Korean J. of Pharmacology
Vol. 32, No. 3, pp. 323~334, 1996

Changes of Polyamine Metabolism and Delayed Neuronal Degeneration of Hippocampus after Transient Cerebral Ischemia in Mongolian Gerbils

Kyung-Ho Shin, Hwa-Jung Shin, Young-Jae Lee, Hyung-Gun Kim, Sang-Hyun Choi, Yeon-Sook Chun, and Boe-Gwun Chun*

Department of Pharmacology, Korea University College of Medicine
Anam-dong 5-ka 126-1, Sungbuk-ku, Seoul 136-705, Korea

ABSTRACT

Male Mongolian gerbils (60~80 g) were given DL-difluoromethylornithine (DFMO; 250 mg/kg, ip) and methylglyoxal bis(guanylhydrazone) (MGBG; 50 mg/k, ip), respectively, 1 h prior to transient (7 min) occlusion of bilateral common carotid arteries (OBC7) and a daily dose of one of them for 6 days after recirculation, and the polyamine contents, activities of ornithine and S-adenosylmethionine decarboxylases (ODC and SAM-DC), and light microscopic findings of the hippocampus were evaluated.

The hippocampal putrescine (PT) levels of the control gerbils treated with saline (STGr), markedly increased after OBC7, showing a peak level at 24 h after recirculation. The peak PT level was reduced in DFMO treated gerbils (DTGr) and in MGBG treated gerbils (MTGr). And 7 days after recirculation, the PT level of DTGr was decreased to about 75% of the PT level in the sham operated group (nonTGr) and to about 55% of the STGr level, respectively. The hippocampal spermidine (SD) level of STGr tended to decline, showing the lowest value at 8 h after recirculation. But the spermidine (SD) level of DTGr was somewhat higher at 8 h after OBC7 than those of STGr and MTGr. The hippocampal spermine (SM) levels of all the experimental groups were little changed for 7 days after OBC.

OBC7 markedly increased the hippocampal ODC activity, reaching a maximum (about 3 times higher than preischemic level) at 8 h and rapidly recovered to the control value by 24 h in STGr gerbils, and the OBC7-induced increase of ODC activity was significantly attenuated by DFMO or MGBG treatment. Whereas OBC7 induced a rapid decrease of the hippocampal SAM-DC activity follwed by gradual recovery to the preischemic level, and the decrease of the SAM-DC activity was slightly attenuated by DFMO or MGBG treatment.

7 Days after OBC7 the histological finding of the hippocampal complex stained with cresyl violet showed an extensive delayed neuronal damage in the CA1 region and to a lesser extent, in the dentate gyrus, sparing the CA3 region. And the neuronal death was aggrevated by DFMO but significantly attenuated by MGBG. The immunochemical reactivity of hippocampus to anti-GFAP antibody was significantly increased in the CA1 region and to a lesser extent, in the dentate gyrus 7 days after OBC7, but was little changed in the CA3. And the increase of the anti-GFAP immunoreactivity was moderately enhanced by DFMO and significantly suppressed by MGBG.

These results suggest that the polyamine metabolism may play a modulatory role in the ischemic brain damage.

Key Words: brain ischemia, polyamines, hippocampus, delayed neuronal death, DFMO, MGBG

This work was supported by a grant for basic medical sciences from the Ministry of Education in 1995.

INTRODUCTION

The mongolian gerbil has been extensively used as a global brain ischemia model due to its defect of vascular connections between the carotid and basilar circulations (Levine and Payan, 1966; Kahn, 1972). The occlusion of bilateral common carotid arteries for 7 min (OBC7) in Mongolian gerbils resulted in the delayed neuronal death (DND) of the hippocampus CA1 region, whereas the adjacent CA3 region and the dentate gyrus were little affected (Kirino, 1982; Kirino and Sano, 1984). However, when OBC was prolonged or combined with hyperglycemia, the neurons of the CA3 and dentate gyrus regions were also destroyed (Kirino *et al.*, 1984; Smith *et al.*, 1988).

This ischemia-induced DND of CA1 neurons has been ascribed to increase of hippocampal glutamate release in response to ischemia (Benveniste *et al*, 1989; Mitani *et al*, 1990) associated with over-activation of postsynaptic N-methyl-D-aspartate (NMDA) receptor (Gass *et al.*, 1993).

Many studies revealed that a recognition site for polyamines exists as part of the NMDA receptor: Ca^{++}-channel complex. The activation of NMDA receptor has been known to be positively modulated by several endogenous substances, such as glycine and polyamines (putrescine, PT; spermidine, SD; spermine, SM; etc) (Lazarewicz *et al*, 1992; Nussenzveig *et al.*, 1991; Williams *et al.*, 1991; Araneda *et al.*, 1993).

These polyamines play an important role in cellular proliferation and differentiation (Milam *et al.*, 1989; Pegg, 1986) as well as biological response to brain ischemia (Paschen, 1992; Paschem *et al.*, 1987; 1988a; 1988b; 1992). It is well known that tissue ischemia and recirculation provoked transient and rapid increases of ornithine decarboxyase (ODC) activity and polyamine levels. Unlike the cerebral cortex, striatum, and thalamus not to be susceptible to ischemic tissue injury, the PT content of the hippocampal CA1 region vulnerable to ischemic insults, were increased and then kept up the plateau values for 4 or more days after ischemia and recirculation (Paschen, 1992; Paschem *et al.*, 1987; 1992).

In an our study using a global ischemia model of Mongolian gerbils, the PT levels of the hippocampal microdialysates were rapidly increased up to a plateau level by about 2 days, and then the plateau level was remained for additional 2 or more days (Shin *et al.*, 1994).

PT activated synaptosomal Ca^{++}-influx (Komulainen and Bondy, 1987) and neuronal liberation of excitatory amino acids (Reed and de Belleroche, 1990), suggesting that PT might be a putative neurotoxic substance (Paschen *et al*, 1992). And the ischemia-induced PT increase in the hippocampus seems to contribute to the development of DND of CA1 region (Paschen, 1992; Paschem *et al.*, 1987; 1992). Kindy *et al.* (1994) reported that DL-difluoromethylornithine (DFMO), an irreversible ODC inhibitor (Seiler, 1987), blocked the ischemia-dependent DND in the CA1 region.

