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14,15-EET promotes mitochondrial biogenesis and protects cortical neurons against oxygen/glucose deprivation-induced apoptosis

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ABSTRACT

14,15-Epoxyeicosatrienoic acid (14,15-EET), a metabolite of arachidonic acid, is enriched in the brain cortex and exerts protective effect against neuronal apoptosis induced by ischemia/reperfusion. Although apoptosis has been well recognized to be closely associated with mitochondrial biogenesis and function, it is still unclear whether the neuroprotective effect of 14,15-EET is mediated by promotion of mitochondrial biogenesis and function in cortical neurons under the condition of oxygen–glucose deprivation (OGD). In this study, we found that 14,15-EET improved cell viability and inhibited apoptosis of cortical neurons. 14,15-EET significantly increased the mitochondrial mass and the ratio of mitochondrial DNA to nuclear DNA. Key makers of mitochondrial biogenesis, peroxisome proliferator activator receptor gamma-coactivator 1 alpha (PGC-1α), nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (TFAM), were elevated at both mRNA and protein levels in the cortical neurons treated with 14,15-EET. Moreover, 14,15-EET markedly attenuated the decline of mitochondrial membrane potential, reduced ROS, while increased ATP synthesis. Knockdown of cAMP-response element binding protein (CREB) by siRNA blunted the up-regulation of PGC-1α and NRF-1 stimulated by 14,15-EET, and consequently abolished the neuroprotective effect of 14,15-EET. Our results indicate that 14,15-EET protects neurons from OGD-induced apoptosis by promoting mitochondrial biogenesis and function through CREB mediated activation of PGC-1α and NRF-1.

1. Introduction

Epoxyeicosatrienoic acids (EETs) are arachidonic acid metabolites formed endogenously via the cytochrome P450 pathway. There exist four regioisomers of EETs including 5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET, which can be further metabolized to supposedly less active diols by the soluble epoxide hydrolase [1]. It has been reported that 14,15-EET is enriched in the brain cortex and exerts neuroprotective effect on neuronal cells in ischemia and reperfusion. For instance, administration of 14,15-EET lessens infarct size in global cerebral ischemia, inhibits astrocyte apoptosis under oxygen–glucose deprivation (OGD) and reduces neurodeficit score after middle cerebral artery occlusion (MCAO) [2–6]. However, the potential neuroprotective effect and underlying mechanism of 14,15-EET against apoptosis in cortical neurons under the condition of ischemia and reperfusion remain to be elucidated.

Lines of evidence have shown that the pathophysiological process of cerebral ischemia is closely associated with the reduction of mitochondrial biogenesis and function. For example, cerebral ischemia results in declines of mitochondrial DNA (mtDNA) copy numbers, ATP production and the mitochondrial membrane potential, whereas the level of reactive oxidative species (ROS) was surged in rodent models [7,8]. The molecular markers of mitochondrial biogenesis include peroxisome proliferator activator receptor gamma coactivator 1α (PGC-1α), nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (TFAM). PGC-1α, NRF-1 and TFAM are critical for the replication of mtDNA and the transcription of nuclear-encoded genes relevant to mitochondrial function [9]. Mitochondrial biogenesis relies on the up-regulation of

Abbreviations: 14,15-EET, 14,15-epoxyeicosatrienoic acid; OGD, oxygen–glucose deprivation; mtDNA, mitochondrial DNA; PGC-1α, peroxisome proliferator activator receptor gamma coactivator 1α; NRF-1, nuclear respiratory factor 1; TFAM, mitochondrial transcription factor A; ROS, reactive oxidative species; CREB, cAMP response element-binding protein.

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PGC-1α, NRF-1 and TFAM in normal or ischemic conditions [10–12]. On the other hand, ischemia inhibits the expressions of PGC-1α, NRF-1 and TFAM, and then reduces mitochondrial biogenesis [10,13]. Recent studies report that the physiological role of EETs is linked to the function of mitochondria, for instance, EETs protect mitochondrial functions by limiting ROS generation and reducing the loss of mitochondrial membrane potential, as well as preserving ATP synthesis in astrocyte, H9c2 cell and carcinoma cell line [14–16]. Based on the above data, we hypothesized that 14,15-EET may exert neuroprotective effect on cortical neurons against OGD-induced apoptosis by enhancing the biogenesis and function of mitochondria.

In the present study, we investigated the effects of 14,15-EET on mitochondrial biogenesis, mitochondrial function, and apoptosis in cultured cortical neurons induced by oxygen–glucose deprivation.

