Neurochemistry International 129 (2019) 104501

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/neuint

Chronic oleoylethanolamide treatment attenuates diabetes-induced mice encephalopathy by triggering peroxisome proliferator-activated receptor alpha in the hippocampus

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ARTICLE INFO

Keywords:
Oleoylethanolamide
Diabetic encephalopathy
Spatial cognitive function
Neuroprotection
Peroxisome proliferator-activated receptor alpha

ABSTRACT

Brain is a site of diabetic end-organ damage. Diabetes-associated cognitive dysfunction, referred as "diabetic encephalopathy" (DE) has been coined for the patients with type 2 diabetes mellitus showing decline in their cognitive function, especially weak episodic memory, cognitive inflexibility and poor psychomotor performance leading towards Alzheimer's disease. Current evidence supported that aberrant synapses, energy metabolism imbalance, advanced glycation end products (AGEs) accumulation and Tau hyperphosphorylation are associated with cognition deficits induced by diabetes. Oleoylethanolamide (OEA), an endogenous peroxisome proliferator-activated receptor alpha (PPARα) agonist, has anti-hyperlipidemia, anti-inflammatory and neuroprotective activities. However, the effect of OEA on DE is unknown. Therefore, we tested its influence against cognitive dysfunction in high fat diet and streptozotocin (HFD + STZ)-induced diabetic C57BL/6J and PPARα$^{-/-}$ mice using Morris water maze (MWM) test. Neuron staining, dementia markers and neuroplasticity in the hippocampus were assessed to evaluate the neuropathological changes. The results showed that chronic OEA treatment significantly lowered hyperglycemia, recovered cognitive performance, reduced dementia markers, and inhibited hippocampal neuron loss and neuroplasticity impairments in diabetic mice. In contrast, the changes in MWM performance and neuron loss were not observed in PPARα knockout mice via OEA administration. These results indicated that OEA may provide a potential alternative therapeutic for DE by activating PPARα signaling.

1. Introduction

Type 2 Diabetes mellitus (T2DM) is one of the most common endocrine diseases(Whiting et al., 2011). Without control of glycemic imbalance, the diabetic patients often accompany with auxiliary complications, such as hepatopathy, retinopathy, and nephropathy (Lukovits et al., 1999). The central nervous system (CNS) is also very susceptible to hyperglycemia. Diabetes-related cognitive dysfunction was first reported in 1922. A series of neurochemical, neurophysiological and structural abnormalities in diabetic CNS represent a condition referred to as 'diabetic encephalopathy' (DE) or 'diabetes-associated cognitive decline' (DACD)(Biessels et al., 2002; Mijnhout et al., 2006). The pathological mechanism of DE includes neurotransmission obstruction, synaptic damage and cognition impairment (Awad et al., 2004; Bhutada et al., 2011). As previous studies showed that the main pathological markers of Alzheimer's disease (AD), such as the advanced

Abbreviations: T2DM, Type 2 Diabetes mellitus; CNS, central nervous system; DE, diabetic encephalopathy; DACD, diabetes-associated cognitive decline; AD, Alzheimer's disease; AGEs, advanced glycation end products; PPARα, peroxisome proliferator-activated receptor α; OEA, oleoylethanolamide; FBG, fasting blood glucose; HOMA-IR, homeostatic model assessment of insulin resistance; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MWM, morris water maze; RAGE, reports of advanced glycation end products; GAP 43, growth associated protein-43; SYN, synaptophysin; NT3, recombinant human neurotrophin-3; BDNF, brain-derived neurotrophic factor

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https://doi.org/10.1016/j.neuint.2019.104501
Received 2 April 2019; Received in revised form 1 July 2019; Accepted 8 July 2019
Available online 09 July 2019

glycation end products (AGEs) and Tau hyperphosphorylation, are implicated in the cognitive impairments of diabetes patients(Gasparotto et al., 2018). In addition, the pathogenesis of AD is characterized by an imbalance of cellular energetic metabolism(Steen et al., 2005). Neuron loss leads to neurotransmitter blockage, synaptic dysfunction and cognitive impairment (Rostami et al., 2013; van den Berg et al., 2010). The chronic neuronal loss is a common pathological process of diabetes and AD. Therefore, the relationship between hyperglycemia-induced spatial memory impairment and neuronal loss has attracted well attention (Moreno et al., 2004; Pugazhenthi et al., 2017). Peroxisome proliferator-activated receptor α (PPARα) is distributed in all neural cell types and regulates many physiological processes, such as energy metabolism, neurotransmission, redox homeostasis, autophagy and the cell cycle(Feige et al., 2006; Heneka and Landreth, 2007; Oveisi et al., 2004). Recent studies have reported that ligand-activated PPARα inhibits the amyloidogenic pathway, Tau hyperphosphorylation, and neuroinflammation(D'Orio et al., 2018; Santos et al., 2005). However, the role of PPARα pathway in diabetic encephalopathy has not been tested in diabetic mice.

Oleoylethanolamide (OEA) is a potent endogenous ligand for PPARα(Astarita et al., 2006). The protective effects of OEA have been confirmed in different neuropathologic models(Lombardi et al., 2007; Sun et al., 2007). Our previous studies have demonstrated that OEA improved cognitive impairments in cerebral ischemic rats(Yang et al., 2015) and propane-2-sulfonic acid octadec-9-enyl-amide (N15), a structural analogue of OEA, played a therapeutic role in high-fat diet (HFD) and streptozotocin (STZ)-induced T2DM mice(Ren et al., 2018). Meanwhile, we also found that OEA inhibited ischemic stroke-induced neuronal cell apoptosis(Yang et al., 2019; Zhou et al., 2017). The anti-apoptosis effects of OEA were interceded by PPARα pathway, which stabilizes the resting membrane potential during energy failure to control the glycemic level in the brain. These findings indicated that OEA may be considered as a potential therapeutic agent for diabetes induced encephalopathy syndrome. Accumulating data from clinical and experimental studies demonstrated that CNS complications being in both type 1 and type 2 diabetic patients(Kodl and Seaquist, 2008; Luchsinger, 2012; Meek and Morton, 2012; Zhang et al., 2013). Male C57BL/6 J treated with HFD and low-dose STZ are considered an ideal type 2 diabetes animal model(Gilbert et al., 2011). Therefore, in the present study, we investigated the effects of OEA on cognitive deficits in HFD + STZ-induced diabetic mice and revealed the underlying mechanism.

