Neuroprotective Effect of Agmatine on Hypoxia-Induced Apoptosis of Retinal Ganglion Cells

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Neuroprotective Effect of Agmatine on Hypoxia-Induced Apoptosis of Retinal Ganglion Cells

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ABSTRACT

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Agmatine is an endogenous polyamine formed by the decarboxylation of L-arginine. We investigated the protective effect of agmatine on the hypoxia-induced apoptosis of transformed rat retinal ganglion cells (RGCs). RGC cells were cultured in a closed hypoxic chamber (5% O_2). With or without agmatine, the cell viability was determined by lactate dehydrogenase (LDH) assay and apoptosis was established by annexin V and caspase-3 assays. The expression and activity of mitogen-activated protein kinases (MAPKs; JNK, ERK p44/42, and p38) and nuclear factor-kappa B (NF-kB) were investigated by Western immunoblot analysis. The effect of agmatine was compared to that of brain-derived neurotrophic factor (BDNF), a well-known

protective neurotrophin for RGCs. After 48 hours of hypoxic culture, 52.3% cell loss was evident and the cell loss was reduced to 25.6% and 30.1% when agmatine and BDNF were administered, respectively. The observed cell loss was due to apoptotic cell death, as established by annexin V and caspase-3 assays. The phosphorylated MAPKs and NF-κB were increased, and agmatine reduced phosphorylated JNK and NF-κB, while BDNF suppressed phosphorylated ERK and p38. Our results show that agmatine has a neuroprotective effect against hypoxia-induced RGC damage that is more powerful than that of BDNF and this effect is associated with the JNK and NF-κB signaling pathways. Our data suggest that agmatine may lead to a novel therapeutic strategy to reduce RGC injury related hypoxia.

Key words: agmatine, apoptosis, glaucoma, hypoxia, MAPK, neuroprotection, NF-κB, retinal ganglion cell, RGC-5

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I. INTRODUCTION

Agmatine is an endogenous polyamine that is synthesized from the decarboxylation of L-arginine by mitochondrial arginine decarboxylase[1,2], and it is widely and unevenly expressed in the brain and other tissues of mammals[3,4]. Agmatine has been reported to have various biological actions; it stimulates the release of catecholamines from the adrenal chromaffin cells[3], insulin from the pancreatic islets[5], and luteinizing hormone-releasing hormone from the hypothalamus[6]. Also, it enhances the analgesic effect of morphine[7], inhibits the activity of inducible nitric oxide synthase (NOS)[8], and contributes to polyamine homeostasis[2,9]. It is known that agmatine is an agonist for the α_2-adrenergic and imidazoline receptors[3], and an antagonist for the N-methyl-D-aspartate (NMDA) receptor[10]. However, the precise

cellular mechanism by which agmatine acts is not well established.

Currently, a large body of experimental evidence has demonstrated the neuroprotective effects of agmatine. Agmatine reduced the infarct area and neuronal loss in cerebral ischemic and ischemic-reperfusion injury models[11-13]. It protected the neurons from cell death after exposure to NMDA and glutamate[14,15]. Its neuroprotective effects also include attenuating the extent of neuronal loss following spinal cord injury[16,17]. Additionally, agmatine protected neurons from glucocorticoid-induced neurotoxicity[18], and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-related dopaminergic toxicity[19].

On the base of these neuroprotective effects of agmatine and its roles as an α_2-adrenergic agonist[3], NMDA receptor antagonist[10], and suppressor of inducible NOS[8], we hypothesize that agmatine may protect to the retinal ganglion cells (RGCs). In present investigation, we examined the neuroprotective effect of agmatine on hypoxia-induced apoptosis of RGCs. And this neuroprotective effect of agmatine was compared to that of brain-derived neurotrophic factor (BDNF), a well-known protective neurotrophin for RGCs. In addition, we studied some molecular pathways associated with the neuroprotective effects of agmatine.

II. MATERIALS AND METHODS

1. Chemicals and antibodies

Agmatine sulfate and recombinant human BDNF were purchased from

Sigma-Aldrich (St. Louis, MO, USA) and R&D System, Inc. (Minneapolis, MN, USA), respectively. Rabbit polyclonal antibodies against c-Jun N-terminal kinase (JNK) p54/46, extracellular signal-regulated kinase (ERK) p44/42, p38 kinase (p38), nuclear factor-kappa B (NF-κB) p65, phospho-JNK p54/46, phospho-ERK p44/42, phospho-p38, phospho-NF-κB p65, and histone 3 were purchased from Cell Signaling Technology, Inc. (Boston, MA, USA). Mouse monoclonal anti-β-actin antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

2. Cell culture

The RGC-5 cell line, a retinal ganglion cell line developed from post-natal Sprague-Dawley rats, was grown in modified Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum and 100 U/mL of penicillin and 100 μg/mL of streptomycin. The cells were passaged every 2 to 3 days, and the cultures incubated at 37 ℃ in 5% CO_2 and air. During cultivation, the cells exhibited the same morphological phenotype. For all experiments, cells were used at an 80% confluence.

