Korean J Physiol Pharmacol
Vol 3: 237–244, June, 1999

Magnesium Suppresses the Responses of Dorsal Horn Cell to Noxious Stimuli in the Rat

Hong Kee Shin, Jin Hyuk Kim, and Kee Soon Kim

Department of Physiology, School of Medicine, Institute of Biomedical Sciences, Hanyang University, Seoul 133–791, Korea

Magnesium ion is known to selectively block the N-methyl-D-aspartate (NMDA)-induced responses and to have anticonvulsive action, neuroprotective effect and antinociceptive action in the behavioral test. In this study, we investigated the effect of Mg^{2+} on the responses of dorsal horn neurons to cutaneous thermal stimulation and graded electrical stimulation of afferent nerves as well as to excitatory amino acids and also elucidated whether the actions of Ca^{2+} and Mg^{2+} are additive or antagonistic. Mg^{2+} suppressed the thermal and C-fiber responses of wide dynamic range (WDR) cell without any effect on the A-fiber responses. When Mg^{2+} was directly applied onto the spinal cord, its inhibitory effect was dependent on the concentration of Mg^{2+} and duration of application. The NMDA- and kainate-induced responses of WDR cell were suppressed by Mg^{2+}, the NMDA-induced responses being inhibited more strongly. Ca^{2+} also inhibited the NMDA-induced responses current-dependently. Both inhibitory actions of Mg^{2+} and Ca^{2+} were additive, while Mg^{2+} suppressed the EGTA-induced augmentation of WDR cell responses to NMDA and C-fiber stimulation. Magnesium had dual effects on the spontaneous activities of WDR cell. These experimental findings suggest that Mg^{2+} is implicated in the modulation of pain in the rat spinal cord by inhibiting the responses of WDR cell to noxious stimuli more strongly than innocuous stimuli.

Key Words: Noxious inputs, Excitatory amino acids, Dorsal horn cell response, Mg^{2+}

INTRODUCTION

Magnesium ion is known to have anticonvulsive action and neuroprotective effects in the cerebral ischemia, traumatic brain injury and excitatory amino acid (EAA)-induced neurotoxicity. Low extracellular Mg^{2+} ($[Mg^{2+}]_o$) increases the spontaneous epileptiform discharges and synaptically evoked responses in the hippocampal neurons, which are suppressed by the increase in extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$), DL-2-amino-5-phosphonovaleric acid and verapamil (Walther et al, 1986; Mody et al, 1987; Pohl et al, 1992). Intracerebroventricularly administered EAAs such as NMDA (N-methyl-D-aspartate), kainate, quinolinate also induce typical convulsion and neurotoxicity, which are aggravated by low $[Mg^{2+}]_o$ (Garthwaite & Garthwaite, 1987; Cox et al, 1989; Wolf et al, 1991) and glucose deprivation (Cox et al, 1989; Lysko et al, 1989), while relieved by EAA antagonists (Finkbeiner & Stevens, 1988; Lysko et al, 1989) and Mg^{2+} (Finkbeiner & Stevens, 1988; Cox et al, 1989; Wolf et al, 1990). In the rat cerebellar slices, substitution of Na^+ and Cl^- in the medium respectively with choline and isethionate did not prevent EAA-induced neurotoxicity, but in a Ca^{2+}-free medium, EAA did not induce any neurotoxicity (Garthwaite et al, 1986). All these findings suggest that excess Ca^{2+} influx through NMDA/Mg^{2+}-gated channels is responsible for the EAA-induced neurotoxicity. Magnesium was also reported to have protective effects against the traumatic nerve injury (McIntosh et al, 1989; Girard et al, 1993) and anoxic neuronal death (Rothman, 1983).

The responses of cat dorsal horn neurons to iontophoretically applied NMDA, but not kainate, were selectively inhibited by Mg^{2+} (Davies & Watkins,

Corresponding to: Hong Kee Shin, Department of Physiology, School of Medicine, Hanyang University, 17 Hengdang-Dong, Sungdong-Gu, Seoul 133-791, Korea

1977). The actions of Ca^{2+} and Mg^{2+} were reported to be additive in some papers (Davies & Watkins, 1977; Evans et al, 1977; Ault et al, 1980) while antagonistic to each other in the others (del Castillo & Engbaek, 1954; Hutter & Kostial, 1954). In in vitro experiment in which employed patch clamp technique in the mouse spinal cord and mesencephalic neurons, Mg^{2+} voltage-dependently suppressed the NMDA-induced inward current by reducing the opening time and frequency and increasing the close time of channels, but did not have any inhibitory effect on kainate- and quisqualate-induced currents (Mayer et al, 1984; Nowak et al, 1984). These experimental findings suggest that Mg^{2+} has a selective action on the NMDA-induced responses.

