Palmitic Acid Affects Proliferation and Differentiation of Neural Stem Cells In Vitro

Zhen Wang,1,2 Dexiang Liu,2,3 Qun Zhang,1 Jianmei Wang,1 Jingmin Zhan,1 Xiuying Xian,1 Zhaoxia Du,2 Xueer Wang,1,4 and Aijun Hao*1

1Department of Physiology, Shandong University School of Medicine, Shandong, People’s Republic of China
2Key Laboratory of the Ministry of Education for Experimental Teratology, Shandong Provincial Key Laboratory of Mental Disorders, Department of Histology and Embryology, Shandong University School of Medicine, Jinan, Shandong, People’s Republic of China
3Department of Medical Psychology, Shandong University School of Medicine, Jinan, Shandong, People’s Republic of China
4Institute of Bioscience, Luoyang Normal University, Luoyang, Henan, People’s Republic of China

High-lipid diet composed of saturated fatty acids (SFAs) has significant detrimental effects on brain homeostasis, and deleterious effects of SFAs on various cells have been well documented. However, the effects of SFAs on neural stem cells (NSCs) function have not been fully explored. The aim of this study was to determine whether palmitic acid (PA) affected the proliferation and differentiation of murine-derived NSCs. The results showed that PA dose dependently suppressed viability of NSCs and was cytotoxic at high concentrations. The toxic levels of PA inhibited the proliferation of NSCs as shown by reduced bromodeoxyuridine labeling of NSCs, which is correlated with reactive oxygen species generation. Pretreatment of the cells with the antioxidant N-acetyl-L-cysteine significantly attenuated the effects of PA on the proliferation of NSCs. Furthermore, nontoxic levels of PA promoted astrocytogenesis in the differentiated NSCs, associated with Stat3 activation and altered expression of serial of basic helix–loop–helix transcription factor genes. Altogether, our data have demonstrated that PA has a significant impact on proliferation and differentiation of NSCs in vitro and may be useful for elucidating the role of SFAs in regulating NSCs fate in physiological and pathological settings. © 2014 Wiley Periodicals, Inc.

Key words: palmitic acid; neural stem cells; proliferation; differentiation

Neural stem cells (NSCs) are endowed with the capacity to self-renew and differentiate into neurons, astrocytes, and oligodendrocytes in predictable proportions. They are present in the newborn and adult in special brain areas (McKay, 1997). In the adult mammalian brain, NSCs are maintained in two neurogenic niches, the forebrain subventricular zone around the lateral ventricles and the subgranular zone of the dentate gyrus (DG). Furthermore, many stimuli have been shown to exert their effects on NSCs, influencing their proliferation, apoptosis, and differentiation under physiological and pathophysiological conditions (Gritti et al., 1999).

Both experimental and human studies have shown that brain function is sensitive to saturated fatty acids (SFAs). For example, it has been reported that greater intake of saturated fat increased the risk for impaired cognitive function in middle-aged or aged populations (Granholm et al., 2008; Kanoski and Davidson, 2011), and rodents fed high levels of SFAs also show impaired learning and memory performance and develop Alzheimer’s disease (AD)-like pathophysiological changes in their brains (Greenwood and Winocur, 1996; Granholm et al., 2008). Fatty acid is free for transport across the blood–brain barrier (Dhopeshwarkar and Mead, 1973). Therefore, brain fatty acid homeostasis may be dependent on its levels in the periphery. It is therefore conceivable that diets rich in SFA may increase brain uptake of intact free fatty acids from the plasma through the blood–brain barrier to affect brain function (Wang et al., 1994).

Contract grant sponsor: National Natural Science Foundation of China; Contract grant numbers: 81200879; 81071057; Contract grant sponsor: Ministry of Education of China; Contract grant number: 20110131110036; Contract grant sponsor: National Science Foundation of Shandong Province; Contract grant numbers: ZR2011HQ035; ZR2012HM021; 2012JQ006; Contract grant sponsor: Postdoctoral Science Foundation of China; Contract grant numbers: 2013T60672; 2012MS11514; 2013MS31610

