



β -Amyrin Ameliorates Alzheimer's Disease-Like Aberrant Synaptic Plasticity in the Mouse Hippocampus

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Abstract

Alzheimer's disease (AD) is a progressive and most frequently diagnosed neurodegenerative disorder. However, there is still no drug preventing the progress of this disorder. β -Amyrin, an ingredient of the surface wax of tomato fruit and dandelion coffee, is previously reported to ameliorate memory impairment induced by cholinergic dysfunction. Therefore, we tested whether β -amyryn can prevent AD-like pathology. β -Amyrin blocked amyloid β (A β)-induced long-term potentiation (LTP) impairment in the hippocampal slices. Moreover, β -amyryn improved A β -induced suppression of phosphatidylinositol-3-kinase (PI3K)/Akt signaling. LY294002, a PI3K inhibitor, blocked the effect of β -amyryn on A β -induced LTP impairment. In *in vivo* experiments, we observed that β -amyryn ameliorated object recognition memory deficit in A β -injected AD mice model. Moreover, neurogenesis impairments induced by A β was improved by β -amyryn treatment. Taken together, β -amyryn might be a good candidate of treatment or supplement for AD patients.

Key Words: β -amyryn, Amyloid β , Alzheimer's disease, Synaptic plasticity

INTRODUCTION

Alzheimer's disease (AD) is a progressive and degenerative brain disease with dementia (Dubois *et al.*, 2007). Although early studies found that senile plaques, aggregations of amyloid β (A β), and tangles, aggregations of tau protein, are the major hallmarks of AD and they could induce AD-like pathology (Selkoe, 1991), pharmacological prevention or supplements of AD progression are still not developed.

A β , which can be produced by the cleavage of amyloid precursor protein, a membrane protein, is toxic when it is aggregated into oligomeric and fibril forms (Hardy and Selkoe, 2002; Viola and Klein, 2015). Extracellular oligomeric A β can bind to various receptors and ion channels, resulting in their dysfunction (Hardy and Selkoe, 2002; Viola and Klein, 2015).

Moreover, oligomeric A β can penetrate into intracellular space and cause dysfunction of various intracellular signaling systems (Hardy and Selkoe, 2002; Viola and Klein, 2015). These various dysfunctions induce neuronal and synaptic dysfunction resulting in dementia (Hardy and Selkoe, 2002; Viola and Klein, 2015). Therefore, an agent that can prevent A β -induced dysfunction of various neuronal functions could be developed as a treatment or supplement for preventing the progression of AD.

Oligomeric A β is reported to induce aberrant synaptic plasticity, a cellular model of learning and memory (Walsh *et al.*, 2002). Oligomeric A β blocks long-term potentiation (LTP) and facilitates long-term depression (LTD). Previous findings indicated that agents preventing A β -induced aberrant synaptic plasticity could ameliorate AD-like pathologies, includ-

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ing memory impairment (Fu *et al.*, 2014; Yan *et al.*, 2016). Therefore, trials to find agents that can modulate A β -induced aberrant synaptic plasticity could be a good method for finding medicines or supplements for AD.

β -Amyrin is natural chemical compound of the triterpene class. β -Amyrin is widely distributed in plants and is a component of glycyrrhizin, which is a major bioactive compound with a wide range of pharmacological properties and is used worldwide as a natural sweetener. Moreover, β -amyrin is also present in the surface wax of the tomato fruit (Szakiel *et al.*, 2012). Previous studies revealed that β -amyrin has anti-fibrotic effects on liver injury models (Krishnan *et al.*, 2014; Thirupathi *et al.*, 2017), and anti-inflammatory (Okoye *et al.*, 2014), anti-diabetic (Nair *et al.*, 2014), anti-hyperglycemic, and hypolipidemic effects (Maurya *et al.*, 2012; Santos *et al.*, 2012). Moreover, β -amyrin induces angiogenesis (Ishii *et al.*, 2015). Recent findings indicated that β -amyrin has neurological effects including regulating sleep (Jeon *et al.*, 2015), memory (Park *et al.*, 2014), and nociception (da Silva *et al.*, 2011; Chicca *et al.*, 2012). However, the effect of β -amyrin on AD has not been studied before. In the present study, we tested whether β -amyrin has anti-AD effects using electrophysiological and behavioral studies. Minocycline is a broad-spectrum tetracycline antibiotic drug. Although minocycline has various side effects in clinical uses, various reports suggested minocycline as potential therapeutics with various positive effects in AD models (Noble *et al.*, 2009; Ferretti *et al.*, 2012; Amani *et al.*, 2019). Therefore, we used minocycline as positive control.

MATERIALS AND METHODS

Materials

A β was purchased from AnaSpec (CA, USA). Anti-phospho-phosphatidylinositol-3-kinase (pPI3K), PI3K, phospho-Akt (pAkt), and Akt antibodies were obtained from Cell Signaling Technology (MA, USA). β -amyrin and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). LY294002 and U0126 were obtained from Tocris Bioscience (MO, USA).