In behavioral tests, subcutaneous injection of Mg^{2+} induced long-lasting inhibition of heat hyperalgesia and mechanical allodynia resulting from unilateral chronic constriction injury of the peripheral nerve (Xiao & Bennett, 1994). Pretreatment with Mg^{2+} dose-dependently reduced autotomy score, delayed autotomy onset and decreased the percentage of animals which showed high autotomy behaviors (Feria et al, 1993). However, there was no report on the effects of Mg^{2+} on the responses of dorsal horn neurons to noxious inputs. The present study was undertaken to investigate the effect of Mg^{2+} on the responses of dorsal horn neurons to cutaneous thermal stimulation and graded electrical stimulation of peripheral nerve as well as EAA and to elucidate whether the actions of Ca^{2+} and Mg^{2+} are additive or antagonistic.

METHODS

Sprague-Dawley male rats (300~450 gm) anesthetized with urethane (1.2 gm/kg i.p.) were used in this experiment. External jugular vein was cannulated with polyethylene tube (PE-60) through which pancuronium bromide (0.3 mg/kg/hr) was continuously infused to paralyze the musculature. The rats were artificially ventilated and end-tidal CO_2 level was maintained between 3.5 and 4.5%. Rectal temperature was maintained near 37°C with an electrical heating blanket (Harvard Apparatus, USA). A laminectomy was carried out to expose lumbar enlargement of spinal cord between T13 and L3. The common peroneal and tibial nerves were dissected from the surrounding tissues at popliteal fossa. Liquid paraffin pool was made around the exposed spinal cord and peripheral nerves to prevent drying. After finishing all these surgical procedures, the rats were placed in a stereotaxic apparatus.

The activities of dorsal horn neurons activated by electrical stimulation of the afferent nerves were extracellulary recorded with the central barrel of seven-barreled microelectrode which contained a low-impedence carbon filament. Once single unit activity of dorsal horn neuron was recorded, the type of neuron was classified according to the response pattern to mechanical stimuli applied to the receptive field. Wide dynamic range (WDR) cells which responded to both innocuous and noxious mechanical stimuli were used in this experiment. One outer barrel of 7-barreled microelectrode was filled with 0.15 M NaCl and used for current balancing. The other barrels were used to apply the following substances by microiontophoresis; Mg^{2+} (0.2 M), EGTA (0.1 M), Ca^{2+} (0.1 M), NMDA (0.05 M), and kainate (0.02 M). All solutions were at pH 7.5~8.0. All drugs were purchased from Sigma chemicals or Research Biochemicals International. Retaining currents (5~10 nA) were used between drug applications to prevent drug leakage.

In each figure, the number next to the name of each drug applied shows the amount of ejection current. For example, Mg5 means that Mg^{2+} was ejected at the current of 5 nA. We first recorded the responses of WDR cells to iontophoretically applied NMDA, kainate, graded electrical stimulation of afferent nerves and thermal stimulation of the receptive field and then investigated the changes in the WDR cell responses induced by single or combined iontophoretical application of Mg^{2+}, EGTA or Ca^{2+}. In 11 experiments, we observed the effects of direct spinal application of Mg^{2+} on the responses of WDR cells to electrical stimulation of afferent nerve and to thermal stimulation of the receptive field. NMDA and kainate were periodically ejected for 5 sec every 15 or 20 sec. The A- or C-fiber responses were compiled from 10 consecutive stimulation of afferent nerves with a single pulse (0.1 msec) or train of three pulses (0.5 msec, 50 Hz), respectively. The intensity of stimuli was adjusted to activate only A fibers (10 T: T is times the threshold for activation of Aβ fibers) or all A and C fibers (200~300 T). Thermal stimuli (50°C) were applied to the receptive field for 10 sec, using a thermo-stimulator equipped with a contact thermode which can regulate set temperature in a

feedback fashion.

All induced electrical activities were amplified (WPI DAM80), displayed on an oscilloscope and fed into a window discriminator (Frederic Haer & Co.) whose output was used by a computer to compile the poststimulus time histogram. Because the size of evoked responses varied from one unit to another, data were expressed as percentage of discharges in the control state before single or combined iontophoretic application of drugs. The data were expressed as mean±SE. Statistical significance was determined by student's t test. P values less than 0.05 were considered significant.

RESULTS

The responses of 95 WDR cells were extracellularly recorded in 35 experiments. Recordings were generally made in the depth between 550 μm and 950 μm below the surface of lumbar enlargement of the spinal cord, suggesting that the cells were located in the laminae IV~VI. Because there were no differences in the response characteristics of WDR cells with or without C-fiber inputs, all the data obtained were pooled together.

Iontophoretically applied Mg^{2+} current-dependently suppressed NMDA- and kainate-induced responses of WDR cells and NMDA responses were more strongly inhibited (Fig. 1A & 1B). Mg^{2+} ejected with the current of 10 nA inhibited NMDA responses by 58.8±6.5% (N=12) while suppressed kainate responses by 21.0±4.7% (N=10). The inhibition of kainate responses by Mg^{2+} with the ejection current of 100 nA (62.4±11.2%) was less than that of NMDA responses induced by Mg^{2+} with the ejection current of 20 nA (79.4±3.8%).