2. Material and methods

2.1. Reagents

14,15-EET was purchased from Cayman Chemical Company (Cayman, USA). Maxima SYBR green qPCR master mix and bicinchoninic acid (BCA) were purchased from Thermo Fisher Scientific (Rockford, IL). The antibodies against PGC-1α, β-actin, phospho-CREB and CREB were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The antibodies against TFAM and NRF 1 were purchased from Bioworld Technology (Minneapolis, USA). Lysis buffer were purchased from Beyotime (Jiangsu, China). ECL Western blotting reagent kits was purchased from Millipore (Billerica, MA). Other chemicals were obtained from Life Technologies (Gaithersburg, MD) unless indicated otherwise.

2.2. Primary cortical neuron culture

All experiments were approved by the Institutional Animal Care and Use Committee of Peking University. Primary cultures of cortical neuron were prepared from the cortex of less than one day old neonatal C57BL/6 mice. Brains were removed rapidly and placed in cold Ca^{2+} and Mg^{2+}-free dulbecco's phosphate-buffered saline (D-PBS). The cortex were dissected and were digested with 0.1% trypsin–EDTA at 37 °C for 10 min, followed by termination of the digestion with DMEM media containing 10% FBS. The mixture were triturated with fire-polished glass pipettes and centrifuged at 1000 rpm for 5 min. The cells were plated in the poly-D-lysine-coated dishes at a density of 70,000 cells/cm^2 and maintained at 37 °C in a humidified 5% CO_2 and 95% air atmosphere incubator. The medium was replaced with neurobasal media supplemented with 2% B27 and 1% L-glutamine after 4 h, and changed every 3 days. The cells were used at Day 7.

2.3. Oxygen–glucose deprivation and administration of 14,15-EET

The media were removed and cells were washed twice with warm D-PBS. The cells were incubated with DMEM without glucose in an oxygen-free incubator (95% N_2 and 5% CO_2) for 2 h. After that, the cells were incubated with neurobasal/B27 in a humidified 95% air and 5% CO_2 at 37 °C for 24 h reoxygenation and glucose restoration. The controls were incubated with high glucose DMEM plus 10% FBS in atmosphere incubator (95% air and 5% CO_2) for 24 h. The effective concentration of 14,15-EET, 20 nM, which was determined by dose-dependent assay, was used 30 min before OGD exposure.

2.4. Measurement of cell apoptosis

Cell apoptosis was detected by flow cytometry using Annexin V Alexa Fluor 647 according to manufacturer's protocol. Cultured neurons were trypsinized and centrifuged at 1500 rpm for 5 min, and washed twice by PBS. The cells were suspended, and incubated with Annexin V-FITC and PI for 15 min in the dark at room temperature, then analyzed with flow cytometry (BD Biosciences).

2.5. Total mRNA extraction and real-time PCR

Total mRNA was extracted using Trizol reagent, and quantified with ultraviolet spectrophotometry. The cDNA was constructed with First-Strand cDNA Synthesis Kit according to the manufacturer's instructions. The real-time PCR was performed with Maxima SYBR Green qPCR Master Mix in Mx3000 multiplex quantitative PCR system (Stratagene, La Jolla, CA, USA). The following primers were used: PGC-1α: sense, 5′-GACGGATTGCCCTCATTT-3′, antisense, 5′-TTGTGGCTTTTGCTGTTG-3′; NRF-1: sense, 5′-ACGATGACTCGGACATACTC-3′, antisense, 5′-TCGGATAG ATGGGTTAGACT-3′; TFAM: sense, 5′-GAAACGCCTAAAGAAGAAAG-3′, antisense, 5′-TCCAAGCCTGA TTTACAAG-3′; 18s rRNA: sense, 5′-AACTTTCGATGGTAGTCGC-3′, antisense, 5′-TTCCTTGGATGTGGT AGCC-3′.

2.6. siRNA transfection

The CREB and scrambled siRNA were purchased from GenePharma (Shanghai, China). The oligonucleotide sequences were as follows. CREB siRNA: sense: 5′-GGUACUACCAUUCUACAAUTT-3′, antisense: 5′-AUUGUAGAAUGGUAGUACCTT-3′; Scrambled siRNA: sense: 5′-UUCUCCGAACGUGUCACGUTT-3′, antisense: 5′-ACGUGACACGUUCGGAGAATT-3′. Seven days after cultured in vitro, the cells were transfected with 50 nM CREB siRNA using lipofectamine RNAiMAX according to the manufacturer's instructions [17].

2.7. Measurement of mitochondrial DNA (mtDNA)

The total DNA was extracted using GeneRay DNA kit (Shanghai, China) according to the manufacturer's instructions. The mtDNA copy number was measured by real-time PCR with mtDNA primers (cytochrome C oxidase subunit I) (sense, 5′-TCGGAA CCCTC TACCTATT-3′, antisense, 5′-GGCTGTGACGATGACATTA-3′) and 18s rRNA primers (sense, 5′-AACTTTCGATGGTAGTCGC-3′, antisense, 5′-TTCCTTGGATGTGGTAGCC-3′). The ratio of mtDNA to 18s rRNA was calculated, and served as the mtDNA copy number in every group.

