

The Effect of Agmatine on Expression of IL-1 β and TLX Which Promotes Neuronal Differentiation in Lipopolysaccharide-Treated Neural Progenitors

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Differentiation of neural progenitor cells (NPCs) is important for protecting neural cells and brain tissue during inflammation. Interleukin-1 beta (IL-1 β) is the most common pro-inflammatory cytokine in brain inflammation, and increased IL-1 β levels can decrease the proliferation of NPCs. We aimed to investigate whether agmatine (Agm), a primary polyamine that protects neural cells, could trigger differentiation of NPCs by activating IL-1 β in vitro. The cortex of ICR mouse embryos (E14) was dissociated to culture NPCs. NPCs were stimulated by lipopolysaccharide (LPS). After 6 days, protein expression of stem cell markers and differentiation signal factors was confirmed by using western blot analysis. Also, immunocytochemistry was used to confirm the cell fate. Agm treatment activated NPC differentiation significantly more than in the control group, which was evident by the increased expression of a neuronal marker, MAP2, in the LPS-induced, Agm-treated group. Differentiation of LPS-induced, Agm-treated NPCs was regulated by the MAPK pathway and is thought to be related to IL-1 β activation and decreased expression of TLX, a transcription factor that regulates NPC differentiation. Our results reveal that Agm can promote NPC differentiation to neural stem cells by modulating IL-1 β expression under inflammatory condition, and they suggest that Agm may be a novel therapeutic strategy for neuroinflammatory diseases.

Key words: agmatine, neural progenitor cell (NPC), differentiation, lipopolysaccharide, IL-1 β , TLX

INTRODUCTION

In the central nervous system (CNS), inflammation is common sign of neural diseases such as stroke, Alzheimer's disease, and infection. The regulating and inhibiting neuroinflammation

are therapeutic targets to cure a variety of CNS diseases [1-6]. Agmatine (Agm) is an endogenous peptide, synthesized by arginine decarboxylase (ADC) that is reported to be present in glia and neuronal cells. Agm, a neurotransmitter or neuromodulator, is neuroprotective in various models of CNS injury, including neurotrauma, neonatal ischemia, and spinal cord injury [7-10]. Also, Agm has been reported to attenuate oxidative stress in mouse cortical neural stem cells (NSCs) [11]. Agm has been reported to protect neurons by reducing the size of ischemic infarctions or preventing the loss of neurons after focal or global ischemia in vivo. Additionally, Agm protected against neuronal damage

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caused by glucocorticoids and glutamate toxicity in primary hippocampal neuron cultures and cell lines [12-15]. Therefore, Agm can enhance neurogenesis by regulating ERK1/2 expression in subventricular zone NSCs [16]. The phosphatidylinositol 3-kinase (PI3K)/Akt and extracellular signal-regulated kinase (ERK) pathways have been reported to mediate the migration of neuroblasts, which enhances brain ischemia-induced neurogenesis [17]. PI3K and MEK inhibitors reduce carbachol-induced DNA synthesis in progenitor cells [18]. Sung et al. reported that a PI3K inhibitor decreases neural progenitors in hypoxic conditions [19]. In ischemic brain inflammation, a variety of cytokines and chemokines are secreted from damaged brain tissue. Interleukin-1 beta (IL-1 β) is the most common pro-inflammatory cytokine in the brain. Previous studies have reported that elevated IL-1 β levels decrease cell proliferation and neurosphere growth. Also, when neural precursors cells (NPCs) were differentiated in the presence of IL-1 β , there was a significantly lower percentage of new or post-mitotic neurons. Also, some studies have demonstrated that IL-1 β has an anti-proliferative, gliogenic effect on hippocampal neuronal differentiation and survival [20-23]. IL-1 β signaling through the IL-1 receptor type 1 is deleterious to hippocampal neurogenesis. Also, the orphan nuclear receptor tailless homolog (TLX) has recently been identified to regulate neurogenesis and maintain NPCs' undifferentiated state [24, 25]. Previous studies also demonstrated negative relationship between IL-1 β and TLX in NPCs. These studies indicate that IL-1 β reduces NPC proliferation and TLX expression in NPCs [23, 26]. Thus, we aimed to investigate whether Agm modulates IL-1 β and elevated IL-1 β levels regulate TLX expression known as NPC differentiation marker in brain inflammation state. This study suggests that Agm enhances the differentiation of NPCs during brain inflammation *in vitro*.

MATERIALS AND METHODS

Animal and reagents

ICR mice were purchased from Samtako Co. (Kyunggi, Korea). Hank's balanced salt solution (HEPES) was purchased from and Gibco BRL stem cell basal medium was obtained from Stem Cell Technologies. Fetal bovine (FBS) and horse serum were supplied by the Hyclone Company. Agmatine and LPS were obtained from the Sigma Chemical Company. Primary antibodies against PI3K, phospho-ERK, Cleaved caspase 3, IL-1 β , IL-1R1, MAP2, SOX2, GFAP, DCX, TLX and β -actin were purchased from Santa Cruz Biotechnology, Cell Signaling Biotechnology and Millipore Biotechnology. Agmatine was purchased from Sigma Aldrich Biotechnology. All the plastic ware was bought from Nunc.

