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Oroxylin A Induces BDNF Expression on Cortical Neurons through Adenosine $A2_A$ Receptor Stimulation: A Possible Role in Neuroprotection

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Abstract

Oroxylin A is a flavone isolated from a medicinal herb reported to be effective in reducing the inflammatory and oxidative stresses. It also modulates the production of brain derived neurotrophic factor (BDNF) in cortical neurons by the transactivation of cAMP response element-binding protein (CREB). As a neurotrophin, BDNF plays roles in neuronal development, differentiation, synaptogenesis, and neural protection from the harmful stimuli. Adenosine $A2_A$ receptor colocalized with BDNF in brain and the functional interaction between $A2_A$ receptor stimulation and BDNF action has been suggested. In this study, we investigated the possibility that oroxylin A modulates BDNF production in cortical neuron through the regulation of $A2_A$ receptor system. As expected, CGS21680 ($A2_A$ receptor agonist) induced BDNF expression and release, however, an antagonist, ZM241385, prevented oroxylin A-induced increase in BDNF production. Oroxylin A activated the PI3K-Akt-GSK-3β signaling pathway, which is inhibited by ZM241385 and the blockade of the signaling pathway abolished the increase in BDNF production. The physiological roles of oroxylin A-induced BDNF production were demonstrated by the increased neurite extension as well as synapse formation from neurons. Overall, oroxylin A might regulate BDNF production in cortical neuron through $A2_A$ receptor stimulation, which promotes cellular survival, synapse formation and neurite extension.

Key Words: Oroxylin A, BDNF, CREB, Adenosine $A2_A$ receptor, CGS21680, ZM241385

INTRODUCTION

Oroxylin A (5,7-dihidrixy-6-methoxyflavone) is a flavonoid originated from the root of *Scutellaria baicalensis Georgi*, which acts as a γ-aminobutyric acid ($GABA_A$) receptor antagonist (Huen *et al.*, 2003). Oroxylin A ameliorated memory dysfunction induced by scopolamine (Kim *et al.*, 2007), and Aβ (25-35) (Kim *et al.*, 2008) and also increased the number of phosphorylated cAMP response element-binding protein (CREB) and brain derived neurotrophic factor (BDNF) positive cells (Kim *et al.*, 2006), which might be associated with the neuroprotective effects of oroxylin A in cultured rat primary neuron (Jeon *et al.*, 2011).

BDNF is a member of the neurotrophin family (Lewin, 1996) which plays important roles in central nervous system (CNS) such as protection of neuronal degeneration (Lindholm *et al.*, 1993), differentiation of hippocampal and cortical neurons (Ip *et al.*, 1993; Croll *et al.*, 1994; Nawa *et al.*, 1994; Marty *et al.*, 1996) and synaptogenesis (Shen *et al.*, 2006). It is also well known that BDNF is involved in the regulation of neurite out-

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growth as well as directional movement *via* a variety of different mechanisms (Bartrup *et al.*, 1997; Winckler, 2007; Sasaki *et al.*, 2010).

Adenosine is a purine nucleoside, which transmits its physiological signal through adenosine receptors. Four subtypes of adenosine receptors have been described to date, A1, $A2_A$, $A2_B$, and A3 subtypes (Tucker and Linden, 1993), which are classified as 2 categories by functions; A1 and A3 which are negatively coupled to adenylate cyclase *via* G proteins and $A2_A$ and $A2_B$ which are positively coupled to the same effectors (Proll *et al.*, 1986; Moser *et al.*, 1991). Among them, $A2_A$ receptor modulates tonic expression of BDNF as well as synaptic actions of BDNF on hippocampal neurons (Diógenes *et al.*, 2004; Tebano *et al.*, 2008). $A2_A$ receptor also activates one of BDNF receptors, tropomyosin-related kinase B (TrkB), and Akt signaling molecule, which promotes motor neuron survival (Wiese *et al.*, 2007) and modulates neurite outgrowth in several different cell types (Cheng *et al.*, 2002; Canals *et al.*, 2005; O'Driscoll and Gorman, 2005). Greengard group showed that $A2_A$ receptor-mediated modulation of PC12 cell differentiation and neurite extension in collaboration with FGF receptors (Flajolet *et al.*, 2008). These strong trophic actions of $A2_A$ receptor activation make it one of the promising targets for several psychiatric and neurodegerative diseases (Cunha *et al.*, 2008).

The positive relationship of $A2_A$ receptor activation and increased BDNF action as well as our preliminary results suggesting that oroxylin A may bind to $A2_A$ receptor (our unpublished results) prompted us to investigate whether oroxylin A might regulate BDNF production *via* modulating $A2_A$ receptor on cultured rat primary neurons using a pharmacological agonist and antagonist. We also investigated the possible intracellular signaling pathway mediating the increased BDNF production by oroxylin A as well as the role of oroxylin A on neurite extension.

