

일차 배양 해마신경세포에서 NMDA- 및 Glutamate- 유도전류의 특성

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= Abstract =

Characteristics of NMDA- and Glutamate-Induced Currents in Primary Cultured Rat Hippocampal Neurons

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Objectives : This study was performed in cultured rat hippocampal neurons to investigate the acute electrop-
hysiological features of ionotropic glutamate receptors which act as a major excitatory neurotransmitter in
mammalian brain.

Method : Glutamate receptor agonists were applied into the bath solution embedding in whole - cell patch - clamp
recording of single hippocampal neuron.

Results : In voltage - clamped at -60mV and the presence of 1mmol Mg^{2+} , extracellularly applied NMDA did not in-
duce any inward current. Both the elimination of Mg^{2+} and addition of glycine in bath, however, elicited a NMDA -
induced inward current. Mg^{2+} block current was increased gradually in more negative potentials from -30mV,
showing a negative slope in I - V plot with Mg^{2+} . Glutamate - induced current represented an outward rectification. A
non - NMDA receptor component occupied about 40% of glutamate - induced current in the voltage range of -80mV
to +60mV.

Conclusion : Present study suggests that glutamate activates acutely the non - NMDA receptors which induces an
inward current in the level of resting membrane potential. This makes the membrane potential increase and can
activate the NMDA receptors that permit calcium influx against Mg^{2+} block. At the depolarized state of neuron, there
may be recovery mechanisms of membrane potential to repolarize irrespective of voltage - dependent potassium
channels in the hippocampal neurons.

KEY WORDS : Hippocampal neuron · Patch clamp · Electrophysiology · Glutamate NMDA receptor · Non - NMDA re-
ceptor.

서 론

(hippocampus)

term potentiation) ³⁾⁹⁾.

glutamate

⁸⁾²⁵⁾. Glutamate

(long -

NMDA - Glutamate -

channels) (ligand-gated ion channels) NMDA (N-methyl-D-aspartate) non-NMDA NMDA glutamate Ca²⁺ 5). glutamate (necrosis) (apoptosis) 1)22)24). NMDA glutamate glycine 가 1000rpm 2 Mg²⁺ 가 17) Mg²⁺ glycine AMPA(- amino - 3 - hydroxy - 5 - methyl - 4 - isox - azole propionate) kainate (G-protein) Ca²⁺ 가 10). 가 4)16). Non - NMDA glutamate 가 in vitro 가 glutamate가 가 NMDA non - NMDA

Hank's balanced salt solution [Ca²⁺, Mg²⁺ 1mmol pyruvate 10mmol HEPES(N-2-hydroxyethylpiperazine-N¹-2-ethane sulfonate) 가 pH 7.4)(HBSS) HBSS 0.25% trypsin - EDTA(ethylenediamine tetraacetic acid) 37 25 HBSS 가 1000rpm 2 2 3 가 glutamate(25 μmol) 가 (Neurobasal medium[®])⁷⁾ 10 μL trypan blue(25 μL) HBSS(15 μL) hemocytometer 가 5 Poly - D - lysine 1 15 × 2mm 10 35mm , 2 × 10⁶ 37 , 5% CO₂ 가 4 glutamate가 10mL B - 27(200 μL), 200mmol L - glutamine(25 μL), 14.3mmol mercaptoethanol(17.5 μL) 100mg/mL penicillin streptomycin (1 μL) 가 10 가 2 Neurobasal medium[®] B - 27 Gibco - BRL Lab () Sigma ()

2. 전기생리학적 측정

whole - cell patch - clamp ¹¹⁾ , 2mmol CaCl₂, 1mmol MgSO₄, 5mmol KCl, 135mmol NaCl, 17mmol glucose, 10mmol HEPES 50mmol sucrose NaOH pH 7.4가 30 가 2mL 가 20