2.8. Mitochondrial mass analysis

To analyze mitochondrial mass, the cortical neurons were plated on coverglass bottom dish. After treatments indicated in each group, cells were washed twice with PBS, and mitochondria stained with 30 nM MitoTracker-Red according to the manufacturer's instructions. Cell nuclei were determined by Hoechst 33342. The fluorescent images were captured by confocal laser scanning microscopy (Leica TCS SP5, Wetzlar, Germany). The average red fluorescent intensity in every cell was analyzed by Image J software (NIH), to indicate mitochondrial mass.

2.9. Measurement of ROS

The production of ROS within mitochondria was measured using reactive oxygen species assay kit according to the manufacturer's instructions. Briefly, the cortical neurons were incubated with 10 μM DCFH-DA for 20 min at 37 °C, and then washed three times with warm serum-free DMEM. The fluorescent images were

captured by confocal laser scanning microscopy (Leica TCS SP5, Wetzlar, Germany) with identical exposure settings. The fluorescent intensity in each group was analyzed by Image J software. Ten fields were taken randomly in each group, and quantified for average fluorescent intensity.

2.10. Measurement of mitochondrial membrane potential

The mitochondrial membrane potential ($\Delta\Psi m$) was measured using JC-1 kits according to the manufacturer's instructions, which is specific fluorescent probe for $\Delta\Psi m$. The cortical neurons were washed by warm PSB and incubated with JC-1 at a final concentration 2 μg/ml for 25 min at 37 °C. The images were captured by confocal laser scanning microscopy (Leica TCS SP5, Wetzlar, Germany) with both red and green channels using identical exposure settings. The ratio of fluorescent intensity of the red to green was analyzed by Image J software, and represented $\Delta\Psi m$ level.

2.11. Measurement of intracellular ATP

The intracellular ATP levels were measured using a luminescent cell viability assay (Promega, USA) according to the manufacturer's instructions.

2.12. Western blotting analysis

The cortical neurons were washed with ice-cold PBS, and lysed in RIPA buffer supplemented 1 mM PMSF on ice. The lysates were centrifuged with 12,000 rpm for 15 min at 4 °C, and determined protein concentration using the BCA kit. After separated by SDS–PAGE, the extracted protein were transferred to a PVDF membrane, and blocked with 5% non-fat milk for 1 h at room temperature. The PVDF membranes were incubated with primary antibodies overnight at 4 °C, and washed with TBST three time. Then the blots were incubated with horseradish peroxidase-conjugated secondary antibodies (1:4000 dilution) for 1 h at room temperature. Finally, the bands were detected with ECL reagent kits, and exposed to film.

2.13. Statistics and data analysis

Data are expressed as the mean ± SEM. The significance difference among multiple groups were evaluated by one-way analyses of variance (ANOVA), and $P < 0.05$ were considered significant.

3. Results

3.1. 14,15-EET promoted mitochondrial biogenesis and inhibited apoptosis in cortical neurons under OGD

The copy number of mtDNA was significantly raised by 14, 15-EET pretreatment and remarkably reduced by OGD. The OGD-induced reduction of mtDNA levels was effectively prevented by 14,15-EET (Fig. 1A). Mito-Tracker Red Staining showed that the fluorescence intensity (mitochondrial mass) was diminished in OGD-treated neurons and restored by treatment with 14,15-EET (Fig. 1B). The expression level of mitochondrial protein, voltage-dependent anion channels (VDAC), was significantly decreased in cortical neurons after OGD injury and recovered by 14,15-EET pretreatment (Fig. 1C). OGD remarkably increased apoptosis in