Animals

We obtained male CD-1 mice (26-28 g, 6 weeks old) from SAMTAKO biokorea (Osan, Korea). Mice were housed in an animal facility for 1 week prior to the study for adaptation to the new environment. Four mice were kept in a cage and allowed free access to water and food (temperature: $23 \pm 1^\circ\text{C}$, humidity: $60 \pm 10\%$). The lights were on from 07:00 to 19:00. The institutional Animal Care and Use Committee of Dong-A University approved protocols for all animal experiments. All animal experiments followed the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Hippocampal slice preparation and field EPSP recording

Artificial cerebrospinal fluid (ACSF) was comprised of 124 mM NaCl, 3 mM KCl, 26 mM NaHCO₃, 1.25 mM NaH₂PO₄, 2 mM CaCl₂, 1 mM MgSO₄, and 10 mM D-glucose. We rapidly removed the brain and isolated the mouse hippocampus. Mouse hippocampal tissues were sliced using a Mcllwain tissue chopper. 400- μm -thick hippocampal slices were made

and incubated in ACSF (20-25°C) for 1 h before the experiment.

Field potential responses were recorded in the Schaffer collateral-commissural pathway in area CA1. Stimuli (constant voltage) were delivered at 30 s intervals. The slope of the evoked field potential responses (fEPSP) was averaged from four consecutive recordings evoked at 30 s intervals. To induce LTP, two trains of high frequency stimulation (HFS: 100 Hz, 100 pulses in 1 s, 30 s interval) were introduced at 20 min after the initiation of a stable baseline. LTP was quantified by comparing the mean fEPSP slope at 80 min after the HFS period with the mean fEPSP slope during the baseline period and calculating the percentage change from the baseline. For the experiments, slices were incubated in ACSF containing vehicle or drugs for 30 min, and then further incubated in ACSF containing A β (1 μM) and/or drugs for 2 h before recording.

Western blot

Hippocampal slices were incubated in β -amyrin-containing ACSF for 30 min. Then the slices were further incubated in A β and β -amyrin-containing ACSF for 2 h. Afterwards hippocampal slices were homogenized in ice-chilled M-PER buffer (Thermo, Rockford, IL, USA), a containing protease inhibitor, and phosphatase inhibitor cocktail (Thermo). Debris was removed by microcentrifugation (4200 \times g, 20 min). Proteins from whole-cell lysates were quantified using a BCA protein assay kit following the manufacturer's instructions. Samples (30 μg of protein) were then subjected to SDS-PAGE (12% gel) under reducing conditions. Proteins were transferred to PDVF membranes using transfer buffer (25 mM Tris-HCl, pH 7.4 containing 192 mM glycine and 20% v/v methanol) at 400 mA for 2 h (4°C). Next, blots were incubated for 2 h with blocking solution (5% skimmed milk for total proteins, 5% BSA for phosphorylated proteins) and then placed at 4°C overnight with 1:1000 dilutions of primary antibody. After serial washing, blots were incubated with a 1:5000 dilution of horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology) for 1 h at room temperature.

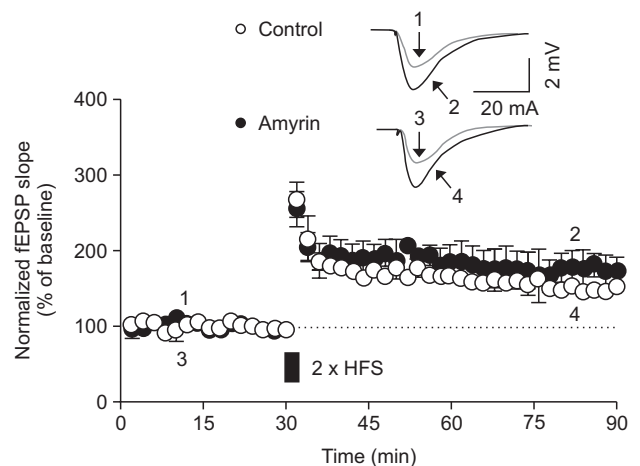


Fig. 1. The effect of β -amyrin on hippocampal long-term potentiation (LTP). LTP was measured in Schaffer-collateral pathway of the hippocampus. β -amyrin was pretreated for 2 h to hippocampal slices. Data represent as mean \pm SD ($n=7$ /group).

Aβ injection and drug administration

We added 1.0% NH₄OH directly to the Aβ₁₋₄₂ (35-40 μl to 0.5 mg peptide or 70-80 μl to 1 mg peptide). This solution was immediately diluted with 1X phosphate-buffered saline (PBS) to a concentration of 1 mg/ml. The solution was gently vortexed and sonicated at room temperature until fully miscible. Aβ₁₋₄₂ (10 μM) was incubated at 37°C for 24 h to obtain various soluble oligomeric species, and then 5 μl of Aβ or vehicle (PBS) was acutely injected into the left lateral ventricle by hand (Kim *et al.*, 2016). β-Amyrin (4 mg/kg, p.o.) and vehicle (10% tween 80 solution) was administered from 1 day to 5 day after the Aβ injection (Park *et al.*, 2014). Minocycline (30 mg/kg, i.p.) was used as a positive control (Jiang *et al.*, 2015).