재료 및 방법

- 1. 해마신경세포의 분리와 배양
 - 17 18 Sprague - Dawley

Mg^{2+} MgSO₄ Sigma
 borosilicate (Narishige ,)
 micropipette puller(Narishige ,)
 (fabrication)
 sylgard
 3 5M
 112mmol KCl, 2mmol MgCl₂, 0.1mmol CaCl₂, 11
 mmol EGTA, 10mmol HEPES 2mmol ATP_{Na}

가 , 가 가
 가 ramp pulse
 Axopatch 200A(Axon ,)
 Digidata 1200A(Axon ,)
 VR - 10B pulse modulator(Instrutech ,)
 가 pClamp
 6.04(Axon ,)
 sampling rate 2KHz, filter 5KHz

3. 약물처리에 따른 시냅스전류의 기록
 가
 가 . Glutamate
 glutamate 가
 . NMDA
 NMDA glycine
 Mg^{2+}
 , non - NMDA
 가
 (I - V plot) 가

NMDA 2 - amino - 5 - phosphonopen -
 tanoic acid(AP5) non - NMDA 6 -
 cyano - 7 - nitroquinoxaline - 2,3 - dione(CNQX) RBI
 , L - Ca²⁺ Nifedipine

결 과

1. 세포외액에 Mg^{2+} 존재시 Glutamate 및 NMDA 유도 전류

-60mV 가 가 ,
 (excitatory postsynaptic curr -
 ent)가
 NMDA Mg^{2+} (1mmol)
 10 μ mol NMDA 가 10 μ mol glutam -
 ate 가
 glutamate (Fig. 1).

2. Mg^{2+} 의 NMDA 수용체 전류에 대한 영향

Mg^{2+} (1mmol)
 -60mV NMDA
 5 μ mol glycine 10 μ mol NMDA (NMDA +
 glycine) Fig. 1 glutamate
 NMDA + glycine
 (Fig. 2A). NMDA + glycine
 NMDA + glycine 가
 Mg^{2+} NMDA +
 glycine Fig. 1 glutamate

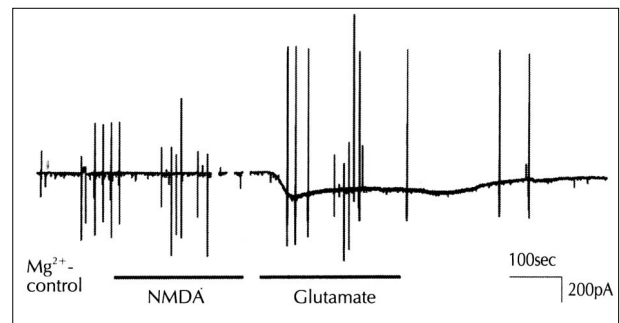


Fig. 1. Inward currents activated by glutamate receptor agonists in a cultured rat hippocampal neuron. NMDA (10 μ mol) did not elicit a noticeable current but glutamate(10 μ mol) induced an inward current in the presence of 1mmol Mg^{2+} . Whole-cell configuration was clamped at -60mV. Vertical bars are on time when ramp pulse from -80mV to +60mV was injected.

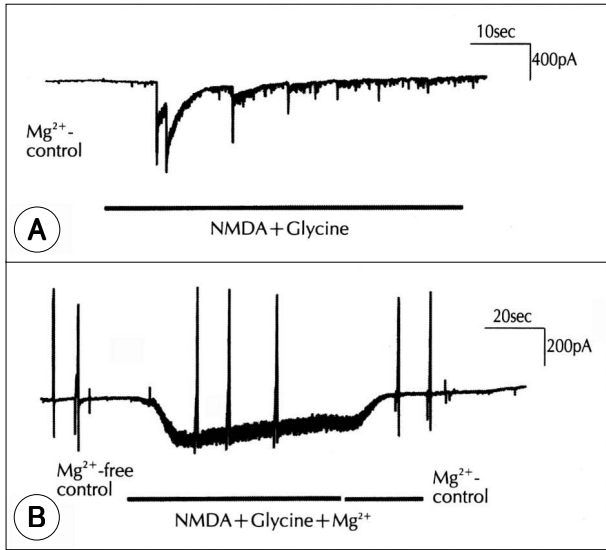


Fig. 2. Both NMDA (10 μ mol) and glycine (5 μ mol)-induced current with (A) or without (B) 1 mmol Mg^{2+} in a cultured rat hippocampal neuron. Whole-cell configuration was clamped at -60 mV. Vertical bars in B are on time when ramp pulse was injected.