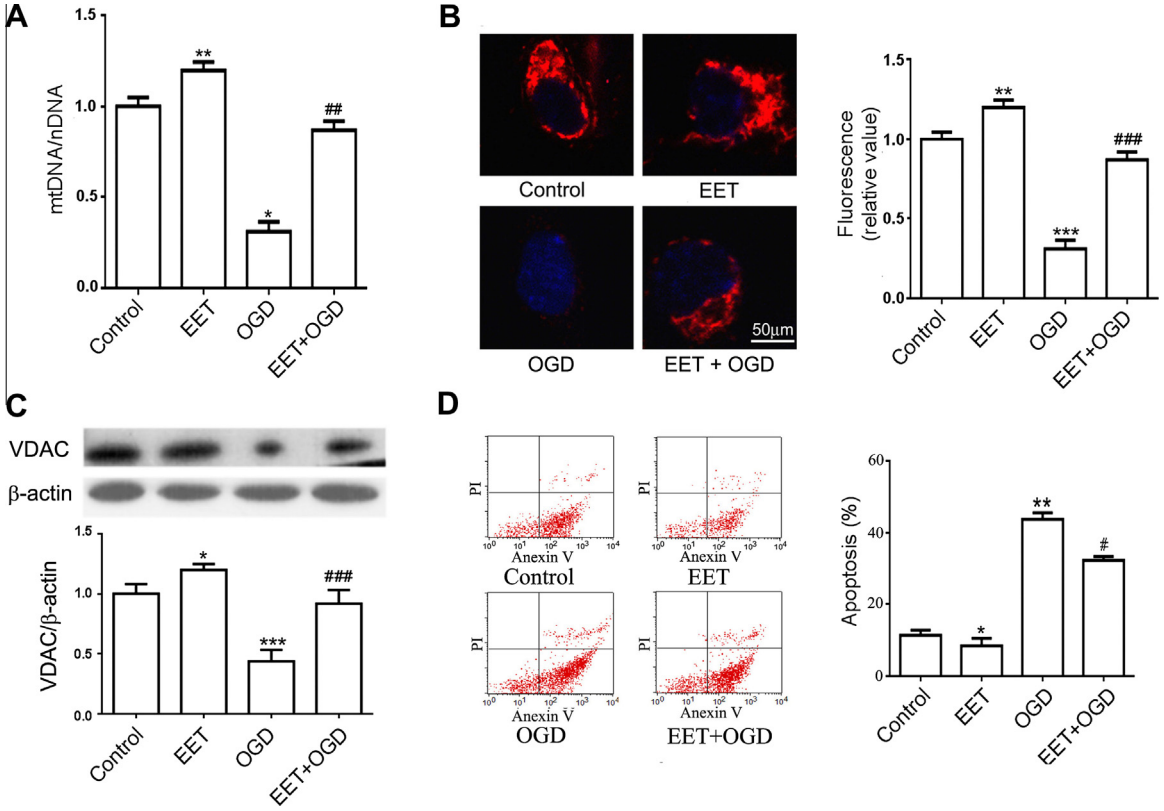

Fig. 1. Effects of 14,15-EET on mitochondrial biogenesis and apoptosis in cortical neurons. (A) The mtDNA copy number was evaluated by quantitative real-time PCR. (B) Mitochondrial mass was measured by Mito-Tracker Red Staining with confocal laser scanning microscopy. (C) Levels of VDAC were analyzed by Western blotting. (D) Apoptosis was detected by flow cytometry with Annexin-V-FITC + PI dual parameters. Data are expressed as means ± SEM of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *versus* Control, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ *versus* OGD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