In the present study, we examined the effects of DFMO and methylglyoxal bis(guanylhydrazone) (MGBG), an inhibitor of S-adenosylmethionine decarboxylase (SAM-DC) and diamine oxidase (DAO), on the OBC7-induced changes of the hippocampal polyamine contents and ODC and SAM-DC activities, refferring the results to the histological findings of the hippocampus.

MATERIALS AND METHOD

Materials

1,8-Diaminooctane, 4-fluoro-3-nitrobenzotrifluoride (FNBT), and dimethyl sulfoxide were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Histidine, sodium carbonate, pyridoxal 5-phosphate (PLP), dithiothreitol (DTT), DL-ornithine, MGBG, potassium phosphate, putrescine, and S-adenosyl-L-methionine (SAM) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). DFMO was kindly provided by Hoescht-Marion-Roussel (Cincinnati, OH, USA). Other chemicals were analytical or HPLC grade.

Animals and ischemia procedure

Male Mongolian gerbils (*Meriones unguiculatus*, Harlan Sprague Dawley Inc., USA) weighing from 60 to 80 g were anesthetized with chloral

hydrate (360 mg/kg, ip inj). Through a ventral middle cervical incision, both common carotid arteries were exposed and separated free of surrounding soft tissues, and a 4-0 silk suture was looped around each artery (Mitani and Kataoka, 1991). The suture silks around the two common carotid arteries were pulled by 10 g weight to occlude the circulation (Suzuki *et al.*, 1983; Mitani *et al.*, 1989). Following 7 min of ischemia, the sutures were removed to allow recirculation and the skin incision was sutured. Sham operations in which both carotid arteries were exposed but not occluded were conducted. During operative procedures, the body temperature was maintained at 37℃ by a electrically feed-back controlled heating pad with monitoring rectal temperature. After awakening, a gerbil were placed to one cage in a room maintained at constant temperature 30℃, under a daily cycle of 12-h light;12-h dark and was allowed access to food and water *ad libitum*.

Drug treatments and hippocampus sampling

DFMO and MGBG were dissolved in 0.9% NaCl. Animals were given one dose of DFMO (250 mg/kg/day) or MGBG (50 mg/kg/day) intraperitoneally 1 h prior to occlusion of bilateral carotid arteries and then the same dose for the following 6 days after recirculation. To estimate the effects of each treatment on the OBC7-induced changes of the hippocampal activities of ODC and SAMDC, polyamine contents, and histological findings, gerbils were decapitated at 1 h, 4 h, 24 h, and 7 days after the last injection of drugs or recirculation, and hippocampi were extirpated

Polyamine assay

The hippocampi were weighed and homogenized using a teflon glass homogenizer in 10 volumes of 0.4 M perchloric acid containing 2 mM disodium EDTA and diaminooctane, 100 ng as an internal standard. Homogenates were centrifuged at 15,000 g for 10 min, and the obtained supernatants were evaporated to dryness with a Speed Vac Concentrator (Savant SVC 200H, Farmingdale, USA). The dry residues obtained were dissolved in 100 ul of 1 M sodium carbonate, were mixed and reacted with 300 ul of FNBT reagent (10 ul FNBT/ml dimethyl sulfoxide). The polyamine derivatives were analyzed according to the HPLC method formulated by Spragg and Hutchings (1983). The FNBT reaction was allowed to proceed at 60℃ for 20 min. A mixture (40 ul) of 1 M histidine in 1 M sodium carbonate was added to stop the reaction and then the incubation continued for a further 5 min. After cooling the mixture, the N-2′-nitro-4′-trifluoromethylphenyl polyamine (NPT-polyamine) derivatives were extracted twice with 2 ml of 2-methylbutane. After centrifugation at 1,500 g for 5 min, the organic phase was evaporated to dryness, and the residue was redissolved in 1 ml of HPLC-grade methanol. The 20 ul of the methanol solution was applied on a reversed-phase HPLC system equipped with an ERMA ODS 1161 column (3 um: 6×100 mm, Tokyo, Japan), and the separation of NPT-polyamines was accomplished by an iso-cratic elution of acetonitrile-water (80:20, v/v) mobile phase at the flow rate of 1.2 ml/min within 20 min. The A_{242} of effluent was monitored by a UV/VIS spectromonitor (Knauer, Berlin, FRG) and a 2-channel flat bed chart recorder (LKB 2210 potentiometric recorder, Bromma, Sweden).

Assays of ODC and SAM-DC activities

The hippocampal tissues were homogenized by using a teflon glass homogenizer in 3 volumes of ice cold 30 mM sodium phosphate buffer (pH 7.1) containing 0.1 mM EDTA, 0.1 mM pyridoxal 5-phosphate (PLP), and 5.0 mM dithiothreitol (DTT). Homogenates were centrifuged at 105,000 g for 60 min at 2℃, and the supernatants were assayed for ODC activity according to the procedure of Gaines *et al.* (1989) by measuring the release of $^{14}CO_2$ from [1-^{14}C] ornithine (final concentration, 50 uM; 5 uCi/umol). Forty microliters of tissue supernatant was added to an Eppendorf microcentrifuge tube containing 40 ul of incubation buffer (30 mM sodium phosphate, pH 7.1, 0.1 mM PLP, and 6.35 mM DTT) and 20 ul of substrate (cold DL-ornithine and 1 uCi of L-[1-^{14}C]ornithine). The reaction solution was mixed lightly and placed in a 20 ml glass scintillation vial with a polypropylene center well (Kontes Co., NJ, USA) containing 200 ul of a trapping agent (Solvable™, NEN, Boston, USA). The vial was closed with a

silicone septum-lined plastic screw cap (Wheaton Scientific, USA). After 1 h incubation with shaking at 37℃, the vials were removed and placed in an ice-water bath. One hundred ul of 0.67 N HCl was injected by a syringe through the septum into each Eppendorf tube. After a second 1 h incubation of the vials with shaking at room temperature, the caps were removed from the scintillation vials, the Eppendorf tubes were discarded, and 4 ml of Atomlight (NEN, Boston, USA) was added. The vials were recapped and the radioactivity was counted by β-counter (LKB 1214, Rackbeta). The protein concentration of supernatants was determined by the method of Peterson (1977). Results are expressed as picomoles of $^{14}CO_2$ released per milligram of protein for 1 h incubation at 37℃.