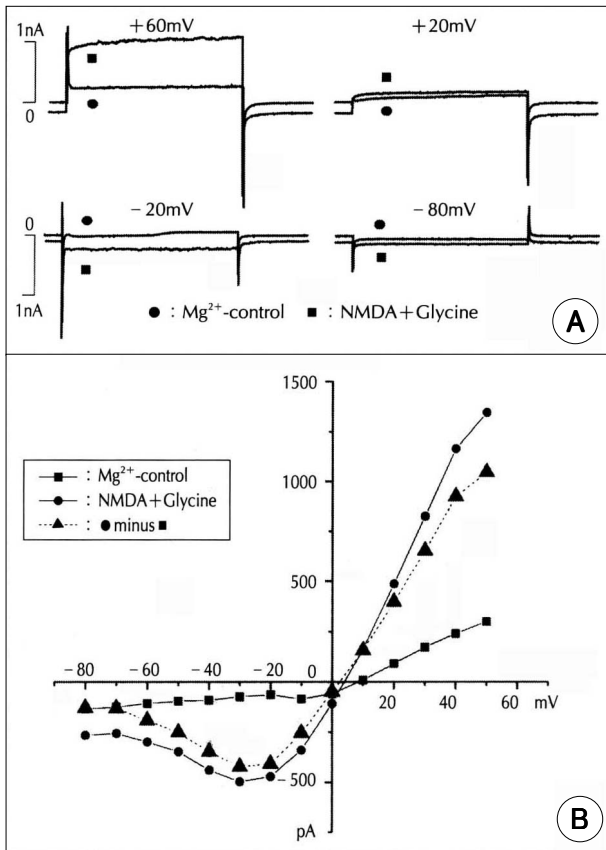


Fig. 3. Both NMDA (10 μ mol) and glycine (5 μ mol)-induced current in the presence of 50 μ mol Mg in a cultured rat hippocampal neuron. A: Inward or outward currents according to the change of membrane potentials elicited by NMDA and glycine application. B: I-V plot for NMDA and glycine-induced current with Mg^{2+} .

가 ,
 Mg^{2+} NMDA
 (Fig. 2B).
 3. 세포막전압 변화에 의한 Mg^{2+} 의 NMDA 수용체 전류의 차단
 - 60mV 50 μ mol
 Mg^{2+} 10 μ mol NMDA 5 μ mol gly -
 cine 가 .
 - 100mV +60mV 10mV 가 ,
 - 30 mV
 0mV

