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The effects of pinoresinol on cholinergic dysfunction-induced memory impairments and synaptic plasticity in mice

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ABSTRACT

Dementia is a category of brain diseases that cause a decrease in cognitive functions. Alzheimer's disease (AD) is the most frequently mentioned neurodegenerative disease showing dementia. Although many useful drugs for dementia were developed, we still need better and safer drugs. Here, we tested pinoresinol, a lignan found in sesame seed and olive oil, whether it could be a candidate for this purpose. Pinoresinol (25 mg/kg, p.o.) ameliorated memory impairment in dementia model induced by cholinergic blockade in the passive avoidance test in a dose-dependent manner. Moreover, pinoresinol (50 μM) facilitated induction of hippocampal long-term potentiation, a cellular model of learning and memory. Pinoresinol blocked acetylcholinesterase (AchE), an acetylcholine-degrading enzyme, activity in a concentration-dependent manner. Moreover, pinoresinol (50 μM) facilitated calcium influx into neuro2a cell. These results suggest that pinoresinol improves memory impairment and facilitates hippocampal LTP induction and these results might be related to the effect of pinoresinol on AChE and calcium influx.

1. Introduction

Dementia describes a class of brain disorders involving intellectual dysfunctions including cognitive problems. Alzheimer's disease (AD), a degenerative brain disease first described by German neurologist Alois Alzheimer in 1907, is the most common cause of dementia (Vinters, 2015). Neuritic plaques and neurofibrillary tangles are commonly observed, and overall brain atrophy due to nerve cell loss can be visually confirmed in the AD brain (Arnold et al., 1991; Michael et al., 2017). This results in a gradual loss of cognitive function, especially learning and memory (Theofilas et al., 2018). Due to the inability to perform the activities of daily living, AD affects not only individual patients but also their families. Although dementia in AD is caused by dysfunction of various neurotransmitter systems and neuronal degeneration, only a small number of therapeutic drugs have been approved (Cummings et al., 2014; Gribkoff and Kaczmarek, 2017). NMDA receptor-modification and acetylcholinesterase (AChE) inhibitors have been approved for the treatment of AD; nevertheless, the need for better and safer alternative therapies for dementia symptoms in AD remains.

Various neurotransmitter systems regulate learning and memory, of which glutamate is one such principle neurotransmitter (Hao et al., 2016; Riedel et al., 2003). Acetylcholine, a major target of treatment in AD, is a positive modulator in learning and memory (Grossberg, 2017; Lombardo and Maskos, 2015). Monoamines including serotonin, norepinephrine and dopamine, may also regulate learning and memory systems (Johansen et al., 2011; Robbins and Arnsten, 2009). Therefore, agent(s) that can increase the capacity of these systems through regulation of these neurotransmitter systems can be potential treatment candidates for AD.

Synaptic plasticity is the ability of synapses to strengthen or weaken over time in response to changes in their activity (Bannerman et al., 2014; Hughes, 1958). Functional synaptic plasticity is regulated by and regulates various neurological phenomena including learning and memory (Bannerman et al., 2014; Takeuchi et al., 2014). Deficits in synaptic plasticity have been observed in several brain disorders, and restoration of synaptic plasticity could potentially overcome these abnormalities (Duman et al., 2016; Lee et al., 2014; Maggio and Vlachos, 2014). Therefore, agents capable of influencing the regulatory mechanisms involved in synaptic plasticity may be promising candidates to treat brain disorders.

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Pinoresinol is a lignan found in sesame seed and olive oil. Previous studies have reported that pinoresinol inhibits α-glucosidase and, may therefore, act as a hypoglycemic agent (Potipiranun et al., 2018). Moreover, pinoresinol-containing extra virgin olive oil has demonstrated *in vitro* chemopreventive properties (Reboredo-Rodriguez et al., 2018). A recent study reported that pinoresinol, which was isolated from the seeds of *Catalpa bungei*, inhibited butyrylcholinesterase, an acetylcholine-degrading enzyme (Tang et al., 2016), suggesting that pinoresinol may increase acetylcholine levels in the brain and regulate learning and memory. However, its effect on brain function remains unclear. Therefore, the present study, investigated the effects of pinoresinol on memory and synaptic plasticity.

2. Materials and methods

2.1. Animals

Male ICR mice weighing 25–30 g (6 weeks old) were purchased from Samtako (Osan, Korea). The animals were housed in an animal room (light/dark cycle, 12 h; temperature, 23 ± 2 °C; humidity, 50 ± 10%) *ad libitum* access to food and water except during testing. All experimental procedures were performed with the approval of the IACUC of Dong-A University.

2.2. Chemicals

Pinoresinol, donepezil hydrochloride monohydrate (DNZ), (−)-scopolamine hydrobromide, acetylthiocholine iodide and 5,5′-dithiobis-[2-nitrobenzoic acid] (DTNB), and human erythrocyte AChE were purchased from the Sigma Chemical Co. (St. Louis, MO). Scopolamine was dissolved in 0.9% saline. Pinoresinol and DNZ were dissolved in 10% Tween 80 solution. All other materials were obtained from normal commercial sources and were of the highest grade available.