MATERIALS AND METHODS

Materials

Neurobasal medium was purchased from GIBCO BRL (NY, USA) and B-27 supplements was obtained from Invitrogen (CA, USA). The BDNF ELISA kit was from Promega (Madison, WI). Specific primary antibody against BDNF was purchased from Santa Cruz Biotechnology Inc. (sc-546, Santa Cruz, CA), and Tuj-1 was from Covance (Richmond, CA). Other phospho- and total- form of antibodies (CREB, ERK, Akt, and GSK-3β) were obtained from Cell Signaling Technology (Beverly, MA, USA). Oroxylin A was obtained from Korea food & drug administration. U0126 and wortmannin were obtained from Calbiochem (San Diego, CA). CGS21680 and ZM241385 were purchased from Tocris Bioscience (Bristol, UK), and all other chemical reagents were purchased from Sigma (St. Louis, MO, USA).

Methods

Cell culture: Primary cortical neurons were isolated from embryonic cerebral cortex of Sprague-Dawley rat (SD rat) as previously described (Jeon *et al.*, 2011). Cerebral cortex obtained from E16 pups was digested and re-suspended in NBM containing B-27 and then cells were placed on poly-D-lysine (PDL) pre-coated plates. The cultures were kept in a humidified 10% CO_2 atmosphere at 37°C for 10 days and media were half-replaced with fresh media every 3 days.

Drug treatment: Inhibitors were pre-treated 1 hr before the oroxylin A treatment. As an ERK1/2 phosphorylation inhibitor, U0126 was used at 10 μM and wortmannin was used as an Akt inhibitor at 100 nM. To modulate the activity of adenosine $A2_A$ receptor, an antagonist (50 nM of ZM241385) and an agonist (20 nM of CGS21680) was used.

Western blot: Treated cells were lysed with 2× sample buffer (4% w/v SDS, 20% glycerol, 200 mM DTT, 0.1 M Tris-HCl, pH 6.8, and 0.02% bromophenol blue) and the samples were fractionated by 8-12% SDS-PAGE and electrotransferred to nitrocellulose (NC) membrane. The NC membrane was blocked with 1 μg/ml polyvinyl alcohol (PVA) for 0.5 hr at room temperature (RT) and incubated overnight at 4°C with the appropriate primary antibodies which were diluted at 1:5000 in 5% skim milk (Roth, Germany). After washing with Tris-buffered saline containing 0.1% Tween20 (TBS-T), NC membranes were incubated with peroxidase conjugated secondary antibody for 2 hr at RT. Following three times of washings with TBS-T, blots were detected by enhanced chemiluminescence (Amersham, Buckinghampshire, UK).

Reverse transcription polymerase chain reaction (RT-PCR): Cellular total RNA was extracted from primary neurons using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and 2 μg of them was converted to cDNA (Maxime RT PreMix Kit, iNtRON Biotechnology, Seoul) according to the manufacturer's protocol. The PCR amplification was performed using Maxime PCR premix Kit (iNtRON Biotechnology, Seoul) and was consisted of 26 cycles with the oligonucleotide primers for BDNF (accession number EF125679.1, Tm=60°C, (Kobayashi *et al.*, 2008)), $GABA_AR2$ (accession number NM_001135779.1, Tm=60°C), $GABA_AR5$ (accession number NM_017295.1, Tm=55°C), $A2_AR$ (accession number L08102, Tm=55°C), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, accession number M17701, Tm=60°C). The following primers were used for amplification reactions:

for BDNF,
forward primer : 5'-ATA GGA GAC CCT CCG CAA CT-3'
reverse primer : 5'-CTG CCA TGC ATG AAA CAC TT-3'

for GAPDH,
forward primer : 5'-TCC CTC AAG ATT GTC AGC AA-3'
reverse primer : 5'-AGA TCC ACA ACG GAT ACA TT-3'

for $GABA_AR2$,
forward primer : 5'- CGG TGC CAG CGA GAA CTG TGT-3'
reverse primer : 5'- GGG CGT AGT TGG CAA CGG CT-3'

for $GABA_AR5$,
forward primer : 5'-GCC CGG AAT TCG CTG CCC AA-3'
reverse primer : 5'- GTC CCG CCT GGA AGC TGC TC -3'

for $A2_AR$,
forward primer : 5'-CCA TGC TGG GCT GGA ACA-3'
reverse primer : 5'-GAA GGG GCA GTA ACA CGA -3'

The amplified products were analyzed on 1% agarose gel and stained with EtBr. The expected size of the amplified DNA fragments was 280 base pairs for BDNF, 297 base pairs for $GABA_AR$ alpha subunit 2, 275 base pairs for $GABA_AR$ alpha

subunit 5, and 308 base pairs for GAPDH.