2. Materials and methods

2.1. Animal experiments

Male C57BL/6 J mice (6–8 weeks old, weighing 20–22 g) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). The PPAR α knockout mice (129S4/SvJae-Pparatm1Gonz/J) (7–9 weeks old) weighing 20–22 g were from the Jackson Laboratory (Bar Harbor, ME, USA). Because Sv129 mice are immunodeficient and have a very low reproductive rate, so we hybridized PPARα and C57BL/6 J mice to further obtain PPARα knockout mice. All animals were housed in specific pathogen-free (SPF) conditions in a 12/12-h dark/light cycle environment with unrestricted food and water. All animal studies (including the mouse euthanasia procedure) were performed in compliance with the institutional animal care regulations and guidelines of Xiamen University and according to AAALAC and IACUC guidelines. In addition, maximum efforts were made to minimize the pain and suffering of the animals.

Previous studies indicated that both C57BL/6 J and PPARα knockout mice can be used to induce diabetic models(Yuan et al., 2019). T2DM (or DE) was induced in mice with a mix of HFD and low-dose STZ. After a week of adaptation in the controlled living conditions, C57BL/6 J mice were randomly divided into five groups (n = 6 for each group): Normal, DE, DE + OEA-15, DE + OEA-30, or DE + OEA-60. The PPARα$^{-/-}$ mice were randomly divided into three groups (n = 6 for each group): Normal, DE, DE + OEA-60. The normal groups were fed a normal pellet diet and received an intraperitoneal injection of vehicle throughout the experiment. Other groups were fed an HFD (30% fat, 20% sugar, 15% protein, 2.5% cholesterol, 1% sodium cholic acid and 31.5% custom carbohydrate) for 6 weeks and received an intraperitoneal injection of STZ (40 mg/kg)(Gilbert et al., 2011). Beginning the next day, these mice received a single daily intraperitoneal injection of OEA (15, 30 or 60 mg/kg, respectively, dissolved in Tween-20/saline: 10/90) or vehicle (Tween-20/saline: 10/90) for 56 days.

2.2. Measurement of the fasting blood glucose, insulin resistance and lipoprotein

The fasting blood glucose (FBG) levels were measured using a blood glucose meter (Johnson & Johnson New Brunswick, NJ), once a week, after 8 h of fasting. The serum insulin level was determined using an ELISA kit (ALPCO, Windham, NH). Insulin resistance was assessed by calculating the homeostatic model assessment of insulin resistance (HOMA-IR), which was previously described(Antunes et al., 2016). The serum concentrations of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and the levels of glucose and lactic acid in hippocampal tissue were measured by the commercial assay kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions.

2.3. Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed on C57BL/6J mice following overnight-fasting period at the 6th week post-treatment. Mice were administered glucose orally at 2.0 g/kg and tail blood samples were obtained at 0, 30, 60 and 120 min post glucose load using glucometer.

2.4. Morris water maze test

The Morris water maze (MWM) was performed during days 43–48 and 56 after T2DM(Morris et al., 1982; Peng et al., 2007). The maze was carried out in a white circular container (120 cm in diameter and 60 cm in height) filled with water at 25 ± 1 °C. A submerged white circular platform with a diameter of 6 cm was placed 1.0 cm below the surface of the water. During the maze test, the position of the cues remained unchanged for spatial orientation.

Phases I. Acquisition: Acquisition training was carried out from day 43–47 after T2DM. The mice received four consecutive daily training trials in the following 5 days. For each trial, mice were placed in the water from the same starting position, heading toward the pool wall with a ceiling time of 60 s. If the mouse found the platform, it was allowed to remain on it for 10 s. The time to reach the platform (escape latency) was recorded at the same time. If the mouse failed to find the platform, it was gently guided onto the platform for 20 s, and the escape latency was recorded as 60 s.

Phases II. Spatial probe trials: On day 48, the platform was removed, and the mice were given 60 s to swim freely for the spatial probe trials. The swimming distance, percentage of swimming distance in the former target or target quadrant, and time spent in the target or target quadrant were recorded to measure the spatial memory retention.

Phases III. Memory consolidation test: A probe trial as phase II was performed on day 56 to measure long-term memory retention.

In brief, the schedule is shown in Supplementary Fig. 1.

2.5. Western blot analysis

Western blot analyses were performed as previously described. Briefly, primary antibodies to phospho-Ser214-Tau (1:1000, Abcam), RAGE (1:1000, Cell Signaling Technology), growth associated protein 43 (GAP43) (1:1000, Cell Signaling Technology), synaptophysin (SYN) (1:1000, Cell Signaling Technology), recombinant human neurotrophin-3 (NT3) (1:1000, Cell Signaling Technology), brain-derived neurotrophic factor (BDNF) (1:1000, Cell Signaling Technology) and β-actin (1:10,000; Abcam) at 4 °C overnight. Bound antibodies were incubated with HRP-conjugated goat anti-rabbit secondary antibodies (ZSGB-BIO, China) at room temperature for 1 h. The protein bands were visualized and quantified by scanning densitometry using ImageStation 4000 R (Rochester, New York, USA).

