Korean J Physiol Pharmacol
Vol 2: 279–286, June, 1998

Effects of Glutamate Receptor Antagonists and Protein Synthesis Inhibitor on Delayed Neuronal Death Induced by Transient Global Ischemia in Rat Brain

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It has been well documented that transient forebrain global ischemia causes selective neuronal degeneration in hippocampal CA1 pyramidal neurons with a delay of a few days. The mechanism of this delayed hippocampal CA1 pyramidal neuronal death (DND) is still controversial. To delineate the mechanisms of the DND, the effects of treatment with MK-801, an NMDA receptor antagonist, kynurenic acid, a NMDA/non-NMDA receptor antagonist, and/or cycloheximide, a protein synthesis inhibitor, on the DND were investigated in male Wistar rats. To examine the participation of apoptotic neuronal death in the DND, TUNEL staining was performed in ischemic brain section. Global ischemia was induced by 4-vessel occlusion for 20 min. All animals in this study showed the DND 3 and 7 days after the ischemic insult. The DND that occured 3 days and 7 days after the ischemia were not affected by pretreatment with MK-801 (1 mg/kg), but markedly attenuated by the pretreatment with kynurenic acid (500 mg/kg). Treatment with cycloheximde (1 mg/kg) also markedly inhibited the DND. The magnitudes of attenuation by the two drugs were similar. The magnitude of attenuation by co-treatments with kynurenic acid and cycloheximide was not greater than that with any single treatment. TUNEL staining was negative in the sections obtained 1 or 2 days after the ischemic insults, but it was positive at hippocampal CA1 pyramidal cells in sections collected 3 days after the ischemia. These results suggested that the DND should be mediated by the activation of non-NMDA receptor, not by the activation of NMDA receptor and that the activation of AMPA receptor should induce the apoptotic process in the DND.

Key Words: Global cerebral ischemia, Delayed neuronal death, MK-801, Kynurenic acid, Cycloheximide, Apoptosis

INTRODUCTION

Transient forebrain ischemia causes selective neuronal degeneration in hippocampal CA1 pyramidal neurons with a delay of a few days. Kirino (1982) observed this phenomenon in mongolian gerbils and named it delayed neuronal death (DND). The DND was observed in many species including human (Petito et al, 1987), rat (Pulsinelli et al, 1982; Kirino et al, 1984; Petito & Pulsinelli, 1984a, b; Freund et al, 1990; Deshpande et al, 1992) and gerbil (Ito et al, 1975; Kirino, 1982; Crain et al, 1988; Araki et al, 1989).

It has been well known that glutamate, a representative excitatory amino acid neurotransmitter of central nervous system (CNS), could kill the CNS neurons at high concentration, and it was named as excitotoxicity (Olney, 1986). The excitotoxicity is mediated through the overstimulation of ionotropic glutamate receptors, which are subdivided into NMDA and non-NMDA receptors. The excitotoxic neuronal death has been regarded as an important component of ischemic neuronal death (Auer et al, 1984; Benveniste et al, 1984; Simon et al, 1984; Wieloch,

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1985a, b; Choi, 1991, 1992). The involvement of excitotoxicity in the process of DND is still controversial.

Ischemic neuronal death has seemed a prototype for necrosis; indeed Wyllie et al (1980), who coined the term 'apoptosis', used ischemic insults as an example of necrosis. Some recent reports suggested that apoptosis should take part in the process of DND (Goto et al, 1990; Shigeno et al, 1990).

This study was performed to delineate the mechanism of the DND, focusing on the relationship between excitotoxicity and apoptosis in the process of DND. The effects of treatment with MK-801, an NMDA receptor antagonist, kynurenic acid, a NMDA /non-NMDA receptor antagonist, and/or cycloheximide, a protein synthesis inhibitor, on the DND were investigated in male Wistar rats. To examine the participation of apoptotic neuronal death in the DND, TUNEL (nick end-labelling of biotin-dUTP using terminal deoxynucleotidyltransferase) staining was performed in ischemic brain section.

METHODS

Transient forebrain ischemia

This study was performed in male Wistar rats supplied from the animal facility of the Korean Food and Drug Administration weighing 280~330 g. Transient forebrain ischemia was induced by four-vessel occlusion (4-VO), as described by Pulsinelli et al (1982). Briefly, the animals were anesthetized with intramuscular injection of ketamine (90 mg/kg) and xylazine (10 mg/kg), and both vertebral arteries were electrocauterized at the first cervical vertebrae. The common carotid arteries were isolated and a loose snare was placed around each artery. Twenty-four hours later, the common carotid arteries were re-exposed under the anesthesia with 1% halothane in 100% O_2. And then the anesthesia was discontinued and both carotid arteries were clamped with aneurysmal clips for 20 min. The animals showed righting reflex during the 20 min-ischemia were discarded. The rectal temperature of the animals was maintained at $37\pm1^{\circ}C$ using Homeothermic Blanket Control Unit (Harvard, UK) during the operation period and until righting reflex was restored.