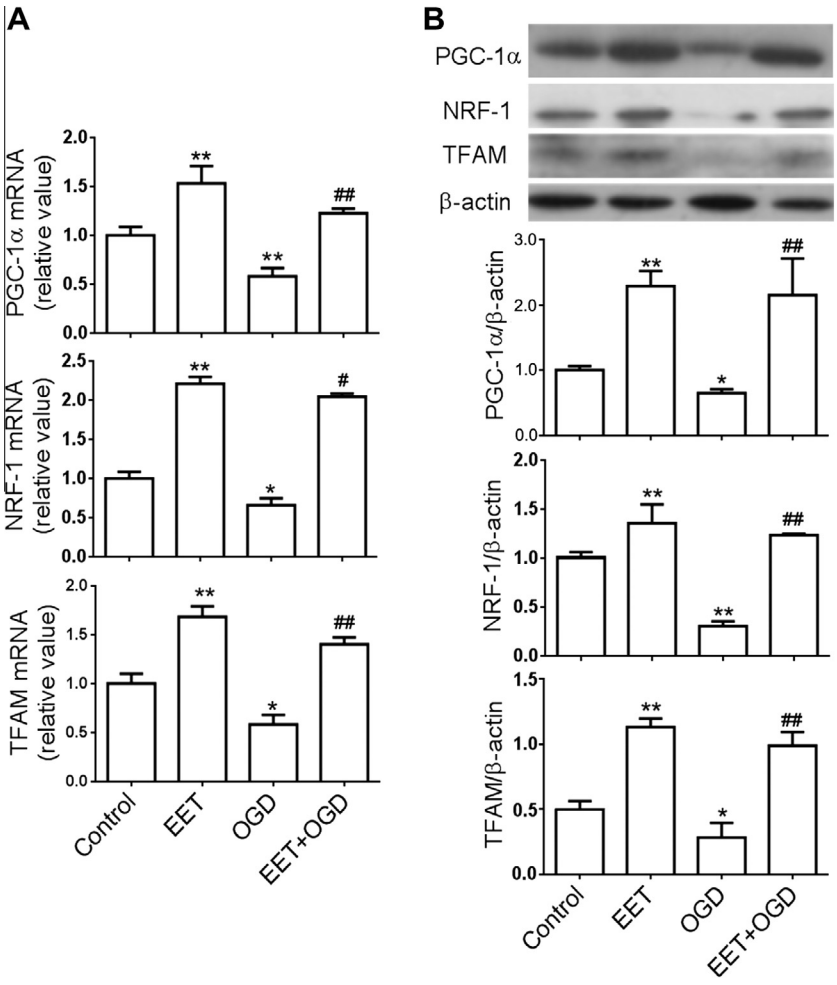

Fig. 2. Effects of 14,15-EET on the expressions of PGC-1α, NRF-1 and TFAM. (A) mRNA levels of PGC-1α, NRF-1 and TFAM were measured by Real-time PCR. (B) Protein levels of PGC-1α, NRF-1 and TFAM were quantified by Western analysis. Data are expressed as means ± SEM of three independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ *versus* Control, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *versus* OGD.

cultured cortex neurons. The proapoptotic effect of OGD was abolished by preincubation with 14,15-EET (Fig. 1D).

3.2. 14,15-EET enhanced the expressions of PGC-1α, NRF-1 and TFAM

Activations of PGC-1α, NRF-1 and TFAM are responsible for the mitochondrial biogenesis, we thus measured expressions of these transcription factors at mRNA and protein levels. Real-time PCR showed that mRNA levels of PGC-1α, NRF-1 and TFAM were significantly increased by the treatment of 14,15-EET. Oxygen–glucose deprivation markedly suppressed the mRNA levels of PGC-1α, NRF-1 and TFAM, which was effectively prevented by pre-incubation with 14,15-EET (Fig. 2A). Western analysis demonstrated a similar effect of 14,15-EET (Fig. 2B).

3.3. 14,15-EET preserved mitochondrial functions in neurons under OGD

To evaluate the protective effect of 14,15-EET on mitochondrial functions, we measured the capacity of ATP production, alteration of mitochondrial membrane potential and generation of reactive oxygen species (ROS) of cultured cortical neurons following OGD. The elevation of ATP content and mitochondrial membrane potentials were observed in the 14,15-EET treated neurons as measured by Luminescent Cell Viability Assay and JC-1 Staining. A significant decrease in ATP content and mitochondrial membrane potential was detected in the condition of OGD, which was effectively restored by 14,15-EET pre-treatment (Fig. 3A and B). The fluorescent intensity of ROS was elevated in cortical neurons under OGD condition, which was significantly inhibited by 14,15-EET pre-treatment (Fig. 3C).

3.4. CREB Knockdown by siRNA abolished the protective effects of 14,15-EET via reducing mitochondrial biogenesis

CREB signal pathway plays important role in mitochondrial biogenesis [18], therefore we investigated the role of CREB signal pathway in the neuroprotective effects of 14,15-EET. Up-regulation of phosphorylated CREB was observed in the cortical neurons treated with 14,15-EET. In the condition of OGD, the phosphorylation of CREB was decreased, which was remarkably restored by pretreatment of 14,15-EET (Fig. 4A). Treatment with 14,15-EET increased the protein expression levels of PGC-1 and NRF-1, which was blocked by CREB siRNA (Fig. 4B and C). Silencing of CREB gene expression by RNA interference entirely eliminated the neuroprotective effect of 14,15-EET on OGD-induced apoptosis in cultured cortical neurons (Fig. 4D).

4. Discussion

The 14,15-EET is enriched in the brain cortex and exerts neuroprotective effect on neuronal cells in ischemia and reperfusion