2.3. Passive avoidance test

The passive avoidance test was executed over a 2-day period that was divided into training and test trials (Eagle et al., 2016). The passive avoidance box consists of two rooms-dark and illuminated-separated by guillotine door. In a training session, a mouse was placed in illuminated room, and the guillotine doorwas opened 10 s later. When the mouse crossed the guillotine door and entered the dark room, the door closed and a 0.5 mA of electric shock was delivered through a grid floor. The next day, the mouse was, again, placed in the illuminated room and the guillotine door was opened 10 s later. The latency time to enter the dark room was measured for 300 s. For the experiment, which tested the effect of pinoresinol alone in normal memory function, 0.25 mA of electric shock was delivered through grid floor and 600 s was used as the celling. The passive avoidance apparatus was cleaned using 70% ethanol between trials. Mice were administered pinoresinol (5, 10, or 25 mg/kg) per os (p.o.), DNZ (1 mg/kg, p.o.) (Yang et al., 2009), or the same volume of vehicle (10% Tween 80 solution, p.o) 1 h before the training trial, and intraperitoneal (i.p.) administration of scopolamine (1 mg/kg) (Yang et al., 2009) or 0.9% saline 30 min before the training trial. DNZ (1 mg/kg) was used as a positive control. In addition, sensitivity to foot shock was measured. Each point was measured according to the reaction of the mouse, such as scream, running, or jumping.

2.4. Electrophysiology

Field recordings were made from acute coronal hippocampal slices of the mouse brain (6 weeks old) (Lee et al., 2018). Field excitatory postsynaptic potential (fEPSP) was recorded from stratum pyramidale in area CA1. A stimulating electrode was placed on the Shaffer collateral-commissural pathway. The input-output (I/O) curve was obtained from recording serial responses induced by 1, 2, 4, 8, 10, 15, and 20 V of stimulation. Paired pulse ratio (PPR) was measured from the ratio of responses induced by 4 V stimulation at intervals of 25, 50, 100, and 500 ms. To induce long-term potentiation (LTP), high frequency stimulation (HFS, 100 pulses at 100 Hz, 2 trains) was introduced at 20 min of stable baseline.

2.5. Western blot

The effects of pinoresinol on the expression of phosphorylated extracellular signal-regulated kinase (pERK), phosphorylated Akt (pAkt) and phosphorylated cAMP-responsive element binding (pCREB) in the hippocampus were investigated. Mice were treated with pinoresinol (50 mg/kg, p.o.) or vehicle 1 h before and scopolamine (1 mg/kg, i.p.) or vehicle at 30 min before euthanization by cervical dislocation. Hippocampal tissues were homogenized in an ice-chilled Tris–HCl buffer (20 mM, pH 7.4) containing 0.32 M sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ethylene glycol bis(2-aminoethyl ether)-N,N,N′,N'-tetraacetic acid (EGTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM sodium orthovanadate and one tablet of protease inhibitor (Roche, Seoul, Korea) per 50 mL of buffer. Samples of homogenates (30 μg of protein) were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. Proteins were transferred to polyvinylidene fluoride membranes in the transfer buffer. After transferring, the membranes were blocked in phosphate buffered saline (PBS) with 0.01% Tween 20 (PBST) containing 5% non-fat dried milk for 1 h at room temperature and incubated 1 h with primary antibodies including pERK (1:1000, Cell Signaling, Beverly, MA), ERK (1:1000, Santa Cruz biotechnology, Santa Cruz, CA), pAkt (1:1000, Cell Signaling, Beverly, MA), Akt (1:1000, Santa Cruz biotechnology, Santa Cruz, CA), pCREB (1:1000, Cell Signaling, Beverly, MA), CREB (1:1000, Santa Cruz biotechnology, Santa Cruz, CA). After three washes in PBST for 30 min, the membranes were incubated for 30 min with secondary antibodies. After three washes, the protein bands were visualized using an enhanced chemiluminescence detection system according to manufacturer's instructions (Amersham Corp., Newark). The membrane was analyzed using bioimaging software installed on the imager device (LAS-4000 mini, FUJIFILM Lifescience U.S.A., Stanford, CT, U.S.A.). Total forms of each proteins were used to normalize the results.

2.6. AChE activity assay

The inhibitory activities of the AChEs were measured using a modified Ellman's method. The source of AChE was entire mouse brain (homogenate) or human erythrocyte (Sigma-Aldrich, Corporation, St. Louis, MO). A mixture including sodium phosphate buffer (0.1 M), acetylthiocholine iodide (75 mM), 5,5-dithiobis-2-nitrobenzoate (10 mM) and various concentration of pinoresinol was incubated at 25 °C. Mouse brain homogenate or human erythrocyte AChE was added to the mixture and absorbance was then measured at wavelength of 412 nm. In addition, AChE inhibition was measured using mouse brain plus ZINC 12613047, a butyrylcholinesterase (BuChE) inhibitor.