Histological assessment of DND

For histological assessment of DND at day 3 or 7 following the ischemic insult, the animals were anesthetized with pentobarbital sodium (100 mg/kg, i.p.) and sacrificed by transcardiac perfusion fixation with 4% paraformaldehyde in phosphate buffer (pH 7.4) preceded by a brief rinse with physiologic saline. The brains were immediately removed and fixed in a same fixative for 16~18 h. The brains were embedded in paraffin and cut into 15 μm coronal sections. The sections were deparaffinized and stained with hematoxylin and eosin. Measurements of neuronal damage were focused on the CA1 area of dorsal hippocampus in both hemisphere between 3.7~4.1 mm posterior to bregma. The histologic score of DND was graded using the Pulsinelli et al (1982) scale modified as follows: 0 = absence of lesion, 1 = less than 10% of damaged neurons, 2 = 10~50% of damaged neurons, 3 = 50~90% of damaged neurons, 4 = over 90% of damaged neurons. The grading was conducted by an observer who was blinded to experimental protocol. Sections from at least 2 different coronal levels of dorsal hippocampus from each animal were examined.

TUNEL staining

Nick end-labelling of biotin-dUTP using terminal deoxynucleotidyltransferase (TdT) was performed to detect DNA fragmentation in paraffin section 24 and 72 h after the ischemic insults. Briefly, after deparaffinizing sections and digesting protein in sections using proteinase K and then quenching endogenous peroxidase activity with 2% H_2O_2 in PBS, the slides were placed in equilibration buffer and then in the mixture of TdT enzyme and biotinylated dUTP, followed by stopping the reaction with 2×SSC (sodium chloride, sodium citrate) buffer. After applying streptavidin peroxidase to the slides, peroxidase was detected with 3-amino-9-ethylcarbazole (AEC), and then the slides were counterstained with hematoxylin.

Experimental protocol

Animals were randomly classified into sham-operation (n=6, bilateral vertebral artery coagulation without common carotid artery occlusion) and ischemia groups. The ischemia group was subclassified into one control (saline-treated) and four drug treated

groups. Each ischemia subgroup was further divided according to the survival time (3 days or 7 days) following the ischemic insults. Drug treated groups were MK-801 (1 mg/kg)- treated (3 days; n=9, 7 days; n=6), kynurenic acid (500 mg/kg) -treated (3 days; n=6, 7 days; n= 6), cycloheximide (1 mg/kg)-treated (3 days; n=8, 7 days; n= 6) and cycloheximide and kynurenic acid- co-treated (3 days; n=6, 7 days; n= 6). All drugs and saline except cycloheximide were injected intraperitoneally 30 min prior to ischemic insults with the volume of 0.1~0.2 ml/100 g. All animals pretreated with cycloheximide received another cycloheximide injection 24 hr after the ischemic insult.

Drug used

(+)-MK-801 hydrogen maleate was purchased from Research Biochemical International (Natick, MA, USA), and cycloheximide and kynurenic acid were from Sigma (St. Louis, MO, USA). MK-801 or cycloheximide was dissolved in physiologic saline, and kynurenic acid was dissolved in 1N NaOH and adjusted to pH 7.4 with HCl.

Statistical analysis

The data were expressed as the mean±standard error of the mean (SEM). To compare the multiple means of histologic scores, Kruskal-Wallis analysis of variance (ANOVA), a non-parametric analysis method, was used. Analysis between 2 groups was evaluated by Mann-Whitney U test. Probability value of less than 0.05 was considered to be statistically significant.