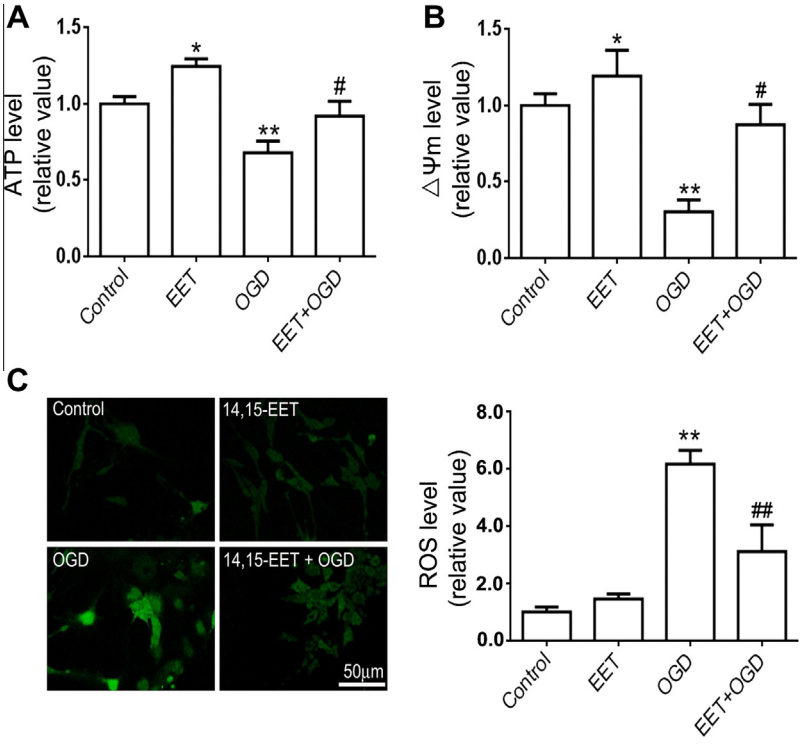

Fig. 3. Effect of 14,15-EET on the mitochondrial function in cortical neurons under OGD condition. (A) ATP levels were detected with Luminescent Cell Viability Assay. (B) The mitochondrial membrane potential was measured by the ratio of red to green fluorescence of JC-1 staining. (C) Generation of ROS was measured with DCFH-DA by confocal laser scanning microscopy. Data are expressed as means ± SEM of three independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ *versus* Control, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *versus* OGD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

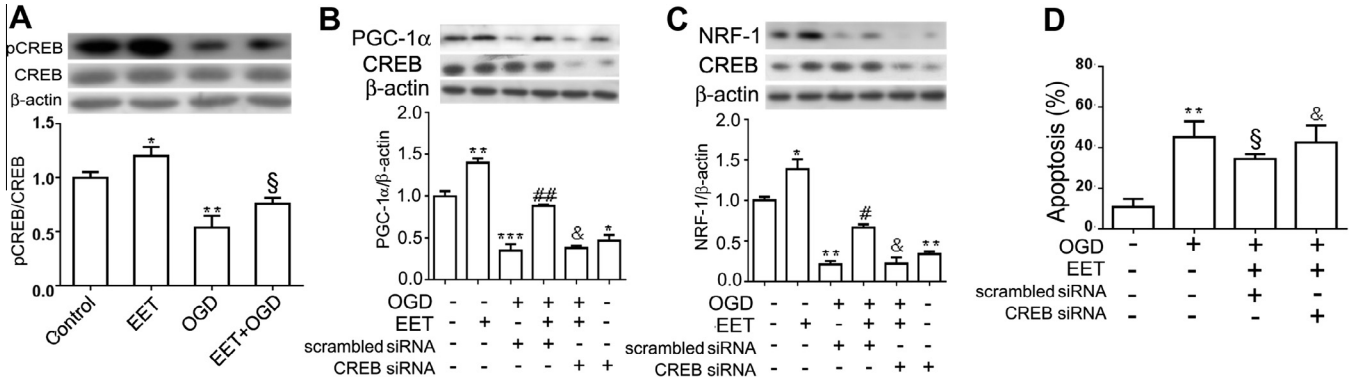

Fig. 4. 14,15-EET enhanced expressions of PGC-1α and NRF-1 through CREB signal pathway. (A) The phosphorylation level of CREB was detected by Western analysis. (B and C) Expressions of PGC-1α and NRF-1 were detected and quantified by Western analysis. (D) Apoptosis was measured by flow cytometry. Data are expressed as means ± SEM of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *versus* control; $^{\S}P < 0.05$ *versus* OGD; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *versus* OGD + scrambled siRNA; $^{\&}P < 0.05$ *versus*14,15-EET + OGD + scrambled siRNA.

[2–6]. Numerous studies demonstrate that the decline in mitochondrial biogenesis and suppression of mitochondrial function are involved in the essential pathological processes of the neuronal injury induced by ischemia and reperfusion [19–23]. Our study further shows that 14,15-EET protects cortical neurons from oxygen/glucose deprivation induced-apoptosis by enhancing mitochondrial biogenesis and function.