2.6. Immunofluorescence and cell counting

Double-labeling of DAPI and neuron was performed using frozen slides from mice hippocampus as described previously(Zhou et al., 2017). Briefly, the sections were incubated with mouse monoclonal anti-NeuN (1:100; Millipore, Billerica, MA, USA) at 4 °C overnight. After several PBS rinses, sections were incubated with Alexa Flour 594 donkey anti-mouse IgG (1:200; Invitrogen, Carlsbad, CA, USA) for 2 h at room temperature.

2.7. Statistical analysis

The results were expressed as the mean ± S.E.M. Statistical analysis was performed using one-way ANOVA and two-way ANOVA with Tukey's post hoc test for comparisons between two groups followed by the Student-Newman-Keuls test (Prism 5 for Windows, GraphPad Software Inc., USA). P values < 0.05 were considered statistically significant.

3. Result

3.1. OEA suppressed plasma glucose and lipid profiles in diabetic mice

We investigated OEA' s potential in improving glucose metabolism in HFD + STZ-induced T2DM mice. OEA administration effectively reduced plasma glucose concentration after 8 h fasting in HFD + STZ T2DM mice (Fig. 1 A). After six weeks of OEA administration, OGTT assay was conducted to measure the influence of OEA on the whole-body glucose homeostasis, and the results demonstrated that OEA treatment effectively improves glucose tolerance in three dosage groups with a dose-dependent therapeutic effect (Fig. 1 B and Supplemental Fig. 3). OEA also reduced serum insulin and HOMA-IR levels in a dose-dependent manner (Fig. 1 C and D). chronic treatment with OEA (30 and 60 mg/kg) significantly reduced the body weights and water intake level in diabetic mice (see Supplemental Fig. 2). As shown in Fig. 1 E–H, the OEA treated mice exhibited less TC, TG, LDL-C and higher HDL-C levels comparing to the vehicle group, thus indicating the suppressed hyperlipidemia in T2DM mice.

3.2. OEA attenuated diabetes-induced spatial cognitive deficits

Next, we performed MWM test to examine the effect of OEA on T2DM induced cognitive dysfunction. On day 47, we found that the swimming traces of the DE (T2DM) group were distributed around the edges of the four quadrants (Fig. 2 A). Meanwhile, there was a significant difference in escape latency between the diabetic and non-diabetic control mice. Thus, this model could be used to investigate the effects of OEA for improving diabetes-induced cognitive ability. Compared to the diabetic mice, the swimming traces of OEA (30 or 60 mg/kg)-treated mice were concentrated in the target zone where the platform had been set (Fig. 2 A). Moreover, OEA-30 or 60-treated group displayed a shorter latency to find the platform (Fig. 2 B), suggesting that OEA treatment effectively improved the spatial learning ability across the 5-day training period.

During the spatial probe trials, in which the platform was removed, there was no difference in swimming distance among the groups of mice (Fig. 2 C). Simultaneously, OEA (30 or 60 mg/kg) treatment effectively elevated the percentage of time and journey spent on the target quadrant (Fig. 2 D and E).

To investigate the effects of OEA on long-term memory recovery, we tested a probe trial one week after training (Phases III on day 56). The data showed that OEA (30 or 60 mg/kg) treatment markedly reduced the latency compared with the diabetes group (Fig. 2 F). Similar to the spatial probe trials, there was no difference in swimming distance among the groups of mice (Fig. 2 G). After 30 or 60 mg/kg OEA treatment, the percentage of time and journey in the target quadrant were markedly increased in diabetic mice (Fig. 2 H and I). This finding indicated that the decline in cognitive function observed in diabetes mice evidently improved following OEA administration in a dose-dependent manner, especially in long-term memory consolidation.

3.3. OEA decreased hippocampal disturbance of glycometabolism and phosphorylation of tau induced by diabetes

The proper levels of glucose and lactic acid in hippocampal tissue have been shown to be essential for learning and memory consolidation. As illustrated in Fig. 3, the concentrations of glucose and lactic acid were increased in the hippocampus of diabetic mice. However, OEA-60 treatment significantly reduced the level of glucose compared with that in the vehicle group (Fig. 3 A). Additionally, the level of lactic acid was reduced by 30 or 60 mg/kg OEA treatment in the hippocampus of diabetic mice (Fig. 3 B). The results from Fig. 2 show that both 30 and 60 mg/kg OEA have protective effects against cognitive impairment, while the effects of OEA at dose of 60 mg/kg were more potent than those of 30 mg/kg. Therefore, we chose 60 mg/kg to further study the molecular mechanism. It is clear that hyperglycemia triggers the production of AGEs, followed by the induction of Tau hyperphosphorylation(Li and Schmidt, 1997; Ramasamy et al., 2008). There was a significant increase in phospho-Ser214 (p-Ser214) of Tau in the hippocampus of diabetic mice. In contrast, OEA-60 mg/kg treatment significantly decreased Tau phosphorylation at Ser214 (Fig. 3 C and D). Moreover, OEA exhibited a robust decrease in RAGE protein expression (Fig. 3 C and E), suggesting that OEA treatment efficiently improved the diabetes-induced high levels of AGEs.

3.4. OEA inhibited neuron loss in the hippocampus of diabetic mice

Hippocampal neuron loss is closely related to hippocampus-dependent memory. As shown in Fig. 4, immunostaining for NeuN revealed that HFD + STZ administration decreased the number of $NeuN^+$ cells in CA1 hippocampal areas compared with the normal group. In contrast, the number of $NeuN^+$ cell in the CA1 hippocampal areas was robustly increased by OEA-treatment after T2DM. These data suggested that chronic treatment with OEA significantly inhibited hippocampal neuron loss induced by HFD + STZ.