*Correspondence to: Dr. Aijun Hao, Key Laboratory of the Ministry of Education for Experimental Teratology, Department of Histology and Embryology, Shandong University School of Medicine, 44# Wenhua Xi Road, Jinan, Shandong, 250012 People’s Republic of China. E-mail: aijunhao@sdu.edu.cn

Received 18 July 2013; Revised 28 October 2013; Accepted 12 November 2013

Published online 21 January 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jnr.23342

© 2014 Wiley Periodicals, Inc.
Studies have shown that specific SFAs such as palmitic acid (PA), a fatty acid produced by adipose tissue and acquired through diet, may play a role in the development of obesity-related disease in the nonpregnant state, such as AD (Granholm et al., 2008; Wang et al., 2012a), diabetes, and coronary heart disease (Micha and Mozaffarian, 2010). In excess, PA can cause insulin resistance and mitochondrial dysfunction, with subsequent apoptosis in various nonadipose cells, including pancreatic beta cells (Chu et al., 2010), cardiac myocytes (Miller et al., 2005), and granulosa cells (Mu et al., 2001), as well as the CNS cells neurons (Almaguel et al., 2009) and astrocytes (Blazquez et al., 2000). Our recent studies have extended the finding that elevated levels of PA lead to the apoptosis of NSCs (Yuan et al., 2013), but little is known of PA's effects on the NSCs proliferation and differentiation. The present study explored the effects and the underlying mechanisms of PA on the proliferation and differentiation of NSCs.

MATERIALS AND METHODS

Primary NSC Culture and Expansion

Brains were removed from mouse embryos at E13.5 according to a previously described method (Wang et al., 2013). After this, the telencephalon was trypsinized for 5 min at 37°C. After three washes with DMEM/F12 (1:1) medium (Invitrogen, Carlsbad, CA) supplemented with 2 mM L-glutamine, cells were resuspended in DMEM/F12 medium (1:1), and cell number was counted. Cells were seeded at 2 × 10^6 cells/ml in DMEM/F12 medium (1:1) supplemented with 2% B27 (Gibco BRL, Grand Island, NY) plus 100 U/ml penicillin, 100 µg/ml streptomycin, basic fibroblast growth factor (bFGF; 20 ng/ml; R&D Systems, Minneapolis, MN), and epidermal growth factor (EGF; 20 ng/ml; R&D Systems). The cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. The culture medium was changed every 2 days, and the cells grew into neurospheres again. Secondary or tertiary neurospheres were dissociated into single cells, and the cells grew into neurospheres again. Secondary or tertiary neurospheres were used for subsequent experiments, and all experimental procedures were carried out with monolayers of NSCs. To examine the proliferation of NSCs, the dissociated cells were cultured in reduced growth medium (DMEM/F12 medium [1:1] supplemented with 2% B27, 5 ng/ml of bFGF, and 20 ng/ml EGF plus 100 U/ml penicillin and 100 µg/ml streptomycin). For differentiation of NSCs, neurospheres were transferred into differentiation medium (DMEM/F12 medium [1:1] plus 100 U/ml penicillin and 100 µg/ml streptomycin, containing 2% fetal bovine serum without the growth factor) and cultured for 3–7 days.

Preparation of Fatty Acid Albumin Complexes

PA was solubilized in ethanol at 70°C. It was then combined with fatty acid-free and low-endotoxin bovine serum albumin at a molar ratio of 10:1 (fatty acid:albumin) in serum-free medium at 50°C for 6 hr, for a final PA concentration of 25–400 µM as described previously (Lee et al., 2001). Fatty acid–albumin complex solution was freshly prepared prior to each experiment. The final concentration of ethanol was <0.5%. In most of the experiments, NSCs were treated with individual PA at a 25–400 µM concentration, and the controls received BSA and vehicle only.

To evaluate the possible contamination of PA with LPS, the endotoxin content was determined by the chromogenic Limulus amebocyte lysate test, following the manufacturer’s instructions (Cambrex Bio Science, Walkersville, MD). The endotoxin content in the 400 µM PA solution was = 3.15 × 10⁻¹ pg/ml, which is far below the concentration required to induce NSC death under our assay conditions.