Iontophoretical application of EGTA augmented NMDA responses of WDR cells and the mean increase in NMDA responses was 250.0±19.5% of

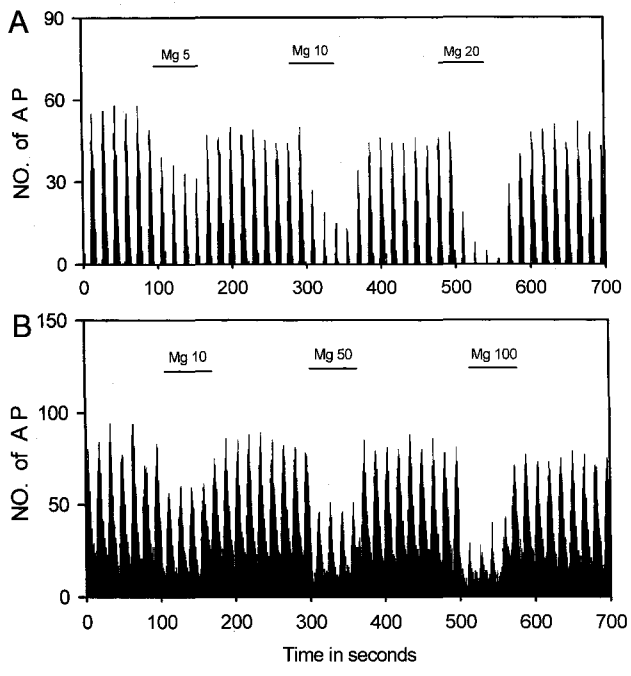

Fig. 1. Magnesium ions currect-dependently suppressed the responses of WDR cells to iontophoretical application of NMDA (A) and kainate (B). NMDA or kainate was periodically ejected for 5 seconds every 15 seconds. Mg5 or Mg100 denotes that magnesium ions were ejected at the current of 5 or 100 nA, respectively.

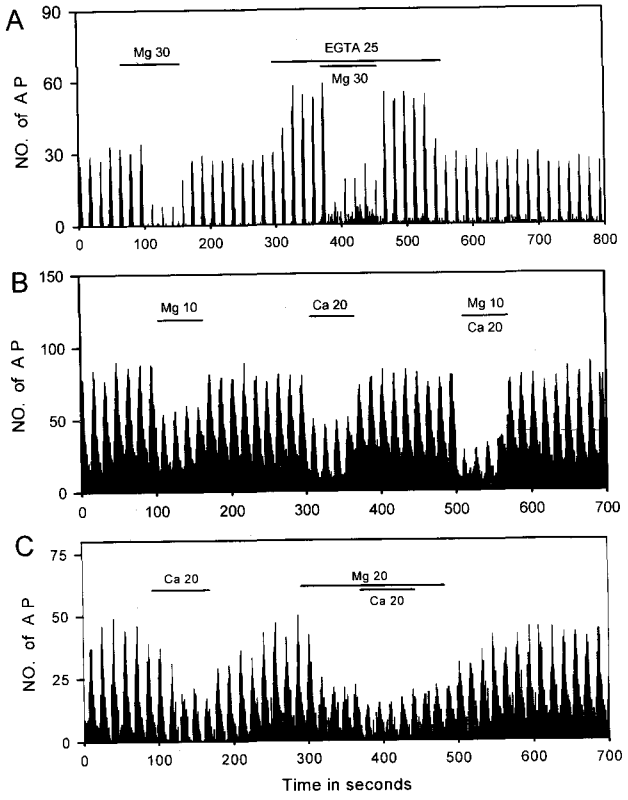

Fig. 2. Effects of Ca^{2+} and EGTA on the inhibitory action of Mg^{2+} on NMDA-induced responses of WDR cell. EGTA augmented the response of WDR cell to NMDA which was strongly inhibited by iontophoretically applied Mg^{2+} (A). Mg^{2+} or Ca^{2+} alone reduced WDR cell responses to NMDA but combined administration of Ca^{2+} and Mg^{2+} did not have synergistic effect.

the control (N=10) when EGTA was applied at the ejection current of 25 nA. After iontophoretical application of Mg^{2+} (25 nA), the EGTA-augmented responses of WDR cells were strongly suppressed by 80.8±7.3% of the augmented responses (Fig. 2A). Ca^{2+} by itself inhibited NMDA responses of WDR cells by 40.0±3.6% (N=14) when Ca^{2+} was iontophoretically applied at the ejection current of 20 nA. As shown in Fig. 2B and 2C, the inhibition induced by combined application of Ca^{2+} and Mg^{2+} was less than simple addition of those evoked by Ca^{2+} or Mg^{2+} alone.