3.5. OEA promoted hippocampal neuroplasticity

To further investigate the mechanism by which OEA improves diabetes-induced cognitive dysfunction, we assessed whether chronic OEA treatment was able to promote hippocampal neuroplasticity. There was a significant increase in the protein expression of GAP43 and SYN in the OEA-treated diabetic mice, suggesting that chronic OEA treatment could enhance hippocampal neuroplasticity after T2DM (Fig. 5 A–C). Additionally, the levels of NT3 and BDNF were markedly upregulated by OEA treatment compared with diabetic mice (Fig. 5 A, D and E). These data indicated that chronic OEA treatment could enhance

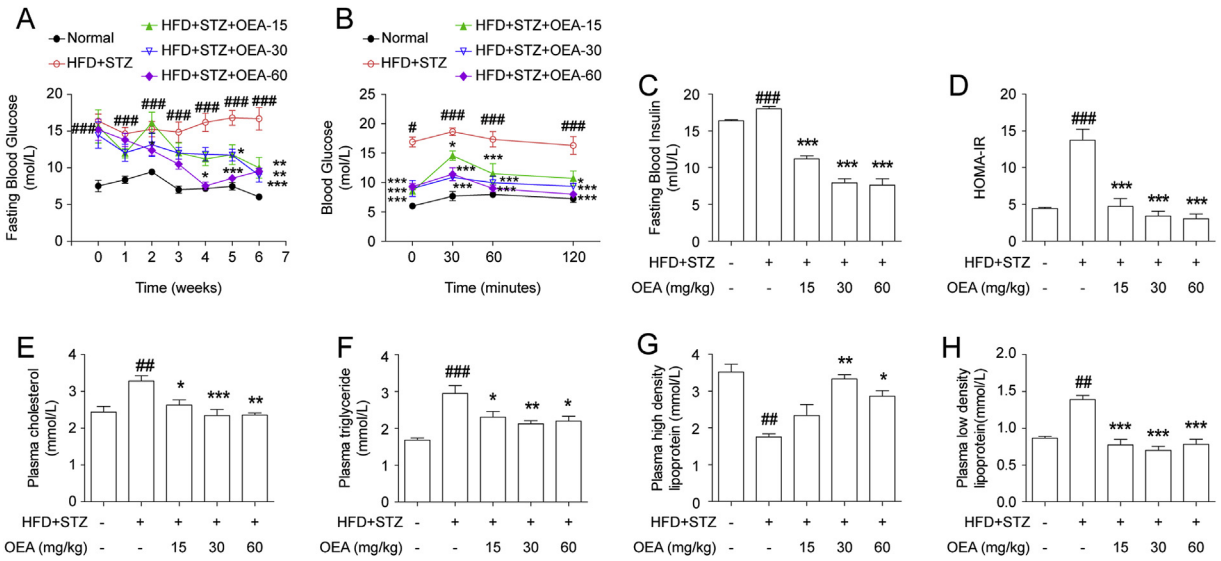

Fig. 1. OEA attenuated plasma glucose and lipid profiles in diabetic mice. OEA-treated groups received single daily intraperitoneal injections of OEA (15, 30 or 60 mg/kg), while the normal and HFD + STZ groups were treated with vehicle on a daily basis. Sequential monitoring of blood glucose after 8 h of fasting (A), glucose tolerance (B), fasting blood insulin (C), HOMA-IR (D), and levels of total cholesterol, total triglycerides, high-density lipoprotein and low-density lipoprotein in serum (E–H) were measured after 6 weeks of treatment. Values are means ± S.E.M. (n = 6 per group). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs.* the normal group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* the DE group.

hippocampal neuroplasticity after T2DM.

3.6. OEA improved neuron loss and spatial cognitive deficits through PPARα

To confirm whether OEA treatment could reduce neuron loss and improve cognitive function through PPARα, we further employed PPARα$^{-/-}$ mice to induce T2DM. We found that the number of NeuN^{+} cells was markedly decreased after T2DM in PPARα knockout mice. Interestingly, the number of NeuN^{+} cells was not increased after OEA treatment in CA1 hippocampal areas of diabetic PPARα$^{-/-}$ mice (Fig. 6 A). However, the NeuN positive cells was increased in C57BL/6 J mice after OEA treatment (Fig. 4). These data suggested that PPARα was essential for OEA-improved neuron loss.

We further tested spatial memory using the MWM in PPARα knockout mice after 56 days of OEA treatment. Consistent with the C57BL/6 J mice tests, no significant difference was observed in swimming distance among the groups (Fig. 6 D), and diabetic mice had a poorer memory regarding the platform location. As the latency of diabetic PPARα$^{-/-}$ mice to find the platform was significantly increased compared with the normal group, the percentage of time and journey in the target quadrant were markedly decreased in diabetic PPARα$^{-/-}$ mice. However, the roles of OEA in reducing latency time and increasing target quadrant time or journey were not present in diabetic PPARα$^{-/-}$ mice (Fig. 6 C, E, and F), indicating that OEA alleviated spatial cognitive deficits in a PPARα-dependent manner.

4. Discussion

Our previous study documented the positive effects of OEA in stroke-induced cognitive deficits(Yang et al., 2015). Here, we found that OEA provided protection against diabetes-induced encephalopathy in HFD + STZ-treated mice. OEA reversed spatial cognitive impairment and reduced hippocampal hyperglycation after T2DM. Additionally, OEA exerted a potent anti-DE action by inhibiting neuron loss and consequently promoting neuroplasticity, and, more importantly, that the mechanism underlying these effects is dependent on PPARα, particularly in terms of neuron loss.