Primary culture

Embryos (E14) were extracted from placental tissue. The cortices were aseptically dissected out of the brains and placed in Hank's balanced salt solution (Gibco, USA). The tissues were triturated by repeated passage through a fire-polished, constricted Pasteur pipette. The dispersed tissues were allowed to settle for 3 min. The supernatant was transferred to a fresh tube and centrifuged at 1,000 g for 5 min. The pellet was resuspended in NPC basal medium with proliferation supplement (Stem Cell Technologies), 20 ng/ml epidermal growth factor, and fibroblast growth factor (Invitrogen). Trypan blue-excluding cells were counted. The cells were plated in a 35-mm dish at a density of 0.3×10^6 cells/ml. The cells were cultured for 3 days and then treated as indicated. NPCs were used 3 days after treatment [27]. Cultures were maintained in a humidified atmosphere of 95% air and 5% CO₂ at 37°C.

Western blot analysis

Western blots were performed to elucidate the molecular mechanism by which Agmatine affects NPC differentiation. NPCs from each group cultured for 6 DIV were washed with PBS and collected by scraping the neurospheres from the plate. The collected NPCs were homogenized in lysis buffer (1% Triton X-100, 0.5% NP-40, 150 mM NaCl, 10 mM Tris (Ph 7.4), 1 mM Ethylene-diamine tetra acetic acid (EDTA), 1 mM EGTA pH 8.0, 0.2 Mm sodium orthovanadate, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), and protease inhibitor cocktail) and centrifuged (12,000 rpm at 4°C) for 15 min. Equal amounts of protein (20 μ g) from the supernatants were separated on 10% acrylamide gels, and proteins were electrophoretically transferred onto nitrocellulose membranes. The membranes were blocked with Tris-buffered saline with Tween-20 and incubated with primary antibody overnight. The primary antibodies used were PI3K (1: 2000, Millipore, USA), phospho-ERK (1:2000, Millipore, USA), cleaved caspase 3 (1:1000, Cell signaling, USA), IL-1 β (1:2000, Santa Cruz, USA), IL-1R1 (1:2000, Santa Cruz, USA), MAP2 (1:2000, Santa Cruz, USA), SOX2 (1:2000, Santa Cruz, USA), GFAP (1:2000, Santa Cruz, USA), DCX (1:2000, Santa Cruz, USA), TLX (1:2000, Millipore, USA), β -actin (1:1000, Santa Cruz, USA) at 4°C overnight. Later the membranes were washed 3 times for 5 mins each with TBST. The secondary antibodies were anti-rabbit and anti-mouse (1:3000, New England Bio labs, USA) and were used for 1 hour at room temperature. After washing with TBST (0.05% with Tween 20) 3 times, immunoreactive signals were detected by chemiluminescence with an ECL detection system (Amersham Life Science, UK) by using the LAS 4000 program.

Immunocytochemical staining of NPCs

Immunocytochemistry was performed to confirm Agmatine's effect on NPCs. NPCs at 6 DIV were washed 3 times with PBS for immunostaining and were blocked for 30 min. After blocking, NPCs were incubated with primary antibodies prepared in PBS containing 10% FCS and 0.1% Triton X-100, overnight at 4°C. Primary antibodies used were anti-SOX2 (goat polyclonal, 1:300), anti-MAP2 (mouse monoclonal, 1:300), anti-GFAP (rabbit monoclonal, 1:300). After incubating the NPCs with primary antibodies, plates were washed 3 times with PBS for 5 min and were incubated with secondary antibodies which were prepared in PBS containing 10% FCS and 0.1% Triton X-100 for 1 hour at room temperature. The secondary antibodies used were rhodamine rabbit anti-mouse IgG (1:300) and fluorescein isothiocyanate mouse anti-rabbit IgG (1:600). NPCs were counterstained with DAPI for 10 min at room temperature. Immunostained NPCs were visualized using a Carl-Zeiss confocal microscope LSM 700 (Carl Zeiss, Germany).

Statistical analysis

Statistical comparisons were performed by using an independent t-test for 2 groups or analysis of variance with the SPSS software. Values are expressed as mean±S.E.M of 3-6 experiments. Differences were considered significant at #p<0.1, *p<0.05, **p<0.01.

RESULTS

Agmatine promotes ERK signaling under LPS-induced inflammation

To confirm induced signaling pathway induced by Agm under mild inflammation, we conducted a western blot analysis. In this study, LPS was used at 10 ng/ml and Agm at 100 μM on the basis of previous results [28]. Fig. 1A shows that the expression of cleaved caspase 3 protein decreased slightly in the Agm-treat group compared with that in a control group at the same LPS concentration (10 ng/ml). For confirming relatively high caspase 3 levels in control group, we analyse the expression of protein between 0 DIV (at primary culture day) and 6 DIV (our control group). Fig. 1B shows that the expression of cleaved caspase 3 did not differ between 0 DIV and 6 DIV. As this data, we indirectly infer that Agm 100 μM and LPS 10 ng/ml treatment not induces severe cell death to NPCs. For checking differentiation of NPCs under minor inflammation condition, concentrations of Agm 100 μM and LPS 10 ng/ml are suitable in this study. Figs. 1A and 1B show that 10 ng/ml LPS induced mild inflammation. Figs. 1C and 1D show that Agm promoted PI3K and ERK signaling compared with a control group under treated with LPS. Fig. 1E shows the

phosphorylation of AKT and also indirectly activity of PI3K in all conditions. These data indicate that Agm reduces NPC cell death and boosts ERK signaling under inflammation. #p<0.1, *p<0.05, **p<0.001.