Several studies have shown that the mitochondrial mass, the copy number of mtDNA, ATP production, and ROS generation are aberrant in the processes of hypoxia/reoxygenation or ischemia/reperfusion [20–23]. We found that 14,15-EET protected the cortical neurons from oxygen/glucose deprivation-induced apoptosis by increasing mitochondrial mass, mtDNA copy number, ATP synthesis as well as reducing generation of ROS. These observations suggest that 14,15-EET protects cortical neurons from apoptosis by enhancing mitochondrial biogenesis and function. Contradicting to our finding, recent studies have shown that the mitochondrial density, the expression levels of PGC-1α, NRF-1, TFAM, HSP60 and COXIV were elevated in a rat model in the hypoxic/ischemic brain injury [24]. Expressions of mitochondrial voltage-dependent anion channel and Complex V, as well as mitochondrial DNA levels have also been reported to be increased following the oxygen–glucose deprivation in cultured rat cortical neurons [25]. Reasons accounting for these conflicting results are currently unknown, but may be related to the difference in the ischemic models, animal strains and the procedure of the experiment.

A recent study reports that elevated expressions of NRF-1, TFAM, and increased mtDNA copy number are induced by pretreatment

with sublethal lipopolysaccharide, which attenuates apoptosis of cortical neurons in ischemic model in vitro [26]. Overexpression of TFAM stimulates mitochondrial biogenesis to reduce neuronal apoptosis after transient forebrain ischemia in mice [13]. Consistent with these reports, our study suggests that increased expressions of PGC-1α, NRF-1 and TFAM are critical for the neuroprotective role of 14,15-EET. The 14,15-EET may enhance the expressions of nuclear gene-encoded mitochondrial proteins through PGC-1α and NRF-1 signaling pathways, and the expressions of mitochondrial gene-encoded proteins by activating TFAM pathway, respectively. Hence, 14,15-EET may antagonize apoptosis by promoting the expressions of mitochondrial proteins through PGC-1α, NRF-1 and TFAM pathways under the condition of shortage of oxygen and nutrients.

It is well documented that PGC-1α, the master regulator of mitochondrial biogenesis, is regulated by cAMP response element-binding protein (CREB) [18]. Activation of PGC-1α stimulates the expression of NRF-1 which consequently upregulates the function of TFAM to promote the mitochondrial biogenesis [9]. Our results show that phosphorylation of CREB was robustly up-regulated by treatment of 14,15-EET. Silencing CREB gene expression by RNA interference markedly inhibits the expressions of PGC-1α and NRF-1 stimulated by 14,15-EET, and subsequently abolished the neuroprotective effect of 14,15-EET on cortical neurons against apoptosis. These results demonstrate that 14,15-EET promotes mitochondrial biogenesis and function by activating PGC-1α and NRF-1 through CREB pathway, which contributes to the neuroprotective actions of 14,15-EET.

In conclusion, our study suggests that 14,15-EET protects cortical neurons from oxygen–glucose deprivation-induced apoptosis through enhancing mitochondrial function and biogenesis via CREB signaling pathway.