Cell Viability Assay

Cell viability was determined by using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Dissociated NSCs were plated into 96-well culture plates at a density of 5 × 10^3 cells/well with 200 µl redowth medium per well in triplicate. After this, the cells were incubated with test substances or vehicle for 24 and 48 hr, respectively. Then, 20 µl MTT (5 mg/ml, Sigma-Aldrich, St. Louis, MO) was added to each well and incubated at 37°C for 4 hr. The culture medium was aspirated, followed by addition of 200 µl dimethyl sulfoxide. The absorbance value was measured in a microplate reader (Bio–Rad, Hercules, CA) at 490 nm. Values were expressed as a percentage relative to controls.

Immunocytochemistry

The cells were fixed in 4% paraformaldehyde for 20 min and blocked with 10% goat serum in PBS. Slides were incubated overnight in a humid chamber at 4°C with the following primary antibodies: antiligtrial fibrillary acidic protein (GFAP; 1:500, mouse monoclonal; Millipore, Billerica, MA), antinestin (1:200, rabbit polyclonal; Sigma-Aldrich), antimicrotubule-associated protein 2 (MAP-2; 1:200, mouse monoclonal; Millipore). After primary antibody incubation, samples were washed again and incubated in the appropriate fluorescent-conjugated secondary antibody (goat anti-mouse/rabbit IgG, 1:500 dilution; Sigma-Aldrich) for 1 hr. The cells were counterstained by 4',6-diamino-2-phenylindole dihydrochloride (DAPI; Sigma-Aldrich), and images were captured with a Nikon TE2000U microscope.

Quantitative Real-Time PCR

Total RNA was extracted from induced cell cultures using the Trizol reagent (Gibco, Invitrogen) according to the manufacturer’s instructions. RNA concentration was determined by a spectrophotometer (Bio–Rad) at 260 nm. Identical amounts of RNA (2 µg) were reverse transcribed into cDNA by using a commercial reverse transcription–polymerase chain reaction (RT–PCR) kit (Fermentas, Vilnius, Lithuania) according to the manufacturer’s instructions. cDNA was subsequently amplified by PCR with specific primers (Table I). Real-time PCR was carried out with 1 µl cDNA on real-time system (Analtik, Jena, Germany) using an UltraSYBR mixture (CoWin Bioscience, Beijing, China) and normalized to β-actin. The protocol of the real-time PCR was as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles at 95°C for 10 sec, 58°C for 30 sec. At the end of the PCR, a melting
curve was obtained by holding at 95°C for 15 sec, cooling to 60°C for 1 min, and then heating slowly at 0.5°C/sec until 95°C.

**Western Blot Analysis**

Cells were washed with cold PBS and lysed in ice-cold RIPA buffer containing protein inhibitors. Cell lysates were incubated at 4°C for 20 min. The sample was centrifuged at 12,000 rpm for 10 min at 4°C. Protein concentration in the supernatants of cell extract was determined by using a BCA protein assay kit (Pierce, Rockford, IL). A quantity of 30 µg total proteins was loaded onto a 4–20% gradient polyacrylamide gel, electrophoretically transferred to polyvinylidene difluoride membrane, and probed with the following primary antibodies: GFAP (1:1,000; Millipore), MAP-2 (1:1,000; Millipore), Stat3 (1:2,000; Cell Signaling, Danvers, MA), and Phospho-Stat3 (Tyr705; 1:1,000; Cell Signaling). The percentage of Phospho-Stat3 was confirmed by RT-PCR. The Stat3 silencing for immunocytochemical staining, and protein or RNA was collected for Western blot or real-time PCR. The Stat3 silencing was confirmed by RT-PCR.

**Proliferation Index by Bromodeoxyuridine Labeling**

Cell proliferation was evaluated by bromodeoxyuridine (BrdU) incorporation assay. Dissociated NSCs were transferred into 12-well plates with poly-D-lysine-coated coverslips and cultured in NSC growth medium. After this, NSCs were treated with test substances or vehicle in reduced growth medium for 24 hr and incubated with BrdU (10 µmol/liter; Sigma-Aldrich) was used as an internal control. Secondary antibodies were horseradish peroxidase conjugated to goat/mouse anti-rabbit IgG (1:8,000; Sigma-Aldrich). The membranes were developed using an enhanced chemiluminescence detection system (Pierce).

