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ORIGINAL ARTICLE

# Roflumilast ameliorates cognitive impairment in APP/PS1 mice via cAMP/CREB/BDNF signaling and anti-neuroinflammatory effects

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## Abstract

Phosphodiesterase type 4 (PDE4) inhibitors can prevent the breakdown of the second messenger cyclic adenosine monophosphate (cAMP) and improve cognitive performances in several animal models of cognition. However, the clinical development of PDE4 inhibitors has been seriously hampered by severe side effects, such as vomiting and nausea. In this study, we investigated the effect and mechanism of roflumilast, an FDA-approved PDE4 inhibitor for treatment of chronic obstructive pulmonary disease (COPD), on learning and memory abilities in the APP/PS1 mouse model of Alzheimer's disease (AD). APP/PS1 transgenic mice received 3 intragastric doses of roflumilast (0.1, 0.2 and 0.4 mg/kg) daily for 3 weeks followed by behavioral tests. Chronic administration of roflumilast significantly improved the learning and memory abilities of APP/PS1 transgenic mice in the novel object recognition task, Morris water maze, and the step-down passive avoidance task. In addition, roflumilast increased the cAMP, phosphorylated cAMP response-element binding protein (p-CREB) and brain-derived neurotrophic factor (BDNF) levels, and reduced the nuclear translocation of nuclear factor-kappa B (NF-κB) p65, and proinflammatory cytokine (IL-6, TNF-a and IL-1β) levels in the hippocampus of APP/PS1 transgenic mice. In conclusion, these findings suggest that roflumilast can enhance cognitive function in APP/PS1 transgenic mice, which may be related to its stimulation of the cAMP/CREB/BDNF pathway and anti-neuroinflammatory effects.

**Keywords** Roflumilast · Phosphodiesterase-4 · Alzheimer's disease · cAMP-response element binding protein (CREB) · Neuroinflammation · Cognition

## Introduction

Alzheimer's disease (AD) is a highly prevalent neurodegenerative disorder characterized by a progressive loss of cognition (Daniilidou et al. 2011). The pathologic hallmarks of AD include senile plaque, neurofibrillary tangles (NFTs), synaptic loss with glial cell proliferation, inflammation and oxidative stress (Swerdlow 2012). The number of dementia patients has been estimated to rise to 65.7 million by 2030 and 115.4 million by 2050, in which 60–70% is caused by AD (Wortmann 2012). Unfortunately, the currently available drugs for AD, acetylcholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists, are not totally effective and do not influence disease progression (Zemek et al. 2014). Therefore, it is crucial to identify new therapeutic targets and develop novel anti-aging agents against AD.

Enhancement of central availability of the second messenger cyclic adenosine monophosphate (cAMP) is a promising approach to improve cognitive function. cAMP binds to and activates protein kinase A (PKA), which phosphorylates and activates the subsequent downstream target cAMP-response element binding (CREB) protein. As a transcription factor, phosphorylated CREB (p-CREB) can regulate expression of many genes involved in signal transduction and synaptic plasticity, and the most representative of which is brain-derived neurotrophic factor (BDNF) (Lonze and Ginty 2002; Dworkin and Mantamadiotis 2010). BDNF is required for hippocampal long-term potentiation (LTP), which is the foundation of mediating memory (Bambah-Mukku et al. 2014). It has also been well established that cAMP modulates the response of

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immune cells to a variety of stimuli (Haraguchi et al. 1995). An increase in intracellular cAMP have been generally associated with inhibition of immune cell activation and proinflammatory cytokines'-production (Jiang et al. 2013; Veremeyko et al. 2018). In addition, studies have suggested that patients with AD as well as animal models of AD experience an impaired in cAMP signal transduction in the brain (Yamamoto et al. 2000; Yamamoto-Sasaki et al. 1999). These suggest that cAMP signaling may be a potential therapeutic target for the treatment of AD.

Phosphodiesterase type 4 (PDE4), a PDE enzyme that hydrolyzes cAMP is expressed abundantly in the brain, and is considered as a promising target for cognition enhancement (Lakics et al. 2010; Garcia-Osta et al. 2012). PDE4 inhibitors can prevent the degradation of cAMP and has protective effects on the central nervous system. For example, studies have shown that rolipram, a classical PDE4 inhibitor, enhances memory (Akar et al. 2015, 2014) and reverses cognitive impairment induced by β-amyloid peptide, muscarinic acetylcholine receptor antagonist scopolamine, tryptophan depletion, and the mutant Amyloid precursor protein (APP) and Presenilin-1 (PS1) transgenes in mice (APP/PS1 mice) (Rutten et al. 2007; Wang et al. 2012; Guo et al. 2017; Rutten et al. 2006). Unfortunately, the emetic side effect have seriously hampered the clinical development of PDE4 inhibitors, and no PDE4 inhibitors were approved for clinical application in AD by the FDA (Press and Banner 2009; Robichaud et al. 2002). Thus, it remains a challenge to reduce the emetic effect of possible PDE4 inhibitors that enhance cognition.