2.7. Cell culture and measurement of intracellular calcium

Mouse neuroblastoma (Neuro2a) cells were cultured in Minimum Essential Medium (WelGENE Co., Daegu, Korea) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in an incubator (37 °C, 5% CO_2).

Fluo-3AM dye was prepared at 100 μM. Cells were seeded at 1×10^4 cells/ml and incubated at 37 °C for 24 h. After removing the medium, 100 μl of HBSS and 5 μl of dye were applied to the cells, which were incubated at room temperature for 30 min. After washing the cells with HBSS, 100 μl of pinoresinol (50, 20, 10, 5, or 1 μM) in HBSS was applied to the cells. Fluorescence was measured using a fluorescence

spectrometer (Excitation, 488/20 nm; Emission, 525/20 nm; and sensitivity 70).

2.8. MTT assay

Cells were seeded at 2×10^4 cells/ml in a 24-well plate and incubated at 37 °C for 24 h. After removing the medium, pinoresinol (50 μM, 20 μM, 10 μM, 5 μM, 1 μM) was added to the medium containing 2% FBS and incubated for 2 h. MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was made up to a final concentration of 0.5 mg/mL. After 24 h of reaction, the medium was removed and treated with 0.5 mg/ml MTT reagent and incubated for 30 min. It was then removed, reacted with dimethyl sulfoxide and transferred to a 96-well plate, which was read at a wavelength of 540 nm.

2.9. Statistics

Latency in the passive avoidance test was analyzed using a Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA) corrected for ties, followed by a two-tailed Mann-Whitney *U* test ($P < 0.05$). The data are expressed as medians (interquartile range). Other data are expressed as means ± standard error of the mean (SEM). Data were analyzed using one-way ANOVA, followed by followed Tukey test statistical differences between groups ($P < 0.05$). The *t*-test was used for electrophysiological data. All statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad, San Diego, CA).

3. Results

3.1. Pinoresinol ameliorated scopolamine-induced memory impairment in the passive avoidance test

The effect of pinoresinol (Fig. 1) on cholinergic dysfunction-induced memory impairment was examined using a passive avoidance test. Before that, however, the effect of pinoresinol on normal memory function was tested. In this experiment, pinoresinol (1, 10, 25 mg/kg, p.o.) demonstrated no effect on latency time or response to foot shock (Fig. 2A, B and C). The effect of pinoresinol in scopolamine-induced memory impairment was tested next. In the training trial, the latency time was not statistically different and did not exceed 60 s ($F_{5,48} = 1.011$, $P > 0.05$, Fig. 2D). In the test trial, there was a significant group effect ($F_{5,48} = 4.267$, $P < 0.05$, Fig. 2E). Scopolamine resulted in cholinergic dysfunction-induced memory impairment when compared with controls, which demonstrated no relevant function ($P < 0.05$, Fig. 2E). Compared with scopolamine alone, a statistically significant increase was observed between the groups receiving 25 mg/kg of pinoresinol and DNZ-positive group after administration of scopolamine ($P < 0.05$, Fig. 2E). Response scores were measured to determine whether pinoresinol increased the sensitivity to foot impact (Fig. 2F). Pinoresinol did not alter sensitivity to foot shock ($F_{3,36} = 0.1304$, $P > 0.05$, Fig. 2F), but improved memory dysfunction in the cholinergic blockade-induced memory impairment model.

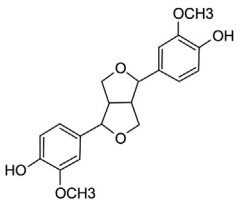

Fig. 1. Structure of pinoresinol.

3.2. Pinoresinol facilitated hippocampal LTP

The effect of pinoresinol on synaptic function was tested. Basal synaptic transmissions, including I/O curves and PPR, were first measured using mouse hippocampal slices. As a result, presynaptic (fiber) volley (two-way ANOVA, drug, $F_{1,112} = 0.8928$, $P > 0.05$; stimulation intensity, $F_{6,112} = 488.5$, $P < 0.05$; interaction, $F_{6,112} = 0.9597$, $P > 0.05$, Fig. 3A) and fEPSP slope (two-way ANOVA, drug, $F_{1,112} = 0.03715$, $P > 0.05$; stimulation intensity, $F_{6,112} = 44.95$, $P < 0.05$; interaction, $F_{6,112} = 0.3474$, $P > 0.05$, Fig. 3B) scale were not affected by pinoresinol. Moreover, there was no significant change in PPR with pinoresinol treatment (two way ANOVA, drug, $F_{1,76} = 1.157$, $P > 0.05$; stimulation interval, $F_{6,76} = 41.66$, $P < 0.05$; interaction, $F_{3,76} = 0.6368$, $P > 0.05$, Fig. 3C). These results suggest that the pinoresinol did not affect basal synaptic transmission in the hippocampus.