Fig. 3 shows inhibitory effect of Mg^{2+} on the WDR cell responses to thermal stimulation of the receptive field (N=11). The inhibitory effect of Mg^{2+} on the WDR cell responses to thermal stimulation was not so strong as that on NMDA responses. Mg^{2+} -induced inhibition did not increase above a certain level even when the ejection current was continuously increased. Even at the ejection current of 200 nA, Mg^{2+} inhibited thermal responses only by 40.2± 10.2%. EGTA also augmented the thermal responses of WDR cells which were strongly inhibited by Mg^{2+} (Fig. 3B). In 5 experiments, 30 mM Mg^{2+} solution was applied onto the spinal cord after recording the control responses of WDR cell to thermal stimulation (50°C). The thermal responses of WDR cell were gradually suppressed with time (Fig. 3C).

In Fig. 4, we observed the changes in A- and C-fiber responses of WDR cells after iontophoretical

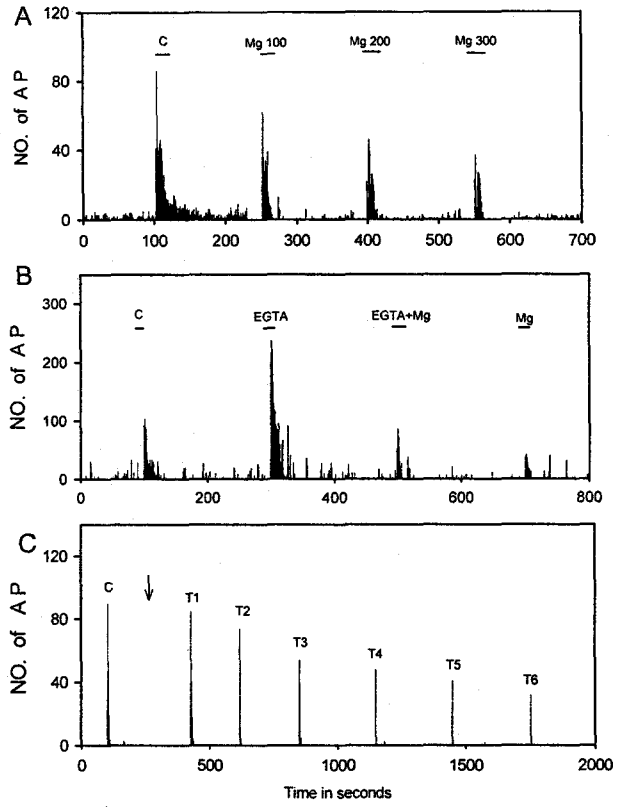

Fig. 3. Changes in the responses of WDR cell to thermal stimulation sfter single or combined application of Mg^{2+} and EGTA. C in fig. A, B and C denotes the control responses of WDR cell to thermal stimulation. Thermal responses of WDR cell were inhibited by iontophoretic application of Mg^{2+} (100, 200 & 300 nA, A). Iontophoretically applied EGTA (35 nA) augmented thermal responses of WDR cells which were suppressed by Mg^{2+} (300 nA, B). C shows that thermal responses of WDR cells were time-dependently reduced after spinal application of Mg^{2+} (30 mM). T1 to T6 denote thermal responses of WDR cells recorded 3, 6, 10, 15, 20 and 30 min after spinal application of Mg^{2+}, respectively. Arrow in fig. C is the time at which Mg^{2+} was applied onto the spinal cord.

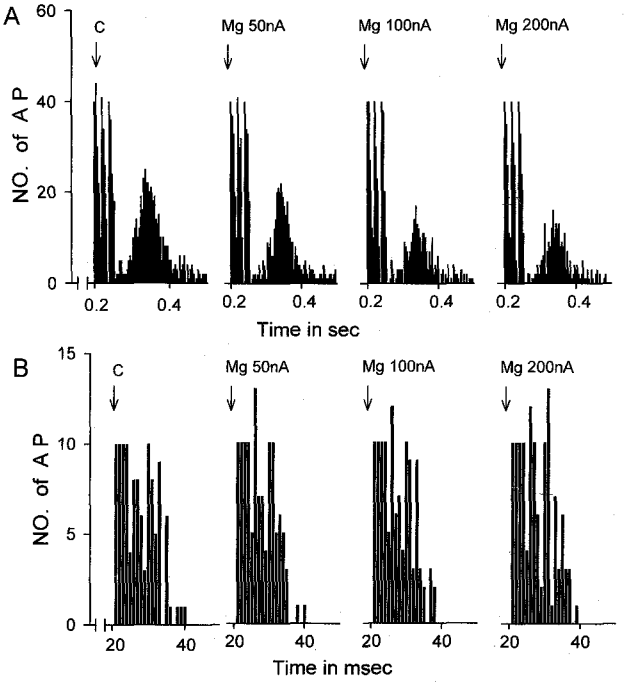

Fig. 4. C-fiber responses of WDR cell were current-dependently reduced after iontophoretic application of Mg^{2+} (A) whereas the A-fiber responses were not significantly changed by Mg^{2+} (B). Arrows indicate the time at which single or 3 train stimuli were applied to the afferent nerves. C is the control C- or A-fiber responses of WDR cell to graded electrical stimulation before and during graded electrical stimulation of afferent nerve at the current indicated above each figure.