Agmatine promotes NPC differentiation by regulating IL-1β under mild inflammatory conditions

To measure the protein expression difference of IL-1β between Agm treatment group and control group under LPS induced inflammatory condition, we conducted western blot analysis. Fig. 2A and Fig. 2B show that Agm treatment increases the expression level of IL-1β and IL-1R1 relatively compare with control group on the same LPS concentration (10 ng/ml). Fig. 2C shows that Agm treatment decreases more the expression level of TLX relatively than control group. Fig. 2 shows that Agm treatment induces increase of IL-1β and IL-1R1 and decrease of TLX in spite of the same LPS concentration (10 ng/ml). *p<0.05, **p<0.001.

Agmatine is related to the differentiation of NPCs into both neuron and glial cells

To assess the cell fate in all groups, we conducted immunocytochemistry using Sox2, MAP2, and GFAP antibodies. Fig. 3A, B shows that Sox2 expression in neurosphere decreased in the LPS only group and in the Agm with LPS group compared to that in the normal control group. Fig. 3C shows that the number of MAP2-positive cells (neurons) in neurosphere tended to increase and the number of GFAP positive cells (astrocytes) in neurosphere tended to decrease in the Agm with LPS treated group compared with levels in the control group. Fig. 3C indicates that neurons tended to increase and astrocytes tended to decrease in cells treated with LPS and Agm compare with control cells. Fig. 3D, E show the quantified MAP2 and GFAP protein levels. These data show neuron and astrocyte patterns in all groups. *p<0.05, **p<0.001.

DISCUSSION

In the CNS, inflammation is a key sign of neuroinflammatory diseases, such as stroke, Alzheimer's disease, and infection. Therapeutic targets for neuroinflammatory diseases include regulating, inhibiting, and preventing neuroinflammation [1-6]. Agm, a polycationic amine synthesized by the decarboxylation of L-arginine, has neuromodulatory and neuroprotective functions in the CNS [29, 30]. We previously demonstrated that Agm protects neurons and astrocytes against ischemic brain injury [15, 31-33]. Agm, as an NMDA receptor blocker, affects calcium homeostasis, modulating various functions in the heart, brain, and vasculature