**Statistical Analysis**

Quantitative data were presented as the mean ± SD of at least three independent experiments. Statistical analysis of data was by Student’s t-test or by one-way ANOVA using the LSD test in multiple comparisons of means. Differences were considered statistically significant at P<0.05.

**RESULTS**

**PA Inhibited Proliferation of NSCs**

The effects of various concentrations of PA (0, 25, 50, 100, 200, and 400 µM) on cell viability were assessed. MTT results showed that 200 and 400 PA µM yielded significant effects on cell viability (0.85±0.056 and 0.57±0.023 of control group for 24 hr, 0.82±0.057 and 0.50±0.012 of control group for 48 hr), whereas PA (0, 25, 50, 100 µM) had no effect on cell viability.

We then took another approach to quantify proliferation cell ratio, the BrdU staining assay. The results
showed that the ratio of BrdU-/nestin-positive cells against total NSCs had decreased from 0.28 ± 0.038 to 0.20 ± 0.032 ($P < 0.001$) at 24 after 200 µM PA treatment compared with the untreated cells (Fig. 1), suggesting that the decrease in cell numbers resulted from a decrease of stem cells.

PA Decreased Proliferation by Elevated ROS

It has been reported that excess SFA-induced ROS production participates in different pathological conditions (Almaguel et al., 2009; Wang et al., 2012b), and oxidative stress is a factor has been shown to affect stem cell development, function, and survival (Kamata and...
We next examined whether PA treatment could affect intracellular ROS levels in NSCs. The cells were treated with 10 μM H₂DCFDA (green) or 2 μM DHE (red) and counterstained with DAPI (blue); the level of ROS was detected with a fluorescence microscope. Images are representative of triplicate sets. B: Quantification of the ROS was determined by fluorescence plate reader. C,D: NSCs were cultured in reduced growth medium for 24 hr and then treated with PA (200 μM), NAC (1 mM), or PA (200 μM) + NAC (1 mM) for 24 hr. NSC proliferation was measured by MTT (C) and BrdU (D) analyses. Values were expressed relative to the fluorescence signal of controls. Values represent the mean ± SD of three independent experiments. **P < 0.01, ***P < 0.001, significantly different from control, ##P < 0.01, ###P < 0.001 vs. PA alone. Scale bar = 20 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

PA Did Not Affect the Neuronal Differentiation of NSCs

We next determined the effect of PA on the differentiation of NSCs. Single cells dissociated from

[578 Zhen et al. Journal of Neuroscience Research]
neurospheres were reseeded and treated with PA in differentiation medium without the growth factors. MTT showed that PA (25, 50, and 100 μM) had no cytotoxic effect on NSCs in differentiation medium, whereas 200 and 400 PA μM yielded significant effects on cell viability in differentiation medium (data not shown). In view of this, we have used PA (25, 50, and 100 μM) in most of the subsequent experiments on NSCs differentiation. The expression of MAP-2 was tested by using Western blotting and immunocytochemistry. The results showed PA did not appear to affect the differentiation of NSCs into neuron compared with untreated control (Fig. 3).

PA Promoted the Astrocytic Differentiation of NSCs

GFAP expression was examined by Western blot and immunocytochemistry. Western blot analysis showed that the protein levels of GFAP in the PA (50 and 100 μM)-treated group were significantly higher than those in the corresponding control group at day 5 (Fig. 4A). Moreover, GFAP expression in PA (50 μM) treatment at days 3, 5, and 7 were significantly higher than those in the matching control (Fig. 4B). In agreement with these results, immunocytochemistry showed that the percentage of GFAP-/nestin-positive cells in NSCs treated with PA (50 μM) was significantly higher compared with untreated control at day 5 (Fig. 4C).

PA Affects Expression of Notch and Basic Helix–Loop–Helix Transcription Factors in Differentiated Cells

It is well documented the determination of neural cell fate involves Notch and basic helix–loop–helix (bHLH) transcription factors (Iso et al., 2003; Ross et al., 2003). To determine whether PA influenced the expression of Notch1 and bHLH transcription factors, the mRNA expression levels of neurogenin1 (Ngn1), NeuroD2, mammalian achaete-scute homologue (Mash) 1, Notch1, hairy enhancer of split (Hes) 1, and Hes5 in differentiated cells were analyzed. Single cells dissociated...
from neurospheres were reseeded and treated with PA (50 µM) in differentiation medium for 1, 2, and 3 days, and the mRNA expression levels were then tested by using real-time PCR. The results showed that the mRNA levels of Notch1, Hes1, and Hes5 were significantly increased in PA-treated groups compared with those in the untreated groups, whereas levels of Ngn1 and Mash were decreased, and levels of NeuroD2 were not altered (Fig. 5).