application of Mg^{2+}. Mg^{2+} selectively inhibited C-fiber responses (N=20) (Fig. 4A) without any significant effect on A-fiber responses (N=12) (Fig. 4B). As in Mg^{2+}-induced inhibition of thermal responses, relatively high ejection current was needed for Mg^{2+} to induce inhibition of C-fiber responses. Even when Mg^{2+} was ejected at the current of 200 nA, C-fiber response of WDR cell was suppressed by 48.2±5.3%.

Mg^{2+} also suppressed the responses of WDR cells to C-fiber stimulation which were augmented by EGTA (N=11) (Fig. 5A). The mean increase in C-fiber responses was 165.6±12.8% of the control when EGTA was iontophoretically applied at the current of 30 nA. Mg^{2+} (100 nA) inhibited the EGTA-augmented responses to 60.1±5.4% of the control (Fig. 5A). Mg^{2+} (100 nA) or Ca^{2+} (25 nA) alone induced 28.8±5.1% and 35.2±4.0% inhibition of C-fiber responses, respectively. However, no synergistic action was observed after Mg^{2+} and Ca^{2+} were simultaneously applied (N=9) (Fig. 5B).

In 6 experiments, we observed the effect of spinal application of Mg^{2+} (50 mM) on A- and C-fiber responses of WDR cells (Fig. 6). C-fiber and A-fiber responses were time-dependently suppressed after spinal application of Mg^{2+}. In contrast to iontophoretical application, Mg^{2+} strongly inhibited the A-fiber responses of WDR cells as well (Fig. 6B). However, no any significant inhibitory action was observed with 25 mM Mg^{2+} (data not shown). In 10 min after spinal application of Mg^{2+}(50 mM), C-fiber and A-fiber responses were suppressed to 12.2±4.1% and 35.1±6.7% of the control, respectively.

The spontaneous activities of WDR cells were also changed after iontophoretical application of Mg^{2+} (Fig. 7). In the majority of WDR cells (10/13 units), application of Mg^{2+} with the ejection current of 50

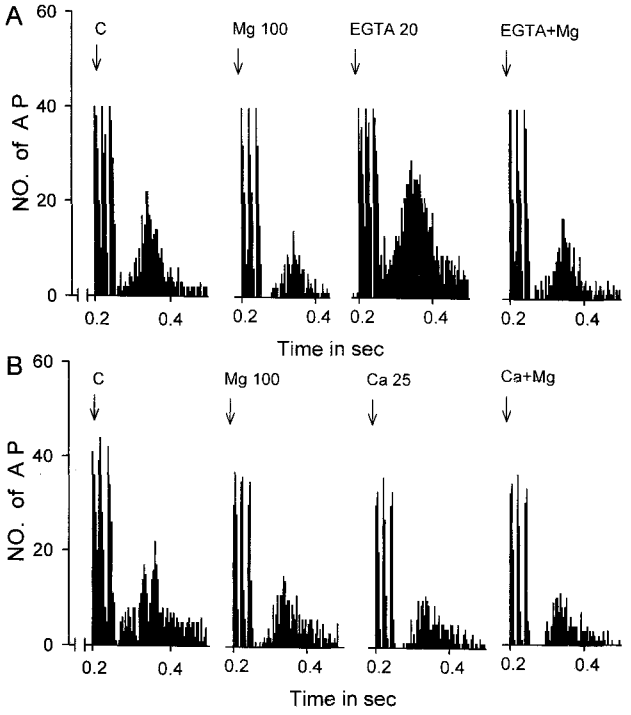

Fig. 5. Effects of Ca^{2+} and EGTA on the Mg^{2+}-induced inhibition of C-fiber responses of WDR cells. Arrows indicate the time at which 3 train stimuli were applied to afferent nerves. C is the control C-fiber responses of WDR cells to electrical stimulation of afferent fibers. Intophoretically applied EGTA (20 nA) augmented the C-fiber responses which were greatly suppressed by Mg^{2+} (100 nA, A). Mg^{2+} (100 nA) or Ca^{2+} (25 nA) alone induced inhibitory effect on the C-fiber responses but combined administration of Mg^{2+} and Ca^{2+} did not have synergistic action.