ELISA assay: The amount of released BDNF was quantified from medium of treated neuron using the Emax ImmunoAssay system (Promega, Madison, WI). BDNF enzyme-linked immunosorbent assay (ELISA) was performed according to the manufacturer's manual. Briefly, 96 well plates were pre-coated with anti-BDNF mAb diluted with carbonate coating buffer at 4°C for 24 hr. After 1 hr blockade with supplied Blocking buffer, the plates were incubated with standard and culture medium sample for 2 hr at RT followed by incubation with anti-human BDNF pAb. After 1 hr incubation of Anti-IgY HRP conjugate, the reaction was developed with tetramethylbenzidine (TMB One Solution) and the absorbance was read at 450 nm with a microplate reader (Tecan Trading AG, Switzerland) after stopping the reaction with 1N HCl.

Immunocytochemistry: DIV 2 primary cortical neurons plat-

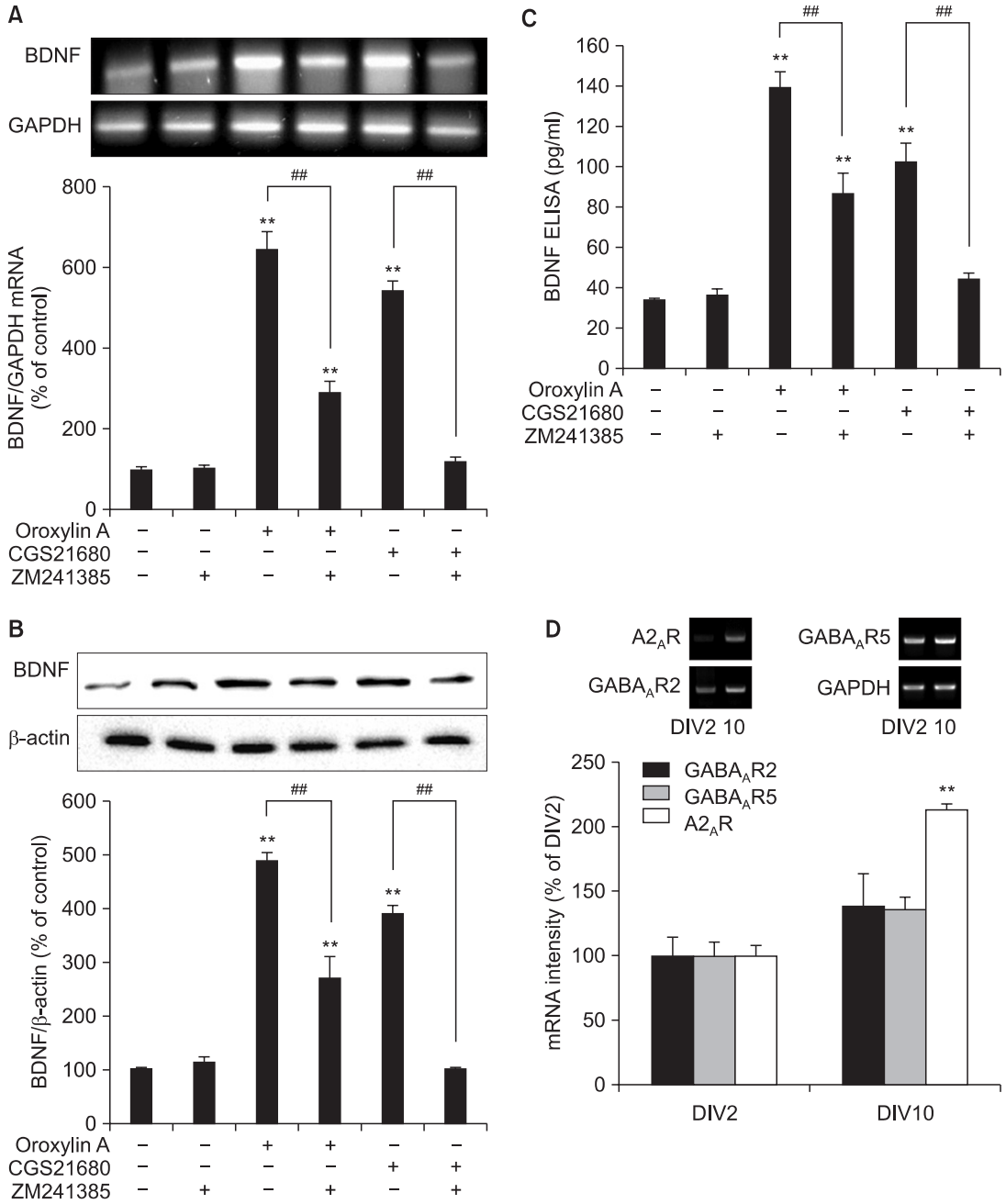

Fig. 1. Oroxylin A induced BDNF production in cortical neurons through adenosine $A2_A$ receptor. (A) Oroxylin A (20 μM), CGS21680 (20 nM), and ZM241385 (50 nM) was treated to primary cortical neurons for 24 hr and cells were lysed and analyzed by RT-PCR. (B) In case of BDNF protein level, samples were analyzed by Western blot. (C) Released BDNF was quantified by ELISA assay. (D) Cortical neurons were collected at DIV2, and DIV10 to confirm the expression of $A2_AR$ and $GABA_AR$, respectively. Each graph represents quantification of RT-PCR and Western blot band intensity, respectively. Data represent Mean ± S.E.M. **Significantly different as compared with control and ##significantly different as compared with oroxylin A or CGS21680 stimulation (p<0.01, n=4).