The activity of SAM decarboxylase (SAMDC) was measured as outlined by Hietala *et al.* (1983). The assay mixture (a total volume of 0.15 ml) were constituted of 0.1 M potassium phosphate (pH 7.4 at 20), 2.5 mM putrescine, 0.16 mM SAM (1.07 mCi/mmol), 6.7 mM DTT, 1 mM EDTA, and 0.1 ml of the 105,000 g supernatant fraction . After 30 min incubation at 37℃, the samples were processed as described above for ODC measurement. Trichloroacetic acid was added to SAM-DC blanks before starting the reaction.

Cresyl violet staining

On the seventh day after the ischemic insult, the animals were anesthetized with sodium pentobarbital sodium (60 mg/kg, ip inj). Animals were thoracotomized, and a cannula was inserted into the ascending aorta. The animals were perfused with heparinized saline (2 IU/0.9% saline ml) and then with 10% formalin in 0.1 M phosphate buffer (pH 7.4). Two hours after perfusion fixation, the brains were removed, divided into coronal sections (about 0.5 cm in thickness) and kept in the same fixative overnight at 4℃. After fixation, each tissue block was dehydrated in ethanol and embedded in paraffin. Serial coronal sections were cut at 6 um by a microtome (AO 820 rotary microtome) and mounted on gelatine-coated slides and were stained with cresyl violet. Stained sections were examined with a light microscope (Zeiss photomicroscope III).

Immunochemical staining of GFAP

Immunostaining of GFAP was carried out according to the procedure of DeArmond *et al.* (1983), using the avidin-biotin-peroxidase system obtained from Signer Laboratories, Inc. (USA). Paraffin-embedded slices of 6 um thickness were adhered to glass slides and dried for 2~16 hours at room temperature. Slices were deparaffinized with xylene, ethanol, and 3% H_2O_2 in methanol, which blocks endogenous peroxidase. After all further steps, slices were washed three times in phosphate-buffered saline (PBS, pH 7.4), followed by incubation with 5% normal serum of 10 mM PBS. The sections were incubated with rat anti-GFAP antibody (diluted to 1:200 with PBS), kept in a moist chamber for 20 min, and then incubated with peroxidase labelled ultrastreptavidin in 0.01 M PBS. Finally, the slices were reacted with 2% 3-amino-9-ethylcarbazole (AEC) and H_2O_2 for color development and then were counter-stained by hematoxylin. Negative controls were prepared by substituting primary antibody with nonimmune serum, and positive controls for GFAP by staining of subpial and subependymal astrocytes on the same tissue section.

Data analysis

The statistical significance of differences between the experimental groups was assessed by two-tailed Student's t-test. Significance was set at $p<0.05$.

RESULT

Changes of hippocampal polyamines contents

The hippocampal contents (mean±SM) of putrescine, spermidine. and spermine in untreated gerbils were 139.3±7.4, 2613.3±460.1 and 1820.0 ±115.0 ng/mg wet weight, respectively.

Putrescine (PT) content: The hippocampal PT content of saline treated group (STGr) increased gradually after 7 min occlusion of bilateral carotid arteries (OBC7), reaching a peak level (about 224% compared to the preischemic value) at 24 h after OBC7 ($p<0.05$) and gradually decreased down to 130% of the preischemic

value in 7 days (Fig. 1). The animal group treated with DFMO (DTGr) or MGBG (MTGr) 1 h prior to OBC7 did not show any significant differences in the increase of the PT content occurred for the first 8 h after OBC7, compared to that of STGr. However, the peak PT level appeared in STGr the at 24 h after OBC7 was reduced in DTGr by about 55% and in MTGr by 32%, respectively (Fig. 1). The hippocampal PT content obtained 7 days after OBC7 was lowered in DTGr by about 25% but was somewhat higher in MTGr, compared to the preischemic control value (Fig. 2).

Spermidine (SD) content: The hippocampal SD content of STGr tended to decline initially down to 76% of the preischemic control value at 8 h after OBC7, and then gradually recovered by 7 days after OBC7. The SD content of DTGr showed a initial rapid decrease of the SD content but unlike in STGr, rather increased up to 120% of the preischemic value at 24 h after OBC7. However, the change of hippocampal SD content in MTGr was little different from that observed in STGr. The SD content measured 7 day after OBC7 were lowered by about 20% in both DTGr and MTGr, respectively, compared to the preischemic value (Fig. 3).

Spermine (SM) content: The hippocampal SM content of STGr was slightly decreased for the initial 8 h after OBC7 and recovered to the preischemic level by 7 day after recirculation. The SM content of DTGr decreased by about 38% by 4 h after OBC7 and then returned to the preischemic control value in 8 h after recirculation, whereas the SM content of MTGr showed an initial increase to about 120% of the preischemic value followed by rapid recovery to. The SM contents of DTGr and MTGr measured 7 day after OBC7 were little different from that of STGr (Fig. 4).

Changes of ODC and SAM-DC activities

ODC activity: The preischemic values (mean ±SM) of ODC and SAMDC activities in the hippocampus of STGr were 143.3±16.0 and 549.6 ±99.9 pmol $^{14}CO_2$/mg protein, respec-tively. The hippocampus ODC activity of STGr rapidly increased to reach a peak value (about 240% of the preischemic control value) at 8 h after OBC7 ($p<0.02$) and then returned to the control value by 24 h (Fig. 5). And this ischemia-induced increase of the hippocampus ODC

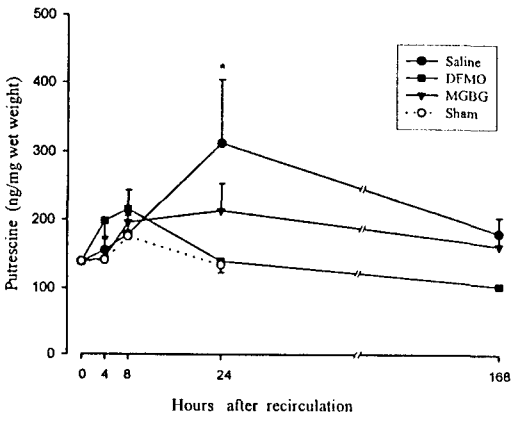

Fig. 1. Effects of DFMO and MGBG on the change of hippocampal putrescine content after transient occlusion of bilateral common carotid arteries for 7 min.
*means $p<0.05$, compared to the data of sham-operated group. Data indicate mean ±S.E..