The effect of pinoresino on the induction of NMDA receptor-dependent LTP (Luscher and Malenka, 2012) was examined next. Similar to a previous study, 2 trains of HFS (100 pulses, 100 Hz) induced LTP in the hippocampus, which was completely blocked by the NMDAR antagonist, AP-5 (50 μM) (Fig. 3D). Pinoresinol facilitated NMDA receptor-dependent LTP (Fig. 3E). Compared with LTP level of control, pinoresinol demonstrated 286 ± 80% LTP level ($t_8 = 2.302$, $P < 0.05$, Fig. 3F). These results suggest that pinoresinol regulates hippocampal LTP without affecting basal synaptic transmission.

3.3. Pinoresinol activated ERK, Akt and CREB in the hippocampus

The effect of pinoresinol on memory-related signaling molecules in the hippocampus (Fig. 4A and B) was examined. Scopolamine treatment significantly decreased the levels of pERK, pAkt and pCREB in the hippocampus ($P < 0.05$). However, pinoresinol (25 mg/kg, p.o.) significantly improved the levels of pERK, pAkt and pCREB reductions by scopolamine in the hippocampus in a dose-dependent manner (pERK, $F_{3,12} = 9.938$, $P < 0.05$; pAkt, $F_{3,12} = 5.034$, $P < 0.05$; pCREB, $F_{3,12} = 7.182$, $P < 0.05$, Fig. 4B).

3.4. Pinoresinol blocked AChE activity

To investigate the inhibitory effect of pinoresinol on AChE, mouse brain and human erythrocyte enzyme were used. A representative experiment using a mouse brain is shown in Fig. 5A. Pinoresinol significantly blocked AChE activity in the mouse whole hippocampus ($IC_{50} = 33.9$ μM, Fig. 5A). The brain also has butyrylcholineasterase (BuChE) as well as AChE. To investigate the effect of pinoresinol on AChE alone, the experiment was performed after treatment with the BuChE inhibitor ZINC12613047 (Fig. 5B). AChE was inhibited by pinoresinol when the BuChE inhibitor was used ($IC_{50} = 125.4$ μM, Fig. 5B). Human erythrocyte AChE was also blocked by pinoresinol ($IC_{50} = 485.4$ μM, Fig. 5C). Pinoresinol is believed to be a leading compound as an AChE inhibitor, even though it may be less effective than DNZ.

3.5. Pinoresinol facilitated calcium influx in neuro2a cells

Intracellular calcium is important in neuronal excitability, and is a requirement for learning, memory, and synaptic plasticity. Therefore, the effect of pinoresinol on calcium influx into neuro2a cells was investigated. Calcium influx increased gradually over the incubation period (Fig. 6A). Two hours after treatment with pinoresinol, calcium influx was significantly increased ($F_{2,6} = 17.02$, $P < 0.05$, Fig. 6B). However, pinoresinol (24 h incubation) did not affect cell viability (Fig. 6C). This increase in calcium influx is believed to increase the release of glutamate, as well as a positive effect of synaptic plasticity in postsynaptic neurons.