Behavioral tests

Object recognition test: Mice were habituated to the open field (25 cm×25 cm×25 cm) with an internal cue on one of the four walls for 10 min. Thirty minutes after the habituation; mice were re-placed in the same box with two distinct objects. The objects consisted of a glass box and a plastic cylinder. Mice were allowed to freely explore the objects freely for 10 min. After 2 h, mice were placed back in the same box for the test phase. The two objects were again present, but one object was now displaced to a novel spatial location. Mice were allowed to freely explore the environment and the objects for 5 min. Time spent exploring the displaced and non-displaced objects were measured. A preference ratio was calculated using the

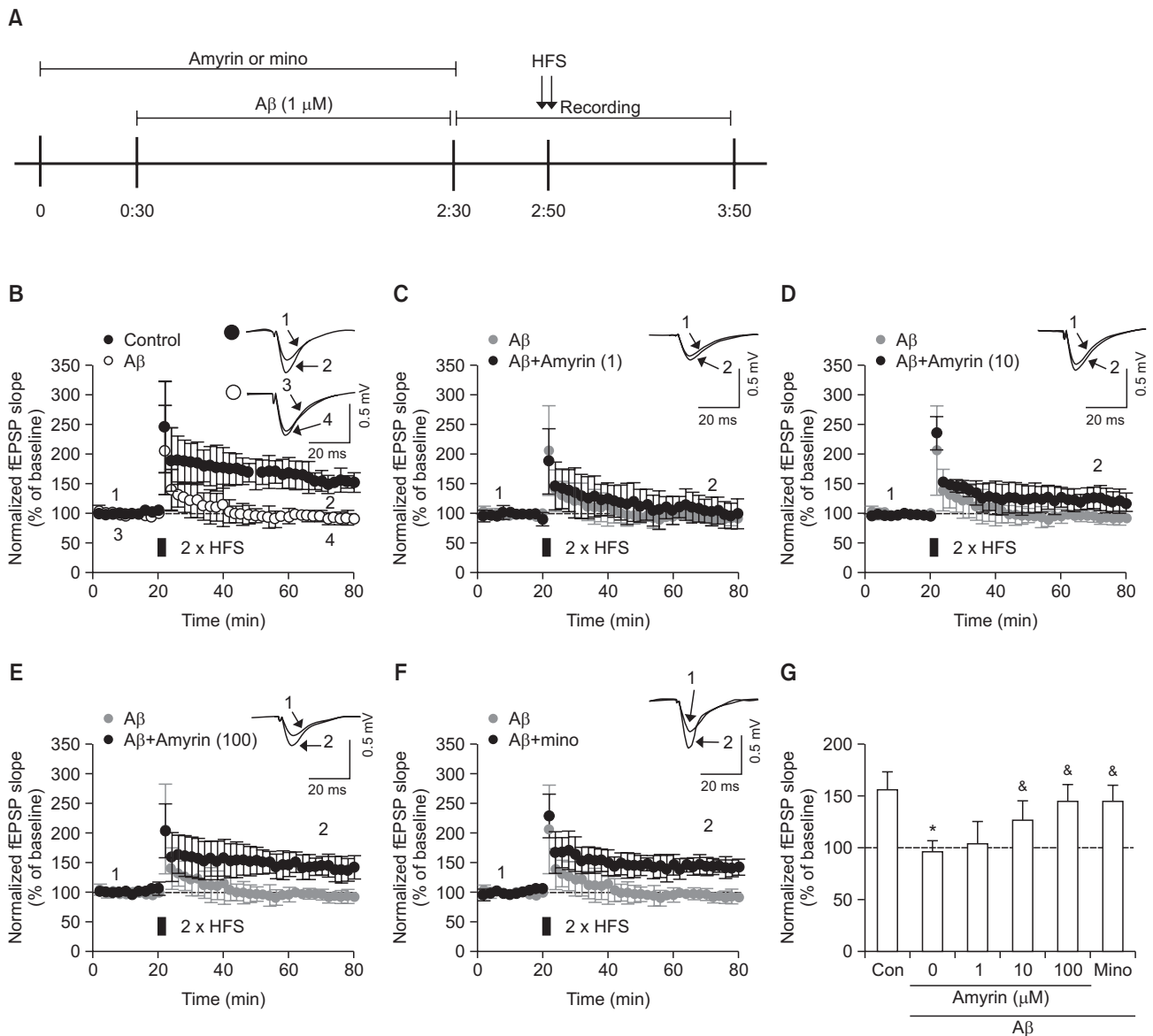


Fig. 2. The effect of β-amyrin on amyloid β (Aβ)-induced LTP impairment. LTP was measured in Schaffer-collateral pathway of the hippocampus. (A) Experimental schedule. (B) Effect of Aβ (1 μM) on hippocampal LTP. (C) Effect of β-amyrin (1 μM) on Aβ-induced LTP impairment. (D) Effect of β-amyrin (10 μM) on Aβ-induced LTP impairment. (E) Effect of β-amyrin (100 μM) on Aβ-induced LTP impairment. (F) Effect of minocycline (1 μM) on Aβ-induced LTP impairment. (G) Bar chart of normalized fEPSP slope at 80 min time points of each groups. Data represent as mean ± SD (n=7/group). *p<0.05 vs. control group. &p<0.05 vs. Aβ-treated group.

following formula: $T_{\text{displaced}}$ or $T_{\text{non-displaced}} / (T_{\text{displaced}} + T_{\text{non-displaced}}) \times 100$, where $T_{\text{displaced}}$ is the time spent exploring the displaced object and $T_{\text{non-displaced}}$ denotes the time spent exploring the non-displaced object.