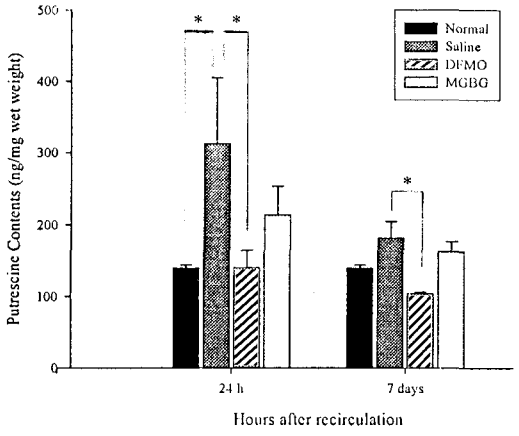

Fig. 2. Effects of DFMO and MGBG on the change of hippocampal putrescine content 24 h and 7 days after transient occlusion of bilateral common carotid arteries for 7 min.
*means $p<0.05$, compared to the data of sham-operated group. Data indicate mean ±S.E.

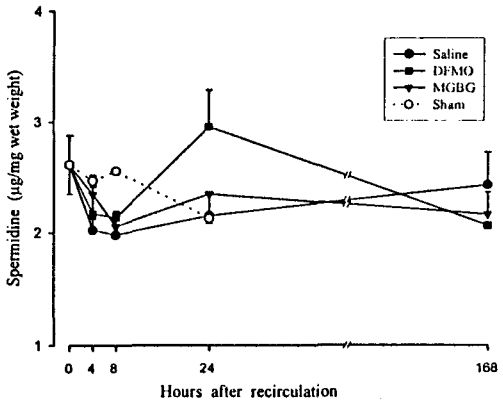

Fig. 3. Effects of DFMO and MGBG on the change hippocampal spermidine content after transient occlusion of bilateral common carotid arteries for 7 min.
*means $p<0.05$, compared to the data of sham-operated group. Data indicate mean ±S.E.

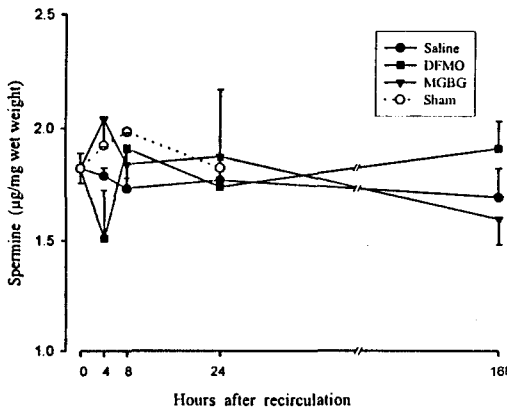

Fig. 4. Effects of DFMO and MGBG on the change hippocampal spermidine content after transient occlusion of bilateral common carotid arteries for 7 min.
*means $p<0.05$, compared to the data of sham-operated group. Data indicate mean ±S.E.

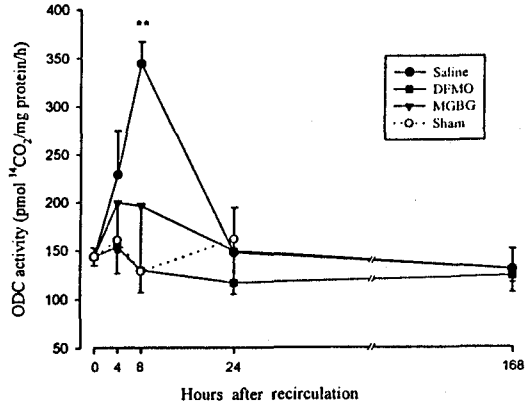

Fig. 5. Effects of DFMO and MGBG on the change hippocampal ODC activity after transient occlusion of bilateral common carotid arteries for 7 min.
*means $p<0.05$, compared to the data of sham-operated group. Data indicate mean ±S.E.

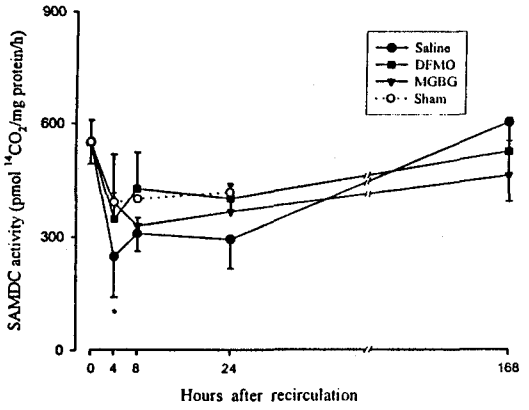

Fig. 6. Effects of DFMO and MGBG on the change hippocampal SAMDC activity after transient occlusion of bilateral common carotid arteries for 7 min.
*means $p<0.05$, compared to the data of sham-operated group. Data indicate mean ±S.E.

activity was significantly attenuated by MGBG and was furthermore suppressed by DFMO. There were no significant differences between the ODC activity of STGR and that of either DTGr or MTGr, if measured 7 day after OBC7 (Fig. 5).

SAM-DC activity: The hippocampus SAM-DC activity of STGr was rapidly decreased down to about 45% of the preischemic control value 4 h after OBC7 and then was remained at the same level for 24 h after recirculation, whereas the lowered activity was gradually recovered to

the preischemic level by 7 day after recirculation. Meanwhile, the decrease of SAM-DC activity was slightly attenuated by DFMO and the recovery rate of the decreased activity was rather attenuated by MGBG. But the SAM-DC activity of the hippocampus was also decreased even in the shame-operated animals by about 30% . And the SAM-DC activities of DTGr and MTGr were not different from that observed in the sham-operated group (Fig. 6).