ed on the PDL pre-coated cover glasses (Fisher Scientific, PA) were treated with 20 μM of oroxylin A for 24 hr. Glasses were washed twice with PBS and fixed with 4% paraformaldehyde (PFA) for 0.5 hr. After three times of washing, samples were permeabilized using 0.3% Triton X-100 solution for 15 min at RT and blocked by blocking buffer (1% BSA, 5% FBS in PBS) for 30 min at RT. Samples were incubated overnight at 4°C with the primary antibody against neuron (Tuj-1, 1:500 in blocking

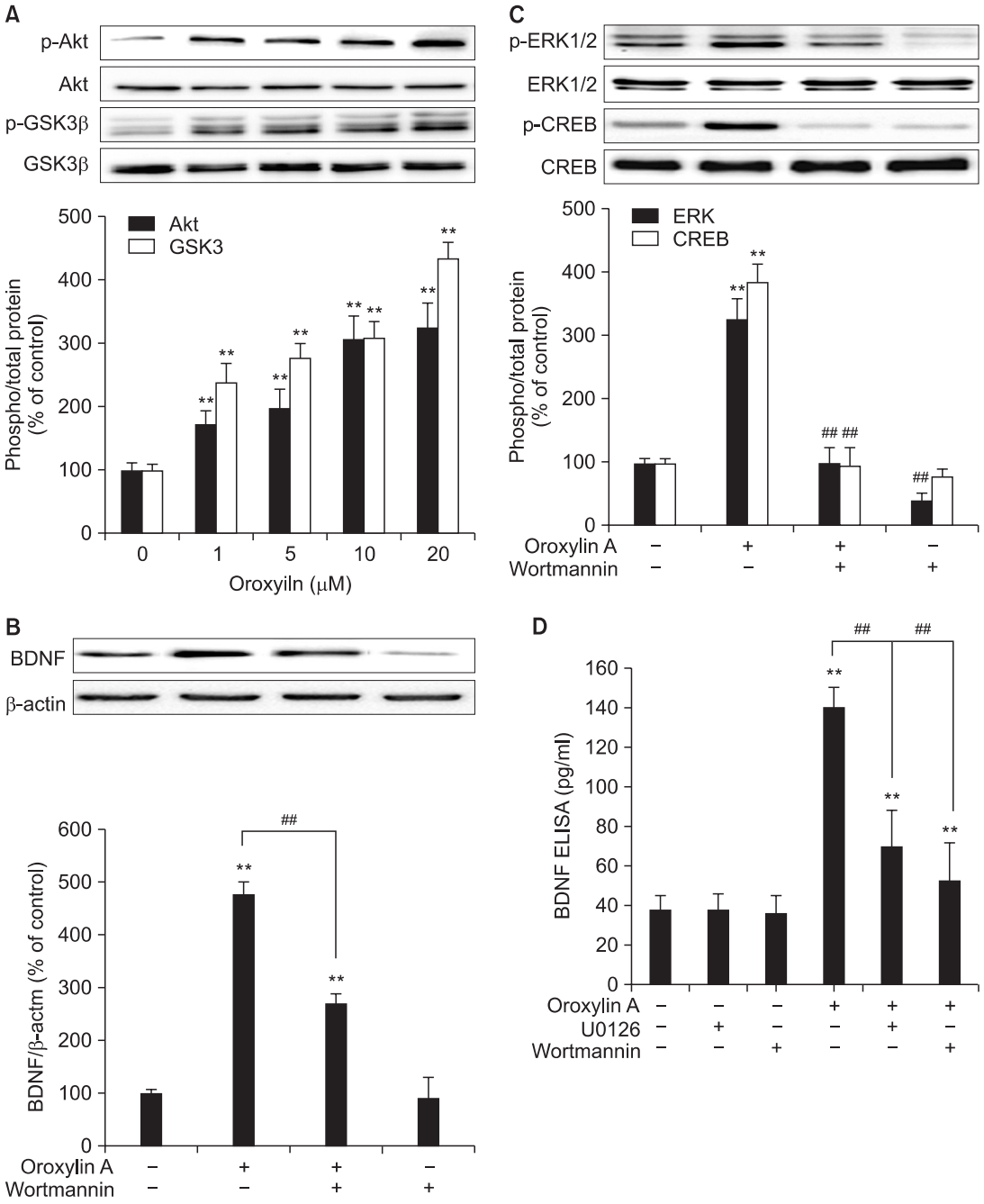

Fig. 2. Oroxylin A induced the activation of Akt, which was necessary for the phosphorylation of ERK1/2-CREB. (A) Rat primary cortical neurons were treated with oroxylin A (1, 5, 10, 20 μM) for 3 hr and analyzed by Western blot. Phospho/total- Akt and GSK-3β ratio was determined by densitometric quantification of Western blot. (B) A PI3K activation inhibitor, wortmannin, was used to investigate the role of Akt and GSK-3β signaling on BDNF production. After 1 hr treatment of wortmannin (100 nM), cells were treated with oroxylin A for 24 hr and the level of BDNF protein expression was measured by Western blot. (C) ERK1/2 and CREB phosphorylation was determined after wortmannin and oroxylin A treatment. (D) The level of BDNF release was measured using ELISA assay from U0126-, wortmannin-, or oroxylin A-treated cell culture supernatants. Detailed protocol was described in Materials and Methods. Each graph represents quantification of Western blot band intensity. Data represent Mean ± S.E.M. **Significantly different as compared with control and ##significantly different as compared with oroxylin A or CGS21680 stimulation (p<0.01, n=4).

buffer). Next day, after washing, samples were incubated for 2 hr with secondary antibodies conjugated with TMRE (diluted at 1:500 in blocking buffer). Then samples were washed and mounted using Vectashield (Vector laboratories, Burlingame, CA, USA). Cellular images were observed by fluorescence microscope (motorized research microscope bx61, Olympus, Japan) and analyzed by Image J software (NIH, USA).

Statistics

Data are expressed as the mean ± standard error of mean (S.E.M.) and analyzed for statistical significance by using one way analysis of variance (ANOVA) followed by Newman-Keuls test as a post hoc test and a *p* value<0.05 was considered significant.

RESULTS

Oroxylin A stimulated BDNF expression and release in rat primary neurons through Adenosine $A2_A$ receptor activation