Passive avoidance test: We conducted passive avoidance test using shuttle box, which is composed of separated dark and illuminating rooms. There was guillotine door between rooms. In a training trial, mouse was located in the illuminating room and then guillotine door was opened 10 s later. When the mouse entered the dark room through the guillotine door, the door closed and an electric shock (0.5 mA for 3 s) was delivered through grid floor (training trial). The following day, mouse was re-introduced in illuminating room. Step-through latency to enter the dark room was measured for a period of 300 s (test trial).

Tissue preparation and immunohistochemistry

Immediately after the behavioral tests, we rapidly removed the brain and washed it with phosphate buffer (100 mM, pH 7.4). The brains were fixed in phosphate buffer (50 mM, pH 7.4) containing 4% paraformaldehyde overnight, then immersed in 30% sucrose solution (in 50 mM PBS) and stored at 4°C until sectioning. Frozen sections were prepared in the coronal plane (30 μm) using a cryostat (Leica, Nussloch, Germany) and kept in storage solution (30% ethylene glycol and 30% glycerol in DW) at 4°C. Forty-five sections were obtained from each mouse. Five sections separated by 9-section intervals (270 μm) were used for each immunohistochemical analysis.

For immunohistochemistry, sections were incubated with blocking solution for 2 h, then with goat anti-doublecortin (DCX, 1:500, Santa Cruz Biotechnology) or rat anti-Ki67 (1:500, Santa Cruz Biotechnology) antibody overnight at 4°C. After washing in PBS, the sections were incubated with biotinylated secondary antibody (1:200 dilution, Vector Laboratories, Inc., Burlingame, CA, USA) for 2 h at room temperature and then with avidin-biotin-peroxidase complex (1:100 dilution, Vector). Thereafter, they were reacted with 0.02% 3,3'-diaminobenzidine and 0.01% H_2O_2 for about 3 min. After each incubation step, the sections were washed three times with PBS. Finally, they were mounted on gelatin-coated slides, dehydrated in ascending alcohol concentrations, and cleared in xylene.

The DCX- or Ki67-positive cells in the SGZ region of the hippocampus were quantified in 5 sections from each mouse by an experimenter who was blind to the identities of the treatment groups. Cell quantification was performed on each marker that was almost entirely included in the section throughout the z-axis at 100 \times magnification (the average cell diameter was $\sim 8 \mu\text{m}$, and cells $< 4 \mu\text{m}$ in diameter were ignored). The average number of immunopositive cells per section was normalized for the entire hippocampus by multiplying this average by the number of 30 μm sections (50 sections) corresponding to the entire hippocampus.

Statistics

Values are expressed as the mean \pm SD. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons and *t*-test for single comparisons. For the inhibition study, two-way ANOVA followed by Bonferroni post hoc test. Statistical significance was set at $p < 0.05$.

RESULTS

β -Amyrin blocked $\text{A}\beta$ -induced synaptic dysfunction

Impairment of synaptic function is shown in the early phase of AD. Oligomeric $\text{A}\beta$ plays important role in this phenomenon. Therefore, we tested the effect of β -amyrin on oligomeric $\text{A}\beta$ -induced LTP impairments in the hippocampal tissues. Before the experiment, we found that β -amyrin did not affect basal LTP in normal hippocampal slices (Fig. 1). In the $\text{A}\beta$ -treated experiments (Fig. 2A), although LTP was induced by HFS in control slices, HFS failed to induce LTP in the oligomeric $\text{A}\beta$ -treated slices, suggesting that oligomeric $\text{A}\beta$ impairs synaptic function in the hippocampus (Fig. 2B). Pretreatment with β -amyrin ameliorated $\text{A}\beta$ -induced LTP impairments in a concentration dependent manner (Fig. 2C-2E). Moreover, minocycline, a positive control, ameliorated $\text{A}\beta$ -induced LTP impairments (Fig. 2F). One-way ANOVA revealed that there is a significant group effect in this experiment ($F_{5,36}=8.072$, $p < 0.05$, $n=7/\text{group}$, Fig. 2G). These results suggest that β -amyrin ameliorates $\text{A}\beta$ -induced LTP impairments.

β -Amyrin ameliorated established LTP impairment induced by $\text{A}\beta$

AD patients already have $\text{A}\beta$ deposits in their brain and established synaptic dysfunction. Therefore, if drugs can be developed for AD patients, the drug should overcome this established synaptic dysfunction. To test this, we examined the effect of delayed treatment with β -amyrin on established LTP impairment induced by $\text{A}\beta$ (Fig. 3A). Two-hour incubation of hippocampal slices with $\text{A}\beta$ blocked LTP induction. However,