Histological findings of hippocampus stained with cresyl violet

In sham-operated gerbils, cell bodies of the

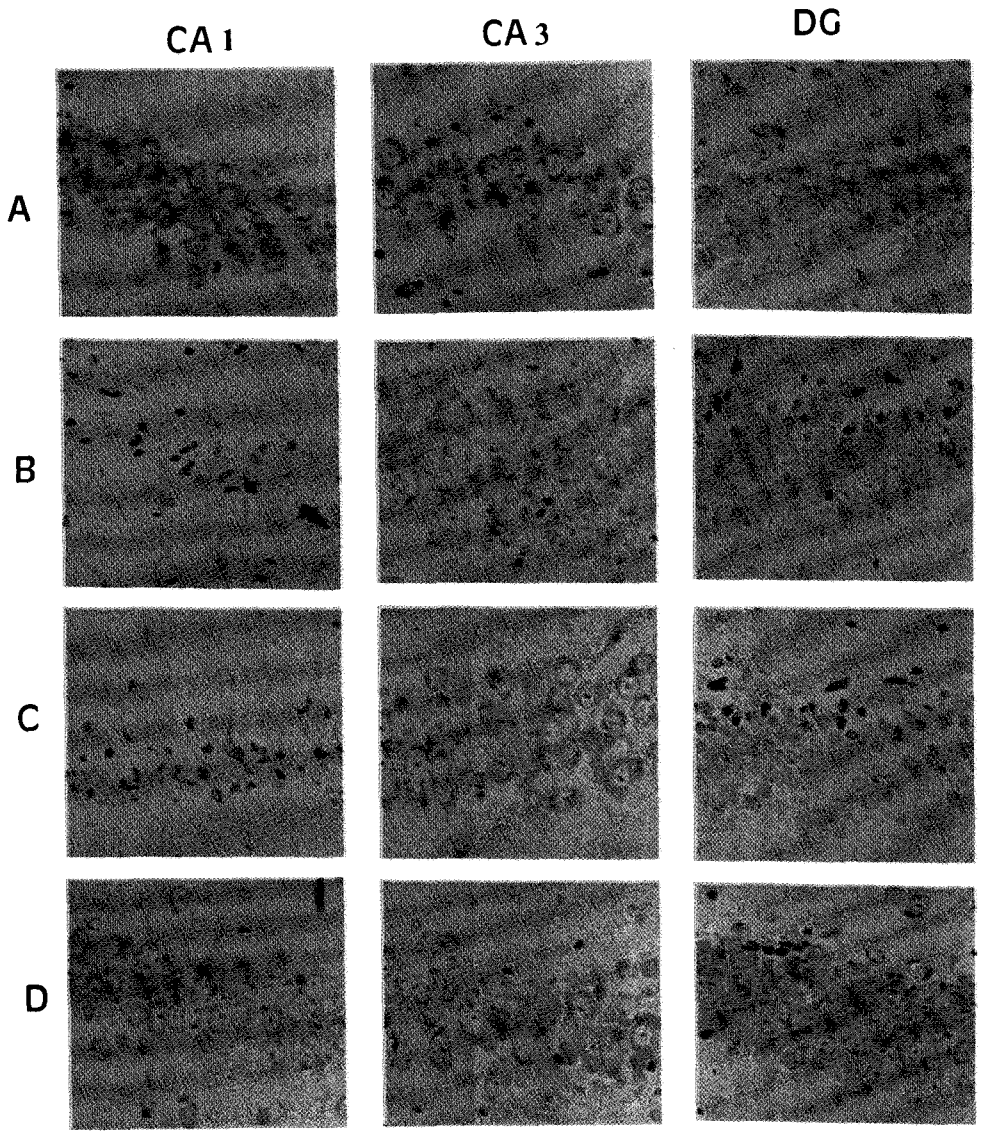

Fig. 7. Light microscopic findings of the CA1, CA3 and dentate gyrus (DG) areas in the hippocampus stained with cresyl violet on the 7th day after transient occlusion of bilateral common carotid arteries for 7 min.
Treatment; A: sham; B: saline; C: DFMO; D: MGBG.

CA1 pyramidal cells were closely packed in the stratum pyramidale. The majority had round nuclei surrounded by relatively scanty cytoplasm. The hippocampus feature of STGr visualized 7 day after OBC7, showed extensive destruction of the pyramidal cells in the CA1 region. Most of CA1 pyramidal cells appeared the characteristic findings of cytolysis, and some CA1 pyramidal cells were slightly swollen. These ischemia-induced neuronal damage were aggrevated by DFMO, whereas MGBG tended to protect the neurons of the CA1 region from ischemic insult (Fig. 7).