3. Hypoxic injury to retinal ganglion cells

Cultures were transferred into a closed hypoxic chamber (Forma Scientific Co., Seoul, Korea) in which oxygen level (5% O_2, 5% CO_2, 90% N_2) and temperature (37 ℃) were controlled. After washing twice with deoxygenated serum-free DMEM, cells were maintained in the hypoxic chamber. Control cells were not exposed to hypoxia. Agmatine or BDNF were added to the culture medium at the start of injury as

indicated.

4. Lactate dehydrogenase assay

Cell viability was quantified by measurement of lactate dehydrogenase (LDH) released by injured cells after hypoxic or normoxic culture for 12, 24, and 48 hours. LDH release is expressed relative to the value of 100, which represented the maximum LDH release that occurred after freezing of each culture at -70 ℃ overnight and rapid thawing, which induced nearly complete cell damage. All experiments were performed in at least quadruplicate and repeated at least eight times using cell cultures derived from different platings. The preliminary studies with the LDH assay tested agmatine concentrations from 10 μM to 1 mM and BDNF concentrations from 5 ng/mL to 100 ng/mL. Cell death was reduced significantly at 100 μM and greater concentrations of agmatine and 10 ng/mL and greater concentrations of BDNF, so we used 100 μM agmatine and 10 ng/mL BDNF for subsequent experiments.

5. Hoechst 33342 and propidium iodide staining

Apoptotic or necrotic cell death was characterized by the use of Hoechst 33342 and propidium iodide (PI) double staining. Cells were stained with 10 μg/mL Hoechst 33342 and 10 μg/mL PI for 30 min at 37 ℃. After washing twice with phosphate buffered saline (PBS), cells were imaged with a digital camera attached to a fluorescence microscope.

6. Annexin V assay

The percentage of cells actively undergoing apoptosis was determined by

flow cytometry using the Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Briefly, cells were harvested and resuspended in binding buffer (10^6 cells/mL). 10^5 cells were mixed with 5 μL of annexin V-FITC and 5 μL of PI. After incubating at room temperature for 15 minutes in the dark, analysis was performed by flow cytometry.

7. Caspase-3 assay

Caspase-3 activity was measured using the CaspACE™ colorimetric assay system (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, cells were harvested and resuspended in cell lysis buffer (10^8 cells/mL). After lysis, 10^6 cells were mixed with 32 μL of assay buffer and 2 μL of 10 mM DEVD-pNA substrate. After incubating at 37℃ for 4 hours, absorbance was measured using a microplate reader at 405 nm. Absorbance of each sample was determined by subtraction of the mean absorbance of the blank from that of the each sample.

8. Western blot analysis

For extraction of whole cellular proteins, cells were washed twice with ice-cold PBS and then lysed with cell lysis buffer (50 mM Tris-HCl pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 10 mM Na_3VO_4, 50 mM NaF, 1 mM PMSF, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin) on ice for 30 minutes. Lysates were sonicated, and the cell homogenates were centrifuged at 15,000*g* for 10 minutes (4 ℃).

For fraction of cytosolic and nuclear proteins, cells were lysed with lysis

buffer A (10 mM HEPES pH 7.4, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 10 mM Na_3VO_4, 50 mM NaF, 1 mM PMSF, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin) on ice for 15 minutes, and 10% NP-40 was added. After vortexing for 10 seconds, lysates were centrifuged at 15,000*g* for 1 minute (4 ℃). The supernatent was collected from the cytosolic fraction. The pellet was resuspended in lysis buffer C (20 mM HEPES pH 7.4, 400 mM NaCl, 1 mM EDTA, 1% glycerol, 1 mM DTT, 10 mM Na_3VO_4, 50 mM NaF, 1 mM PMSF, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin) on ice for 30 minutes. Lysates were centrifuge at 15,000*g* for 15 minutes (4 ℃). The supernatent was collected from the nuclear fraction.