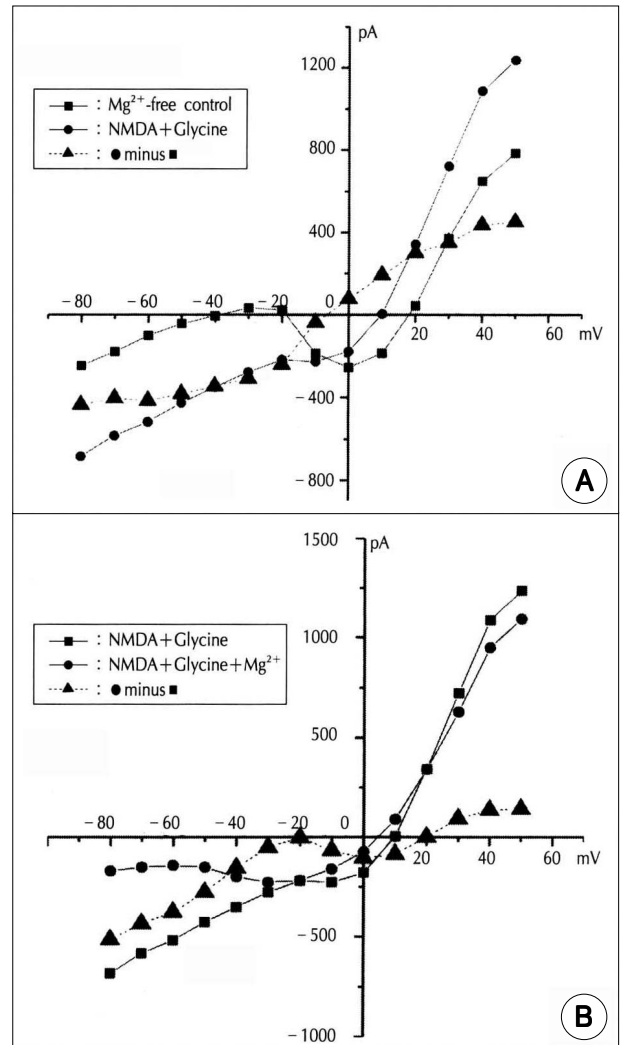


Fig. 4. Effect of 50 μ mol Mg^{2+} on both NMDA (10 μ mol) and glycine (5 μ mol)-induced current. A: I-V plot for NMDA and glycine-current () without Mg^{2+} . B: I-V plot for the amount of Mg^{2+} -sensitive NMDA current.

Mg²⁺ NMDA -30mV
-70
mV (Fig. 3). -30mV

Mg²⁺ NMDA -
Mg²⁺ -30mV
(Fig. 4).

4. Non-NMDA 수용체 전류의 특성
-60mV 10 μmol glutamate
NMDA

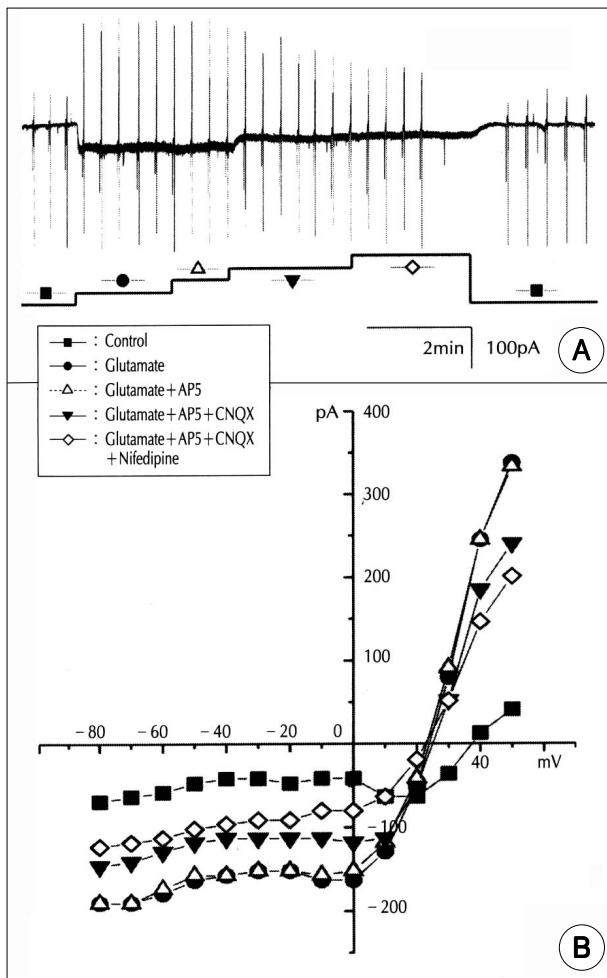


Fig. 5. Effect of glutamate receptor antagonists and nifedipine on glutamate-induced current. A : Glutamate-induced inward current was partially eliminated by its antagonists ; AP5 (50 μmol), CNQX (10 μmol) and nifedipine (10 μmol) were applied subsequently. Vertical bars are on time when ramp pulse was injected. Whole-cell configuration was clamped at -60mV. B : I-V plots recorded from ramp pulse during the ramp pulses in serial drug application.