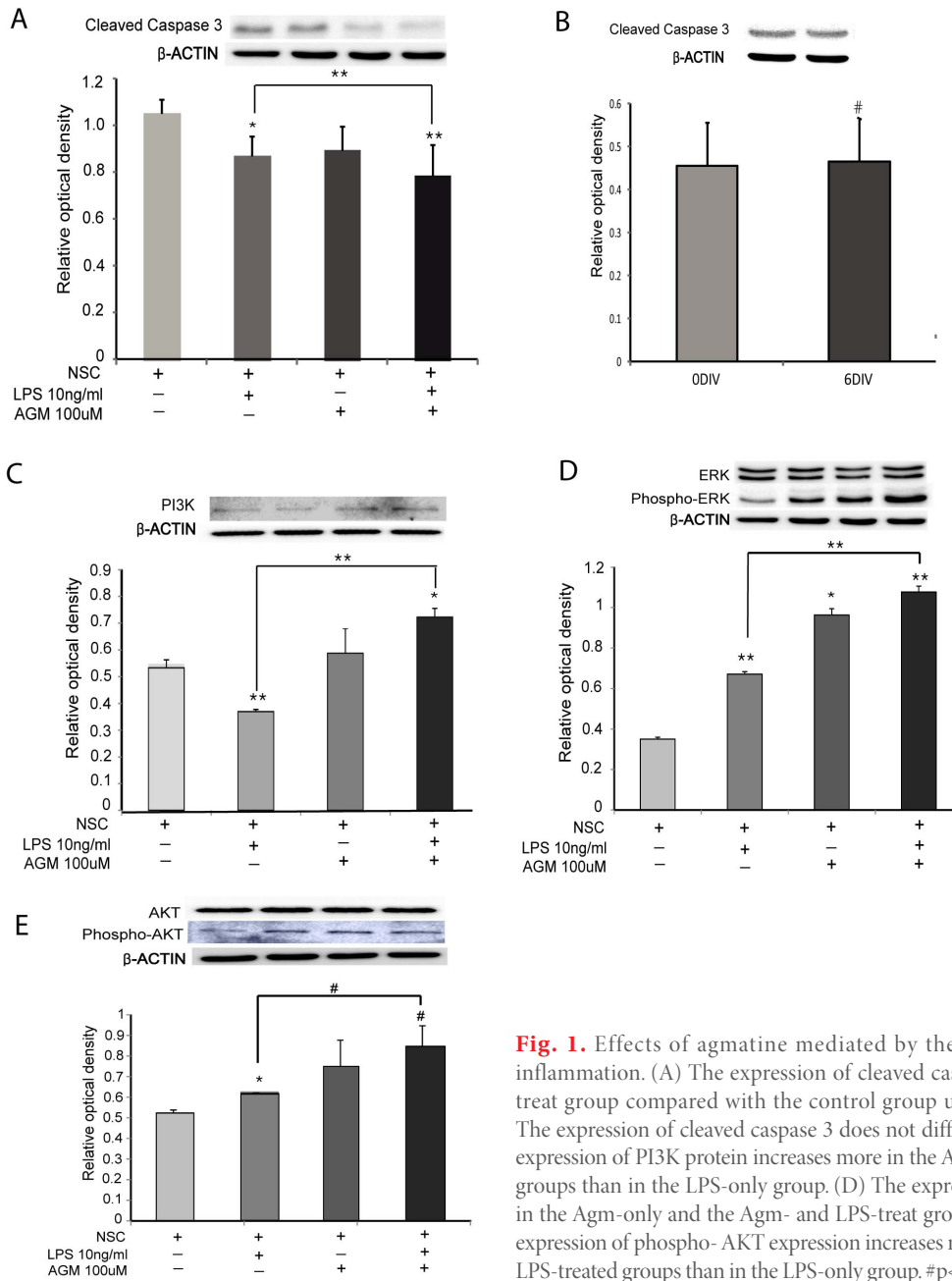


Fig. 1. Effects of agmatine mediated by the ERK pathway under LPS-induced inflammation. (A) The expression of cleaved caspase 3 protein decreases in the Agm-treat group compared with the control group under LPS-induced inflammation. (B) The expression of cleaved caspase 3 does not differ much between 0 and 6 DIV. (C) The expression of PI3K protein increases more in the Agm-only and the Agm- and LPS-treated groups than in the LPS-only group. (D) The expression of phospho-ERK increases more in the Agm-only and the Agm- and LPS-treat groups than in the LPS-only group. (E) The expression of phospho- AKT expression increases more in the Agm-only and the Agm- and LPS-treated groups than in the LPS-only group. #p<0.1, *p<0.05, **p<0.001.

[34]. A previous study reported that Agm treatment (100 μM) up-regulated eNOS expression and maintained functional NO release in bEnd3 cells [35]. We also used 100 μM Agm to study its effects in the cells without toxicity. Our pilot study showed that 10 ng/ml LPS induced mild inflammation (cell viability > 65% following measured data by MTT assay). Therefore, we used LPS to induce mild inflammation and to stimulate IL-1β secretion. Agm has been shown to have anti-proliferative effects in the brain and the peripheral nervous system [36, 37]. Also, previous research found that both ERK1/2 and p38 up-regulate neural differentiation

of ESCs. Song HW et al. reported that Agm regulates ERK1/2 activation and this activation may be sufficient to induce the differentiation of SVZ NSCs. This report suggests that Agm regulates both proliferation and differentiation of specific cell types [37]. Carbachol-induced activation of both PI3K/Akt and the mitogen-activated protein kinase/ERK kinase (MEK) pathways via muscarinic receptors stimulates DNA synthesis in FGF-treated neural progenitors isolated from rat cortical neuroepithelium. PI3K inhibitors (LY294002 and wortmannin) and a MEK inhibitor (PD98059) inhibit carbachol-induced DNA synthesis