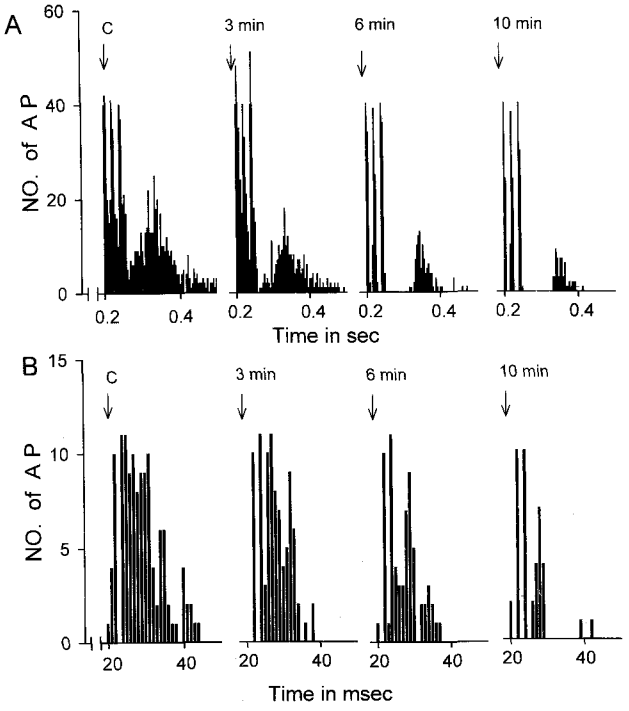

Fig. 6. Effects of spinal application of Mg^{2+} (50 mM) on the responses of WDR cells to electrical stimulation of afferent nerves. Arrows indicate the time at which single or 3 train stimuli were applied to afferent fibers. C is the control C- or A-fiber responses of WDR cell to graded electrical stimulation of afferent fibers. C-fiber (A) and A-fiber (B) responses were time-dependently suppressed after spinal application of Mg^{2+} (50 mM).

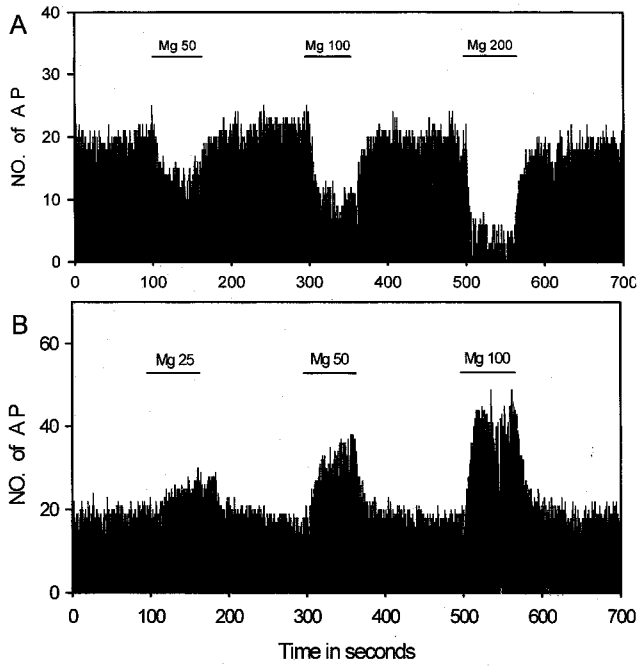

Fig. 7. Changes in spontaneous activities of WDR cells after iontophoretic application of Mg^{2+}. Spontaneous activities were current-dependently reduced after iontophoretic administration of Mg^{2+} (50, 100 & 200 nA) in 10/13 units (A) while Mg^{2+} (25, 50 & 100 nA) increased spontaneous activities in 3/13 units (B).

nA and 100 nA respectively, suppressed the spontaneous activites by 29.4±6.7% and 51.9±8.4%. But in a few cases (N=3), Mg^{2+} rather increased spontaneous activites which was also current-dependent.

DISCUSSION

Magnesium has been known to selectively inhibit the NMDA-gated channels. In the present study, iontophoretically applied Mg^{2+} preferentially inhibited NMDA-induced responses. The responses of dorsal horn neurons to kainate, thermal stimulation and C-fiber stimulation were also suppressed at a relatively high ejection current of Mg^{2+} but the responses to A-fiber stimulation was not affected. These results imply that Mg^{2+} has a selective inhibitory action for NMDA receptors and also for the responses to noxious stimuli. The selective inhibtion of NMDA receptors by Mg^{2+} has already been reported in the isolated spinal cords of frog and rat (Evans et al, 1977; Ault et al, 1980), as well as in the cat dorsal horn neurons (Davies & Watkins, 1977). Nowak et al (1984) suggested that Mg^{2+}-induced selective inhibition is mediated through the decrease in opening time and frequency, and increase in close time of NMDA-gated channels. The different degrees of the Mg^{2+}-induced inhibition observed in NMDA responses and in the responses to thermal and C-fiber stimulation may be due to different kinds of transmitters released. In addition to excitatory amino acids, noxious stimuli such as thermal and C-fiber stimulation have been known to cause the release of substance P and calcitonin gene-related peptide (CGRP) from nociceptive primary afferent fibers (Gibbins et al, 1985; Duggan et al, 1988). Although magnesium has been reported to reduce the release of a few transmitters, there are no experimental findings which elucidated the effect of Mg^{2+} on the responses induced by neurotransmitters other than excitatory amino acids. For this reason, we can not intelligently discuss whether magnesium ions have any affinity with substance P and CGRP receptors. However, both A-fiber and C-fiber responses were time- and concentration-dependently suppressed after spinal application of Mg^{2+} and the selective action of Mg^{2+} on A-fiber and C-fiber responses was not observed. This non-selective inhibition may be due to excessively high concentration of Mg^{2+} which was infiltrated into the spinal cord. This high concentration Mg^{2+} could induce excessive reduction in the transmitter release, decrease in the membrane excitability and increase in the threshold for excitation (del Castillo & Engbaek, 1954; Hutter & Kostial, 1954; Erulkar et al, 1974), resulting in the complete block or partial inhibition of synaptic transmission.