However, the CA3 region of the hippocampus

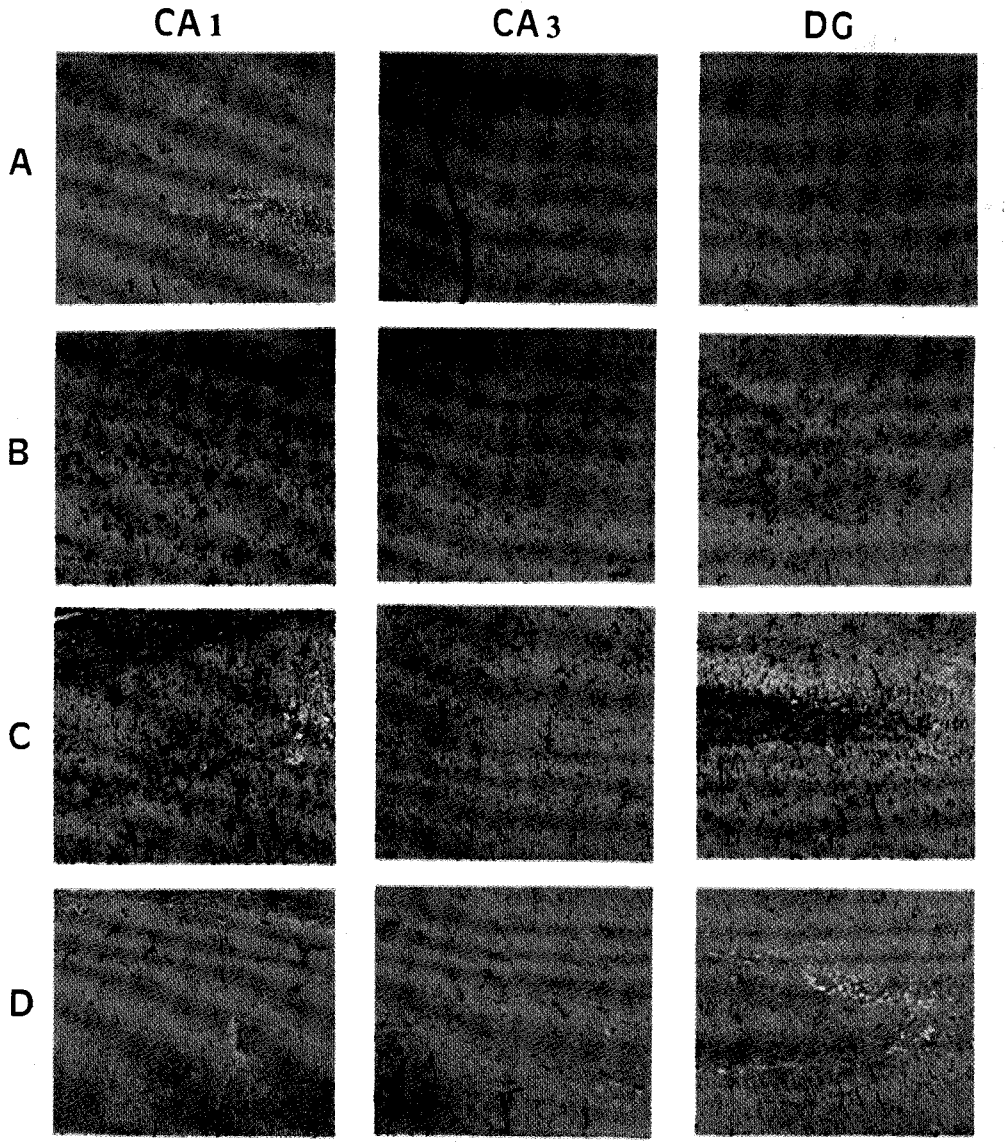

Fig. 8. Immunohistochemistry for the glial fibrillary acidic protein(GFAP) of the CA1, CA3 and dentate gyrus (DG) areas in the hippocampus on the 7th day after transient occlusion of bilateral common carotid arteries for 7 min.
Treatments; A: sham; B: saline; C: DFMO; D: MGBG.

was slightly affected after OBC7, whereas in the dentate gyrus, some neurons were somewhat shrinken and in addition, other neurons showed even cytolytic features.These moderate neuronal damage of the dentate gyrus appeared after OBC7, was slightly aggravated by DFMO but was significantly attenuated by MGBG (Fig. 7)

Immunocytochemical reactivity of hippocampus to anti-GFAP antibody

The hippocampal immunoreactivity to anti-GFAP antibody revealed a homogenous pattern of astrocyte distribution in all hippocampal areas of sham-operated gerbils (Fig. 8). Seven days after OBC7, the strong immunoreactivity appeared in the CA1 area and the dentate gyrus (to a lesser extent) and distributed in the striata oriens and radiatum; whereas little increase of the immunoreactivity were observed in the CA3 area. Particularly, the reactivities observed in the CA1 area and the dentate gyrus indicated a moderate hypertophic features of astrocytes (Fig. 8). The OBC7-induced increase of the anti-GFAP immunoreactivity in the CA1 and detate gyrus areas was markedly enhanced by DFMO but was significantly attenuated by MGBG (Fig. 8).

DISCUSSION

The hippocampus, particlurly the CA1 area, has been known to be one of the most vulnerable regions in brain ischemia (Kirino, 1982 Kirino and Sano, 1984). The ischemia-induced delayed neuronal death (DND) of the CA1 neurons has been ascribed to over-activation of postsynaptic NMDA receptors (Gass *et al.*, 1993) through extracellular glutamate liberated during brain ischemia (Benveniste *et al.*, 1989; Mitani *et al.*, 1990). In this study using a global ischemia model of transient (7 min) occlussion of bilateral common carotid arteries (OBC7) in male Mongolian gerbils, the histological findings of the hippocampus appeared the extensive neuronal loss of the CA1 pyramidal cells and the moderate neuronal loss of the dentate gyrus, while the CA3 area was slghtly affected. These histological findings in similar to those reported by Kirino (1982) and Kirino and Sano (1984). And also, like the hippocampal findings appeared by cresyl violet staining, the hippocampal immunoreactivity to anti-GFAP antibody showed the strong astrocyte reactivity and hypertrophic feature in the CA1 area and, to a lesser extent, in the dentate gyrus, while the immunoreactivity of the CA3 area was slghtly increased. Taken together, these results demostrated that the neuronal vulnerability of the hippocampal regions to global brain ischemia is in susceptible order of the CA1, the dentate gyrus, and finally the CA3.

However, Mitani *et al.* (1992) reported that there was no significant differences in the time-course in glutamate release and in the extracellular glutamate levels of between the CA1 and CA3 regions whereas the DND would be developed only in the field of CA1, suggesting that the ability of glutamate to produce ischemic damage is determined largely by other factors.

The activation of NMDA receptor has been known to be positively modulated by polyamines (Lazarewicz *et al*, 1992; Nussenzveig *et al.*, 1991; Williams *et al.*, 1991; Araneda *et al.*, 1993).

It has been known that polyamines play an important role in biological responses to brain ischemia (Paschen, 1992; Paschem *et al.*, 1987; 1988a; 1988b; 1992). Interestingly, the PT contents of the hippocampal CA1 region vulnerable to ischemic insults, were increased to be kept up the plateau level for 4 or more days after recirculation, while unlike the CA1 region, the tissue PT concent was transiently increased in the cerebral cortex, striatum, and thalamus not to be susceptible to ischemic tissue injury (Paschen, 1992; Paschem *et al.*, 1987; 1992). After global brain ischemia of gerbils for 10 min, the brain ODC mRNA levels were considerably increased, peaking at about 4 h of recirculation and then returned to control values by 12 h after ischemia, and interestingly, the increase in ODC mRNA levels was most prominent in the hippocampus (Dempsey *et al.*, 1991). Also, ODC activity was markedly increased for 8 h after brain ischemia and returned to the control levels by 24 h (Paschen *et al.*, 1987).

