104.

[17] Woolf, C.J., The pathophysiology of peripheral neuropathic pain – abnormal peripheral input and abnormal central processing, Acta Neurochir. Suppl. (Wien), 58 (1993) 125–130.

[19] Yoshimura, M. and Jessell, T., Amino acid-mediated EPSPs at primary afferent synapses with substantia gelatinosa neurones in the rat spinal cord, J. Physiol., 430 (1990) 315–335.