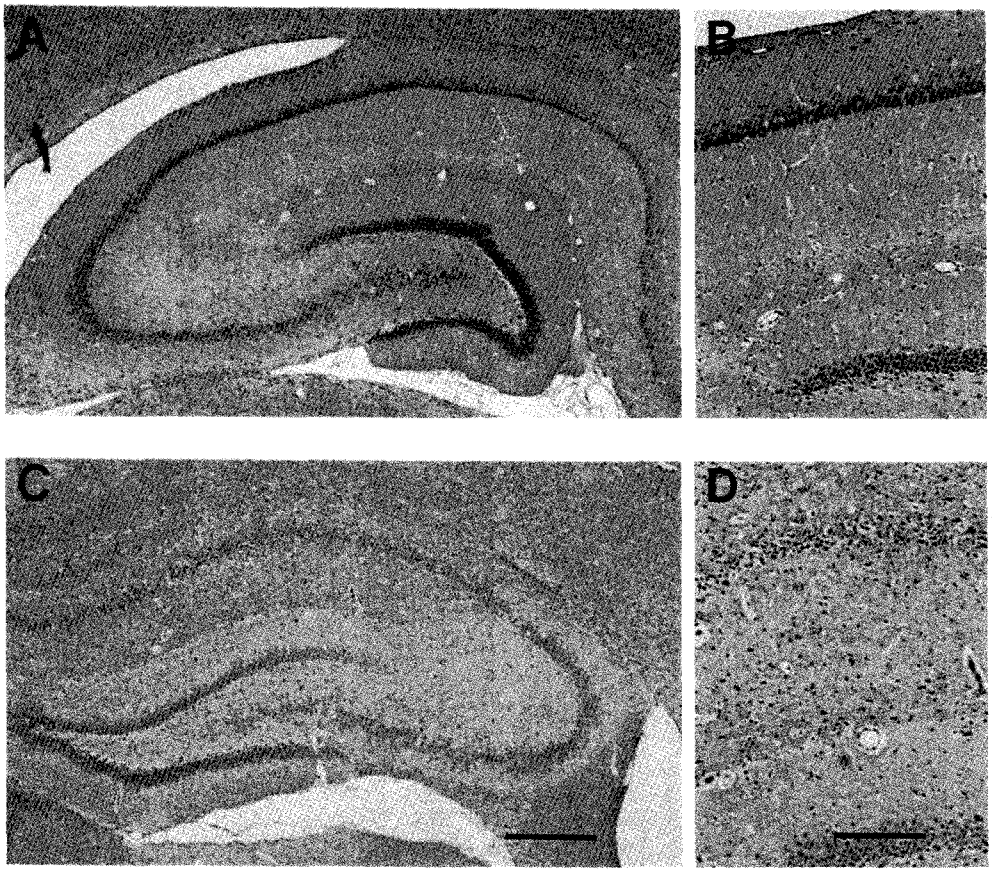

Fig. 1. Light micrographs of 15 μm coronal sections stained with hematoxylin-eosin are showing hippocampal area 3 days following sham operation (A, B), 20 min global ischemia (C, D) in Wistar rat. B and D are pyramical CA1 area in higher magnification. Calibration bars: 500 μm in C and 190 μm in D.

RESULTS

In sham-operated animals (n=6), the hippocampal CA1 pyradimal neurons did not show any morphological abnormalities 3 days after operation (Fig. 1. A, C). Three days following 20 min of 4-VO ischemia, however, selective neuronal death produced in the hippocampal CA1 pyradimal neurons (Fig. 1. B, D). Some of the 3-day survival group (4 out of 10) displayed partial neuronal death (histological scores; 2 or 3), while the rest of the animals showed almost entire neuronal death (histological score; 4). In 7-day survival group, all animals showed histological score 4 (Fig. 2).

The pretreatment with MK-801 (1 mg/kg), a non-competitive NMDA receptor antagonist, had no effect on the DND in either 3-day or 7-day survival group (Fig. 3). However, the pretreatment with kynurenic acid (500 mg/kg), a NMDA/non-NMDA receptor antagonist, significantly attenuated the DND in 3-day or 7-day survival group compared with the corresponding control ischemia group ($p<0.01$) (Fig. 4). The pre- and post-treatment with cycloheximide, a protein synthesis inhibitor, also significantly attenuated the DND in either 3-day or 7-day survival group ($p<0.01$). The inhibitory effect of cycloheximide on DND was comparable to that of kynurenic acid (Fig. 5). These data indicated that not only kynurenic acid, an anti-excitotoxic agent, but also cy-

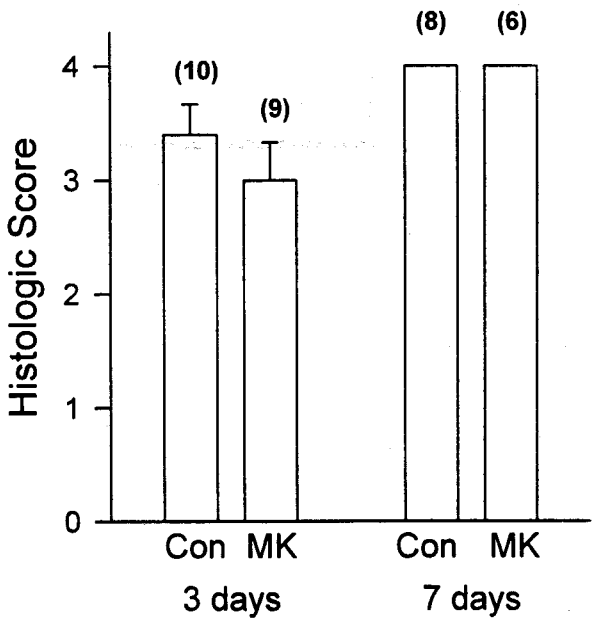

Fig. 3. MK-801 (1 mg/kg) has no protective effect on the ischemia-induced hippocampal CA1 pyramidal cell death. Legends are the same as in Fig. 2.