Iontophoretically applied Ca^{2+} and EGTA had opposite effects on the dorsal horn cell responses. Ca^{2+} inhibited, but EGTA augmented the responses to NMDA, kainate, thermal and C-fiber stimulations. EGTA-augmented responses were strongly inhinited by Mg^{2+}, suggesting that Ca^{2+} fluxes through Mg^{2+}-gated channels are implicated in Mg^{2+}-induced inhibitions. This suggestion agree well with the contention that Mg^{2+} acts as a selective NMDA-blocker (Mayer et al, 1984; Nowak et al, 1984) and activation of NMDA receptor causes Ca^{2+} influx into neuronal cells (MacDermott et al, 1986) and with our previous reports (Shin et al, 1997, 1999), but are in a sharp contrast with other experimental findings that intracerebroventricular (i.c.v.) administration of Ca^{2+} produced hyperalgesia while i.c.v. or intrathecal EGTA

induced dose-dependent analgesia (Schmidt & Way, 1990; Kim et al, 1991). These differences in the effects of Ca^{2+} and EGTA can be attributed to the extent of changes in Ca^{2+} concentration in extracellular fluid (ECF) induced by iontophoretical application of Ca^{2+} or EGTA. Local increase in Ca^{2+} concentration resulting from iontophoretical application of Ca^{2+} can reduce the membrane excitability whereas low Ca^{2+} concentration can decrease the thershold for excitation and then increase the spontaneous discharges (Frankenhaeuser, 1957; Frankenhaeuser & Meves, 1958). Curtis et al (1960) also reported that iontophoretical application of calcium chelators excited the spinal neurons by reducing extracellular Ca^{2+} concentration and by removing Ca^{2+} combined with the membrane proteins. However, excessive decrease in Ca^{2+} concentration by EGTA can block signal transduction mechanisms leading to the inhibition of dorsal horn cell responses (Shin et al, 1999). The inhibitory actions of Mg^{2+} on the neuromuscular and ganglionic transmission were reported to be attenuated by Ca^{2+} (del Castillo & Engbaek, 1954; Hutter & Kostial, 1954). However, antagonistic effects of Ca^{2+} were not observed in the present study. Both effects of Ca^{2+} and Mg^{2+} on the WDR cell responses were inhibitory. Our findings, together with those of others, demonstrate that Mg^{2+} modulates signal transmission in the spinal cord by preferentially reducing the activity of NMDA-gated channels.

ACKNOWLEDGEMENT

The authors wish to acknowledge the financial support of Hanyang University, Seoul, Korea, made in the program year of 1998.

REFERENCES

Ault B, Evans RH, Francis AA, Oakes DJ, Watkins JC. Selective depression of excitatory amino acid induced depolarizations by magnesium ions in isolated spinal cord preparations. *J Physiol* 307: 413−428, 1980

Cox JA, Lysko PG, Hennberry RC. Excitatory amino acid neurotoxicity at the N-methyl-D-aspartate receptor in cultured neurons: role of the voltage-dependent magnesium block. *Brain Res* 499: 267−272, 1989

Curtis DR, Phillips TW, Watkins JC. The excitation of spinal neurones by the iontophoretic application of agents which chelate calcium. *J Neurochem* 6: 1−20, 1960

Davies J, Watkins JC. Effect of magnesium ions on the responses of spinal neurones to excitatory amino acids and acetylcholine. *Brain Res* 130: 364−368, 1977

del Castillo J, Engbaek L. The nature of the neuromuscular block produced by magnesium. *J Physiol* 124: 370−384, 1954

Duggan AW, Hendry IA, Morton CR, Hutchison WD, Zhao ZQ. Cutaneous stimuli releasing immunoreactive substance P in the dorsal horn of the cat. *Brain Res* 451: 261−273, 1988