Stat3 Signal Is Involved in PA’s Effects on Astrocytogenesis of NSCs

It is well known that Stat3 plays crucial roles in the determination of NSC fate (Bonni et al., 1997). Suppression of Stat3 activity in NSCs inhibited astrogliogenesis and promoted neurogenesis (Gu et al., 2005). Next, we examined whether PA regulates Stat3 activation in differentiation medium. The proteins harvested from the cells after different durations (0.5, 1, 2, and 4 hr) of treatment with or without PA (50 µM) were processed for Western blotting to detect intracellular level of phospho-Stat3 (P-Tyr-705). As shown in Figure 6A, the levels of phospho-Stat3 in NSCs induced by PA were increased at 0.5 hr and were significantly higher than the matching untreated control at all time points. However, phospho-Stat3 started increasing in control at 2 hr after PA treatment, and total Stat3 did not change at any time point. The level of P-Tyr-705 Stat3 was also increased after PA treatment (50 and 100 µM) within 1 hr (Fig. 6B). Furthermore, pretreatment with the Stat3 inhibitor JSI-124 (cucurbitacin I, 100 nM) for 60 min prior to exposure of cells to PA (50 µM) significantly reduced the protein level of GFAP, compared with the PA treatment only at day 5 (Fig. 6C), and JSI-124 decreased the levels of PA-induced phospho-Stat3 in NCSs (data not shown).

To substantiate further the role of Stat3 in the differentiation of NSCs, we used RNA silencing to decrease...
Stat3. RT-PCR showed that siStat3 had decreased 53.8% of the Stat3 mRNA expression compared with siCON-transfected NSCs at 24 hr posttransfection (Fig. 7A). Next, we started PA (50 μM) treatment at 24 hr post-transfection for the following experiments. The results showed that knockdown of Stat3 by siRNA inhibited the PA-induced increase of GFAP expression at 5 days post-treatment (Fig. 7B).

Finally, we examined the effects of siStat3 on the expression of Notch1, Hes1, and Hes5, which control the fate of NSCs. After 24 hr post-transfection, we started PA (50 μM) treatment for 2 days. The results showed that the mRNA levels of Notch1, Hes1, and Hes5 were decreased by siStat3 (Fig. 7C). Moreover, knockdown of Stat3 by siRNA inhibited the PA-induced increase of Notch1 and Hes1 mRNA expression (Fig. 7C).

**Effect of Palmitic Acid on Fate of Neural Stem Cells**

Oxidative stress plays a role in differentiation and cell damage (Smith et al., 2000; Le Belle et al., 2011). Next, we treated NSCs with PA (50 μM) with or without NAC (1 mM) in the differentiation medium. The results showed that PA did not affect ROS production in NSCs compared with the control (Fig. 8A), and NAC did not affect the PA-induced GFAP expression (Fig. 8B) at 5 days. Finally, NAC did not reverse the upregulation of phospho-STAT3 by PA at 1 hr treatment (Fig. 8C). These data suggest that ROS is not involved in PA’s effects on astrocytogenesis of NSCs.

**DISCUSSION**

The present study shows that the high concentrations of PA can inhibit the proliferation of NSCs, as shown by reduced BrdU labeling of NSCs in vitro. PA’s adverse effect on proliferation was associated with increased ROS generation. Another major finding was that low concentrations of PA promoted astrocytogenesis in the differentiated NSCs, associated with Stat3 activation and altered expression of differentiation-related genes.