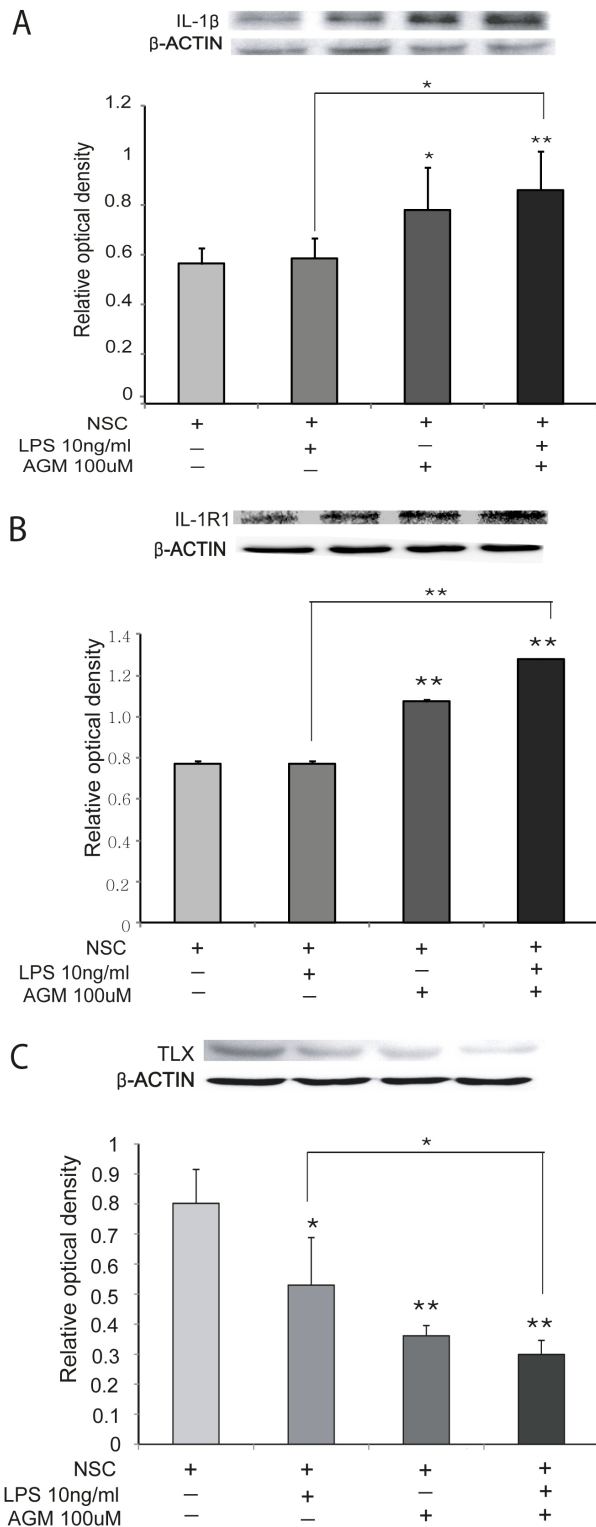


Fig. 2. Effects of agmatine on IL-1β and TLX and subsequent NPC differentiation. (A) IL-1β expression increased more in the Agm-only and the Agm- and LPS-treated groups than in the LPS-only group. (B) IL-1R1 expression increased more in the Agm-only and the Agm- and LPS-treated groups than in the LPS-only group. (C) TLX expression decreased more in the Agm- and LPS-treated group than in the other groups. *p<0.05, **p<0.001.

in progenitor cells [18]. Also, Sung et al. reported that PI3K inhibitors decreased the neural progenitors in hypoxic conditions. The ERK pathway has a central role in differentiation of neural progenitor cells [19]. The ERK pathway, which can act in direct opposition to JNK and p38 MAP kinases, has also been shown to protect against p53-mediated cell death in sympathetic neurons [38, 39]. In this study, the PI3K pathway promoted survival of the Agm treated group and the Agm with LPS group. These data suggest that Agm can induce NPC differentiation by associating with TLX to regulate p21 expression in a p53 dependent manner in inflammation. In ischemic brain inflammation, a variety of cytokines and chemokines are secreted from damaged brain tissue. IL-1β is the most common pro-inflammatory cytokine in the brain. Previous studies have reported that elevated IL-1β levels decrease cell proliferation and neurosphere growth. Also, when NPCs were differentiated in the presence of IL-1β, the percentage of new and post-mitotic neurons decreased significantly. Also, some studies have demonstrated that IL-1β has an anti-proliferative, pro-gliogenic effect on hippocampal neuronal differentiation and survival [20-22, 40]. Our western blots showed increased IL-1β expression in Agm with LPS treated cells compared to LPS-treated cells despite an equal LPS concentration (10 ng/ml). These data means that the co-treatment of Agm (100 μm) and LPS (10 ng/ml) in NPCs could boost the secretion of IL-1β known as neuronal differentiation related cytokine. In brain inflammation, Agm play a role as a booster to induce the secretion of IL-1β for differentiation of NPCs. In addition, recent studies have demonstrated that IL-1 type-1 receptor (IL-1R1) expression is evident in neural progenitors in the DG of the hippocampus in adult rats, E18 rat hippocampal neurons, and NPCs from E16 rat forebrain, E13.5 mouse cortex, and E21 rat hippocampi [21, 41-43]. Previous studies indicate that IL-1R1 is expressed on NSPCs [44, 45]. Therefore, IL-1β expression in proliferating and differentiating NPCs can be prevented by IL-1RA, which prevents IL-1β from binding to IL-1R1 [46]. Other research confirmed that IL-1β it binds to cell surface IL-1R1 [47]. IL-1R1 expression has been shown to be up-regulated in mature hippocampal neurons and astrocytes in the presence of IL-1β [42, 45]. Our results showed increased IL-1R1 protein expression in cells treated with LPS and Agm compared to the LPS-only group. Our western blots for checking the expression of IL-1R1 indicate that Agm increases IL-1β binding to IL-1R1 and the resultant intracellular signaling increases IL-1R1 expression during LPS-stimulated inflammation. The nuclear receptor TLX has recently been identified to regulate adult hippocampal neurogenesis [24, 25] and also has a role in hippocampal-dependent learning and memory [25]. TLX is an essential regulator of proliferation