The protein concentrations in the resultant supernatants were determined with the Bradford reagent, and equal amounts of protein (40 μg) were boiled in Laemmli sample buffer and resolved by 10 or 15% SDS-PAGE. The proteins were transferred to polyvinylidene fluoride membranes and probed overnight with antibodies against JNK, ERK p44/42, p38, NF-κB p65, phospho-JNK, phospho-ERK p44/42, phospho-p38, phospho-NF-κB, β-actin and histone 3 as indicated (diluted 1:1000). The immunoreactive bands were detected with horseradish peroxidase–conjugated secondary antibodies and visualized by enhanced chemiluminescence.

9. Statistical Analysis

Data were analyzed by 2-tailed Student *t*-test or one-way ANOVA using the Statistical Package for Social Sciences 12.0 (SPSS). Differences were considered statistically significant at $p<0.05$.

III. RESULTS

1. Agmatine inhibits hypoxia-induced cell death of RGC-5

We first examined the effect of hypoxia on cultured transformed rat RGCs (RGC-5). As shown in Fig. 1, the effects of hypoxic conditions on RGCs were significant (all $P<0.001$). Exposure to hypoxia for 12, 24, and 48 hours significantly increased LDH by 10.17%, 20.04%, and 52.25%, respectively. This result indicates a hypoxia-induced time-dependent neurotoxicity on RGCs.

Next, we examined possible neuroprotective effects of agmatine on hypoxia-induced RGC damage, and compared these effects to those of BDNF. The results indicate a significant influence of agmatine on hypoxic neuronal damage, and this effect was more powerful than that observed for BDNF. After 12 and 24 hours of hypoxia (Fig. 1A and B), none of the treatment groups showed a significant effect on the increased LDH release produced by hypoxia ($P=0.864$ and $P=0.266$, respectively). As illustrated in Fig. 1C, however, there were significant effects of agmatine and BDNF on LDH release (all $P<0.001$). 100 μM and 500 μM agmatine prevented the hypoxia-induced increase of LDH release to 25.60% and 27.09%. Similarly, 10, 50, and 100 ng/mL BDNF inhibited the release of LDH to 30.10%, 33.67%, and 36.06%, respectively. In addition, 100 μM agmatine was more effective at suppressing LDH release than 10 ng/mL BDNF ($P<0.001$).

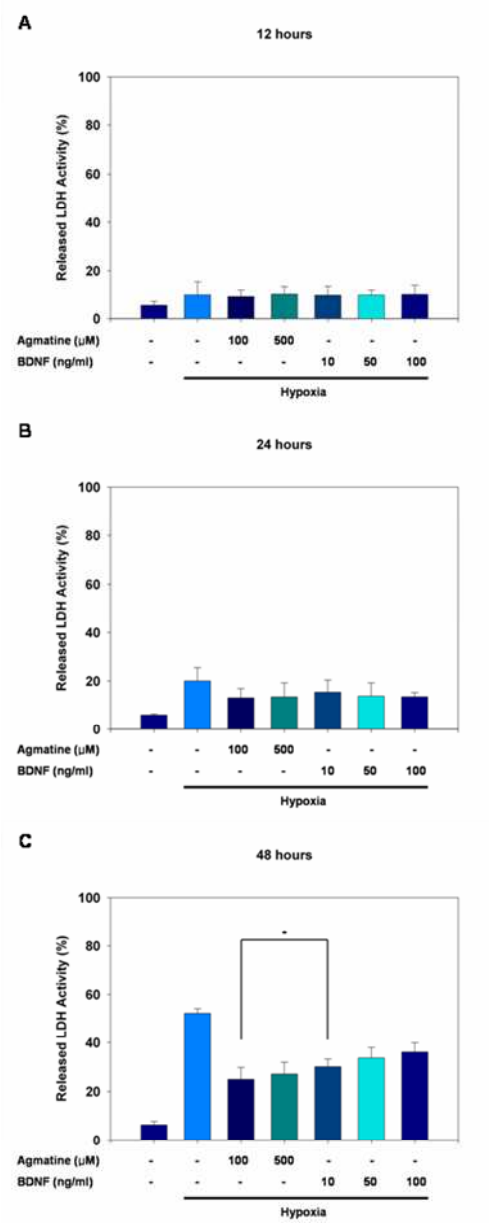

Figure 1. Effect of agmatine and BDNF on the hypoxia-induced LDH release of RGC-5. Cells were exposed to hypoxia injury for 12 hours (A), 24 hours (B), and 48 hours (C). Agmatine or BDNF was added at the start of injury. Data are shown as mean ± S.E.M. of 32 measurements. *P<0.001.