Treatment of oroxylin A or an $A2_A$ receptor agonist, CGS21680 increased BDNF mRNA level 6.24 ± 0.44 and 4.65 ± 0.26 folds compared with control, respectively (Fig. 1A). Co-treatment with an $A2_A$ antagonist ZM241385 inhibited the increased BDNF mRNA expression induced by oroxylin A or CGS21680. Similar pattern of changes was also observed for BDNF protein level (Fig. 1B) as well as BDNF release (Fig. 1C), which was determined by Western blot and ELISA, respectively. nterestingly, while $A2_A$ receptor antagonist ZM241385 completely prevented the increased expression of BDNF induced by $A2_A$ receptor agonist CGS21680, ZM241385 only partially inhibited oroxylin A-mediated increase in BDNF expression. To investigate the expression of $A2_A$ receptor in rat primary neuron, we performed RT-PCR analysis. The expression of $A2_A$ receptor was confirmed ar DIV 2 neuron, which was increased in DIV 10. Similar pattern was also observed with $GABA_A$ receptor (Fig. 1D). These results suggest that oroxylin A may induce BDNF expression by $A2_A$ receptor activation as well as other intracellular signaling pathways.

Oroxylin A-induced BDNF production was mediated by Akt and GSK-3β phosphorylation in rat primary neurons

We previously reported that oroxylin A-induced BDNF production was mediated by CREB and ERK1/2 phosphorylation (Kim *et al.*, 2008; Jeon *et al.*, 2011). Because Akt-GSK3β pathway is a downstream pathway of $A2_A$ receptor activation and is also involved in the modulation of BDNF production (Mai *et al.*, 2002), we next investigated the activation of Akt-GSK3β pathway by oroxylin A (Fig. 2A). Oroxylin A induced phosphorylation of Akt and its downstream target GSK-3β in a concentration dependent manner (Fig. 2A). At the highest concentration of oroxylin A (20 μM), phosphorylation of Akt and GSK-3β reached 319.07 ± 36% and 431.20 ± 28.31% of control level, respectively (Fig. 2A). Oroxylin A-induced BDNF protein expression determined by Western blot was inhibited by pretreatment of an Akt inhibitor, wortmannin (Fig. 2B) and in this condition, ERK1/2 and CREB phosphorylation, a whole mark of transcriptional activation of oroxylin A-induced BDNF expression, was also inhibited as reported previously (Fig. 2C) (Jeon *et al.*, 2011). We next measured the release of BDNF using ELISA assay and either Akt inhibitor wortmannin or