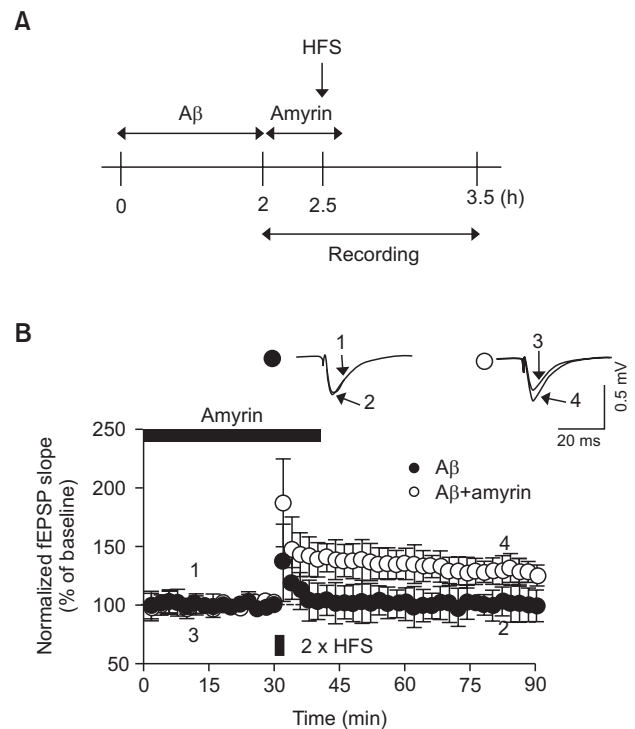


Fig. 3. Effect of delayed treatment of β -amyrin on established LTP impairment induced by amyloid β ($\text{A}\beta$). (A) Experimental schedule. (B) The effect of β -amyrin (100 μM) on established LTP impairment induced by $\text{A}\beta$ (1 μM). Data represent as mean \pm SD ($n=7/\text{group}$).

delayed treatment with β -amyrin improved LTP ($t_{12}=3.177$, $p<0.05$, $n=7$ /group, Fig. 3B). This suggests that β -amyrin restores LTP after established impairment by A β .

PI3K/Akt signaling was involved in the effect of β -amyrin

A previous study reported that glycogen synthase kinase-3 β (GSK-3 β) inhibitors ameliorated established LTP impairment in an AD model (Jo *et al.*, 2011). Moreover, A β impairs PI3K/Akt signaling, an upstream regulator of GSK-3 β (Chen *et al.*, 2009; Reddy, 2013). Therefore, we next tested the effect of β -amyrin on PI3K/Akt signaling. As in previous reports, A β decreased the activities of PI3K and Akt, which were shown by decreases in their phosphorylation levels (Fig. 4A, 4B, 4D, 4E). β -Amyrin (30 μ M) ameliorated the A β -induced reduction of PI3K/Akt signaling (PI3K: $F_{2,9}=4.543$, $p<0.05$, $n=4$ /group, Fig. 4A, 4B; pAkt: $F_{2,9}=8.438$, $p<0.05$, $n=4$ /group, Fig. 4D, 4E). Total forms of PI3K and Akt were not changed by treatment

(PI3K: $F_{2,9}=0.05842$, $p>0.05$, $n=4$ /group, Fig. 4A, 4C; Akt: $F_{2,9}=1.622$, $p<0.05$, $n=4$ /group, Fig. 4D, 4F). These results suggest that β -amyrin ameliorates A β -induced abnormality of PI3K/Akt signaling.

To confirm these results, we conducted a blocking experiment with the PI3K inhibitor LY294002. A β impaired LTP and β -amyrin ameliorated the LTP deficit in the hippocampal slices (Fig. 5A). An off-target inhibitor, U0125 [mitogen-activated protein kinase (MAPK) inhibitor], failed to affect A β -impaired LTP and the effect of β -amyrin (two-way ANOVA: U0126, $F_{1,16}=1.910$, $p>0.05$; amyrin, $F_{1,16}=122.4$, $p<0.05$; interaction, $F_{1,16}=0.007$ $p>0.05$, $n=5$ /group, Fig. 5A, 5B). However, LY294002 blocked the effect of β -amyrin (two-way ANOVA: LY294002, $F_{1,16}=77.95$, $p<0.05$; amyrin, $F_{1,16}=62.15$, $p<0.05$; interaction, $F_{1,16}=57.79$ $p<0.05$, $n=5$ /group, Fig. 5C, 5D). These results suggest that β -amyrin ameliorates A β -induced LTP impairment through PI3K/Akt signaling.