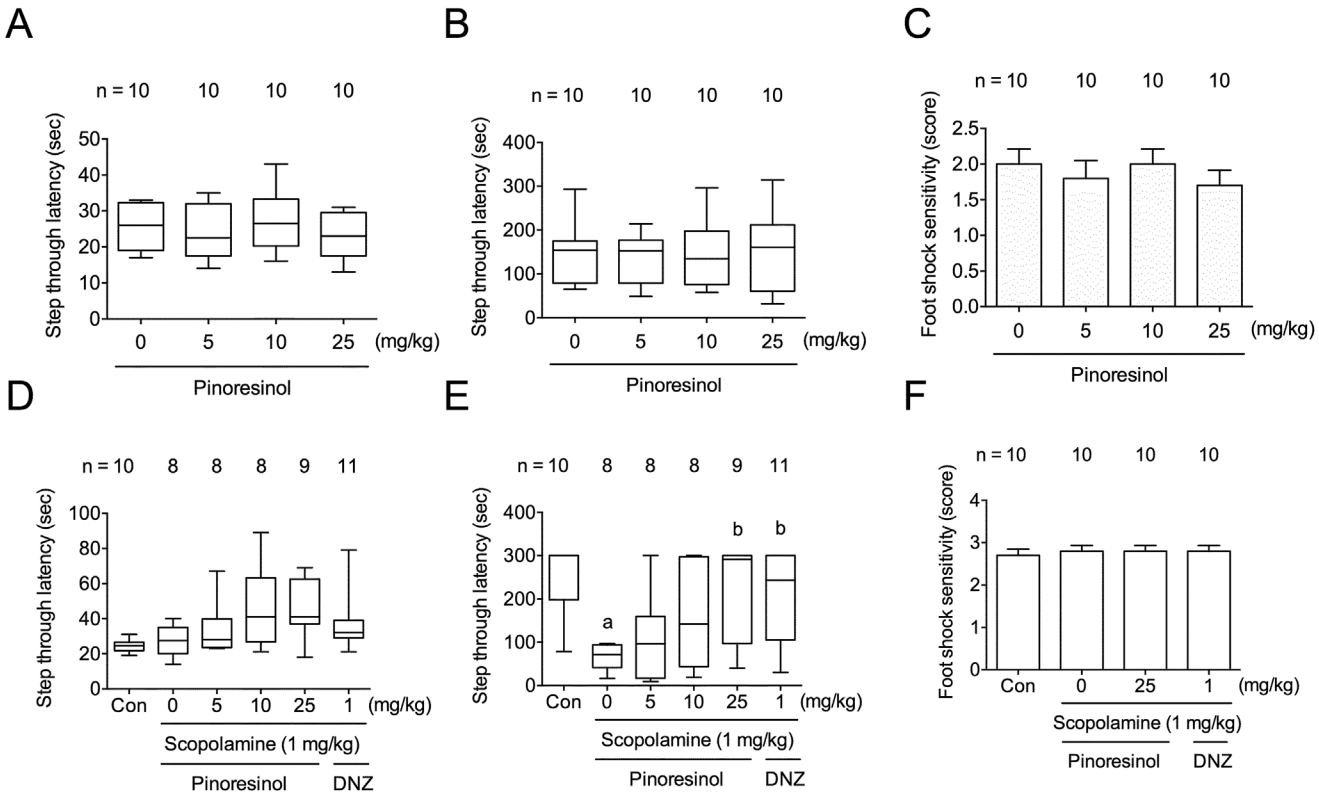

Fig. 2. The effect of pinoresinol in normal memory and scopolamine-induced memory impairment in passive avoidance test. Pinoresinol (5, 10, or 25 mg/kg, p.o.) or donepezil (DNZ, 1 mg/kg, p.o.) was administered 1 h before training trial. Scopolamine (1 mg/kg, i.p.) was administered 30 min before the training trial for the memory impairment experiments. A-C. Step-through latency in training trial (A) and test trial (B) were represented. Foot shock sensitivity in training trial (C) was represented. Data represent mean ± SEM (n = 10/group). D-F. Step-through latency in training trial (D) and test trial (E) were represented. Foot shock sensitivity in training trial (F) was represented. Data represent mean ± SEM (n = 8–10/group). Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA) corrected for ties, followed by a two-tailed Mann-Whitney *U* test. $^{a}P < 0.05$ vs. control group. $^{b}P < 0.05$ vs. scopolamine group. Con, control group.

4. Discussion

In the present study, we demonstrated that pinoresinol ameliorated cholinergic dysfunction-induced memory impairment and facilitated hippocampal LTP. Pinoresinol inhibited AChE in the hippocampus and facilitated calcium influx into neuro2a cells. These results suggest that pinoresinol may improve memory in a model of dementia, and that this effect may be related to its role in synaptic plasticity, AChE activity, and calcium influx.

AD is a typical disease involving memory deficit. Although abnormal aggregations of amyloid β and tau protein have been revealed as major hallmarks, the precise mechanism of memory deficit and the intrinsic role of these proteins remain unclear. Learning and memory is closely related to LTP in the hippocampus (Holscher, 1999; Nabavi et al., 2014). LTP is a process in which the signal transmission of nerve cells is continually improved by external electrical or chemical stimulation. It occurs when the glutamate receptor opens and the intracellular calcium concentration is increased, which activates various protein kinase (Grosshans et al., 2002; Luscher and Malenka, 2012).

The activation of Ca^{2+}/calmodulin-dependent kinase II (CaMKII) exposes the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor to the cell membrane and promotes conductivity through AMPA receptor phosphorylation (Barria and Malinow, 2005; Szapiro et al., 2003). Akt, ERK, and mitogen-activated protein kinase facilitate protein synthesis for new spine formation (Szapiro et al., 2003). This series of processes increases the efficiency of synapses and finally results in LTP. LTP has been reported to be abnormal in the AD brain (Chapman et al., 1999; Zhang et al., 2015). Hippocampal LTP deficits are evident in AD animal models and human patients (Baglietto-Vargas et al., 2018; Di Lorenzo et al., 2016). Normalizing hippocampal deficits ameliorated memory deficits in AD models (Cavanagh et al., 2016; Jin et al., 2017). These studies demonstrated that agents that can facilitate hippocampal LTP may be good candidate drugs for therapy in AD patients. In the present study, pinoresinol facilitated hippocampal LTP and, therefore, could be considered as a leading compound for developing therapy for AD patients.

cAMP response element-binding protein (CREB), one of the most commonly mentioned proteins in relation to memory, is a key transcription factor that controls gene expression needed to form new spine in the nucleus (Frank and Greenberg, 1994; Johansen et al., 2011; Yin and Tully, 1996). Many previous studies have investigated possible correlations between CREB and memory. CREB overexpression in the dorsal CA1 ameliorated long-term memory deficits in aged rats (Yu et al., 2017). Moreover, downregulation of CREB was observed in various brain diseases causing memory impairment (Pugazhenthi et al., 2011). These findings suggest that CREB is an essential factor in memory formation and may represent a treatment target for brain diseases. In the present study, pinoresinol ameliorated cholinergic blockade-induced reduction in CREB activity and, therefore, may be a leading compound for developing therapy for AD patients.