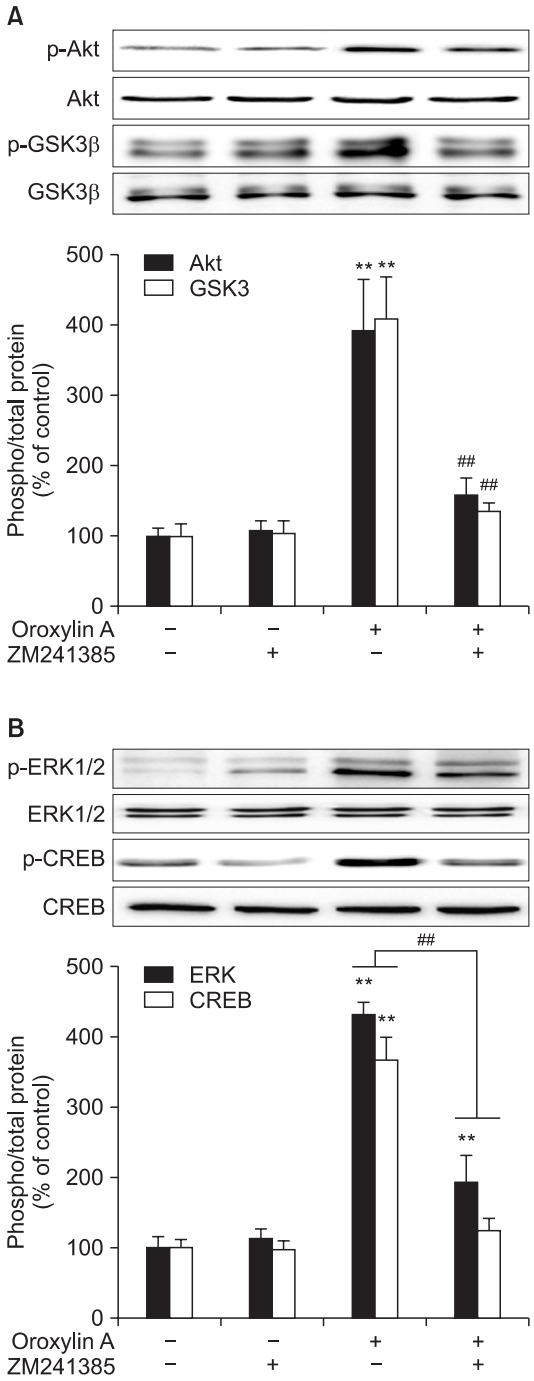

Fig. 3. Adenosine $A2_A$ receptor mediates oroxylin A-induced Akt and ERK1/2 phosphorylation and BDNF production. To identify the signaling pathway involved in the Adenosine $A2_A$ receptor-mediated stimulation of BDNF production, phosphorylation level of Akt and ERK1/2 were analyzed using Western blot. (A, B) Cells were treated with ZM241385 (50 nM) for 1 hr before the treatment of oroxylin A (20 μM). After 1 hr, cells were harvested to analyze the level of phospho/total Akt-GSK-3β (A) and ERK1/2- CREB (B) by Western blot. Each graph represents quantification of Western blot band intensity. Data represent the mean ± S.E.M. **Significantly different as compared with control and ##significantly different as compared with oroxylin A stimulation (p<0.01, n=4).