The neuroprotective effects of agmatine were further studied using Hoechst 33342 and PI double staining. After 48 hours, the control normoxic culture exhibited confluent Hoechst-positive cells with homogeneous and compact nuclear morphology, and sparse numbers of PI-labeled cells (Fig. 2A). Exposure of cultures to hypoxia for 48 hours resulted in a significant loss of Hoechst-positive cells and the appearance of many PI-positive cells with distorted and condensed nuclei (Fig. 2B). The RGC loss was prevented by the addition of 100 μM agmatine (Fig. 2C) or 10 ng/mL BDNF (Fig. 2D) to the cultures.

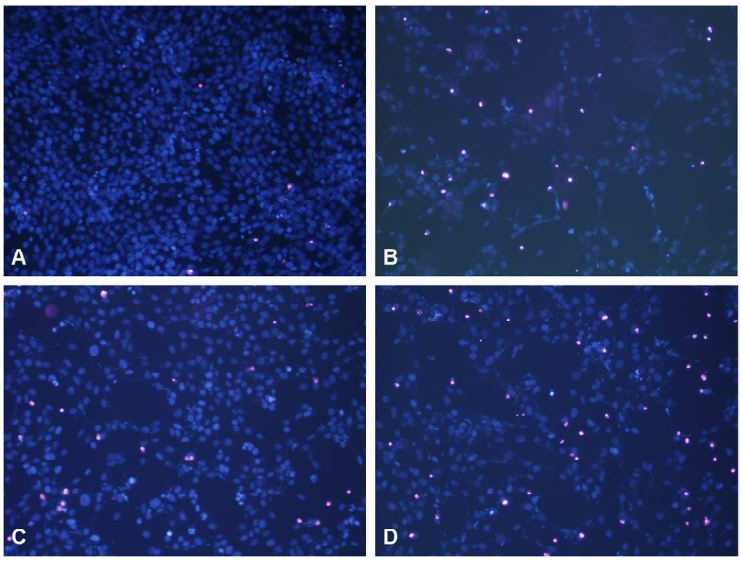

Figure 2. Effect of agmatine and BDNF on the hypoxia-induced cell death of RGC-5. Cells were exposed to hypoxia for 48 hours either alone (B) or in the presence of agmatine (100 μM) (C) or BDNF (10 ng/mL) (D). A control normoxic culture is shown in (A). The cultures were stained with Hoechst 33342 and propidium iodide. The magnification is X 400.

2. Agmatine protects RGC-5 from hypoxia-induced apoptosis

In order to verify whether agmatine had protective effects on the hypoxia-induced apoptotic death of RGCs, we tested these cells using an annexin V assay. While there was no significant difference in the proportion of apoptotic cells after 12 hours of hypoxia (Fig. 3), there was a significant decrease in apoptotic cells in the presence of agmatine or BDNF after 24 hours of hypoxia (Fig. 4).

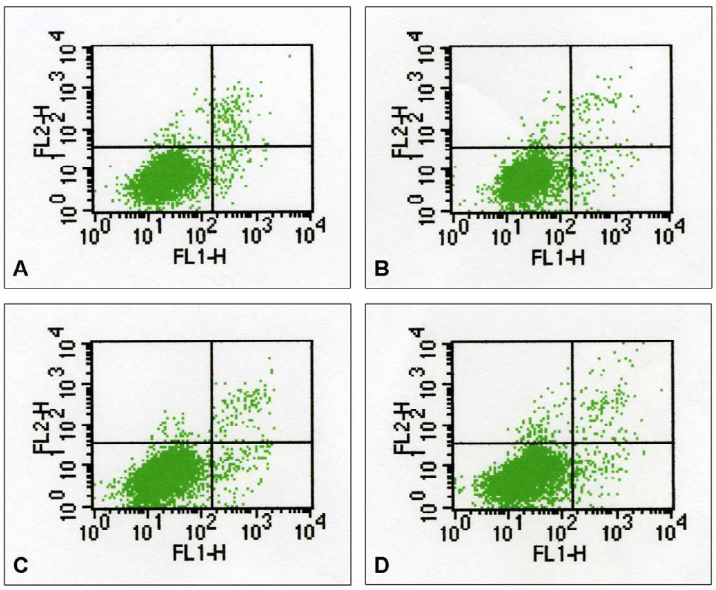

Figure 3. Effect of agmatine and BDNF on the 12 hours hypoxia-induced apoptosis of RGC-5. Cells were exposed to hypoxia for 12 hours either alone (B) or in the presence of agmatine (100 μM) (C) or BDNF (10 ng/mL) (D). A control normoxic culture is shown in (A). The cultures were stained with annexin V-FITC and propidium iodide (PI). The cells of high reactivity with FITC and low reactivity with PI (right lower area) are the apoptotic cells.