Diabetes encephalopathy has a complex pathophysiology. Epidemiological studies have reported that diabetes is associated with a greater lethality of stroke and Alzheimer's disease(Lukovits et al., 1999). Conversely, T2DM increases the risk of atherosclerosis and is viewed as an autonomous risk factor for stroke(Bruno et al., 2008). Therefore, diabetes treatment is an important goal for prevention of DACD morbidity. OEA is well known to induce weight loss and satiety via activation of PPARα receptor. OEA displays diverse properties and involves peripheral and/or central mechanisms. The effects of OEA on receptors such as TRPV1, GPR119 or PPAR-α receptor have been described(Sarro-Ramirez et al., 2013). These suggested that OEA may have anti-atherosclerosis, anti-oxidant, neuroprotective and anti-diabetic potential effect. The neuroprotective effect of OEA has been verified in our previous study(Yang et al., 2015; Zhou et al., 2012, 2017). In present study, our data showed that OEA exerted a potent anti-insulin resistance action. Moreover, OEA effectively inhibited atherosclerotic plaque formation in HFD-fed ApoE$^{-/-}$ mice(Ma et al., 2015). The compounds of antihyperglycemics and insulin-sensitizing ameliorate memory deficit in the diabetic state. With these backgrounds, the present study was attempted to examine the roles of OEA in diabetes-induced cognitive impairment and revealed the underlying mechanism involving these roles. As shown in the MWM test, OEA considerably decreased the escape latency during task acquisition of diabetic mice, implying that OEA treatment adequately improved the spatial learning abilities of diabetic mice. The probe trials were tested one week after training, in which OEA was effective in long-term memory recovery after T2DM, as indicated by the decreased escape latency and increased time and journey spent in the target quadrant. These data confirmed that OEA treatment attenuated learning and memory impairment caused by T2DM.

Hippocampal glucose metabolism is also crucial for the development of diabetes, impairing brain functions. In our study, 56 days OEA administration abolished the enhanced glucose level in the hippocampus of mice with T2DM, indicating that OEA could facilitate the efficiency of hippocampal glucose metabolism in diabetic mice. It is known that a higher level of glucose can inhibit mitochondrial oxidative phosphorylation, which in turn elevates nonoxidative glucose utilization and then accelerates the production of lactic acid(Belanger et al., 2011). Indeed, we found that the level of lactic acid was increased in diabetic mice hippocampus. However, OEA treatment decreased the