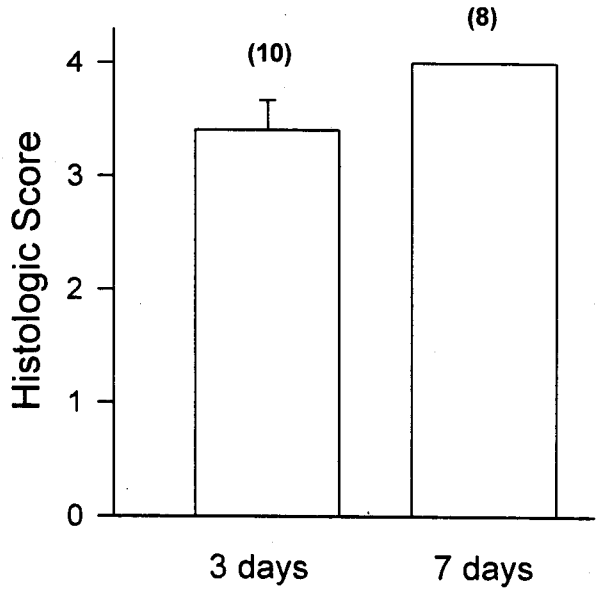

Fig. 2. Bar graph illustrating the histologic score 3 days and 7 days following 20 min-ischemia. Verticals bars are mean±SEM. Numerals in parenthesis are number of animals.

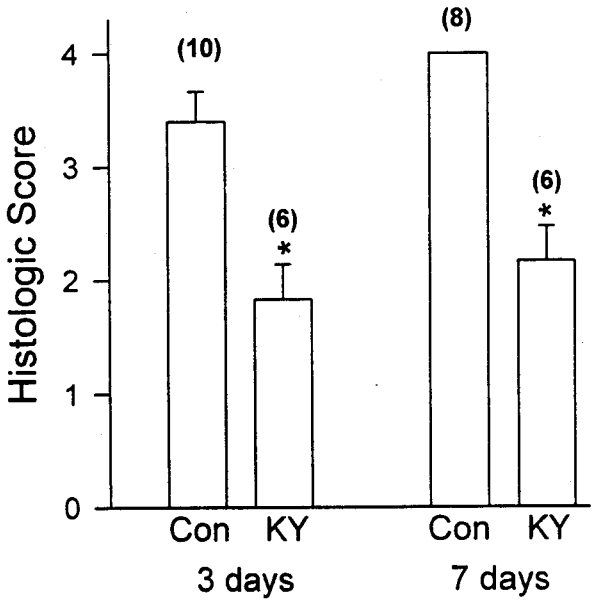

Fig. 4. Neuroprotective action of kynurenic acid (500 mg/kg) on the ischemia-induced hippocampal neurodegeneration. Legends are the same as in Fig. 2. Asterisk denotes significant difference comparing with control ischemic insults ($p<0.01$).

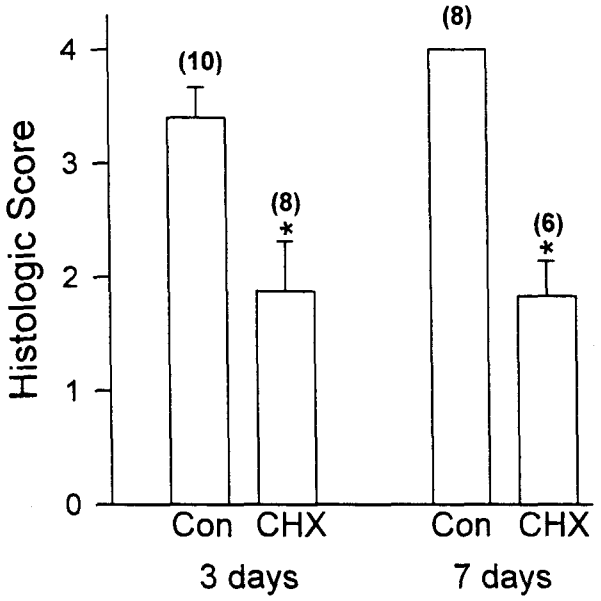

Fig. 5. Neuroprotective action of cycloheximide (1 mg /kg) on the ischemia-induced hippocampal neurodegeneration. Legends are the same as in Fig. 2. Asterisk denotes significant difference comparing with control ischemic insults ($p<0.01$).