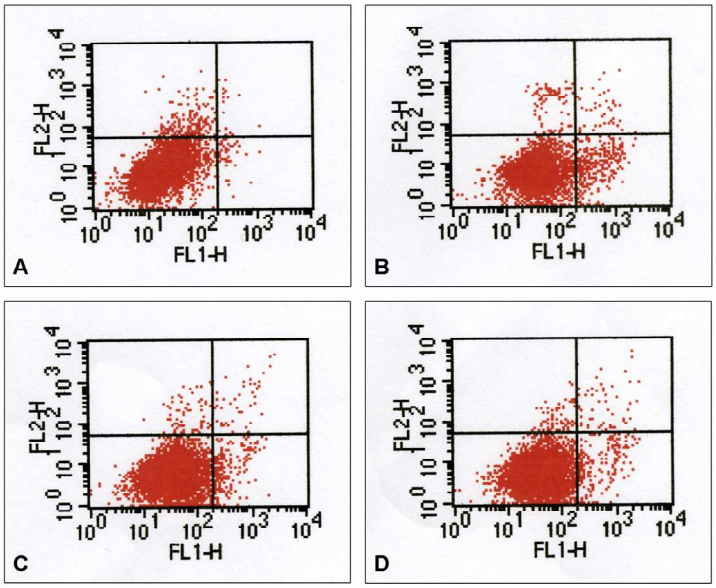

Figure 4. Effect of agmatine and BDNF on the 24 hours hypoxia-induced apoptosis of RGC-5. Cells were exposed to hypoxia for 24 hours either alone (B) or in the presence of agmatine (100 μM) (C) or BDNF (10 ng/mL) (D). A control normoxic culture is shown in (A). The cultures were stained with annexin-V-FITC and propidium iodide (PI). The cells of high reactivity with FITC and low reactivity with PI (right lower area) are the apoptotic cells.

Using the caspase-3 assay, we studied whether agmatine had an effect on the hypoxia-induced specific activity of caspase-3. The specific activity of caspase-3 was measured by cleavage of the caspase-3 substrate (Ac-DEVD-pNA). After 24 hours of hypoxia, there was a significant induction of caspase-3 activity, which was equally suppressed by treatment with 100 μM agmatine or 50 μM caspase-3 inhibitor Z-VAD-FMK (Fig. 5).

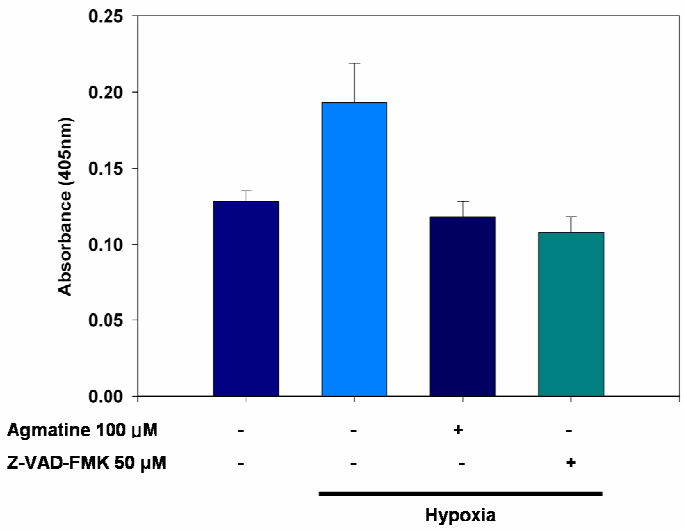

Figure 5. Effects of agmatine on the caspase-3 activity induced by 24 hours hypoxia in RGC-5. Cells were exposed to hypoxia for 24 hours with or without agmatine (100 μM) or Z-VAD-FMK (50 μM). The specific activity of caspase-3 was measured by cleavage of the caspase-3 substrate (Ac-DEVD-pNA).

3. Selective suppression of JNK activation by agmatine

Representative Western blots of the total and phosphorylated mitogen-activated protein kinases (MAPKs), and ß-actin of RGCs after hypoxic injury are shown in Fig. 6.

The antibody against phospho-JNK detected two bands at 54 and 46 kDa, and both bands showed similar changes in this study. Increases of phospho-JNKs in hypoxic RGCs became evident 9 hours after hypoxic injury and remained elevated

(Fig. 6A). Treatment with Agmatine, but not BDNF, significantly suppressed hypoxia-induced phosphorylation of JNKs.