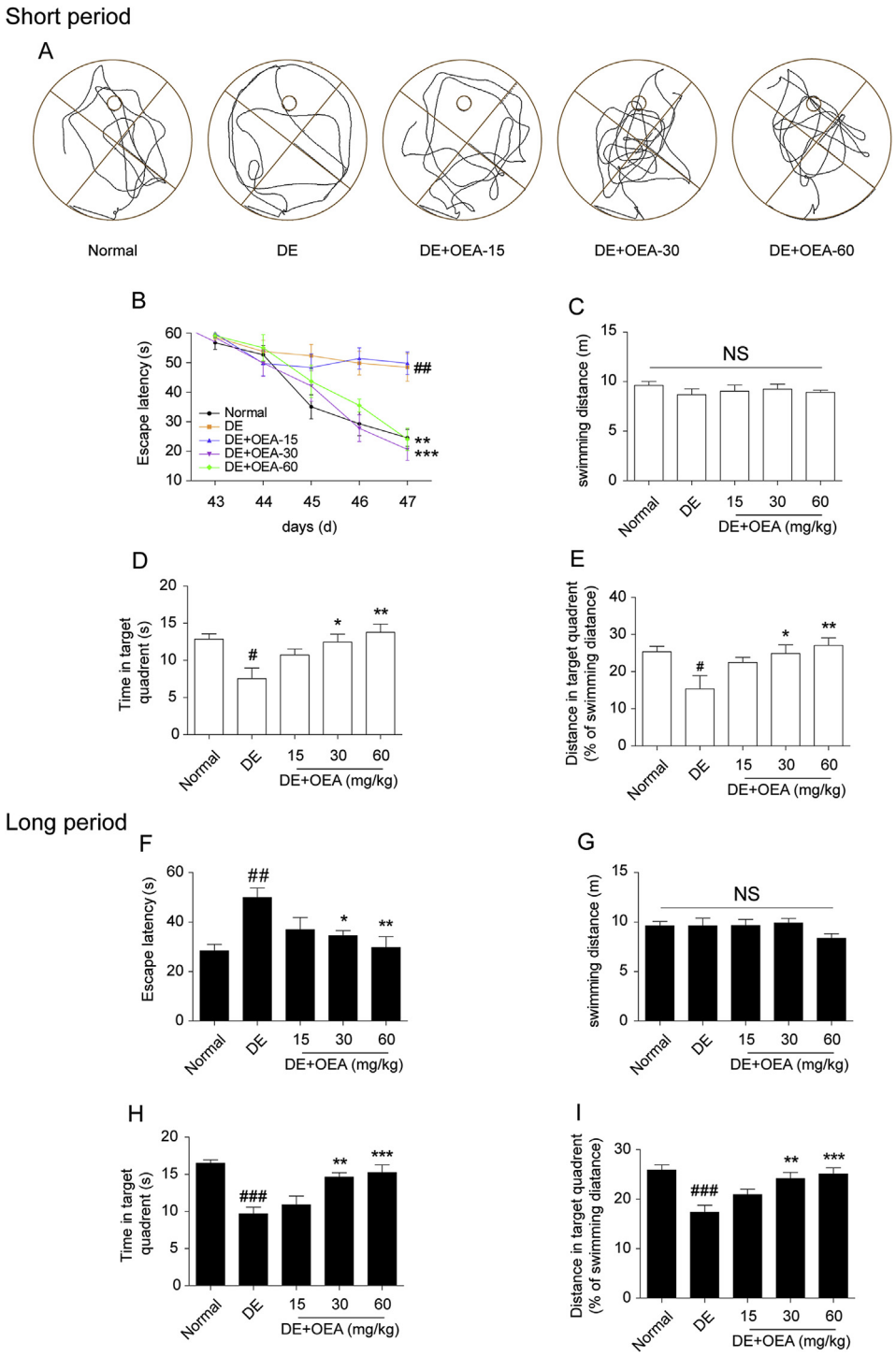

Fig. 2. OEA ameliorated T2DM-induced spatial cognitive deficits. A **representative swimming trace in the spatial probe trials for each group (A). The escape latency (B and F) and swimming distance (C and G) in the hidden platform trials. The time spent (D and H) and the distance (E and I) in the target quadrant. Values are means** ± S.E.M. (n = 6 per group). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs.* the normal group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the DE group.

lactic acid level compared to diabetic mice. Overproduction of lactic acid may provide an energy substrate for neurons to repay for the downregulated glucose oxidative phosphorylation. Moreover, an insufficient energy supply induced by the decline of glucose oxidative phosphorylation would lead to spontaneous apoptosis in neurons and glial cells. Thus, our results suggested that OEA might play an important role in the regulation of hippocampal energy metabolism under high glucose conditions.

Recent evidence has indicated that T2DM is one of the major risk factors for AD development (Yarchoan and Arnold, 2014). Consistent with previous work(Yoon et al., 2010), the level of hyperphosphorylated Tau was markedly increased in the hippocampus of T2DM mice, but this change was reversed by OEA treatment. It has been shown that hyperglycemia promotes Tau hyperphosphorylation in the hippocampus, finally resulting in the dysfunction of neural cells (Gasparotto et al., 2015). Under physiological conditions, RAGE is low

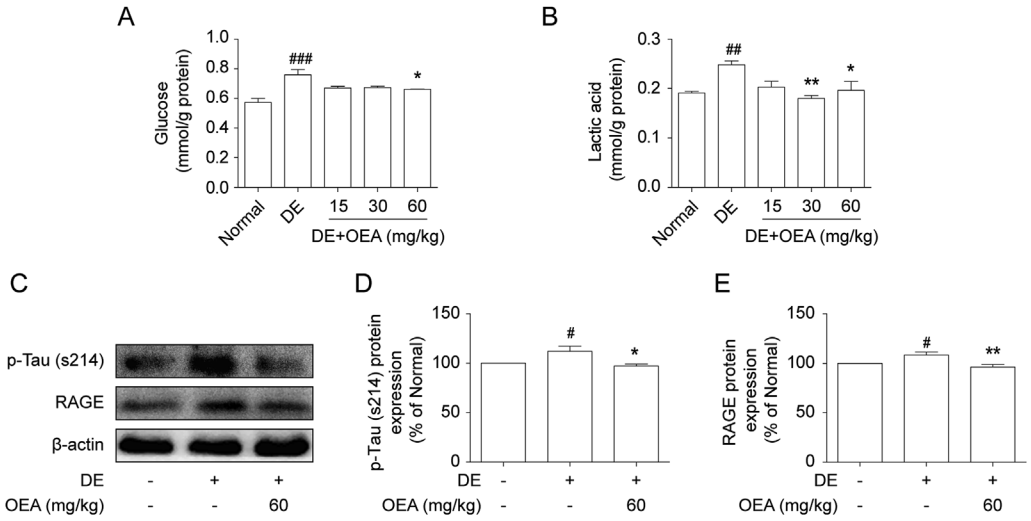

Fig. 3. Effects of OEA on glucometabolism and Tau hyperphosphorylation in the hippocampus. The levels of glucose (A) and lactic acid (B) in the hippocampus were measured after 56 days of treatment. Representative Western blot images (C) and quantitative analysis of protein expression of p-Tau (D) and RAGE (E) in the hippocampus of mice after 56 days of treatment. Values are expressed as percentages compared with the normal group (set to 100%) and are represented as means ± S.E.M. (n = 6 per group). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs.* the normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* the DE group.

expressed in most tissues. However, RAGE expression is significantly elevated in chronic diseases. Here we found that OEA treatment exhibited a robust decrease in the expression of RAGE protein, suggesting that OEA treatment efficiently improved diabetes-induced high levels of AGEs. Tau hyperphosphorylation and RAGE expression were chosen as markers for evaluating the process of DE. Therefore, our results may provide insights into the mechanism of OEA in T2DM-induced cognitive impairments.

It has been observed that learning and memory impairment could result from neuron loss, and thus we next examined the status of neuron loss in each group. In the present study, $NeuN^+$ cells number in the CA1 hippocampal areas was greatly enhanced by OEA-treatment after T2DM. These results suggest that the role of OEA in promoting cognitive function is closely related to enhancing neuron survival in diabetic mice. Growing preclinical literature provides ample evidences that diabetes negatively impacts the morphological integrity of the hippocampus and reduces hippocampal neurogenesis, in concert with deficits of other forms of neuroplasticity, may contribute to comorbid cognitive and mood symptoms in diabetes(Bocchetta et al., 2016). In the present study, the MWM test and NeuN staining were chosen as methods for evaluating the effect of OEA on diabetic-associated cognitive defects. Our data indicated that chronic OEA treatment attenuated damage in the hippocampus after diabetes-induced encephalopathy.