APV (50 μmol) glutamate
glycine glutamate NMDA
CNQX (10 μmol) non-NMDA
40% 가 glutamate
nifedipine (10 μmol)
(Fig. 5).

glutamate
(I glutamate - I control) +20mV

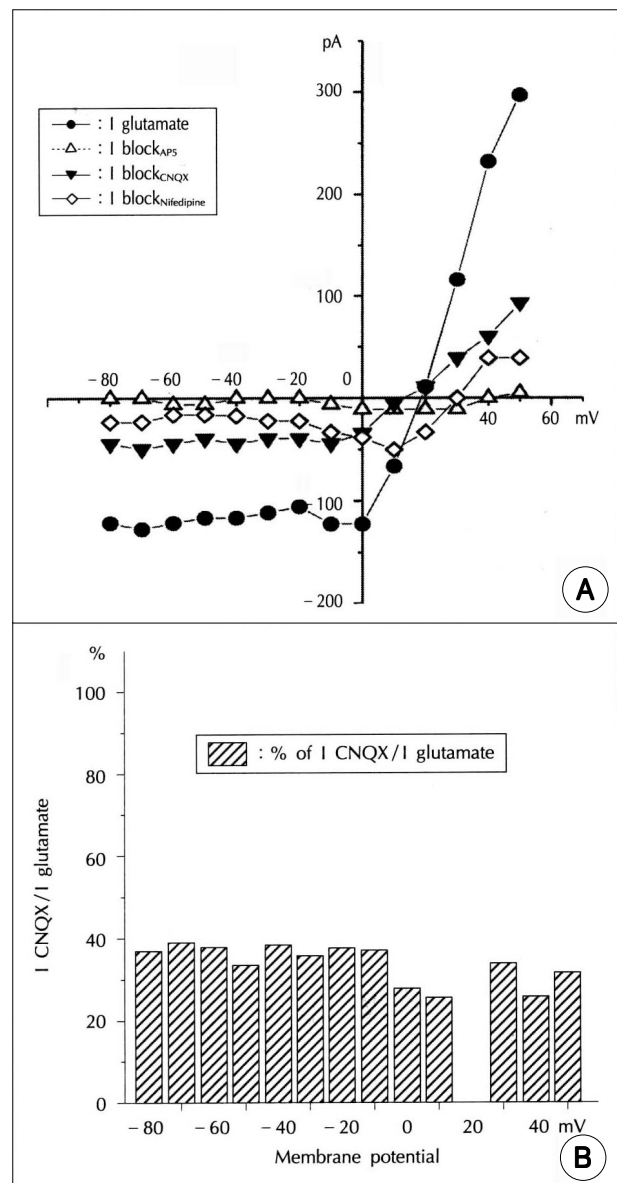


Fig. 6. Effect of glutamate receptor antagonists and nifedipine on glutamate-induced current. A : Current-voltage relationships representing the block currents by each drug. B : Percentage values of CNQX-dependent current in overall glutamate current.

NMDA - Glutamate -
 (out - 가
 ward rectification) 가
 . CNQX non - NMDA . 가
 + 10mV glutamate 가
 . Nifedipine + 30 NMDA + 80mV - 60mV
 mV - Mg²⁺
 (Fig. 6A). - 30mV
 glutamate CNQX 40% 12) Na⁺
 가 (Fig. 6B).
 고 찰 NMDA Mg²⁺
 ate glutamate NMDA .
 glutamate 가
 glutamate NMDA
 . 0mV 가
 . Glutamate non - NMDA
 2)15)21) NMDA 가
 glycine non - NMDA
 13) NMDA non - NMDA
 glycine 14)
 glutamate . Glu -
 tamate NMDA non - NMDA Glycine NMDA 가
 NMDA Glycine glutamate non - NMDA
 19) NMDA 가
 non - NMDA 23)
 NMDA glutamate
 glycine non - NMDA 가
 NMDA glycine NMDA 가
 NMDA NMDA
 . NMDA Mg²⁺ glutamate
 가 non - NMDA
 NMDA Cl⁻ glutamate non -
 NMDA 가 Mg²⁺ NMDA