AChE is an enzyme that degrades acetylcholine, a neurotransmitter involved in learning and memory (Hasselmo, 2006; Solari and Hangya, 2018). The AD brain exhibits low levels of acetylcholine; therefore, enhancing acetylcholine levels by inhibiting acetylcholine degradation could be a promising therapy in AD patients (Muir, 1997). The AChE inhibitors, DNZ, Donepezil, rivastigmine and galantamine have been approved as therapies for patients with AD (Francis et al., 1999). However, because these drugs have several side effects, including insomnia, efforts to find safer drugs are ongoing (Birks, 2006). In the present study, pinoresinol inhibited AChE. Although the IC_{50} level was

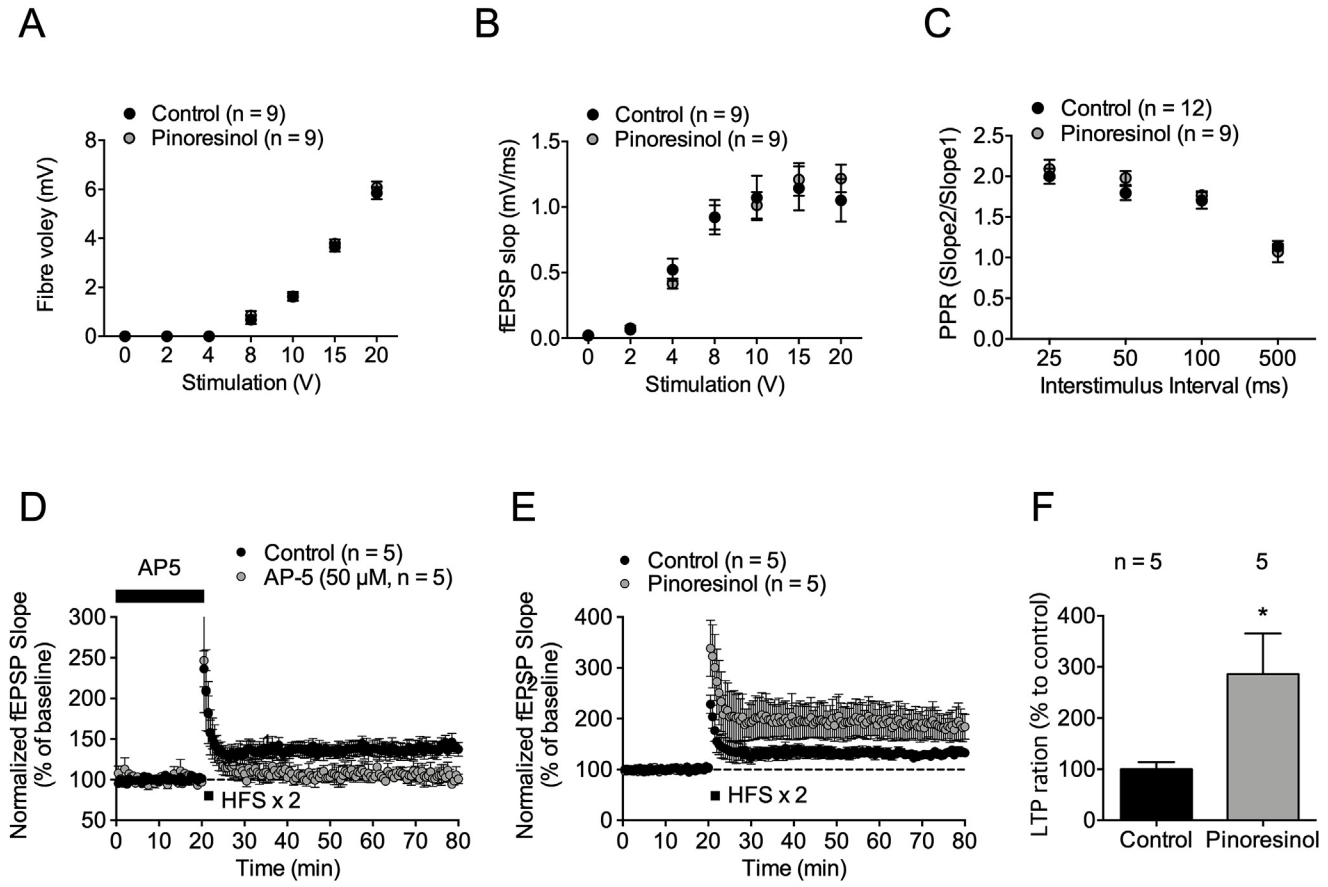

Fig. 3. The effect of pinoresinol on basal synaptic transmission and hippocampal LTP. Acute hippocampal slices were prepared from coronal section of mouse brain. Hippocampal slices were incubated with pinoresinol (50 μM) for 2 h and then introduced into recording chamber. A. Changes in fiber volley scale through changes of stimulation (n = 9/group). B. Changes in fEPSP scale through changes of stimulation (n = 9/group). C. Changes in paired pulse facilitation (n = 12 or 9/group). D. The effect of pinoresinol on hippocampal LTP. E. Bar chart of normalized fEPSP slop at 80 min time points of each groups. Data represent mean ± SEM (n = 5/group). *T*-test was used for electrophysiological data. $^{*}P < 0.05$ vs. control group.