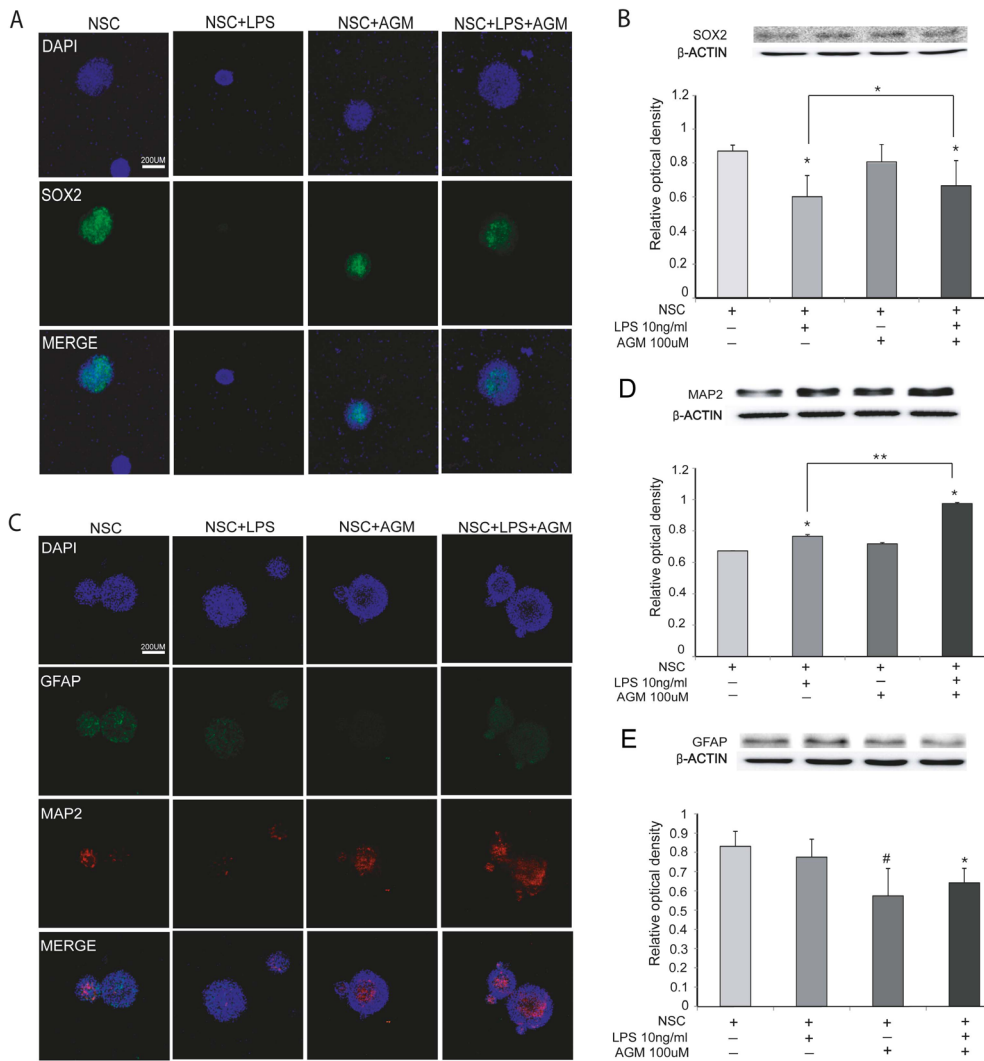


Fig. 3. NPC differentiation measured by photomicrographs and western blot analysis. (A) The number of SOX2-positive cells (green) in neurosphere decreased more in Agm-only and the Agm- and LPS-treated groups than in the LPS only group. DAPI was used to counterstaining (blue). (B) SOX2 protein expression decreased more than in the LPS-only, in the Agm-only, the Agm- and LPS-treated groups than in the control group. (C) The number of MAP2-positive cells (red) in neurosphere increased in the Agm- and LPS-treated group compared to the LPS-only group. The number of GFAP-positive cells (green) in neurosphere was lower in the Agm-only group than in the control group. Scale bar = 200 μ m (\times 100). (D) MAP2 protein expression decreased more in the Agm- and LPS-treated group than in the LPS-only group. (E) GFAP protein expression decreased more in the Agm-only and Agm- and LPS-treated groups than in the LPS-only group. * p <0.05, ** p <0.001.

and its deletion leads to non-proliferative NSCs [48]. TLX is required to maintain neural stem/progenitor cells (NSPCs) in an undifferentiated state and is involved in NSPC fate determination. TLX regulates p21 expression in a p53-dependent manner. The effects of IL-1 β in NSPCs are paralleled by decreased TLX expression, and IL-1 β expression is related to decreased TLX expression [23, 26]. Therefore, Agm promotes ERK signaling, and TLX regulates p53-dependent p21 expression. The network between the ERK and p53 pathways could explain the correlation between Agm and TLX in NPC differentiation. In our study, TLX protein expression decreased more in the LPS and Agm group than in the control group, similar to previous studies. These data indicate that Agm may inhibit IL-1 β -induced TLX expression in NSPCs. Therefore, SRY-box-containing gene 2 (Sox2) a transcriptional regulator in NPC differentiation forms a molecular network with TLX in adult NSCs. Both Sox2 and TLX bind to the upstream region of the Tlx gene. Sox2 binds to the Tlx

5' UTR chromatin and activates the Tlx promoter in adult NSCs [49]. In this study, Sox2 immunocytochemistry and western blot data suggest that Agm may decrease TLX and Sox2 expression for inducing differentiation on NPCs in brain inflammation state. Previous studies indicate that Agm enhances neurogenesis and suppresses astrogensis by decreasing BMP-2 and -4 and SMAD-1, -5, and -8 protein expression [27]. Our immunocytochemistry data means that Agm increases the differentiation of neurons and decreases the differentiation of astrocytes on NPCs in LPS-induced inflammation. However, we could not determine the exact portion of neurons and glial cells. Increasing IL-1 β expression and decreasing TLX expression induces NPC differentiation. Agm could induce NPC differentiation by modulating IL-1 β in brain inflammation condition. The network between ERK signaling activated by Agm and p53 signaling activated by TLX could explain the correlation between Agm and TLX in NPC differentiation. In conclusion, Agm modulates

NPC differentiation by regulating IL-1 β expression under LPS stimulated inflammation condition. Although our data show the synergetic effect of Agm and LPS regarding secreting IL-1 β , we could not suggest the optimal quantitative concentration range of IL-1 β for differentiation of NPCs. A further study to quantify IL-1 β expression is important to determine a therapeutic strategy of Agm for inflammatory brain diseases. This study strongly suggests that Agm regulate the differentiation of NPCs by modulating differentiation related factors including IL-1 β and TLX in spite of this limitation. Hence, Agm may be a promising therapeutic agent to promote NPC differentiation in neuroinflammatory diseases.