The antibody against the phospho-ERK also detected two bands at 44 and 42 kDa, and both bands showed a similar trend in this study. Phospho-ERKs were not detected in normoxic cultures of RGCs, but were highly expressed in RGCs after 3 hours of hypoxia and remained elevated (Fig. 6B). Treatment with BDNF completely blocked the phosphorylation of ERKs after 3 and 6 hours of hypoxia, but had no effect thereafter. In comparison, agmatine did not significantly affect the phosphorylation of ERKs.

The antibody against phospho-p38 detected one band at 38 kDa. Phospho-p38 was not detected in normoxic RGCs until after 12 hours, but was evident in hypoxic RGCs after 3 hours of hypoxia and remained elevated (Fig. 6C). BDNF only blocked the phosphorylation of p38 at 6 hours and agmatine had no effect on phospho-p38 levels.

Total MAPKs (JNK, ERK, and p38) and ß-actin were unaffected by hypoxic injury (Fig. 6). There were no significant changes after treatment with BDNF or agmatine. Thus, phospho-MAPKs showed different activation profiles after hypoxia; ERK and p38 were activated early, and JNK was activated late. BDNF inhibited the activation of ERK (at 3 and 6 hours after hypoxia) and p38 (at 6 hours after hypoxia), while agmatine suppressed the activation of JNK (with a significant increase at 9

hours after hypoxia).

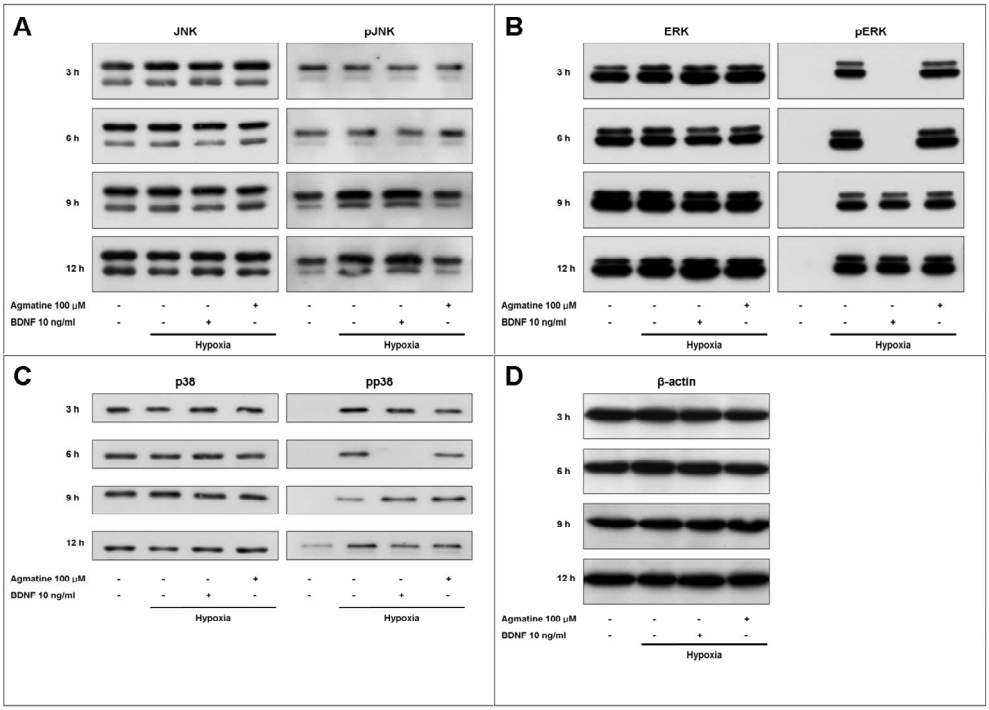

Figure 6. Western blot analysis showing the effect of agmatine and BDNF on MAPKs. Western immunoblots probed with antibodies against JNK and phospho-JNK (A), ERK and phospho-ERK (B), p38 and phospho-p38 (C), and ß-actin (D).

4. Suppression of NF-κB activation by agmatine

The expression and activation of the NF-κB were evaluated from the nuclear and cytosolic fraction of RGCs after hypoxic injury. The representative bands in Western blot analysis are shown in Fig. 7. The antibodies against total and phospho-NF-κB detected their respective bands at 65 kDa.