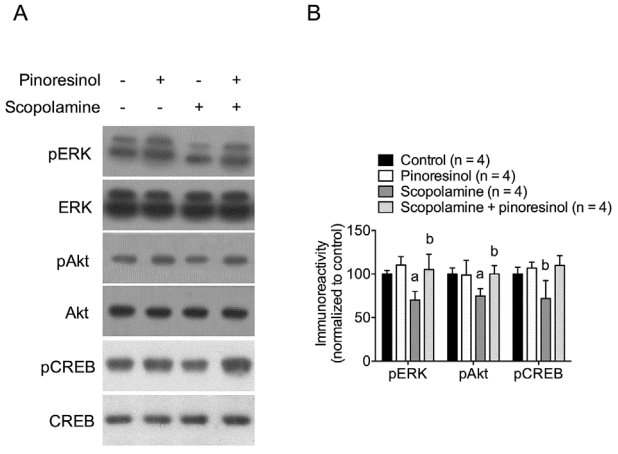

Fig. 4. The effect of pinoresinol on memory-related signaling molecules in the hippocampus. Mouse hippocampus was isolated immediately after Y-maze test. A. Protein expressions of pERK, pAkt, pCREB and GAPDH in the hippocampus. B. Normalized Immunoreactivities of pERK/GAPDH, pAkt/GAPDH and pCREB/GAPDB in the hippocampus. Data represent mean ± SEM (n = 4/group). One-way ANOVA, followed by Tukey for the significance between groups was used. $^{a}P < 0.05$ vs. control group. $^{b}P < 0.05$ vs. scopolamine group. Con, control group.

rather high, we suggest pinoresinol as a lead compound for better effective and safer drug development. Scopolamine induces amnesia through cholinergic blockade. AChE inhibitors could overcome memory impairment induced by scopolamine. In the present study, scopolamine caused severe memory impairment in the passive avoidance test. Pinoresinol abolished the effect of scopolamine in passive avoidance memory, which may be due to the inhibition of AChE. The passive avoidance test uses foot shock for memory formation. Therefore, if a drug can affect the sensory system rather than learning and memory, it could be misunderstood as a memory-regulating drug. However, pinoresinol did not affect foot shock sensitivity. According to a previous study, the bioavailability of pinoresinol di-glucopyranoside (not pinoresinol) is approximately 51.3% (Gong et al., 2017). Accordingly 50 mg/kg of pinoresinol would correspond to a concentration of approximately 25.65 mg/kg, which in a mouse would correspond to a concentration of approximately 58.5 ml/kg in the blood. Therefore, the plasma concentration of pinoresinol is approximately 437 mg/L (the molecular weight of pinoresinol is 358). Therefore, the plasma concentration of pinoresinol is approximately 1 mM. These results suggest that pinoresinol ameliorated cholinergic blockade-induced memory impairment, and it may be due to its AChE inhibiting activity.

In the present study, pinoresinol increased calcium-influx into neuro2a cells. Calcium is required for synaptic transmission (Takahashi and Momiyama, 1993). Presynaptic calcium influx is required for neurotransmitter release and postsynaptic calcium is required for postsynaptic response and synaptic plasticity (Al-Osta et al., 2018; Llinas et al., 1976; Luebke et al., 1993). Otherwise, calcium is also involved in excitotoxicity (Bano and Ankarcrona, 2018; Szydlowska and Tymianski, 2010). Excessive increases in calcium influx initiate cell death mechanisms, which may represent a possible mechanism of neurodegenerative diseases in AD (Bezprozvanny and Mattson, 2008;