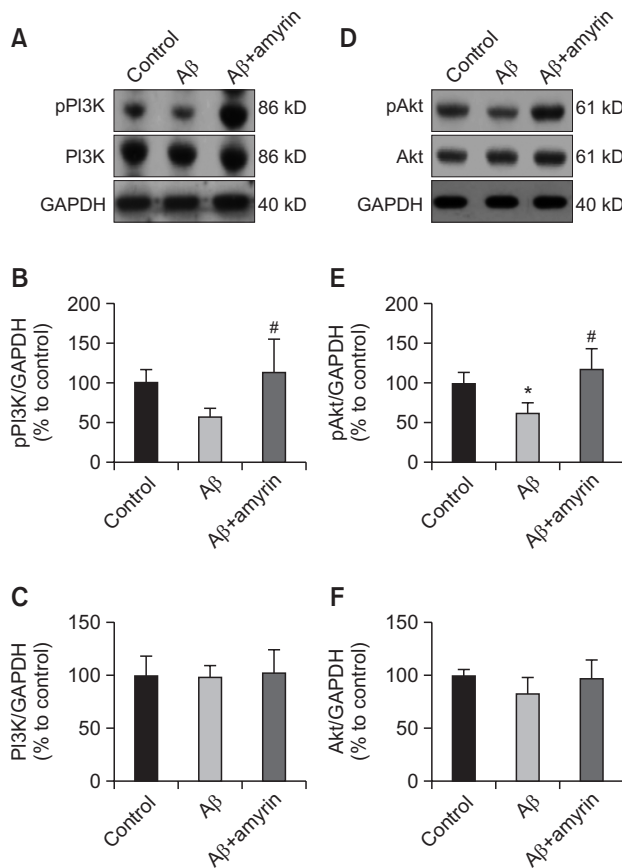


Fig. 4. Effect of β -amyrin on phosphatidylinositol-3-kinase (PI3K)/Akt pathway. Hippocampal slices were incubated in β -amyrin (100 μ M)-containing artificial cerebrospinal fluid (ACSF) for 30 min. Then the slices were more incubated in amyloid β (A β) (1 μ M) and β -amyrin (100 μ M)-containing ACSF for 2 h. (A) PI3K signaling. (B) Quantitative analysis of the level of pPI3K compared to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). (C) Quantitative analysis of the level of PI3K compared to GAPDH. (D) Akt signaling. (E) Quantitative analysis of the level of pAkt compared to GAPDH. (F) Quantitative analysis of the level of Akt compared to GAPDH. Data represent as mean \pm SD ($n=4$ /group). * $p<0.05$ vs. control group. # $p<0.05$ vs. A β -treated group.

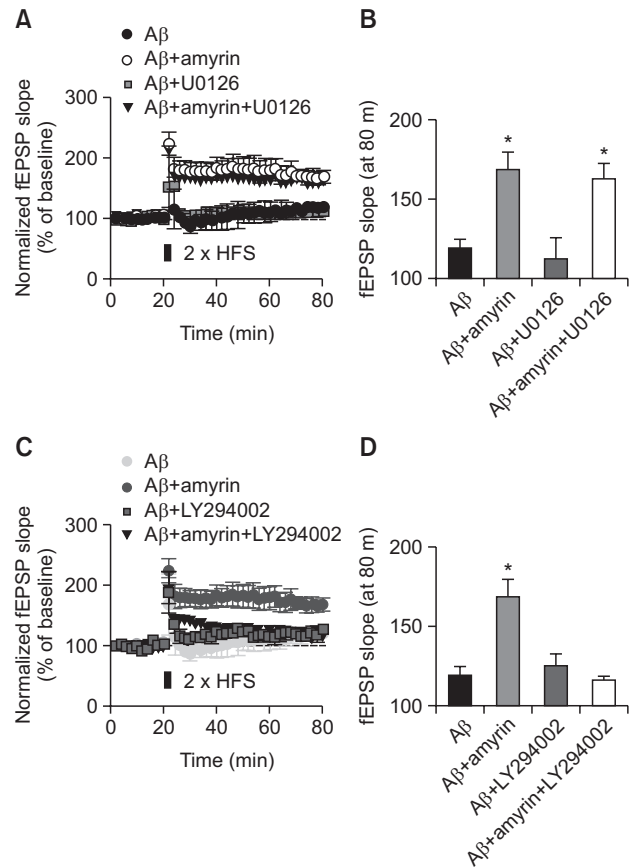


Fig. 5. Effect of phosphatidylinositol-3-kinase (PI3K) inhibitor on the effect of β -amyrin on amyloid β (A β)-induced LTP impairment. Hippocampal slices were incubated in β -amyrin (100 μ M) and inhibitor (U0126 or LY294002)-containing artificial cerebrospinal fluid (ACSF) for 30 min. Then the slices were more incubated in A β (1 μ M) and β -amyrin (100 μ M)-containing ACSF for 2 h. (A, B) Effect of U0126 (20 μ M) on the effect of β -amyrin. (A) Field excitatory postsynaptic potential (fEPSP) at every time points. (B) fEPSP at 80 m point. (C, D) Effect of LY294002 (50 μ M) on the effect of β -amyrin. (C) fEPSP at every time points. (D) fEPSP at 80 m point. Data represent as mean \pm SD ($n=5$ /group). * $p<0.05$ vs. A β -treated group.

β -Amyrin ameliorated memory impairments induced by A β

To test whether β -amyrin ameliorates memory impairments induced by A β , we tested the effect of β -amyrin on memory impairments in an A β -injected mouse AD model (Fig. 6A). A β -injected mice showed memory impairments in an object recognition test (Fig. 6B). However, β -amyrin (4 mg/kg, p.o.) ameliorated A β -induced memory impairments in the object recognition test (Fig. 6B). Minocycline (30 mg/kg, i.p.), a positive control, also ameliorated memory impairments induced by A β ($F_{3,36}=2.657$, $p<0.05$, $n=10$ /group, Fig. 6B). During the tests, the total duration of exploration was not different among groups ($F_{3,36}=0.04404$, $p>0.05$, $n=10$ /group, Fig. 6C). In the training trial of passive avoidance test, there was no significant difference of the step-through latency between groups