Roflumilast, a selective PDE4 inhibitor and a FDA-approved PDE4 inhibitor for treatment of chronic obstructive pulmonary disease (COPD), only has mild emetic side effects (Chong et al. 2017). Previous studies have suggested that roflumilast might possess cognitive enhancement properties with reduced emetic properties (Jabaris et al. 2015; Vanmierlo et al. 2016). Based on previous researches, the aim of the current study was to determine whether chronic treatment with roflumilast can reverse cognitive impairment in APP/PS1 transgenic mouse model of AD. In addition, the potential mechanism underlying the cognitive enhancement of roflumilast was explored through whether it could restore the dysfunction of the cAMP/CREB/BDNF pathway and inhibit the inflammatory responses in the hippocampus.

## Materials and methods

### Animals

APP/PS1 double-transgenic male mice and littermate wild-type (WT) male mice (7 months old) of a congenic genetic background were obtained from the Model Animal Research Center of Nanjing University, China. This type of transgenic mouse has been widely used (Guo et al. 2017; Nagakura et al. 2013). Animals were housed in groups under standard experimental conditions: temperature 21 ± 2 °C, relative humidity 50 ± 10%, and a 12 h light/dark cycle with free access to food and water. All animal experimental procedures and laboratory care were carried out according to the National Institutes of Health guide for the care and use of laboratory animals (NIH Publication No. 86–23; revised 1996) and approved by the Ethics Committee of Southern Medical University (Certificate No. 2016–0167).

### Preparation of drugs and treatments

Roflumilast and rolipram (Sigma-Aldrich St, Louis, USA) were dissolved in the vehicle (1% DMSO, 0.5% methylcellulose) to obtain working solutions, and kept at 4 °C. A total of 6 different experimental groups ($n = 12$) were created by random group allocation: WT mice-vehicle; APP/PS1 mice-vehicle; APP/PS1 mice-rolipram group (0.4 mg/kg) and the three APP/PS1 mice-roflumilast treatment groups (0.1, 0.2 and 0.4 mg/kg). In the present study, roflumilast, rolipram or vehicle was administered intragastrically in a volume of 0.1 ml/10 g at the start of the experiment day. Behavioral tests were performed after three weeks of daily treatment, and all the drugs were continued to administer to mice 1 h before the test each day during the observation period.

### Behavioral tests

#### Novel object recognition test (ORT)

The novel ORT was performed as described previously (Guo et al. 2015) except for minor modifications. The ORT paradigm includes three sections: on the first day, the mice were acclimated to the empty box for 5 min; on the second day, mice were allowed to explore two identical objects for 5 min; on the third day, each mouse was again allowed to explore the box for 5 min in the presence of a familiar object and a novel object with a different color and shape. Exploration was defined as touching the object with the nose and/or directing the nose to the object at a distance less than 2 cm. Standing or sitting on the objects was not considered as exploratory behaviour. To preclude any olfactory/taste cues, the apparatus was then cleaned using detergent and dried after each test. The exploration activity was scored manually using a video camera positioned over the arena by an observer blinded to the treatment condition of the animals. The recognition index (RI) was calculated by dividing the amount of time spent in exploring any one of the two objects or the novel object by the total time spent in exploring both the objects.

### The Morris water maze test

The Morris water maze test was modified from a previously reported method (Scearce-Levie 2011). A circular pool (diameter: 95 cm; height: 25 cm) filled with water (21 ± 2 °C), was divided into four equally spaced quadrants. The water in the pool was dyed white by mixing with milk powder. A transparent platform was placed at one of the four quadrants with its surface 1 cm beneath the water. The mice were allowed to habituate the pool 24 h before the test. Then the mice were subjected to acquisition trial (training to escape to the hidden platform) 3 times a day for 5 consecutive days. On each trial, mice were placed in the pool at different starting points, which were spaced equally around the rim of the pool. The latency to reach the platform was measured using a computer-controlled video-tracking system. In the case of mice failed to find the platform within 60 s, mice were guided to the platform artificially. 24 h after the last acquisition trial, the platform was removed and the mice were subjected to the probe trial test, to test the ability of mice to find the location of the removed platform by memory. In this part of the test, the mice were allowed to swim in the pool for 60 s, and the swim distance and time spent in each quadrant were recorded and analyzed.