Neuroplasticity refers to the ability of the brain to adapt, change and reorganize throughout life. GAP43 is a neuron-specific axon protein that plays an important role in the regulation of neurite outgrowth, growth cone guidance and synaptic plasticity(Liu et al., 2005). SYN is a presynaptic vesicle protein that has a neuroprotective role in synaptogenesis and synapse functions(Maggio and Vlachos, 2014). Our results exhibited that OEA treatment markedly increased the expression of both GAP43 and SYN. It indicated that the role of OEA in improving diabetes-induced cognitive impairment might have occurred through enhancing synaptic plasticity. The neurotrophic factors, such as BDNF and NT3, play critical roles in axonal growth, survival of existing neurons and plasticity (Zakharenko et al., 2003). Our data showed that OEA treatment significantly increased the levels of BNDF and NT3. Therefore, targeting hippocampal neurotrophy and neuroplasticity may be a potential strategy to reduce the risk of DE and improve DE outcomes. Considering that neuron loss is closely related to the neurotrophy deficiency and neuroplasticity impairment, and that OEA promotes protein levels of GAP43, SYN, BDNF and NT3, thus the modulatory effects of OEA on neurotrophy and neuroplasticity after DE may be associated with promoting neuron survival. PPARα is expressed in high levels in the liver, intestinal mucosa, skeletal muscles, heart, and brown adipose tissue, where it plays an important role in fatty acid metabolism, as well as glucose and lipid metabolism(Lagana et al., 2016; Vitale et al., 2016). PPARα is also highly expressed in the brain and is considered the crossroads of obesity, diabetes, inflammation and AD. OEA is a high-closeness agonist of PPARα. Meanwhile, OEA regulates peripheral lipid metabolism through activation of the nuclear receptor PPARα(Fan et al., 2014; Fu et al., 2003). Here, we found that OEA not only regulate blood lipid metabolism in diabetic mice, but also blood glucose metabolism, especially glucose metabolism in hippocampus. In an addition, the data showed that OEA efficiently suppressed neuron loss in CA1 area of diabetic mice hippocampus. In contrast, OEA failed to normalize the number of $NeuN^+$ cells in PPARα

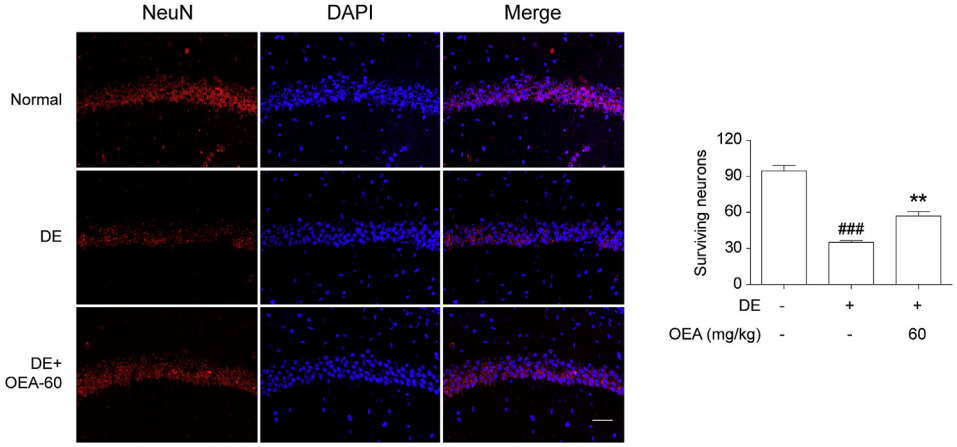

Fig. 4. OEA inhibited neuron loss in the hippocampus of diabetic mice. Representative confocal fluorescence images immunolabeled with NeuN (red) and DAPI (blue) antibodies (A). Quantitative analysis of $NeuN^+/DAPI^+$ (B). Values are means ± S.E.M. (n = 6 per group). $^{\#\#\#}P < 0.001$ *vs.* the normal group; $^{**}P < 0.01$ *vs.* the DE group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

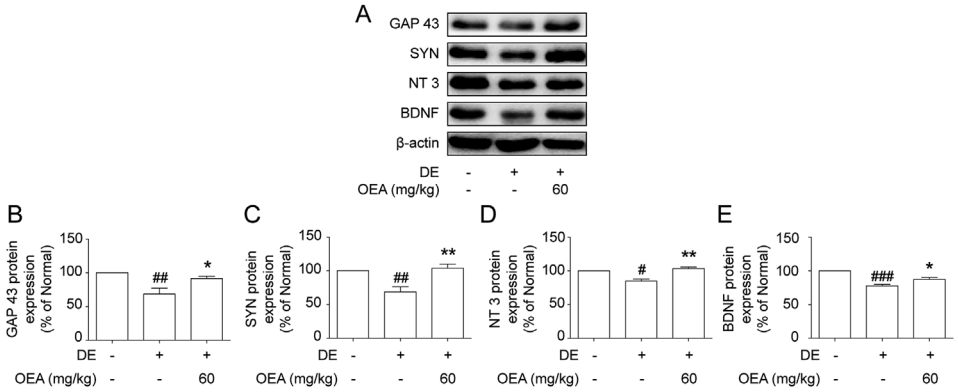

Fig. 5. OEA promoted hippocampal neuroplasticity and neurogenesis. Representative Western blot images (A) and quantitative analysis of protein expression of GAP 43 (B), SYN (C), NT 3 (D) and BDNF (E) in the hippocampus of mice. Values are expressed as percentages compared with the normal group (set to 100%) and are represented as the means ± S.E.M. (n = 6 per group). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs*. the normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs*. the DE group.

knockout mice, suggesting that PPARα is not related to regulation energy metabolism but also to neuroprotection. Meanwhile, our data also revealed the beneficial roles of OEA in the recovery of cognitive ability. Interestingly, these beneficial effects were not discovered in the diabetic PPARα$^{-/-}$ mice, indicating that OEA alleviated spatial cognitive deficits in a PPARα-dependent manner. However, OEA is not only an endogenous agonist of PPARα, but also TRPV1 and GPR119 receptors. Therefore, the inhibitory effect of OEA on encephalopathy in diabetic mice whether is related to TRPV1 and GPR119 receptors need prove by further experiments.