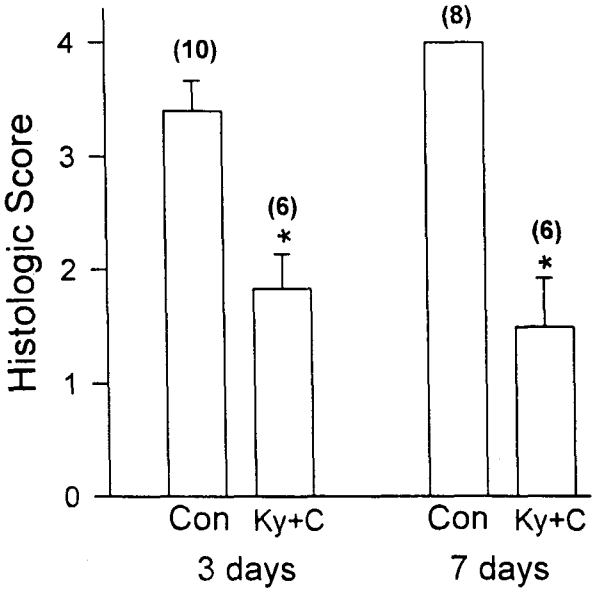

Fig. 6. Neuroprotective action of combined treatment of cycloheximide (1 mg/kg) and kynurenic acid (500 mg/kg) on the ischemia-induced hippocampal neurodegeneration. Legends are the same as in Fig. 2. Asterisk denotes significant difference comparing with control ischemic insults ($p<0.01$).

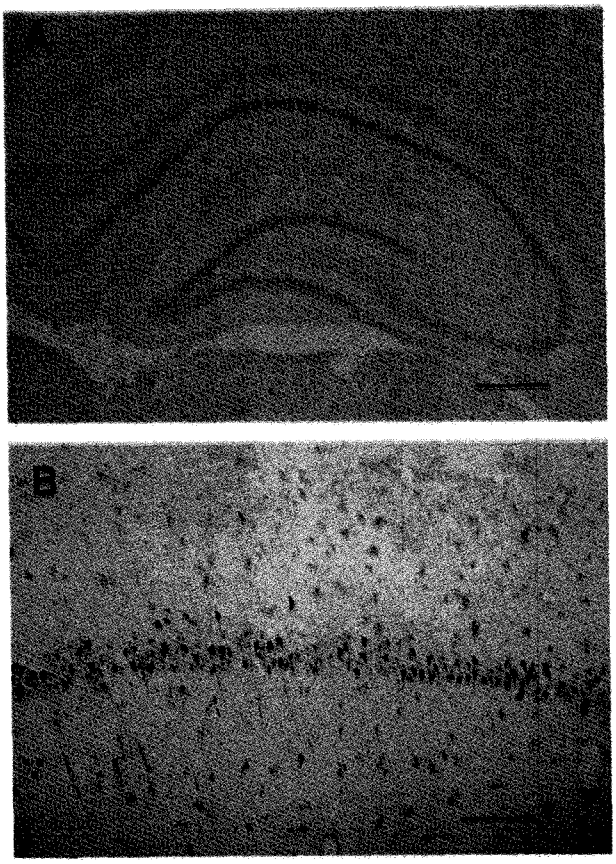

Fig. 7. Light micrographs of positive staining of TUNEL reaction in hippocampal CA1 pyramidal layers 3 days following 20 min-ischemia (A) and in higher magnification (B). Calibration bar: 500 μm in A and 100 μm in B. Labeling was done with 3-amino-9-ethylcarbazole. Hematoxylin counterstaing.

cloheximide, an anti-apoptotic agent, could inhibit DND. Therefore, to examine the existence of synergism between the two drugs, the effect of combined treatment with kynurenic acid and cycloheximide on DND was investigated. The combined treatment with the two drugs also significantly attenuated the DND in either 3-day or 7-day survival group, however the magnitude of attenuation was not additive at all (Fig. 6).

Cycloheximide, which has been widely used as a pharmacological agent for inhibiting apoptotic cell death, significantly attenuated the DND (Fig. 5). We tried to examine the involvement of apoptosis in DND morphologically, using TUNEL staining. TUNEL staining was positive at hippocampal CA1 pyramidal cells in sections obtained 3 days after the ischemia (Fig. 7), but negative in the sections obtained 1 or 2 days after the ischemic insults (data not shown).

DISCUSSION

Present study demonstrated that pretreatment with MK-801, a non-competitive NMDA receptor antagonist, did not protect the DND. In accordance with our data, many laboratories have reported that NMDA receptor antagonists could not attenuate DND (Fleischer et al, 1989; Michenfelder et al, 1989; Lanier et al, 1990; Buchan et al, 1991a). However, it has been reported that treatment with MK-801 significantly attenuated the DND in mongolian gerbils (Gill et al, 1987) and rats (Rod & Auer, 1989; Swan & Meldrum, 1990). This disagreement of neuroprotective effect of MK-801 was explained by Pulsinelli et al (1993). They insisted that body temperature was a major reason for the discrepancy. It has been well known that hypothemia protects the experimental ischemic brain injury (Ginsberg et al, 1993) and that MK-801 induces hypothermia (Buchan & Pulsinelli, 1990). They demonstrated that MK-801 protected the DND with prolonged hypothermia. When normothermia was maintained, however, the neuroprotective effect was lost.