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REFERENCES

- Shastri A, Bonifati DM, Kishore U (2013) Innate immunity and neuroinflammation. *Mediators Inflamm* 2013:342931.
- Kim WG, Mohney RP, Wilson B, Jeohn GH, Liu B, Hong JS (2000) Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. *J Neurosci* 20:6309-6316.
- Streit WJ, Graeber MB, Kreutzberg GW (1988) Functional plasticity of microglia: a review. *Glia* 1:301-307.
- Lee SC, Liu W, Dickson DW, Brosnan CF, Berman JW (1993) Cytokine production by human fetal microglia and astrocytes. Differential induction by lipopolysaccharide and IL-1 beta. *J Immunol* 150:2659-2667.
- Rothwell NJ, Relton JK (1993) Involvement of cytokines in acute neurodegeneration in the CNS. *Neurosci Biobehav Rev* 17:217-227.
- Giulian D (1987) Ameboid microglia as effectors of inflammation in the central nervous system. *J Neurosci Res* 18:155-171.
- Uzbay TI (2012) The pharmacological importance of agmatine in the brain. *Neurosci Biobehav Rev* 36:502-519.
- Halaris A, Plietz J (2007) Agmatine : metabolic pathway and spectrum of activity in brain. *CNS Drugs* 21:885-900.
- Feng Y, Piletz JE, Leblanc MH (2002) Agmatine suppresses nitric oxide production and attenuates hypoxic-ischemic brain injury in neonatal rats. *Pediatr Res* 52:606-611.
- Gilad GM, Salame K, Rabey JM, Gilad VH (1996) Agmatine treatment is neuroprotective in rodent brain injury models. *Life Sci* 58:PL 41-46.
- Bokara KK, Kwon KH, Nho Y, Lee WT, Park KA, Lee JE (2011) Retroviral expression of arginine decarboxylase attenuates oxidative burden in mouse cortical neural stem cells. *Stem Cells Dev* 20:527-537.
- Yang XC, Reis DJ (1999) Agmatine selectively blocks the N-methyl-D-aspartate subclass of glutamate receptor channels in rat hippocampal neurons. *J Pharmacol Exp Ther* 288:544-549.
- Olmos G, DeGregorio-Rocasolano N, Paz Regalado M, Gasull T, Assumpció Boronat M, Trullas R, Villaruel A, Lerma J, García-Sevilla JA (1999) Protection by imidazol(ine) drugs and agmatine of glutamate-induced neurotoxicity in cultured cerebellar granule cells through blockade of NMDA receptor. *Br J Pharmacol* 127:1317-1326.
- Mun CH, Lee WT, Park KA, Lee JE (2010) Regulation of endothelial nitric oxide synthase by agmatine after transient global cerebral ischemia in rat brain. *Anat Cell Biol* 43:230-240.
- Kim JH, Yenari MA, Giffard RG, Cho SW, Park KA, Lee JE (2004) Agmatine reduces infarct area in a mouse model of transient focal cerebral ischemia and protects cultured neurons from ischemia-like injury. *Exp Neurol* 189:122-130.
- Song L, Shen B, Li Y (2001) Association and contribution of ERK to IL-6-induced activation of signal transducer and activator of transcription in a human myeloma cell line. *Chin Med J (Engl)* 114:954-957.
- Wang WP, Iyo AH, Miguel-Hidalgo J, Regunathan S, Zhu MY (2006) Agmatine protects against cell damage induced by NMDA and glutamate in cultured hippocampal neurons. *Brain Res* 1084:210-216.
- Li H, He C, Zheng J (2001) Mechanism of the activation of extracellular signal-regulated kinase (ERK) in prostate cancer cell lines with different metastatic potential. *Zhonghua Yi Xue Za Zhi* 81:197-200.
- Sung SM, Jung DS, Kwon CH, Park JY, Kang SK, Kim YK (2007) Hypoxia/reoxygenation stimulates proliferation through PKC-dependent activation of ERK and Akt in mouse neural progenitor cells. *Neurochem Res* 32:1932-1939.
- Zunszain PA, Anacker C, Cattaneo A, Choudhury S, Musaelyan K, Myint AM, Thuret S, Price J, Pariante CM (2012) Interleukin-1beta: a new regulator of the kynurenine pathway affecting human hippocampal neurogenesis. *Neuropsychopharmacology* 37:939-949.
- Koo JW, Duman RS (2008) IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc*