Therefore, this study was carried out to evaluate the relationship between the changes of brain polyamine metabolim and the DND of the

hippocampus caused by global brain ischemia in the male Mongolian gerbils. The hippocampal activity of ODC, the primary rate-limiting enzyme of polyamine synthesis, was markedly increased by occlusion of bilateral common carotid arteries for 7 min (OBC7) and then recovered to the preischemic level by 24 h after recirculation. On the other hand the hippocampal activity of SAM-DC, the secondary rate-limiting enzyme of polyamine synthesis, was reciprocally decreased compared to that of ODC and was kept up the decreased level for 24 h after recirculation. And the OBC7-induced increase of hippocampal ODC activity was significantly attenuated by DFMO and MGBG, respectively, whereas the decrease of hippocampal SAM-DC activity was little affected by them. However, the hippocampal PT content was markedly increased, reaching a peak level at 24 h after OBC7 followed by recirculation and then gradually returned to the preischemic level by 7 day after recirculation. And the hippocampal PT increase was significantly inhibited by DFMO and MGBG, respectively, like the change of the hippocampal ODC activity. But the hippocampal SD content was moderately decreased by 4 h after OBC7 and remained for 24 h after recirculation. But the hippocampal SM content was little changed in response to OBC7. And DFMO and MGBG did not significantly affect the hippocampal contents of SD and SM, respectively.

The changes of the metabolic products of SAM-DC, such as SD and SM, could be ascribed to the decrease of SAM-DC activity in the hippocampus due to decrease of pyruvate, the cofactor of SAM-DC and partly decrease of S-adenosylmethionine, which were obligatorily developed in ischemic condition. However, the hippocampal features revealed by cresyl violet staining and immunocytochemical staining to GFAP 7 days after OBC7, demonstarated that the vulnerality of hippocampal regions to global brain ischemia is most prominent in the CA1 area and to a lesser extent, in the dentate gyrus, but the CA3 region is relatively resistant to ischemic insult. The ischemic neuronal damage would be attenuated by MGBG, but could be aggravated by DFMO, in contrast to the report of Kindley *et al* (1994).

However, PT has been shown to activate synaptosomal Ca^{++}-influx (Komulainen and Bondy, 1987) and neuronal liberation of excitatory amino acids (Reed and de Belleroche, 1990), and the ischemia-induced PT increase in the hippocampus seems to contribute to the development of DND of CA1 region (Paschen, 1992; Paschem *et al.*, 1987; 1992).

And although the delayed neuronal death in the hippocampus after global brain ischemia has been known to be modulated in part by the ineraction of polyamines on the NMDA:Ca^{++}-channel complex or the other direct action of polyamines on the hippocampal neurons. But, taken together the results obtained in this study and the evidences cited above, the effects of DFMO and MGBG on the ischemic DND of the hippocampus, particularly the CA1 area, may be associated with unkown actions of them, such as intereference of agmatine synthesis (Bey *et al.*, 1987).

REFERENCES

Araneda RC, Zukin RS and Bennett MV: *Effecgts of polyamines on NMDA-induced currents in rat hippocampal neurons: a whole-cell and single-channel study. Neurosci Lett 152: 107-112, 1993*

Benveniste H, Jorgensen MB, Sandberg M, Christensen T, Hagberg H and Diemer NH: *Ischemic damage in hippocampal CA1 is dependent on glutamate release and intact innervation form CA3. J Cereb Blood Flow Metab 9: 629-639, 1989*

Bey P, Danzin C, and Jung M: *Inhibition of basic amino acid decarboxylases involved in polyamine synthesis. In: Inhibition of Polyamine metabolism, ed; by PP McCann, AE Pegg, and A Sjoerdsma, pp 1-31, Academic Press, 1987.*

DeArmond SJ, Fajardo M, Naughton SA and Eng LF: *Degradation of glial fibrillary acidic protein by a calcium dependent proteinase: an electroblot study. Brain Res 262: 275-282, 1983*

Dempsey RJ, Carney JM and Kindy MS: *Modulation of ornithine decarboxylase mRNA following transient ischemia in the gerbil brain. J Cereb Blood Flow Metab 11: 979-985, 1991*

Gaines DW, Friedman L and Braunberg RC: *Facilitated micromethod for measurement of metabolically generated 14CO2, with application to measurement of ornithine decarboxylase. Anal Biochem 178: 52-56, 1989*

Gass P, Muelhardt C, Sommer C, Becker CM and

Kiessling M: *NMDA and glycine receptor mRNA expression following transient global ischemia in the gerbil brain. J Cereb Blood Flow Metab 13: 337-341, 1993*

Hietala OA, Laitinen SI, Laitinen PH, Lapinjoki SP and Pajunen AE: *The inverse changes of mouse brain ornithine and S-adenosylmethionine decarboxylase activities by chlorpromazine and imipramine. Dependence of ornithine decarboxylase induction on beta-adrenoceptors. Biochem Pharmacol 32: 1581-1585, 1983*

Kahn K: *The natural course of experimental cerebral infarction in the gerbil. Neurology 22: 510-515, 1972*

Kindy MS, Hu Y and Dempsey RJ: *Blockade of ornithine decarboxylases enzyme protects against ischemic brain damage. j Cereb Blood Flow Metab 14: 1040-1045, 1994*

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

Kirino T and Sano K: *Fine structural nature of delayed neuronal death following ischemia in the gerbil hippocampus. Acta Neuropathol Berl 62: 209-218, 1984*

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

Komulainen H and Bondy SC: *Tranisient elevation of intrasynaptosomal free calcium by putrescine. Brain Res 401: 50-54, 1987*

Lazarewicz JW, Salinska E and Puka M: *Glycine enhances extracellular 45Ca^{2+} response to NMDA application investigated with microdialysis of rabbit hippocampus in vivo. Acta Neurobiol Exp(Warsz) 52: 83-91, 1992*

Levines S and Payan H: *Effects of ischemia and other procedures on the brain and retina of the gerbil (Meriones unguiculatus). Exp neurol 16: 255-262, 1966*

Milam KM, Deen DF and Marton LJ: *Cell proliferation and polyamine metabolism in 9L cells treated with (2R, 5R)-6-heptyne-2, 5-diamine or alpha-difluoromethylornithine. Cell Tissue Kinet 22: 269-277, 1989*

Mitani A, Andou Y and Kataoka K: *Selective vulnerability of hippocampal CA1 neurons cannot be explained in terms of an increase in glutamate concentration during ischemia in the gerbil: Brain microdialysis study [published erratum appears in neuroscience 1992 Sep; 50(1): 251]. Neuroscience 48: 307-313, '992*