In conclusion, our data demonstrated that chronic OEA treatment debilitated diabetes-induced cognitive deficits and regulated hippocampal energy metabolism in the diabetic mice. Furthermore, the anti-diabetic encephalopathy effects of OEA occur *via* a PPARα-mediated pathway. Accordingly, OEA may be a promising neuronal protective agent for diabetic encephalopathy treatment.

5. Author contributions

L-cY and XJ conceived and designed the experiments. TR, J-fL and

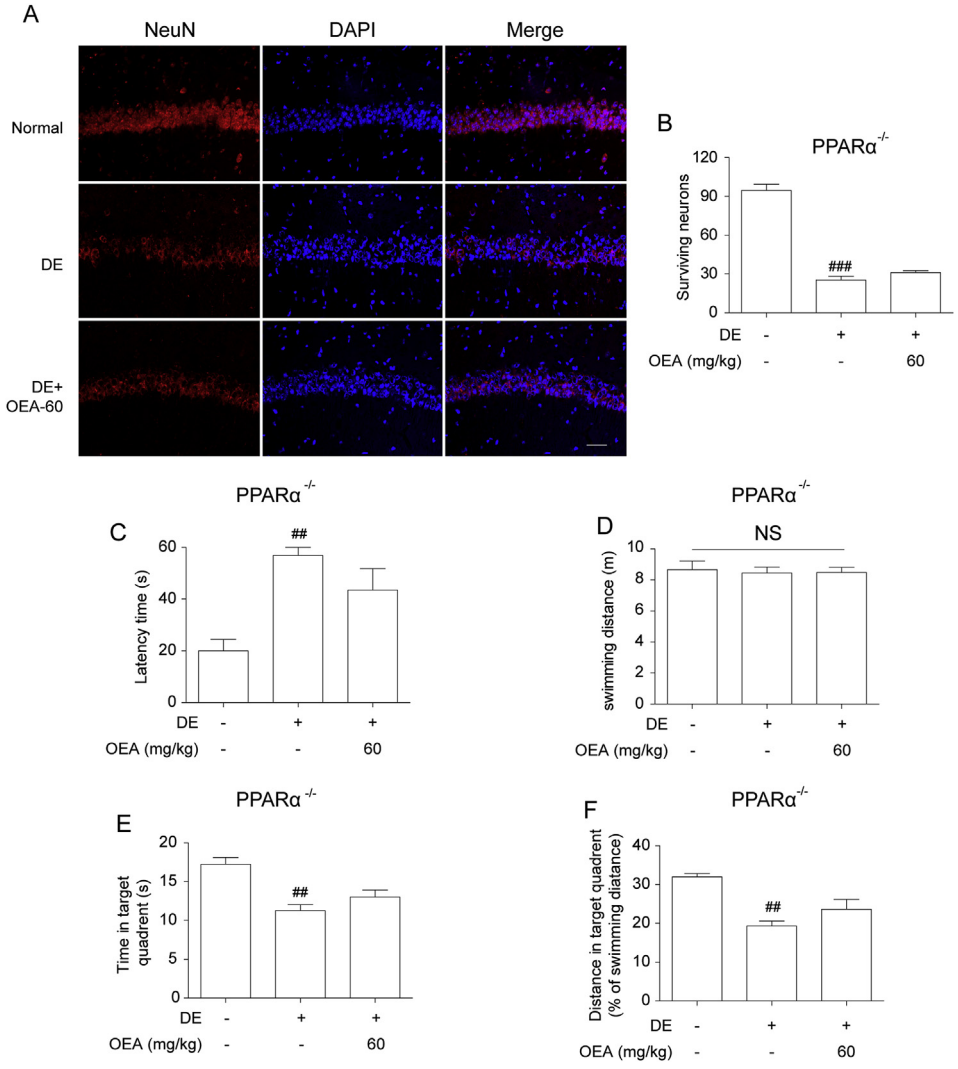

Fig. 6. PPARα was essential for OEA-improved neuron loss and spatial cognitive deficits. PPARα$^{-/-}$ mice were employed to induce type 2 diabetes mellitus. Representative confocal fluorescence images immunolabeled with NeuN (red) and DAPI (blue) antibodies (A). Quantitative analysis of NeuN^{+}/DAPI^{+} (B). The escape latency (C) and swimming distance (D) in the hidden platform trials. The time spent (E) and the percentage of distance (F) in the target quadrant. The time spent (G) and percentage of distance (H) in the platform area. Values are means ± S.E.M. (n = 6 per group). $^{\#\#}P < 0.01$ *vs*. the normal group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Y-lG performed the experiments. PL and R-gZ analyzed the data. L-cY and FL wrote the paper. All authors reviewed and gave final approval.

Disclosure/conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This study was supported by grants from the Fundamental Research Funds for the Central Universities (No. 20720180042), the National Natural Science Foundation of China (No. 81872866, 81601227), the Health Science Research Personnel Training Program of Fujian Province (2018-CXB-30), the Natural Science Foundation of Fujian, China (Nos. 2016J01415, 2016D024), the higher talent start-up fund of Xiang'an hospital and the Science and Technology Project of Xiamen, China (No. 3502Z20174029).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2019.104501.

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