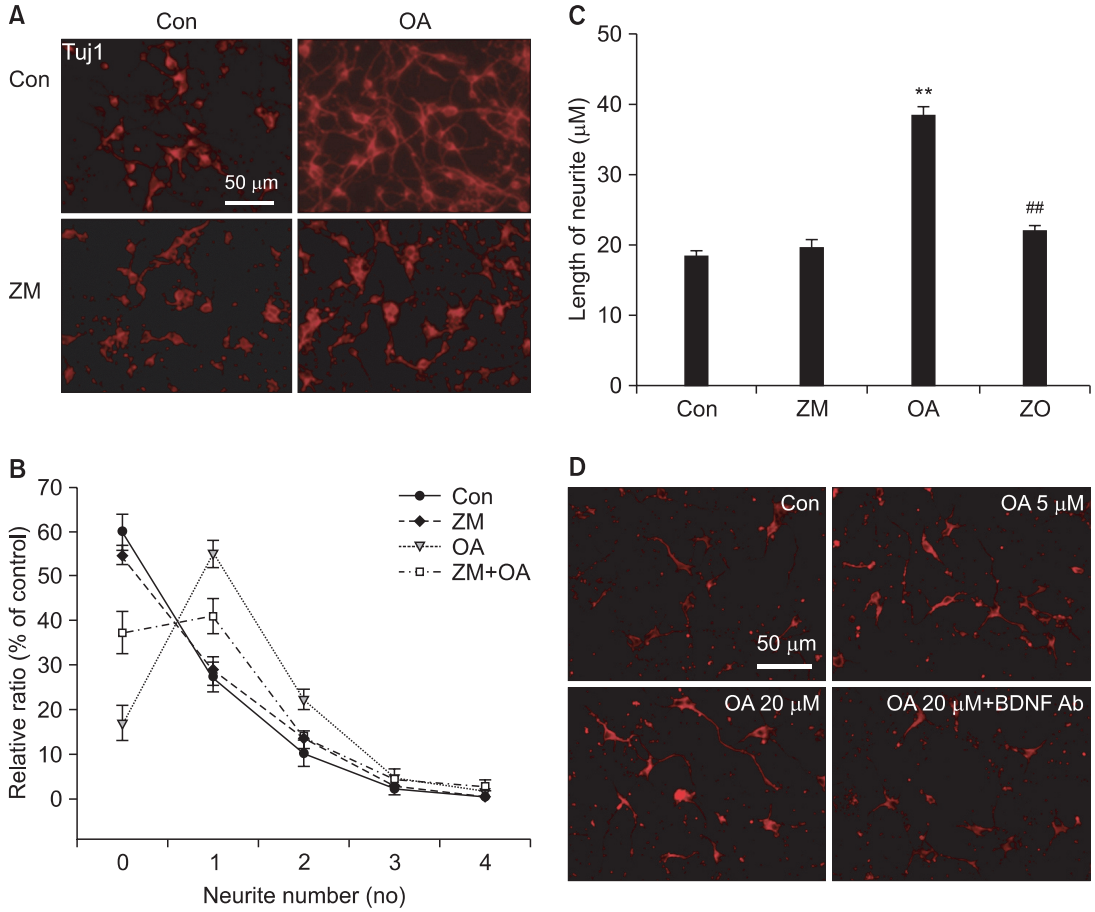

Fig. 4. Oroxylin A-induced up-regulation of BDNF facilitated differentiation of rat primary cortical neuron. Neurons were treated with oroxylin A and were immunostained against neuronal marker Tuj-1 (A). The number of neurite extending from each neuron was analyzed by Image J (B) as described in materials and methods. The length of neurite (C) was also analyzed by Image J analysis of immunostaining data. (D) At DIV2, cortical neurons were treated with oroxylin A (5, 20 μM) and immunostained with Tuj-1 antibody to visualize the neurite extension. Anti-BDNF antibody (2 μg/ml) was used to block BDNF action from oroxylin A treated neurons. Data represent the mean ± S.E.M. Scale bar represents 50 μm. **Significantly different as compared with control and ##significantly different as compared with oroxylin A treated group (p<0.01, n=4).

ERK1/2 inhibitor, U0126 treatment (Fig. 2D) inhibited oroxylin-induced BDNF release. These results suggest that oroxylin A induces phosphorylation of Akt-GSK3β pathway which may modulate, at least in part, the phosphorylation of ERK1/2 and CREB pathways.

Oroxylin A activates Akt-GSK-3β pathways through adenosine $A2_A$ receptor

Next, we investigated whether adenosine $A2_A$ receptor is involved in the activation of Akt pathway induced by oroxylin A (Fig. 3). Pretreatment of adenosine $A2_A$ receptor antagonist, ZM241385, inhibited oroxylin A-induced phosphorylation of Akt and GSK-3β (Fig. 3A) and in this condition, ERK1/2-CREB phosphorylation was also significantly inhibited (Fig. 3B). Taken together with the decreased oroxylin-induced BDNF production by ZM241385 (Fig. 1B), these results suggest that oroxylin A induces phosphorylation of Akt-GSK3β and downstream pathways leading to the BDNF production by modulating the activation of $A2_A$ receptor.

Oroxylin A induces neurite outgrowth in cultured rat primary neuron

Considering the roles of BDNF in neurons, we finally investigated the role of oroxylin A on the neurite outgrowth in immature rat primary cortical neuron. We previously reported the neuroprotective effect of oroxylin A against glutamate, presumably *via* the increased production of BDNF, in rat primary cortical neurons (Jeon *et al.*, 2011). DIV 2 rat primary cortical neurons were treated with oroxylin A and visualized by a neuronal marker, Tuj-1 immunostaining (Fig. 4). Oroxylin A increased the average number of neurite branches extending from a single neuron as well as the length of individual neurite. Interestingly, pre-incubation of ZM241385 prevented oroxylin-induced neurite outgrowth, again suggesting the essential role of $A2_A$ receptor activation in this process (Fig. 4). The increased neurite extension by oroxylin A was also inhibited by the addition of anti-BDNF antibody (Fig. 4D). Overall, these results suggest that oroxylin A enhances neurite outgrowth by regulating BDNF expression and release, at least in part, through $A2_A$ receptor.

DISCUSSION

In this study, we provided evidences that oroxylin A increased BDNF production and neurite outgrowth at least in part by $A2_A$ receptor stimulation followed by activation of Akt-GSK3β pathway. In our previous reports, oroxylin A improved working memory in scopolamine treated animals (Kim *et al.*, 2007) as well as in transiently bilateral common carotid artery occluded animals (Kim *et al.*, 2006), which may also be related to the increased BDNF production and neurogenesis in brain. In addition, we observed increased synaptogenesis by oroxylin A as evidenced by increased expression of synaptic marker proteins in immature rat primary cortical neuron (Jeon *et al.*, 2011). Gasiorowski *et al.* recently showed oroxylin A dramatically increased cognition and memory from aged animals, which was consistent with our data (Gasiorowski *et al.*, 2011). However they did not elucidate the exact mechanism of neuroprotective effects in their report. Nevertheless, these results suggest that using the flavonoids like oroxylin A, as a potential neuroprotective agent may be a potential neuroprotective booster (food additive, drug) target. Our data suggesting the possible role of oroxylin A in neuroprotection, neurogenesis and neural differentiation *via* modulation of BDNF expression by mechanism involving regulation of $A2_A$ activation may support and strengthen this view.