Mitani A, Imon H, Iga K, Kubo H and kataoka K: *Gerbil hippocampal extracellular glutamate and neuronal activity after transient ischemia. Brain Res Bull 25: 319-324, 1990*

Mitani A, Imon H and Kataoka K: *High frequency discharges of gerbil hippocampal CA1 neurons shortly after ischemia. Brain Ees Bull 23: 569-572, 1989*

Mitani A and Kataoka K: *Critical levels of extracellular glutamate mediating gerbil hippocampal delayed neuronal death during hypothermia: Brain microdialysis study. Neuroscience 42: 661-670, 1991*

Nussenzveig IZ, Sircar R, Wong ML, Frusciante MJ, Javitt DC and Zukin SR: *Polyamine effects upon N-methyl-D-aspartate receptor functioning: differential alteration by glutamate and glycine site antagonists. Brain Res 561: 285-291, 1991*

Paschen W: *Polyamine metabolism in reversible cerebral ischemia. Cerebrovasc Brain Metab Rev 4: 59-88, 1992*

Paschen W, Hallmayer J and Rohn G: *Relationship between putrescine content and density of ischemic cell damage in the brain of Mongolian gerbils: effect of nimodipine and barbiturate. Acta Neuropathol Berl 76: 388-394, 1988a*

Paschen W, Rohn G, Hallmayer J and Mies G: *Polyamine metabolism in reversible cerebral ischemia of Mongolian gerbils. Metab Brain Dis 3: 297-302, 1988b*

Paschen W, Schmidt-Kastner R, Djuricic B, Meess C, Linn F and Hossmann KA: *Polyamine changes in reversible cerebral ischemia. J Neurochem 49: 35-37, 1987*

Paschen W, Widmann R and Weber C: *Changes in regional polyamine profiles in rat brains after transient cerebral ischemia (single versus repetitive ischemia): evidence for release of polyamines from injured neurons. Neurosci Lett 135: 121-124, 1992*

Pegg AE: *Recent advances in the biochemistry of polyamines in eukaryotes. Biochem J 234: 249-262, 1986*

Paschen W, Cleef M, Röhn G, Müller M and Pajunen AEI: *Ischemiainduced disturbances of polyamine synthesis. In Neurobiology of ischemic brain damage(ed. K Kogure, KA Hossman and BK Siesjö) Elsevier, Amsterdam, pp 147-160, 1993*

Peterson GL: *A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 83: 346-356, 1977*

Reed LJ and de Belleroche J: *Induction of ornithine decarboxylase in cerebral cortex by excitotoxin lesion of nucleus basalis: association with postsynaptic reponsiveness and N-methyl-D-aspartate receptor activation. J Neurochem 55: 780-787, 1990*

Seiler N: *Inhibition of Enzymes oxidizing polyamines. In: Inhibition of Polyamine methabolism, ed: by PP McCann, AE Pegg and A Sjoerdsma, pp 449-77, Academic Press, 1987*

Shin KH, Kim HG, Choi SH, Cho SH, Chun YS, Chun BG: *Changes of Glutamate and Polyamine Levels of Hippocampal Microdialysates in Response to Occlusion of Both Carotid Arteries in Mongolian Gerbils. Korean J*

Pharmacol 30:; 273-289, 1994

Smith ML, Kalimo H, Warner DS and Siesjo BK: *Morphological lesions in the brain preceding the development of postischemic seizures. Acta neuropathol Berl 76: 253-264, 1988*

Spragg BP and Hutchings AD: *High-performance liquid chromatographic determination of putrescine, spermidine and spermine after derivatisation with r-fluoro-3-nitrobenzotrifluoride. J Chromatogr 258: 289-291, 1983*

Williams K, Romano C, Dichter MA and Molinoff PB: *Modulation of the NMDA receptor by polyamines. life Sci 48: 496-498, 1991*

=국문초록=

뇌허혈 손상에 있어서 Polyamine 대사의 변동이 해마신경세포의 지연성괴사에 미치는 효과에 관한 연구

고려대학교 의과대학 약리학교실

신경호 · 신화정 · 이영재 · 김형건 · 최상현 · 천연숙 · 전보권

웅성 모래쥐의 양경동맥을 7분간 폐쇄하여(OBC7) 뇌의 허혈을 유발하여, 해마의 microdialysate내 polyamine 함량과 조직내 polyamine 생합성효소(ornithine decarboxylase: ODC와 S-adenosylmethionine decarboxylase: SAM-DC)의 활성도를 분석하고, 해마의 cresyl violet(CV) 염색과 glial fibrillary acidic protein(GFAP) 면역염색소견들을 관찰하여, 허혈성 해마의 신경손상과 polyamine대사의 연관성을 검토하였다.

1) OBC부하 후, 해마의 dialysate내 polyamine 변동에서, putrescine(PT)은 현저히 증가되었으나, spermidine과 spermine은 다소 감소되는 경향을 보였고, 이에 해마조직내 ODC활성의 현저한 상승과 SAM-DC활성도의 유의한 저하 동반되었다.

2) Difluoromethylornithine(DFMO)는 OBC에 의한 PT증가와 ODC활성도 상승을 유의하게 억제하였으나, methylglyoxal bis(guanylhydrazone)(MGBG)는 각각 다소 억제하는 경향을 보였다.

3) OBC부하 7일후에 관찰한 조직소견에서, 해마의 CA1 부위의 유의한 신경손상이 유도되었으나 CA3와 dentate gyrus 부위에는 미약한 손상만 보였으며, GFAP 양성반응도 CA1 부위에서만 유의한 증가를 보였다. 이같은 소견들은 DFMO에 의하여 크게 영향을 받지 않았으나 MGBG에 의하여 유의하게 억제되었다.

이상의 성적들은 해마 polyamine의 과도한 허혈성 증가가 허혈성 신경손상에 관여할 수 있으나, 한편으로 polyamine대사의 과도한 억제도 허혈성 신경손상을 악화시킬 수 있으며, 허혈성 뇌손상에 대한 MGBG의 보호작용은 polyamine 대사보다는 다른 작용에 매개되는 것으로 사료된다.