MK-801 failed to protect the DND, but the pretreatment with kynurenic acid (500 mg/kg), a non -selective NMDA/non-NMDA receptor antagonist, protected the DND in this study. These data indicated that the blockade of non-NMDA receptor might protect the DND. The major difference between NMDA and non-NMDA receptors is Ca^{2+} permeability, that is, calcium ion could pass through NMDA receptor channel, but not non-NMDA receptor channel. Non -NMDA receptors are subdivided into AMPA (amino -3-hydroxy- 5-methyl-4-isoxazolepropionic acid) and kainate receptors (Monaghan et al, 1989). Furthermore, AMPA receptors comprise four closely related subunits, GluR-1, 2, 3, 4 (Sommer & Seeburg, 1992). Homomeric or heteromeric channels assembled from GluR-1, GluR-3 and/or GluR-4 subunit are permeable to Ca^{2+}, but homomeric GluR-2 channels as well as heteromeric channels formed with the participation of GluR-2 drastically lose Ca^{2+} permeability (Hollmann et al, 1989; Burnashev et al, 1992). Sustained Ca^{2+} influx through glutamate receptor channels is thought to play a critical role in postischemic cell death (Choi, 1992). In accordance with this calcium hypothesis, Pellegrini-Giampietro et al (1992, 1994) reported that GluR-2 gene expression was preferentially reduced in CA1 hippocampal neurons at a time point that preceded their degeneration following a severe transient forebrain ischemia. In addition to these reports, Buchan et al (1991a, b) demonstrated that MK-801 could not attenuate the DND, while NBQX, a selective AMPA receptor antagonist, could protect the DND. Taken together, the neuroprotective effect of kynurenic acid in this study should be resulted from the blockade of AMPA receptor.

Pre- and post- treatment with cycloheximide (1 mg/kg), a protein synthesis inhibitor, attenuated the DND in the present study. Cycloheximide has been reported as an anti-apoptotic agent in various neuronal apoptosis-related in vitro and in vivo experiments, such as developmental programmed neuronal death (Oppenheim et al, 1990; Oppenheim, 1991), the neuronal deaths induced by the deprivation of neurotrophins (Martin et al, 1988; Scott & Davies, 1990), staurosporin, a protein kinase inhibitor (Koh et al, 1995), irradiation (Inoyue et al, 1992: Ferrer 1992), global cerebral ischemia (Goto et al, 1990; Shigeno et al, 1990; Papas et al, 1992) or focal cerebral ischemia (Linnik et al, 1993). Furthermore, hippocampal CA1 pyramidal neurons were stained with TUNEL staining at sections obtained 3 days after ischemic insults. TUNEL staining is a well-known morphological marker for apoptotic cell death (Gavrieli et al, 1992; Heron et al, 1993). Even though there were several reports contrary to our results (Deshpande et al, 1992; Tortosa et al, 1994), present study strongly suggested that apoptosis should participate in the process of DND.

In this study, cycloheximide and kynurenic acid attenuated the DND with a similar magnitude, and a combined treatment of both drugs did not show any synergism. If the two drugs inhibited the DND through blocking different cell death pathways, the combined treatment would show synergism. In this point of view, these results implied that the activation of AMPA receptor induced by transient ischemia produced DND via apoptosis.

In conclusion, these results suggested that the DND should be mediated by the activation of non-NMDA receptor, but not by the activation of NMDA receptor, and that the activation of AMPA receptor is involved in the apoptotic process of the DND.

ACKNOWLEDGEMENTS

This work was supported by Basic Medical Research Fund from Ministry of Education (95-172),

1995, and Research Grant from Chonnam University Hospital (95-Co-02), 1995.