Acknowledgments

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References

[1] J.D. Imig, A.N. Simpkins, M. Renic, et al., Cytochrome P450 cosanoids and cerebrovascular function, Expert Rev. Mol. Med. 13 (2011) e7.
[2] P. Sarkar, J. Narayanan, D.R. Harder, Differential effect of amyloid β on the cytochrome P450 epoxygenase activity in rat brain, Neuroscience 194 (2011) 241–249.
[3] R. Li, X. Xu, C. Chen, et al., Cytochrome P450 2J2 is protective against global cerebral ischemia in transgenic mice, Prostaglandins Other Lipid Mediat. 99 (3–4) (2012) 68–78.
[4] A.N. Simpkins, R.D. Rudic, D.A. Schreihofer, et al., Soluble epoxide inhibition is protective against cerebral ischemia via vascular and neural protection, Am. J. Pathol. 174 (6) (2009) 2086–2095.
[5] A.M. Dorrance, N. Rupp, D.M. Pollock, et al., An epoxide hydrolase inhibitor, 12-(3-adam antan-1-yl-ureido)dodecanoic acid (AUDA), reduces ischemic cerebral infarct size instroke-prone spontaneously hypertensive rats, J. Cardiovasc. Pharmacol. 46 (6) (2005) 842–848.
[6] W. Zhang, T. Otsuka, N. Sugo, et al., Soluble epoxide hydrolase gene deletion is protective against experimental cerebral ischemia, Stroke 39 (7) (2008) 2073–2078.
[7] W. Liu, F. Tian, T. Kurata, et al., Dynamic changes of mitochondrial fission proteins after transient cerebral ischemia in mice, Brain Res. 1456 (25) (2012) 94–99.
[8] H. Chen, C.J. Hu, Y.Y. He, et al., Reduction and restoration of mitochondrial DNA content after focal cerebral ischemia/reperfusion, Stroke 32 (10) (2001) 2382–2387.
[9] R. Anne Stetler, R.K. Leak, Y. Gao, et al., The dynamics of the mitochondrial organelle as a potential therapeutic target, J. Cereb. Blood Flow Metab. 33 (1) (2013) 22–32.
[10] F. Mohagheghi, A. Ahmadiani, B. Rahmani, et al., Gemfibrozil pretreatment resulted in a sexually dimorphic outcome in the rat models of global cerebral ischemia-reperfusion via modulation of mitochondrial pro-survival and apoptotic cell death factors as well as MAPKs, J. Mol. Neurosci. 50 (3) (2013) 379–393.
[11] G. Ashabi, F. Khodagholi, L. Khalaj, et al., Activation of AMP-activated protein kinase by metformin protects against global cerebral ischemia in male rats: interference of AMPK/PGC-1α pathway, Metab. Brain Dis. 29 (1) (2014) 47–58.
[12] S.D. Chen, D.I. Yang, T.K. Lin, et al., Roles of oxidative stress, apoptosis, PGC-1α and mitochondrial biogenesis in cerebral ischemia, Int. J. Mol. Sci. 12 (10) (2011) 7199–7215.
[13] M. Hokari, S. Kuroda, S. Kinugawa, et al., Overexpression of mitochondrial transcription factor A (TFAM) ameliorates delayed neuronal death due to transient forebrain ischemia in mice, Neuropathology 30 (4) (2010) 401–407.
[14] S.N. Batchu, S.B. Lee, V. Samokhvalov, et al., Novel soluble epoxide hydrolase inhibitor protects mitochondrial function following stress, Can. J. Physiol. Pharmacol. 90 (6) (2012) 811–823.
[15] L. Liu, C. Chen, W. Gong, et al., Epoxyeicosatrienoic acids attenuate reactive oxygen species level, mitochondrial dysfunction, caspase activation, and apoptosis in carcinoma cells treated with arsenic trioxide, J. Pharmacol. Exp. Ther. 339 (2) (2011) 451–463.
[16] P. Sarkar, I. Zaja, M. Bienengraeber, et al., Epoxyeicosatrienoic acids pretreatment improves amyloid β-induced mitochondrial dysfunction in cultured rat hippocampal astrocytes, Am. J. Physiol. Heart Circ. Physiol. 306 (4) (2014) H475–484.
[17] B. Qiu, S. Hu, L. Liu, et al., CART attenuates endoplasmic reticulum stress response induced by cerebral ischemia and reperfusion through upregulating BDNF synthesis and secretion, Biochem. Biophys. Res. Commun. 436 (4) (2013) 655–659.
[18] L. Zuo, Q. Li, B. Sun, et al., Cilostazol promotes mitochondrial biogenesis in human umbilical vein endothelial cells through activating the expression of PGC-1α, Biochem. Biophys. Res. Commun. 433 (1) (2013) 52–57.
[19] J. Du, M. Ma, Q. Zhao, et al., Mitochondrial bioenergetic deficits in the hippocampi of rats with chronic ischemia-induced vascular dementia, Neuroscience 231 (12) (2013) 345–352.
[20] L. Sun, M. Zhao, X.J. Yu, et al., Cardioprotection by acetylcholine: a novel mechanism via mitochondrial biogenesis and function involving the PGC-1α pathway, J. Cell. Physiol. 228 (6) (2013) 1238–1248.
[21] H. Rehman, Y. Shi, Z. Zhong, Ischemia/reperfusion inhibits mitochondrial biogenesis after partial hepatectomy in mice, Transplantation 90 (2010) 839.
[22] J. Bi, H. Li, S.Q. Ye, et al., Pre-B-cell colony-enhancing factor exerts a neuronal protection through its enzymatic activity and the reduction of mitochondrial dysfunction in in vitro ischemic models, J. Neurochem. 120 (2) (2012) 334–346.
[23] A. Valerio, P. Bertolotti, A. Delbarba, et al., Glycogen synthase kinase-3 inhibition reduces ischemic cerebral damage, restores impaired mitochondrial biogenesis and prevents ROS production, J. Neurochem. 16 (6) (2011) 1148–1159.
[24] W. Yin, A.P. Signore, M. Iwai, et al., Rapidly increased neuronal mitochondrial biogenesis after hypoxic-ischemic brain injury, Stroke 39 (11) (2008) 3057–3063.
[25] E.A. Wappler, S. Institoris, P. Dutta, et al., Mitochondrial dynamics associated with oxygen–glucose deprivation in rat primary neuronal cultures, PLoS One 8 (5) (2013) e63206.
[26] R.A. Stetler, R.K. Leak, W. Yin, et al., Mitochondrial biogenesis contributes to ischemic neuroprotection afforded by LPS pre-conditioning, J. Neurochem. 123 (2) (2012) 125–137.