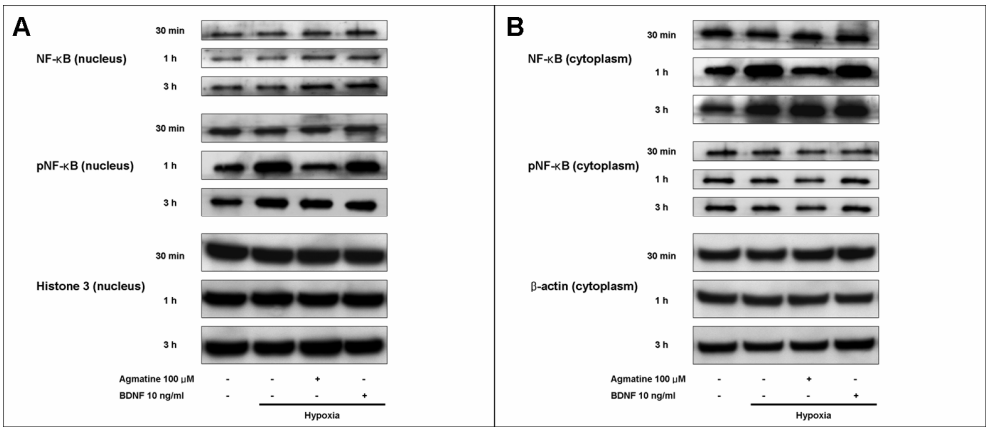

Figure 7. Western blot analysis showing the effect of agmatine and BDNF on NF-κB. Western immunoblots probed with antibodies against NF-κB and phospho-NF-κB from nuclear (A) and cytosolic (B) proteins. Histone 3 (A) and ß-actin (B) were used as internal controls.

In the nuclear fraction, total NF-κB and histone 3 were unaffected by hypoxic injury, and there were no significant changes with the addition of BDNF and agmatine. However, the phospho-NF-κB was significantly increased in RGCs by hypoxia after 1 hour and returned to normal levels after 3 hours. The increase in phospho-NF-κB was suppressed by agmatine, but not BDNF, treatment.

In comparison, in the cytoplasmic fraction, there were no significant changes in levels of phospho-NF-κB and ß-actin in hypoxic RGCs. However, total NF-κB expression increased after 1 hour of hypoxia and returned to normal levels after 3 hours. This increase was inhibited by treatment with agmatine, but not BDNF.

IV. DISCUSSION

Our present study demonstrates that agmatine, an endogenous polyamine with a guanidino group, prevents hypoxia-induced increases in LDH release and apoptotic death in RGCs as measured by annexin V and caspase-3 assays. This neuroprotective effect was similar and even more extensive than with BDNF, a well-known protective neurotrophin for RGCs[20-22]. However, the cellular mechanisms of agmatine- and BDNF-induced protection are different; agmatine suppresses the hypoxia-induced activation of JNK and NF-κB, whereas BDNF inhibits the induction of ERK and p38. Therefore, the ability of agmatine to regulate the JNK and NF-κB pathways may contribute to its neuroprotective effect against hypoxia-induced cell death in cultured RGCs.

Apoptosis, a programmed cell death, has been shown to be the final common pathway for RGC death in glaucoma[23,24]. Several putative mechanisms have been implicated as the cause of RGC apoptosis, including neurotrophic factor deprivation, hypoperfusion/ischemia of the anterior optic nerve, glial cell activation, glutamate excitotoxicity, and abnormal immune response[25]. In the present study, we focused on the hypoxic theory of RGC death in glaucoma. Hypoxia has been postulated to occur in the glaucomatous eye[26,27], and it has been well documented that the hypoxia leads to selective RGC damage[28,29]. Currently, the paradigm of glaucoma therapy is shifting to neuroprotection, so many researchers are seeking drugs with neuroprotective effects.

MAPKs are involved in highly conserved signaling pathways that regulate diverse cellular functions including cell proliferation, differentiation, migration, and apoptosis[30-32].They are activated through phosphorylation by the distinct pathways, depending on the stimulus and the cell type. When they are activated, they phosphorylate a wide range of substrates, including transcription factors and cytoskeletal proteins, resulting in specific cellular responses. In the present study, agmatine regulated the activation of JNK but not ERK and p38 in RGCs after hypoxic injury. This result is discrepant with a previous report using kidney mesangial cells under a high-glucose condition, in which agmatine was involved in the ERK pathway[33]. However, we could not find other reports on the effect of agmatine on the any kind of MAPKs in the literatures, and MAPKs have been known to work differently depending on the stimulus and the cell type. In addition, the characteristics of agmatine as an antagonist for the NMDA receptor[10], suggests that agmatine might regulate the phosphorylation of MAPKs. There is a report demonstrating that another antagonist of the NMDA receptor, MK801, can block the phosphorylation of MAPKs[34].