REFERENCES

Araki T, Kato H, Kogure K. Selective neuronal vulnerability following transient cerebral ischemia in the gerbil: distribution and time course. *Acta Neurol Scand* 80: 548−553, 1989

Auer RN, Wieloch T, Olsson Y, Siesjo BK. Distribution of hypoglycemic brain damage: relationship to white matter and cerebrospinal fluid pathways. *Acta Neuropathol (Berl.)* 64: 177−191, 1984

Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem* 43: 1369−1374, 1984

Buchan A, Li H, Pulsinelli W. The *N*-methyl-D-aspartate antagonist, MK-801, fails to protect against neuronal damage caused by transient, severe forebrain ischemia in adult rats. *J Neurosci* 11: 1049−1056, 1991a

Buchan A, Li H, Cho S-H, Pulsinelli W. Blockade of the AMPA receptor prevents CA1 hippocampal injury following severe but transient forebrain ischemia in adult rats. *Neurosci Lett* 132: 255−258, 1991b

Buchan A, Pulsinelli W. Hypothermia but not the N -methyl-D-aspartate antagonist, MK-801, attenuates neuronal damage in gerbils subjected to transient global ischemia. *J Neurosci* 10: 311−316, 1990

Burnashev N, Monyer H, Seeburg PH, Sakmann B. Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. *Neuron* 8: 189−198, 1992

Choi DW. Excitotoxic cell death. *J Neurobiology* 23: 1261−1276, 1992

Choi DW. Excitotoxicity. In: Excitatory Amino Acid Antagonists. B.S. Meldrum, ed. Blackwell Scientific Publications, London, p216−236, 1991

Crain BJ, Westerkam WD, Harrison AH, Nadler JV. Selective neuronal death after transient forebrain ischemia in the mongolian gerbil: a silver impregnation study. *Neuroscience* 27: 387−402, 1988

Deshpande J, Bergstedt K, Kalimo H, Wieloch T. Ultranstructural changes in the hippocampal CA1 region following transient cerebral ischemia: evidence against programmed cell death. *Exp Brain Res* 88: 91−105, 1992

Ferrer I. The effect of cycloheximide on natural and X-ray-induced cell death in the developing cerebral cortex. *Brain Res* 588: 351−357, 1992

Fleischer JE, Tateishi A, Drummond JC, Scheller MS, Grafe MR, Zornow MH, Shearman GT, Shapiro HM. MK-801, an excitatory amino acid antagonist, does not improve neurologic outcome following cardiac arrest in cats. *J Cereb Blood Flow Metab* 9: 795−804, 1989

Freund TF, Buzsaki G, Leon A, Baimbridge KG, Somogyi P. Relationship of neuronal vulnerability and calcium binding protein immunoreactivity in ischemia. *Exp Brain Res* 83: 55−66, 1990

Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol* 119: 493−501, 1992

Gill R, Foster AC, Woodruff GN. Systemic administration of MK-801 protects against ischemia-induced hippocampal neurodegeneration in the gerbil. *J Neurosci* 7: 3343−3349, 1987

Ginsberg MD, Globus MY, Dietrich WD, Busto R. Temperature modulation of ischemic brain injury - a synthesis of recent advances. *Prog Brain Res* 96: 13−22, 1993

Goto K, Ishige A, Sekiguchi K, Iizuka S, Sugimoto A, Yuzurihara M, Aburada M, Hosoya E, Kogure K. Effects of cyclohemixide on delayed neuronal death in rat hippocampus. *Brain Res* 534: 299−302, 1990

Heron A, Pollard H, Dessi F, Moreau J, Lasbennes F, Ben-Ari Y, Charriaut-Marlangue C. Regional variability in DNA fragmentation after global ischemia evidenced by combined histological and gel electrophoresis observations in the rat brain. *J Neurochem* 61: 1973−1976, 1993

Hollmann M, O'Shea-Sreenfield A, Rogers SW, Heinemann S. Cloning by functional expression of a member of the glutamate receptor family. *Nature* 7: 643−648, 1989

Inouye M, Tamaru M, Kameyama Y. Effects of cycloheximide and actinomycin D on radiation-induced apoptotic cell death in the developing mouse cerebellum. *Int J Rad Biol* 61: 669−674, 1992

Ito U, Spatz M, Walker JT, Klatzo I. Experimental cerebral ischemia in monogolian gerbils. I. Light microscopic observations. *Acta Neuropathol* 32: 209−223, 1975

Kirino T, Tamura A, Sano K. Delayed neuronal death in the rat hippocampus following transient forebrain ischemia. *Acta Neuropathol* 64: 139−147, 1984

Kirino T. Delayed neuronal death in the gerbil hippocampus following ischemia. *Brain Res* 239: 57−69, 1982

Koh JY, Wie MB, Gwag BJ, Sensi SL, Canzonieero LMT, Demaro J, Csernansky C, Choi DW. Staurosporine-induced neuronal apoptosis. *Exp Neurol* 135: 153−159, 1995

Lanier WL, Perkins WJ, Karlsson BR, Milde JH, Scheithauer BW, Shearman GT, Michenfelder JD. The ef-

fects of dizocilpine maleate (MK-801), an antagonist of the N-methyl-D-aspartate receptor, on neurologic recovery and histopathology following complete cerebral ischemia in primates. *J Cereb Blood Flow Metab* 10: 252−261, 1990

Linnik MD, Zobrist RH, Hatfield MD. Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats. *Stroke* 24: 2002−2009, 1993