Erulkar SD, Dambach GE, Mender D. The effect of magnesium at motoneurons of the isolated spinal cord of the frog. *Brain Res* 66: 413−424, 1974

Evans RH, Francis AA, Watkins JC. Selective antagonism by Mg^{2+} of amino aicd-induced depolarization of spinal neurons. *Experientia* 33: 489−491, 1977

Feria M, Abad F, Sánchez A, Abreu P. Magnesium sulphate injected subcutaneously suppresses autotomy in peripherally deafferented rats. *Pain* 53: 287−293, 1993

Finkbeiner S, Stevens CF. Applications of quantitative measurements for assessing glutamate neurotoxicity. *Proc Natl Acad Sci USA* 85: 4071−4074, 1988

Frankenhaeuser B. The effect of calcium on the myelinated nerve fibre. *J Physiol* 137: 245−260, 1957

Frankenhaeuser B, Meves H. The effect of magnesium and calcium on the frog myelinated nerve fibre. *J Physiol* 142: 360−365, 1958

Garthwaite G, Garthwaite J. Receptor-linked ionic channels mediate N-methyl-D-aspartate neurotoxicity in rat cerebellar slices. *Neurosci Lett* 83: 241−246, 1987

Garthwaite G, Hajós F, Garthwaite J. Ionic requirements for neurotoxic effects of excitatory amino acid analogues in rat cerebellar slices. *Neuroscience* 18: 437−447, 1986

Gibbins IL, Furness JB, Costa M, MacIntyre I, Hillyard CJ, Girgis S. Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. *Neurosci Lett* 57: 125−130, 1985

Girard JM, Panizzon KL, Wallis RA. An *in vitro* model of traumatic brain injury with protection with post-trauma magnesium treatment. *Soc Neurosci Abstr* 19: 1876, 1993

Hutter OF, Kostial K. Effect of magnesium and calcium ions on the release of acetylcholine. *J Physiol* 124: 234−241, 1954

Kim JH, Shin HK, Kim KS, Huh C. Analgesic action of calcium modulators in the cat. *J Hanyang Med Coll* 11: 464−473, 1991

Lysko PG, Cox JA, Vigano A, Henneberry RC. Exci-

tatory amino acid neurotoxicity at the N-methyl-D-aspartate receptor in cultured neurons: pharmacological characterization. *Brain Res* 499: 258−266, 1989

MacDermott AB, Mayer ML, Westbrook GL, Smith SJ, Barker JL. NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurons. *Nature* 321: 519−522, 1986

Mayer ML, Westbrook GL, Guthrie PB. Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* 309: 261−263, 1984

McIntosh TK, Vink R, Yamakami I, Faden AI. Magnesium protects against neurological deficit after brain injury. *Brain Res* 482: 252−260, 1989

Mody I, Lambert JDC, Heinemann U. Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. *J Neurophysiol* 57: 869−888, 1987

Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307: 462−465, 1984

Pohl M, Straub H, Speckmann E-J. Low magnesium-induced epileptiform discharges in guinea pig hippocampal slices: depression by the organic calcium antagonist verapamil. *Brain Res* 577: 29−35, 1992

Rothman SM. Synaptic activity mediates death of hypoxic neurons. *Science* 220: 536−537, 1983

Schmidt WK, Way EL. Hyperalgesic effects of divalent cations and antinociceptive effects of a calcium chelator in naive and morphine-dependent mice. *J Pharmacol Exp Ther* 212: 22−27, 1980

Shin HK, Kang SH, Kim KS. Calcium channel blockers suppress the responses of rat dorsal horn cell to nociceptive input. *Kor J Physiol Pharmacol* 1: 625−637, 1997

Shin HK, Kang SH, Chung ID, Kim KS. Calcium modulates excitatory amino acid (EAA)- and substance P-induced rat dorsal horn cell responses. *Kor J Physiol Pharmacol* 3: 35−45, 1999

Walther H, Lambert JDC, Jones RSG, Heinemann U, Hamon B. Epileptiform activity in combined slices of the hippocampus, subiculum and entorhinal cortex during perfusion with low magnesium. *Neurosci Lett* 69: 156−161, 1986

Wolf G, Fischer S, Hass P, Abicht K, Keilhoff G. Magnesium sulphate subcutaneously injected protects against kainate-induced convulsions and neurodegeneration: *in vivo* study on the rat hippocampus. *Neuroscience* 43: 31−34, 1991

Wolf G, Keilhoff G, Fischer S, Hass P. Subcutaneously applied magnesium protects reliably against quinolinate-induced N-methyl-D-aspartate (NMDA)-mediated neurodegeneration and convulsion in rats: are there therapeutical implications? *Neurosci Lett* 117: 207−211, 1990

Xiao W-H, Bennett GJ. Magnesium suppresses neuropathic pain responses in rats via a spinal site of action. *Brain Res* 666: 168−172, 1994