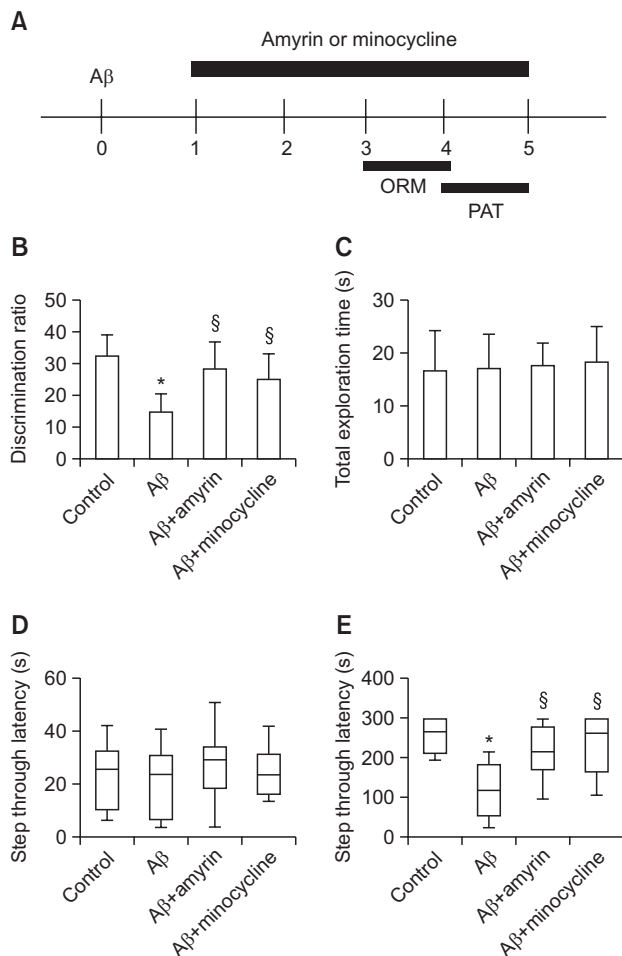


Fig. 6. Effect of β -amyrin on amyloid β (A β)-induced memory impairments. β -amyrin (4 mg/kg/day, p.o.) or minocycline (30 mg/kg/day, i.p.) was administered from 1 day after the A β (10 μ M/5 μ l, i.c.v.) injection for 5 days. (A) Experimental schedule. (B) Discrimination ratio in object recognition test. (C) Total exploration time in test session of object recognition test. (D) Step-through latency in training trials of passive avoidance test. (E) Step-through latency in test trials of passive avoidance test. Data represent as mean \pm SD ($n=10$ /group). * $p<0.05$ vs. control group. $\S p<0.05$ vs. A β -treated group. ORM, object recognition memory test. PAT, passive avoidance test.

($p>0.05$, Fig. 6D). However, β -amyrin or minocycline ameliorated memory impairment induced by A β ($F_{3,36}=9093$, $p<0.05$, $n=10$ /group, $p<0.05$, Fig. 6E). These results suggest that β -amyrin ameliorates A β -induced memory impairments.

β -Amyrin ameliorated neurogenesis impairments induced by A β

PI3K/Akt signaling plays an important role in adult neurogenesis, which is aberrantly regulated in the AD brain (Mu and Gage, 2011; Rodriguez and Verkhatsky, 2011; Kitagishi *et al.*, 2014). Therefore, we examined neurogenesis in the hippocampus of A β -injected AD model mice with immunohistochemistry with DCX, an immature neuron marker, and Ki67, a proliferation marker. As in previous reports, A β treatment decreased neurogenesis, which was confirmed by a reduction of the number of DCX- and Ki67-positive cells in the hippocampus (Fig. 7) (Haughey *et al.*, 2002). β -Amyrin (4 mg/kg, p.o.) and minocycline (30 mg/kg, i.p.) ameliorated the reduction of neurogenesis in our AD model (DCX: $F_{3,16}=4.589$, $p<0.05$, $n=5$ /group, Fig. 7A; Ki67: $F_{3,36}=4.990$, $p<0.05$, $n=5$ /group, Fig. 7B). These results suggest that β -amyrin ameliorates the A β -induced reduction in hippocampal neurogenesis.

DISCUSSION

In the present study, we found that β -amyrin ameliorated A β -induced synaptic dysfunction. PI3K inhibition blocked the effect of β -amyrin, suggesting that β -amyrin ameliorates A β -induced synaptic dysfunction through the PI3K/Akt pathway. Moreover, delayed β -amyrin administration improved memory impairments and the reduction of hippocampal neurogenesis induced by A β injection.

Hippocampal LTP is the enhancement of synaptic efficacy. Many findings suggest that hippocampal LTP is the cellular basis of learning and memory (Nabavi *et al.*, 2014; Penn *et al.*, 2017; Shimshek *et al.*, 2017). In various pathological situations including AD, Parkinson's disease and stroke, hippocampal LTP is aberrantly regulated (Stein *et al.*, 2015; Tozzi *et al.*, 2015; Zhu *et al.*, 2015). Therefore, regulation of hippocampal LTP might be a candidate means of treating these pathological conditions. A β causes over-activation of the NMDA receptor, and results in synaptic dysfunction and cell death (Birnbbaum *et al.*, 2015; Arbel-Ornath *et al.*, 2017). Recent findings indicated that activation of PI3K/Akt signaling could prevent various A β -induced various pathological conditions (Tiwari *et al.*, 2015; Yi *et al.*, 2018). PI3K/Akt activation inhibits GSK-3 β , a key molecule for A β -induced synaptic dysfunction (Beurel *et al.*, 2015), and rescues aberrant synaptic plasticity and cell death by A β (Yi *et al.*, 2018). A previous report suggested that β -amyrin activates extracellular signal-regulated kinase (ERK) and inhibits GSK-3 β in the presence of scopolamine (Park *et al.*, 2014). In the present study, we found that β -amyrin activates PI3K/Akt in the presence of A β (Fig. 4). However, blockade of PI3K/Akt prevented an A β -induced LTP deficit, but ERK prevention failed. These results suggest that β -amyrin prevents A β -induced LTP deficits through PI3K/Akt activation. Interestingly, the present results show that β -amyrin restored established LTP impairment by A β . This suggests that β -amyrin might be a disease-modifying drug. A previous study indicated that regulation of GSK-3 β could restore A β -induced LTP deficits (Jo *et al.*, 2011). Because β -amyrin regulated