### Step-down passive avoidance test

This was performed as described before (Wang et al. 2016) except for minor modifications. The apparatus consisted of a chamber containing a wooden platform on one side of the grid floor, and electric shocks were delivered to the grid by an isolated stimulator. The test consisted of a training and retention test session, and the mice were taken for the prior habituation to the chamber for 5 min before training. During the training session, the mice was placed on the platform and received an electric shock (36 V) once stepped down on the grid floor. This procedure was repeated immediately and again 1 h after the initial training. Mice that stayed on the wooden platform for over 60 s were considered to have learned the task and were removed to their home cages, without being given further shocks. The retention test was performed 24 h after the last training session. In this part of the test, mice were placed on the platform and the step-down latency was recorded. The upper cut-off time in this session was 300 s.

## The cAMP and cytokines assay

Approximately 24 h after behavioral tests, the mice were deeply anaesthetized with chloral hydrate and the hippocampus samples were quickly collected. The concentrations of cAMP, IL-6, TNF-a and IL-1β were determined using the ELISA kit (UBI, Sunnyvale, CA, USA) according to the manufacturer's instructions.

## RNA isolation and quantitative real-time PCR

Total RNA was extracted from the hippocampus by using TRIzol reagent kit (Invitrogen, California, USA). cDNA was synthesized by using TIANScript RT kit (Tiangen Biotech, Beijing, China). Quantitative PCR (q-PCR) was conducted using the SYBR Green PCR Kit (Applied Biosystems, USA) and the ABI Prism 7000 Sequence Detector system. The primers used were BDNF: 5′-TTATTTCATACTTCG GTTG-3′ (forward) and 5′-TGTCAGCCAGTGATGTCG-3′ (reverse); GAPDH: 5′-A GACAGCCGCATCTTCTTGT-3′ (forward) and 5′-TGATGGCAACAATGTCCAC T-3′ (reverse). The relative levels of target genes were calculated using the $2^{-\Delta\Delta Ct}$ method, and the results were normalized to that of GAPDH.

## Western blot analysis

The hippocampal tissues were cut into small pieces and homogenized in ice-cold RIPA lysis buffer containing protease and phosphatase inhibitor cocktail. Lysates were centrifuged at 12,000×g for 30 min at 4 °C, and the supernatant was collected. The samples were separated using SDS-PAGE, transferred to polyvinylidene difluoride membranes and incubated with the following primary antibodies: anti-p-CREB, anti-CREB, anti-BDNF (1:1000; Cell Signaling Technology, USA), anti-p65 subunit of NF-κB, anti-laminin B (1:2000; Abcam, USA) and anti-GAPDH (1:20000; Sigma-Aldrich, USA), overnight at 4 °C. Next, the membranes were washed and probed with horseradish peroxidase conjugated goat anti-rabbit IgG (1:5000; Santa Cruz Biotechnology, USA) for 60 min at room temperature. Then, the bands were visualized using an enhanced chemiluminescence kit (ECL, Amersham, Sweden), and quantified by Image J software 6.0.

## Statistical analysis

Data are presented as the mean ± S.E.M. Two-way repeated-measures ANOVA were used to analyze the data of the acquisition trials in the water-maze test. Other data were analyzed by one-way ANOVA followed by the Newman-Keuls tests for post hoc comparisons between groups using SPSS software (SPSS Inc., Chicago, USA). Differences with $p$ values of less than 0.05 were considered statistically significant for analysis. The figures were obtained by Prism 5 (GraphPad Software, Inc., San Diego, CA, USA).

## Results

### Effect of roflumilast on novel object recognition task in APP/PS1 mice

After being treated with roflumilast for 3 weeks, all the mice were subjected to novel object recognition task. In the training session, there was no significant difference in recognition index (RI) among all groups [$F(5, 66) = 0.166$, $P > 0.05$] (Fig. 1a). On the subsequent trial, one of the objects was replaced with a novel object, a significant difference in RI among various treatments were seen [$F(5, 66) = 5.140$, $P < 0.01$] (Fig. 1b). A post hoc analysis revealed that vehicle-treated APP/PS1 mice had a lower RI compared with the WT mice ($P < 0.01$). This was reversed by chronic treatment with roflumilast, particularly at the dose of 0.4 mg/kg ($p < 0.01$).