The process of neuronal production in offspring is affected by maternal nutritional conditions such as nutrients, stress, experience, and physical activity (Mirescu et al., 2004; Tozuka et al., 2009). For example, Welham et al. (2002) demonstrated that protein restriction in pregnancy is associated with increased apoptosis of mesenchymal cells. Moreover, neurogenesis in the offspring of high-fat diet-fed animals is affected during postnatal development (Lindqvist et al., 2006; Tozuka et al., 2009). The impaired neurogenesis could be related to increased lipid peroxidation; malondialdehyde, a product of peroxidized lipids, has been shown to reduce proliferation of hippocampal progenitor cells in vitro (Tozuka et al., 2009). Moreover, it was reported that an n-3 fatty acid-deficient diet altered neurogenesis in the embryonic rat brain (Coti Bertrand et al., 2006). NSCs retain the ability...
to proliferate and self-renew and have the capacity to give rise to both neuronal and glial lineages (McKay, 1997, 2004). A complete understanding of NSCs and neurogenesis requires the identification of molecules that determine the self-renewal and multipotent character of these cells. These molecules likely include epigenetic regulators, such as histone deacetylases, that coordinate with cell-intrinsic transcription factors and various signaling pathways to regulate stem cell pluripotency, self-renewal, and differentiation (Sun et al., 2010).

Although the physiological mechanisms by which a high-fat diet affects brain development are poorly understood, studies have shown that brain function is sensitive to SFAs. Many recent links have been made between elevated levels of PA and cellular dysfunction, insulin resistance, and cellular death in a number of different tissue types, including cardiac muscle, pancreatic beta cells, and kidney (Listenberger and Schaffer, 2002; El-Assaad et al., 2003; Katsoulieris et al., 2010) as well as the CNS cells (Almaguel et al., 2009). The phenomenon known as lipotoxicity is characterized by lipid accumulation in nonadipose tissues, resulting in increased endoplasmic reticulum stress and subsequent cell death (Almaguel et al., 2009; Katsoulieris et al., 2010). In this study, PA dose dependently suppressed NSC proliferation and was cytotoxic at higher concentrations. Nontoxic levels PA treatment also promoted the differentiation of the NSCs into astrocytes. Moreover, other studies have demonstrated that embryos are capable of metabolizing PA (Haggarty et al., 2006); excess PA exposure in preimplantation embryos altered metabolic and growth patterns (Junghiem et al., 2011; Yuan et al., 2013). Importantly, the increased consumption of saturated fats in a high-fat diet contributes to obesity, neurodegenerative diseases, long-term memory loss,
and cognitive impairment. On the basis of these results, we hypothesize that an adverse effects of excess SFAs on NSCs subsequently manifests in long-term effects on fetal brain restriction.

Oxidative stress induced by ROS is a well-known cause of several physiological and pathophysiological processes, including cell proliferation and differentiation (Kamata and Hirata, 1999). Stem cells are known to maintain a low metabolic status, and the production of ROS is minimal. As the stem cells are stimulated, low levels of ROS are produced, which activate redox-sensitive signaling pathways that favor cell proliferation, whereas, as the ROS levels increase, the rate of proliferation slows, and in turn the intracellular environment favors differentiation (Smith et al., 2000; Le Belle et al., 2011). The toxic levels of ROS production may lead to deleterious alterations in cell physiology by inducing lipid peroxidation, DNA fragmentation, and protein oxidation (Valko et al., 2006). SFA overloading has been associated with ROS generation (Listenberger et al., 2001; Almaguel et al., 2009). This study showed that excess PA treatment increased intracellular ROS levels in NSCs. More shown is a representative of results obtained in three separate experiments. Quantification of GFAP expression over β-actin expression.

C: Posttransfection with siStat3 or siCON for 24 hr, followed by culturing in the differentiation medium with or without 50 μM PA for 2 days and real-time PCR analysis, which demonstrates Notch1, Hes1, and Hes5 mRNA expression in all groups. Values represent the mean ± SD of three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 compared with control. #P < 0.05, ##P < 0.01 vs. PA alone.