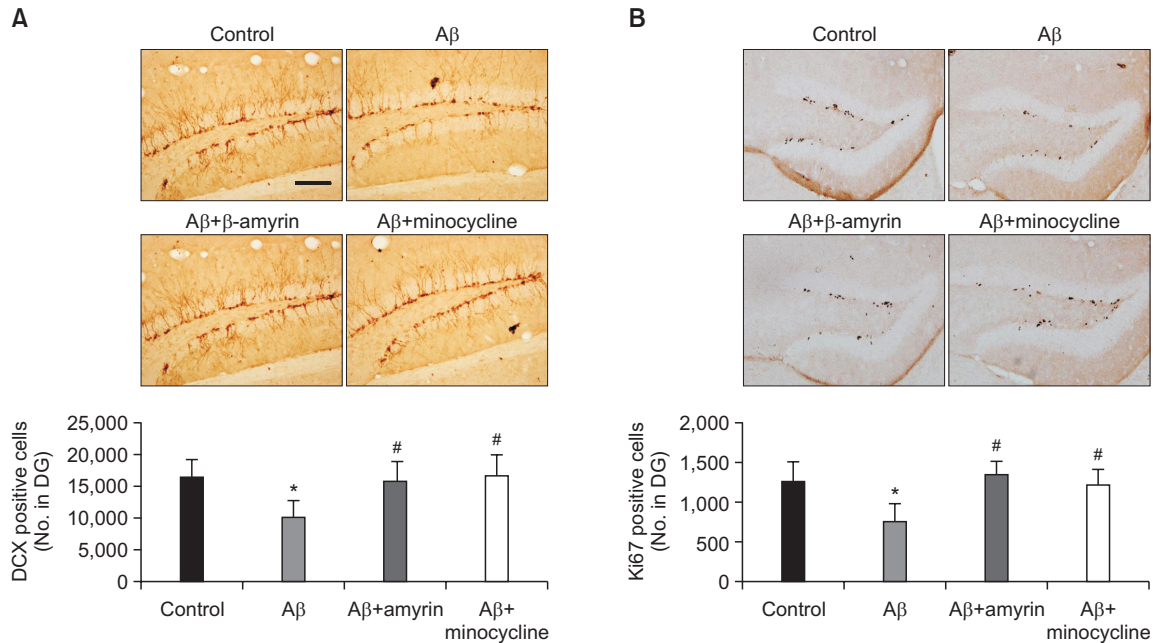


Fig. 7. Effect of β -amyryn on amyloid β ($A\beta$)-induced neurogenesis impairment. (A) Photomicroscopies and quantitative analysis of double-cortin (DCX)-positive cells in the hippocampus. (B) Photomicroscopies and quantitative analysis of Ki67-positive cells in the hippocampus. Bar=150 μ m. Data represent as mean \pm SD (n=5/group). * p <0.05 vs. control group. # p <0.05 vs. $A\beta$ -treated group.

PI3K/Akt signaling in the present study and GSK-3 β activity in previous study (Park *et al.*, 2014), the effect of β -amyryn on an established LTP deficit seems to be due to its effect on PI3K/Akt/GSK-3 β signaling. To test this in an *in vivo* system, we examined the effect of delayed administration of β -amyryn on memory impairments in an AD mouse model. This experiment showed that delayed administration of β -amyryn ameliorated memory impairments in our AD mouse model. These results suggest that β -amyryn could be a drug candidate for moderate to severe AD patients. Moreover, according to guidelines for dose conversion between animals and humans (Nair and Jacob, 2016), 4 mg/kg of β -amyryn in mice could be converted to 0.325 mg/kg in humans. However, β -amyryn failed to activate PI3K/Akt signaling in *in vivo* experiment (data not shown). It could be speculated that β -amyryn may be metabolized in the liver and the metabolite of β -amyryn may act differently in the brain. Further study will be needed to clarify this.

From a functional point of view, hippocampal neurogenesis plays an important role in structural plasticity and network maintenance. Therefore, it is likely to contribute to information storage, as well as learning and memory processes. The hippocampus is affected early in AD. Analysis of post mortem brain tissues from humans clinically diagnosed with AD revealed a reduction of progenitors in the subventricular zone (Ziabreva *et al.*, 2006). Moreover, although it is still controversial, many studies suggested that neurogenic capabilities are impaired in the subgranular zone of the hippocampal DG (Rodriguez *et al.*, 2008; Rodriguez and Verkhatsky, 2011). Voluntary running or environmental enrichment enhanced memory in transgenic mouse models of AD (Rodriguez *et al.*, 2011; Gregoire *et al.*, 2014), as well as increasing hippocampal neurogenesis (Rodriguez *et al.*, 2011; Tapia-Rojas *et al.*, 2016). Moreover, 5-HT facilitation by antidepressants ameliorates AD-like pathology and neurogenesis deficits at late ages (Ma

et al., 2017). In the present study, we found that β -amyryn prevents $A\beta$ -induced reduction in neurogenesis. This suggests that β -amyryn might be another therapeutic candidate for AD.

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