In the present study, agmatine suppressed the activation of NF-κB in RGCs after hypoxic injury. This finding is similar to that of a previous report in which agmatine reduced high glucose-induced activation of NF-κB in kidney mesangial cells[35]. While our observation that NF-κB is activated by hypoxic damage in RGC-5 is different from that of Charles et al.[36], in which the activity of NF-κB was decreased

RGC-5 cells with serum-deprivation-induced apoptosis, our result is similar to that of a study by Wang et al. [37], looking at rat RGCs after ischemia/reperfusion injury.

In the case of glaucoma, to identify the precise cellular mechanisms behind the associated cell death, it is important to study primary cultured RGCs. However, *in vitro* experiments using primary cultured RGCs are not easy to perform, mainly because of the limited yield and the typically postmitotic feature of these neurons. Therefore, we used a transformed rat RGC line (RGC-5) to optimize cell number and survival in culture. It has been well-recognized that RGC-5 cells have similar characteristics to RGCs[36,38-41], and they have been used for glaucoma research. In addition, we used a closed hypoxic culture system to identify specific mechanisms in RGCs that are specifically triggered by hypoxic injury. Thus, many features of our culture system make it a unique tool for *in vitro* studies to identify the specific mechanisms of hypoxic neurodegeneration in RGCs.

Perhaps the most significant finding of the present study was that both the increased annexin V-positive cell number and the increased caspase-3 activity produced by exposure RGCs to hypoxia were counteracted by addition of agmatine into the culture medium, suggesting that agmatine may exert a neuroprotective effect by inhibiting apoptosis in the hypoxia-injured RGCs. To our knowledge, this is the first report regarding the potential anti-apoptotic characteristics of agmatine in RGCs. Even though this study demonstrates that JNK and NF-κB pathways were associated

with the agmatine treatment in the RGCs after hypoxic injury, further studies are needed to understand the precise mechanism by which agmatine blocks apoptosis. A further understanding of the precise mechanisms associated with agmatine protection against hypoxic RGC injury may facilitate efforts to improve the survival of RGCs in glaucoma.

V. CONCLUSION

Agmatine prevents hypoxia-induced increases in LDH release and apoptotic death in RGCs as measured by annexin V and caspase-3 assays, and these neuroprotective mechanisms are associated with the activation of JNK and NF-κB pathway.

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ABSTRACT (IN KOREAN)

저산소 손상에 의한 망막신경절세포의 아폽토시스에 대한
아그마틴의 신경 보호 효과

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아그마틴은 L-아르기닌의 탈탄산화에 의해 생성되는 1차 아민의 일종이다. 본 연구에서는 저산소손상에 의한 망막신경절세포의 아폽토시스에 대한 아그마틴의 보호 효과에 대해 알아보고, 망막신경절세포의 보호인자로 알려진 뇌-유도 향 신경인자의 효과와 비교하였다. 형질전환된 흰쥐의 망막신경절세포를 5% 산소압의 저산소 환경에서 배양하면서 아그마틴 또는 뇌-유도 향 신경인자를 첨가하고, 세포사멸과 아폽토시스의 변화를 유산염탈수소효소 분석, 아넥신 V 분석 및 캐스페이즈-3 분석 등을 이용하여 평가하였다. 미토겐-활성 단백질 키나제와 핵인자-카파B의 활성에 대한 효과는 웨스턴 면역 분석을

이용하여 확인하였다. 48시간의 저산소 손상 후 52.3%의 세포사멸이 관찰되었으며, 이는 아그마틴과 뇌-유도 향 신경인자의 첨가에 의해 각각 25.6% 및 30.1%로 줄어들었다. 아넥신 V 분석 및 캐스페이즈-3 분석에 의해 저산소 손상에 의한 망막신경절세포의 아폽토시스에 아그마틴이 보효 효과가 있음을 알 수 있었다. 이러한 아그마틴의 신경 보호 효과는 JNK 및 핵인자-카파B 인산화의 억제와 연관되어 있었고, 이는 뇌-유도 향 신경인자의 기전과는 차이를 보이는 것이었다. 결론적으로 본 연구에서는 아그마틴이 저산소 손상에 의한 망막신경절세포의 손상에 대해 신경 보호 효과를 가짐을 알 수 있었고, 이는 저산소 기전과 관련되어 망막신경절세포의 손상을 가져오는 질병에서 아그마틴이 효과를 나타낼 수 있는 가능성을 제시하고 있다.

핵심되는 말: 아그마틴, 아폽토시스, 녹내장, 저산소, 미토겐-활성 단백질 키나제, 신경보호, 핵인자-카파B, 망막신경절세포, RGC-5