Fig. 7. siRNA for Stat3 inhibited the effect of PA on differentiation of NSCs. A: NSCs were transfected with siStat3 or siCON for 24 hr. Stat3 mRNA expression was detected by RT-PCR analysis. Quantification of mRNA levels of Stat3 was determined by Image Pro Plus 6.0. β-Actin was used as an internal control. B: Posttransfection with siStat3 or siCON for 24 hr, followed by culturing in the differentiation medium with or without 50 μM PA for 5 days and Western blot analysis, which demonstrates GFAP expression in NSCs in all groups. Levels of β-actin were used to evaluate protein loading. The blot shown is a representative of results obtained in three separate experiments. Quantification of GFAP expression over β-actin expression.
importantly, the antioxidant NAC effectively suppressed PA-induced ROS generation and reversed PA-impaired proliferation of NSCs. However, PA at lower concentration can induce differentiation (astrogliogenesis) rather than increasing ROS level of NSCs under differentiation conditions, and NAC did not affect PA’s effects on astrocytogenesis. It is possible that PA-induced oxidative stress acts as a negative regulator of NSC proliferation and that oxidative stress is not involved in PA’s effects on astrocytogenesis of NSCs.

Accumulating data indicate that, during development, Notch signaling maintains NSCs and inhibits neurogenesis (Gaiano and Fishell, 2002; Iso et al., 2003). Hes1 and Hes5 are target genes for Notch signaling. Both Hes1 and Hes5 also maintain NSCs in the proliferative state and inhibit neurogenesis (Ohtsuka et al., 2001). Importantly, Hes1 and Hes5 are transiently expressed by differentiating astrocytes in the brain and Müller glia in the retina. Misexpression of Hes1 and Hes5 increases generation of astrocytes and Müller glia, suggesting that the repressor-type bHLH genes promote gliogenesis (Hojo et al., 2000; Ohtsuka et al., 2001). The activator-type bHLH genes, Mash1, Ngn, and NeuroD, are involved in neuronal differentiation. Misexpression of these genes in NSCs induces the pan-neuronal gene expression and determines neuronal fate (Ohtsuka et al., 2001). Moreover, the Notch–Hes pathway regulates the normal timing of differentiation by repressing premature onset of the differentiation process.
activator-type bHLH genes. For example, the double mutation of Mash1 and Ngn2 leads to a decrease of neurons and premature generation of astrocytes (Nieto et al., 2001), and overexpression of Ngn1 actively inhibits astrocytic differentiation (Sun et al., 2001). Our data show that PA upregulated the mRNA expression of Notch1, Hes1, and Hes5 and downregulated the mRNA expression of Mash1 and Ngn1 in the differentiated medium. It is possible that PA promotes astrocytogenesis that is partially attributed to the expression of Notch–Hes pathway, which then repressed Mash1 and Ngn1 expression.

It is well known that the Stat3 signaling pathway plays crucial roles in NSCs differentiation, particularly in enhancing astrocytic differentiation and inhibiting neuronal differentiation (Bonni et al., 1997). Enhancement of Stat3 activity in NSCs at embryonic day 14.5 leads to subsequent glial differentiation (Rajan and McKay, 1998), whereas inhibition of Stat3 activity suppresses astroglialogenesis in NSCs (Gu et al., 2005; Cao et al., 2010). Deletion of Stat3 in murine embryonic NSCs inhibits neurosphere formation and self-renewal (Niwa et al., 1998). Cytokines or growth factors activate members of the Jak family, which in turn are activated by tyrosine phosphorylation of Stat3. Dimersed Stat3 translocates to the nucleus and binds to the GFAP promoter, activating the transcription of GFAP (Bonni et al., 1997). This study shows that exposure to PA induces the activation of Stat3 and subsequent astroglialogenesis in differentiation medium. In addition, blockade of Stat3 activation significantly attenuated the effects of PA on differentiation and the expression of Notch1 and bHLH transcription factors. Consistent with these reports, our results indicate that PA is involved in the network of Stat3, Notch, and bHLH transcription factors that determines the fate of different populations of progenitor cells. On the other hand, the mechanism by which PA activates Stat3 remains to be clarified.

In conclusion, this study demonstrates that the toxic levels of PA impaired the proliferation of NSCs via ROS. Nontoxic levels of PA induced Stat3 activity and upregulation of Notch1, Hes1, and Hes5, leading to gliogenesis. Thus our results have provided the cellular basis as well as the mechanisms of the roles of PA in NSCs. Ongoing studies are designed to determine the specific roles of SFAs in the detrimental effects of maternal diet on the brain development of offspring.

ACKNOWLEDGMENTS

The authors have no conflicts of interest to declare.

REFERENCES