Martin DP, Schmidt RE, DiStefano PS, Lowry OH, Carter JG, Johnson EMJr. Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by nerve growth factor deprivation. *J Cell Biol* 106: 829−844, 1988

Michenfelder JD, Lanier WL, Scheithauer BW, Perkins WJ, Shearman GT, Milde JH. Evaluation of the glutamate antagonist dizocilipine maleate (MK-801) on neurologic outcome in a canine model of complete cerebral ischemia: correlation with hippocampal histopathology. *Brain Res* 481: 228−234, 1989

Monaghan DT, Bridges RJ, Cotman CW. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the functionof the CNS. *Annu Rev Pharmacol Toxicol* 29: 365−402, 1989

Olney JW. Inciting excitotoxic cytocide among central neurons. *Adv Exp Med Biol* 203: 631−645, 1986

Oppenheim RW, Prevette D, Tytell M, Homma S. Naturally occurring and induced neuronal death in the chick embryo in vivo requires protein and RNA synthesis: evidence for the role of cell death genes. *Dev Biol* 138: 104−113, 1990

Oppenheim RW. Cell death during development of the nervous system. *Annu Rev Neurosci* 14: 453−501, 1991

Papas S, Crepel V, Hasboun D, Jorquera I, Chinestra P, Ben-Ari Y. Cycloheximide reduced the effects of anoxic insult in vivo and in vitro. *Eur J Neurosci* 4: 758−765, 1992

Pellegrini-Giampietro DE, Pulsinelli WA, Zukin RS. NMDA and non-NMDA receptor gene expression following global brain ischemia in rats: effect of NMDA and non-NMDA receptor antagonists. *J Neurochem* 62: 1067−1073, 1994

Pellegrini-Giampietro DE, Zukin RS, Bennett MV, Cho W, Pulsinelli WA. Switch in glutamate receptor subunit gene expression in CA1 subfield of hippocampus following global ischemia in rats. *Proc Natl Acad Sci USA* 89: 10499−10503, 1992

Petito CK, Feldmann E, Pulsinelli WA, Plum F. Delayed hippocampal damage in humans following cardiorespiratory arrest. *Neurol* 37: 1281−1286, 1987

Petito CK, Pulsinelli WA. Delayed neuronal recovery and delayed neuronal death in rat hippocampus following severe cerebral ischemia: possible relationship to abnormalities in neuronal processes. *J Cereb Blood Flow Metab* 4: 194−205, 1984a

Petito CK, Pulsinelli WA. Sequential development of reversible and irreversible neuronal damage following cerebral ischemia. *J Neuropathol Exp Neurol* 43: 141−153, 1984b

Pulsinelli WA, Brierley JB, Plum F. Temporal profile of neuronal damage in a model of transient cerebral ischemia. *Ann Neurol* 11: 491−498, 1982

Pulsinelli W, Sarokin A, Buchan A. Antagonism of NMDA and non-NMDA receptors in global versus focal brain ischemia. *Prog Brain Res* 96: 125−135, 1993

Rod MR, Auer RN. Pre- and post-ischemic administration of dizocilpine (MK-801) reduces cerebral necrosis in the rat. *Can J Neurol Sci* 16: 340−344, 1989

Scott SA, Davies AM. Inhibition of protein synthesis prevents cell death in sensory and parasympathetic neurons deprived of neurotrophic factor in vitro. *J Neurobiol* 21: 630−638, 1990

Shigeno T, Yamasaki Y, Kato G, Kusaka K, Mima T, Takakura K, Graham DI, Furukawa S. Reduction of delayed neuronal death by inhibition of protein synthesis. *Neurosci Lett* 120: 117−119, 1990

Simon RP, Swan JH, Griffiths T, Meldrum BS. Blockade of *N*-methyl-$_D$-aspartate receptors may protect against ischemic damage in the brain. *Science* 226: 850−852, 1984

Sommer B, Seeburg PH. Glutamate receptor channels: novel properties and new clones. *TiPS* 13: 291−296, 1992

Swan JH, Meldrum BS. Protection by NMDA antagonists against selective cell loss following transient ischaemia. *J Cereb Blood Flow Metab* 10: 343−351, 1990

Tortosa A, Rivera R, Ferrer I. Dose-related effects of cycloheximide on delayed neuronal death in the gerbil hippocampus after bilateral transitory forebrain ischemia. *J Neurological Sci* 121: 10−17, 1994

Wieloch T. Hypoglycemia-induced neuronal damage prevented by an *N*-methyl- $_D$-aspartate antagonist. *Science* 230: 681−683, 1985a

Wieloch T. Neurochemical correlates to selective neuronal vulnerability. *Prog Brain Res* 63: 69−85, 1985b

Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol* 68: 251−306, 1980