Previously, oroxylin A is reported to suppress nitric oxide generation (Jiwajinda *et al.*, 2002) and inhibit LPS induced iNOS and COX-2 expression by modulating NF-κB activation (Chen *et al.*, 2000; Chen *et al.*, 2001). The effect of oroxylin A on the prevention of uterine contraction was also suggested. Oroxylin A may inhibit uterine contractions by opening calcium dependent potassium channels or adenosine triphosphate dependent potassium channel (Shih *et al.*, 2009), which suggest that oroxylin A may modulate cellular membrane channels or receptors, in our cases, $A2_A$ adenosine receptor.

Adenosine receptors control essential brain functions like synaptic plasticity, neurotransmitter transport, and astrogliosis (Sebastião and Ribeiro, 2009) by receptor dimerization. Especially, $A2_A$ receptor-dopamine D2 receptor heterodimers may exist in the striatal GABA pathways, where activation of $A2_A$ receptors inhibits D2 receptor action. As a result of the $A2_A$ receptor-induced reduction of D2 receptor signaling, the activity of GABA neurotransmission is increased, which may provide novel tools to treat Parkinson's disease, schizophrenia, and addiction (Francesco *et al.*, 2008). However, it will be needed further investigations to define whether oroxylin A will be able to modulate the pathophysiology of these disorders.

In addition to the regulation of BDNF expression, the activation of $A2_A$ receptor increases calcium dependent protein secretion by modulating cAMP-PKA, ERK1/2 and PKC pathways in PC12 cells (Huang *et al.*, 2001; Cheng *et al.*, 2002; Flajolet *et al.*, 2008), which is consistent with the increase in BDNF release by oroxylin A as observed in this study. Pousinha *et al.* also showed that increased BDNF affected synaptic transmission from CGS21680 administered Wistar rats (Pousinha *et al.*, 2006), and this $A2_A$ receptor-mediated BDNF action was coupled to phospholipase C-γ (PLC-γ), which was regulated by $A2_A$-activated PKA pathway to act on neuromuscular transmission (Pousinha *et al.*, 2006). It has been also reported that $A2_A$ receptor is coupled to metabotropic glutamate receptor 5 (mGluR5), which facilitates the activation of N-methyl-D-aspartate (NMDA) receptor in hippocampus (Tebano *et al.*, 2005; Rebola *et al.*, 2008). In this regard, we showed NMDA receptor antagonist (MK801) treatment significantly reduced the oroxylin A induced BDNF production as well as MAPK phosphorylation (Jeon *et al.*, 2011). Overall, these suggest that activation of $A2_A$ receptor by oroxylin A may be positively coupled to NMDA receptor activation, which plays a role in the up-regulation of BDNF expression.

$A2_A$ receptor stimulation inhibits primary neurosphere formation and the proliferation of human and rodent neural precursor cells (Scemes *et al.*, 2003; Stafford *et al.*, 2007) suggesting that it may play a regulatory role in neuronal differentiation, which is one of the actions of BDNF during developmental period and is well presented in the increase of neurogenesis by oroxylin A (Terashima *et al.*, 2010; Jeon *et al.*, 2011; Noble *et al.*, 2011).

Interestingly, it has been suggested that removal of endogenous adenosine inhibited the excitatory stimulation of BDNF activation, and on the contrary, administration of CGS21680 potentiated the BDNF actions on LTP suggesting that proper level of endogenous adenosine should be maintained to trigger BDNF action (Fontinha *et al.*, 2008). These results suggest that facilitatory actions of BDNF are essentially dependent on adenosine and adenosine $A2_A$ receptor activation, which underscores the importance of $A2_A$ receptor on BDNF actions.

BDNF protects cortical and hippocampal neurons from the injury (Cheng *et al.*, 1997; Han and Holtzman, 2000) and induces growth and differentiation of new neurons and synapses (Choi *et al.*, 2009; Lee and Son, 2009). In clinical aspects, BDNF has been implicated in the regulation of learning and memory (Lynch *et al.*, 2008; Cowansage *et al.*, 2010), and reported in the therapeutic effects in psychiatric disorders such as depression (Hashimoto *et al.*, 2004; Schmidt and Duman, 2007) and schizophrenia (Angelucci *et al.*, 2005) as well as neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (Pezet and Malcangio, 2004; Pardon, 2010). Considering inefficient transport of BDNF through BBB, one possible approach to take advantage of BDNF as a therapeutic target is to use small molecules to boost endogenous level of BDNF. Interestingly, daily administration of $A2_A$ agonist CGS21680 ameliorates the symptoms of Huntington's disease animal models (Chou *et al.*, 2005). Whether oroxylin A may provide clinical efficacy against neurological diseases such as mood disorder and neurodegenerative diseases by inducing BDNF expression and release through its action on $A2_A$ receptor should be further investigated in future studies.

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