NMDA - Glutamate -

- 6) Bowie D, Smart TG : *Interplay between expressed non-NMDA receptors and endogenous calcium-activated chloride currents in Xenopus laevis oocytes. Neurosci Lett 151 : 4-8, 1993*
- 7) Brewer GJ, Torricelli JR, Evege EK, Price PJ : *Optimized survival of hippocampal neurons in B27 supplemented neurobasalTM : a new serum-free medium combination. J Neurosci Res 35 : 567-576, 1995*
- 8) Castillo PE, Malenka RC, Nicoli RA : *Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. Nature 388 : 182-186, 1997*
- 9) Collingridge GL, Singer W : *Excitatory amino acid receptors and synaptic plasticity. Trends Pharmacol Sci 11 : 290-296, 1990*
- 10) Golshani P, Warren RA, Jones EG : *Progression of change in NMDA, non-NMDA, and metabotropic glutamate receptor function at the developing corticothalamic synapse. J Neurophysiol 80 : 143-54, 1998*
- 11) Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ : *Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflgers Arch 391 : 85-100, 1981*
- 12) Jahr CE, Westbrook GL : *Physiological approaches to the study of glutamate receptors. In Chad J, Wheal H, Molecular Neurobiology : a practical approach. 1st ed. Oxford, Oxford University Press, 1991, pp49-73*
- 13) Johnson JW, Ascher P : *Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325 : 529-531, 1997*
- 14) Joels M, Yool AJ, Gruol DL : *Unique properties of non-NMDA excitatory responses in cultured purkinje neurons. Proc Natl Acad Sci USA 86 : 3404-3408, 1989*
- 15) Karlsson U, Sundgren AK, Nasstrom J, Johansson S : *Glutamate-evoked currents in acutely dissociated neurons from the rat medial preoptic nucleus. Brain Res 759 : 270-276, 1998*
- 16) Kleckner NW, Dingledine R : *Requirement for glycine in activation of NMDA-receptors expressed in Xenopus Oocytes. Science 241 : 835-837, 1988*
- 17) MacDoanld JF, Bartlett MC, Mody I, Pahapill P, Reynolds JN, Salter MW, et al : *Actions of ketamine, phencyclidine and MK 801 on NMDA receptor currents in cultured mouse hippocampal neurones. J Physiol 432 : 483-508, 1991*
- 18) Mayer ML, Vyklicky Jr L : *Concanavalin A selectively reduces desensitization of mammalian neuronal quisqualate receptors. Proc Natl Acad Sci USA 86 : 1411-1415, 1989*
- 19) Mayer ML, Westbrook GL, Vyklicky Jr L : *Sites of antagonist action on N-methyl-D-aspartic acid receptors studied using fluctuation analysis and a rapid perfusion technique. J Neurophysiol 60 : 645-663, 1988*
- 20) Monaghan DT, Bridges RJ, Cotman CW : *The excitatory amino acid receptors : their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu Rev Pharmacol Toxicol 29 : 365-402, 1989*
- 21) Nowak L, Bregestovski P, Ascher P, Herbert A, Prochiantz A : *Magnesium gates glutamate-activated channels in mouse neurones. Nature 307 : 462-465, 1984*
- 22) Simonian NA, Coyle JT : *Oxidative stress in neurodegenerative diseases. Annu Rev Pharmacol 36 : 83-106, 1996*
- 23) Tang C-M, Dichter M, Morad M : *Quisqualate activates a rapidly inactivating high conductance ionic channel in hippocampal neurons. Science 243 : 1474-1477, 1989*
- 24) Ying W : *A new hypothesis of neurodegenerative disease : the deliterious network hypothesis. Med Hypothesis 47 : 307-313, 1996*
- 25) Zafra F, Hengerer B, Leibrock J, Thoenen H, Lindholm D : *Activity dependent regulation of BDNF and NGK mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. EMBO J 9 : 3545-3550, 1990*