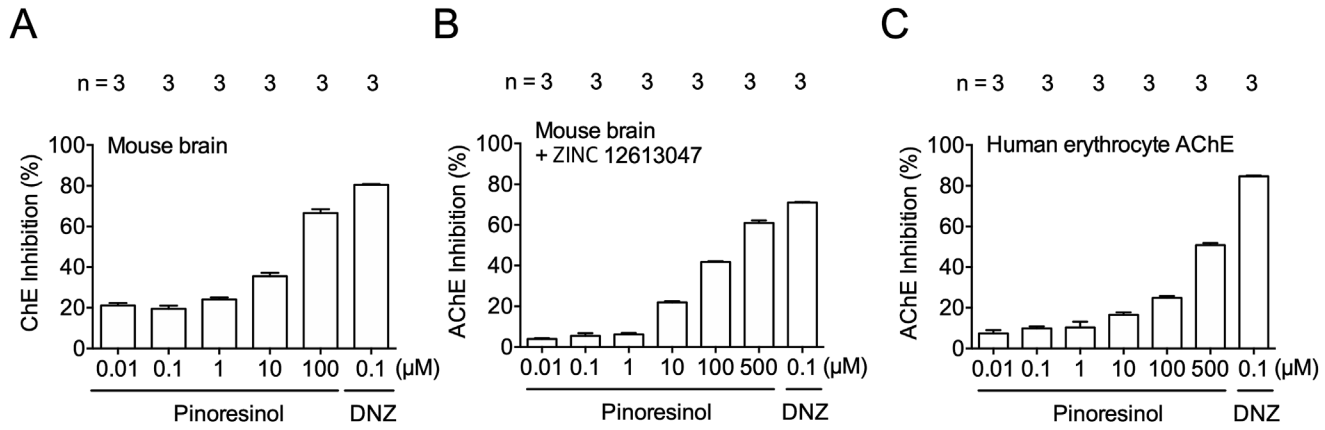

Fig. 5. The effect of pinoresinol on AChE activity. A. The effect of pinoresinol on ChE from mouse hippocampus. B. The effect of pinoresinol on AChE from mouse hippocampus. To block BuChE, ZINC12613047 (50 μM) was added into brain homogenates. C. The effect of pinoresinol on AChE from human erythrocyte. Data represent mean ± SEM (n = 3/group). One-way ANOVA, followed by Tukey for the significance between groups was used.

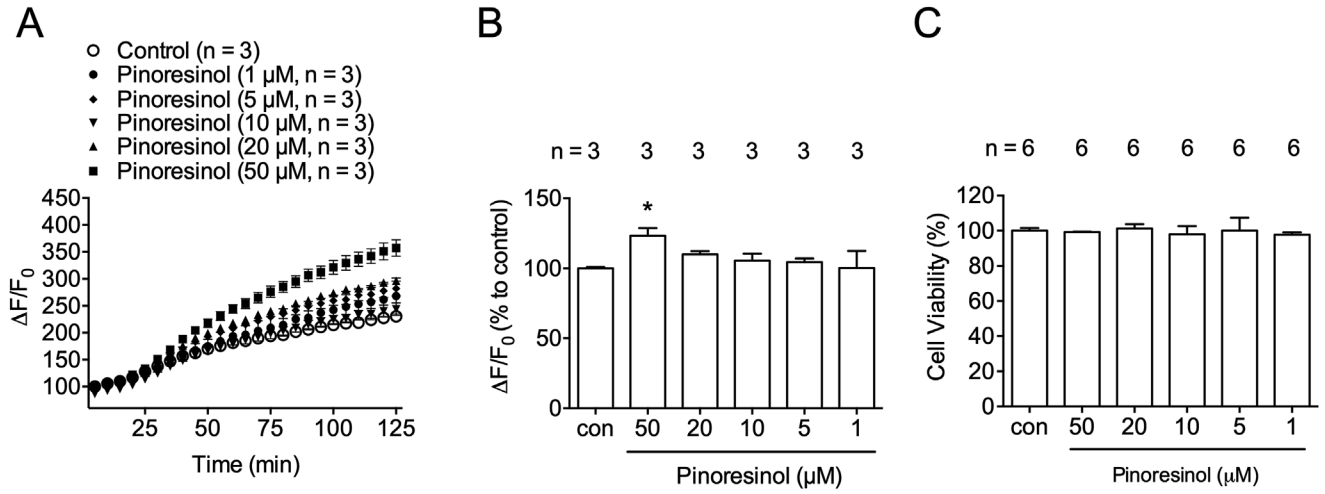

Fig. 6. The effect of pinoresinol on intracellular calcium level in neuro2a cell. A. The effect of pinoresinol on time-dependent changes in calcium level. B. The effect of pinoresinol in intracellular calcium level at 2 h point (n = 3/group). C. The effect of pinoresinol on cell viability (n = 6/group). Data represent mean ± SEM. One-way ANOVA, followed by Tukey for the significance between groups was used. *$P < 0.05$ vs. control group.

Verma et al., 2018). Although pinoresinol gradually increased intracellular calcium levels, it did not induce cell death. This suggests that pinoresinol may sufficiently increase intracellular calcium to facilitate neuronal function rather than induce neuronal cell death. However, higher concentration than 50 μM may induce excitotoxicity, which could be a limitation for using pinoresinol as a treatment for AD. If pinoresinol is, however, developed as a treatment, a way to maintain the plasma levels < 50 μM should be considered.

In the present study, we identified several positive effects of pinoresinol in a mouse model of acute memory impairment. Although our results may be preliminary, and further research investigating the long-term and side effects of pinoresiol is required, we believe that pinoresinol could be a promising candidate for leading compound for developing AD therapy.

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Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.01.017.

Conflicts of interest

There is no conflict of interest to this study.

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