- Natl Acad Sci U S A 105:751-756.
22. Yirmiya R, Goshen I (2011) Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain Behav Immun* 25:181-213.
 23. Green HE, Treacy E, Keohane AK, Sullivan AM, O'Keefe GW, Nolan YM (2012) A role for interleukin-1beta in determining the lineage fate of embryonic rat hippocampal neural precursor cells. *Mol Cell Neurosci* 49:311-321.
 24. Elmi M, Matsumoto Y, Zeng ZJ, Lakshminarasimhan P, Yang W, Uemura A, Nishikawa S, Moshiri A, Tajima N, Agren H, Funa K (2010) TLX activates MASH1 for induction of neuronal lineage commitment of adult hippocampal neuroprogenitors. *Mol Cell Neurosci* 45:121-131.
 25. Shi Y, Chichung Lie D, Taupin P, Nakashima K, Ray J, Yu RT, Gage FH, Evans RM (2004) Expression and function of orphan nuclear receptor TLX in adult neural stem cells. *Nature* 427:78-83.
 26. Ryan SM, O'Keefe GW, O'Connor C, Keeshan K, Nolan YM (2013) Negative regulation of TLX by IL-1beta correlates with an inhibition of adult hippocampal neural precursor cell proliferation. *Brain Behav Immun* 33:7-13.
 27. Song HW, Kumar BK, Kim SH, Jeon YH, Lee YA, Lee WT, Park KA, Lee JE (2011) Agmatine enhances neurogenesis by increasing ERK1/2 expression, and suppresses astrogenesis by decreasing BMP 2,4 and SMAD 1,5,8 expression in subventricular zone neural stem cells. *Life Sci* 89:439-449.
 28. Jung HJ, Yang MZ, Kwon KH, Yenari MA, Choi YJ, Lee WT, Park KA, Lee JE (2010) Endogenous agmatine inhibits cerebral vascular matrix metalloproteinases expression by regulating activating transcription factor 3 and endothelial nitric oxide synthesis. *Curr Neurovasc Res* 7:201-212.
 29. Reis DJ, Regunathan S (1999) Agmatine: an endogenous ligand at imidazoline receptors is a novel neurotransmitter. *Ann N Y Acad Sci* 881:65-80.
 30. Reis DJ, Regunathan S (2000) Is agmatine a novel neurotransmitter in brain? *Trends Pharmacol Sci* 21:187-193.
 31. Hong S, Kim CY, Lee JE, Seong GJ (2009) Agmatine protects cultured retinal ganglion cells from tumor necrosis factor-alpha-induced apoptosis. *Life Sci* 84:28-32.
 32. Kim JW, Kim TD, Hong BS, Kim OY, Yoon WH, Chae CB, Gho YS (2010) A serum-stable branched dimeric anti-VEGF peptide blocks tumor growth via anti-angiogenic activity. *Exp Mol Med* 42:514-523.
 33. Ahn SK, Hong S, Park YM, Choi JY, Lee WT, Park KA, Lee JE (2012) Protective effects of agmatine on lipopolysaccharide-injured microglia and inducible nitric oxide synthase activity. *Life Sci* 91:1345-1350.
 34. Raghavan SA, Dikshit M (2004) Vascular regulation by the L-arginine metabolites, nitric oxide and agmatine. *Pharmacol Res* 49:397-414.
 35. Yang MZ, Mun CH, Choi YJ, Baik JH, Park KA, Lee WT, Lee JE (2007) Agmatine inhibits matrix metalloproteinase-9 via endothelial nitric oxide synthase in cerebral endothelial cells. *Neurol Res* 29:749-754.
 36. Li G, Regunathan S, Reis DJ (1995) Agmatine is synthesized by a mitochondrial arginine decarboxylase in rat brain. *Ann N Y Acad Sci* 763:325-329.
 37. Morrissey J, McCracken R, Ishidoya S, Klahr S (1995) Partial cloning and characterization of an arginine decarboxylase in the kidney. *Kidney Int* 47:1458-1461.
 38. Anderson CN, Tolkovsky AM (1999) A role for MAPK/ERK in sympathetic neuron survival: protection against a p53-dependent, JNK-independent induction of apoptosis by cytosine arabinoside. *J Neurosci* 19:664-673.
 39. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* 270:1326-1331.
 40. Green HE, Nolan YM (2012) Unlocking mechanisms in interleukin-1beta-induced changes in hippocampal neurogenesis--a role for GSK-3beta and TLX. *Transl Psychiatry* 2:e194.
 41. Cunningham ET Jr, Wada E, Carter DB, Tracey DE, Battey JF, De Souza EB (1992) In situ histochemical localization of type I interleukin-1 receptor messenger RNA in the central nervous system, pituitary, and adrenal gland of the mouse. *J Neurosci* 12:1101-1114.
 42. Friedman WJ (2001) Cytokines regulate expression of the type I interleukin-1 receptor in rat hippocampal neurons and glia. *Exp Neurol* 168:23-31.
 43. Ajmone-Cat MA, Cacci E, Ragazzoni Y, Minghetti L, Biagioni S (2010) Pro-gliogenic effect of IL-1alpha in the differentiation of embryonic neural precursor cells in vitro. *J Neurochem* 113:1060-1072.
 44. Crampton SJ, Collins LM, Toulouse A, Nolan YM, O'Keefe GW (2012) Exposure of foetal neural progenitor cells to IL-1beta impairs their proliferation and alters their differentiation - a role for maternal inflammation? *J Neurochem* 120:964-973.
 45. Nolan Y, Martin D, Campbell VA, Lynch MA (2004) Evidence of a protective effect of phosphatidylserine-containing liposomes on lipopolysaccharide-induced impairment of long-term potentiation in the rat hippocampus. *J Neuroimmunol* 151:12-23.
 46. Arend WP, Welgus HG, Thompson RC, Eisenberg SP (1990)

- Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist. *J Clin Invest* 85:1694-1697.
47. Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G (1995) Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. *J Biol Chem* 270:13757-13765.
48. Niu W, Zou Y, Shen C, Zhang CL (2011) Activation of postnatal neural stem cells requires nuclear receptor TLX. *J Neurosci* 31:13816-13828.
49. Shimosaki K, Zhang CL, Suh H, Denli AM, Evans RM, Gage FH (2012) SRY-box-containing gene 2 regulation of nuclear receptor tailless (Tlx) transcription in adult neural stem cells. *J Biol Chem* 287:5969-5978.