### Effect of roflumilast on Morris water maze test in APP/PS1 mice

The spatial learning and memory of APP/PS1 mice were observed in the Morris water maze test. Although during the 5 days of acquisition training, all the mice, regardless of the different treatments, displayed a progressive decrease in the time needed to locate the platform, the groups significantly differed in their latencies to locate the platform. Two-way repeated measures ANOVA revealed significant changes in treatment effect [$F(5, 330) = 7.140$, $p < 0.01$] and time effect [$F(4, 330) = 53.43$, $p < 0.01$], but not for treatment × time interaction [$F(20, 330) = 0.5980$, $P > 0.05$; Fig. 2a]. Post-hoc analysis indicated that vehicle-treated APP/PS1 mice took longer time to reach the platform at day 3 ($P < 0.05$), day 4 ($P < 0.01$) and day 5 ($P < 0.01$) compared with WT mice. These were reversed by roflumilast at doses of 0.4 mg/kg ($p < 0.01$). In addition, the swim speed of mice was not altered by any treatments (data not shown), indicating that the differences in escape latencies were independent of general motor activity of the mice.

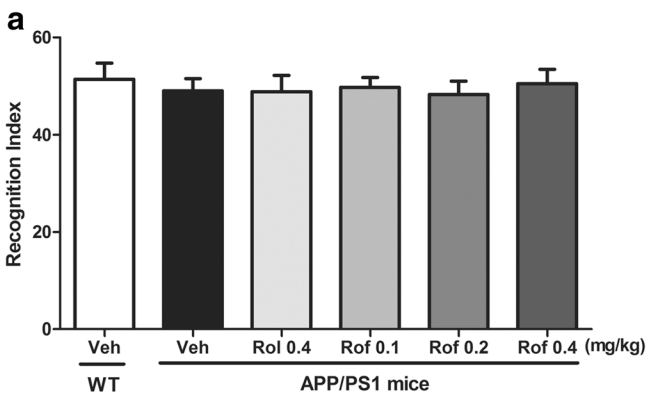
Approximately 24 h after the last training trial, mice were tested for spatial memory in the probe trial test, during which the platform was removed. Vehicle-treated APP/PS1 mice had lesser distance [$F(5, 66) = 9.553$, $P < 0.01$] and exploratory time [$F(5, 66) = 5.556$, $P < 0.01$] in the target quadrant in comparison with WT mice, and administration of roflumilast at doses of 0.2 mg/kg and 0.4 mg/kg reversed these changes ($P < 0.05$, $P < 0.01$) (Fig. 2b&c).

### Effect of roflumilast on step-down passive avoidance task in APP/PS1 mice

To further confirm the cognitive enhancement effect of roflumilast, the mice were tested for memory performance by step-down passive avoidance test (Fig. 3). Retention latency tested 24 h after training indicated that vehicle-treated APP/PS1 mice exhibited a decrease in latency relative to WT mice [$F(5, 66) = 10.42$, $P < 0.01$]. Roflumilast (0.2, 0.4 mg/kg) significantly increased the latency of APP/PS1 mice compared with the vehicle-treated APP/PS1 mice ($P < 0.01$).

### Effect of roflumilast on the cAMP/CREB/BDNF pathway in the hippocampus of APP/PS1 mice

Since the cAMP/CREB/BDNF signaling plays a key role in the cognitive enhancement of PDE4 inhibitors, we investigated the cAMP, p-CREB and BDNF levels in the hippocampus of APP/PS1 mice after treated with roflumilast. The cAMP levels were significantly reduced in the vehicle-treated APP/PS1 mice compared with WT mice [$F(5, 12) = 7.910$, $P < 0.01$] ($P < 0.01$). Roflumilast (0.1, 0.2 and 0.4 mg/kg) effectively raised the cAMP levels in a dose-dependent manner, compared with vehicle-treated APP/PS1 mice ($P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively, Fig. 4a). Additionally, we also found that roflumilast at doses of 0.2 mg/kg and



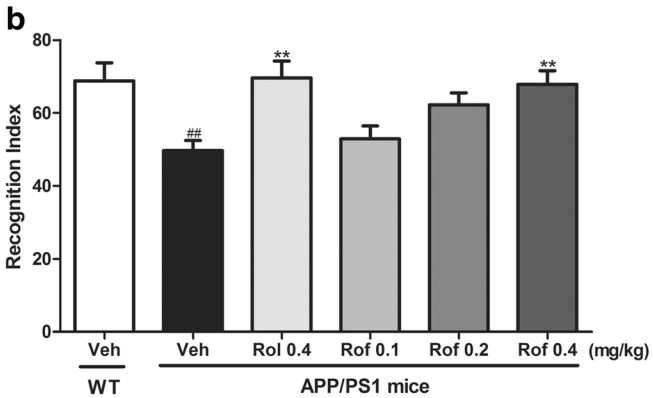




**Fig. 1** Effects of roflumilast on novel object recognition task in APP/PS1 mice. **a** Identical object test. **b** Novel object test. The results are presented as the mean ± SEM ($n = 12$). $^{\#\#}P < 0.01$ compared with the WT mice; $^{**}P < 0.01$ compared with the vehicle-treated APP/PS1 mice. *Rof roflumilast, Rol rolipram*

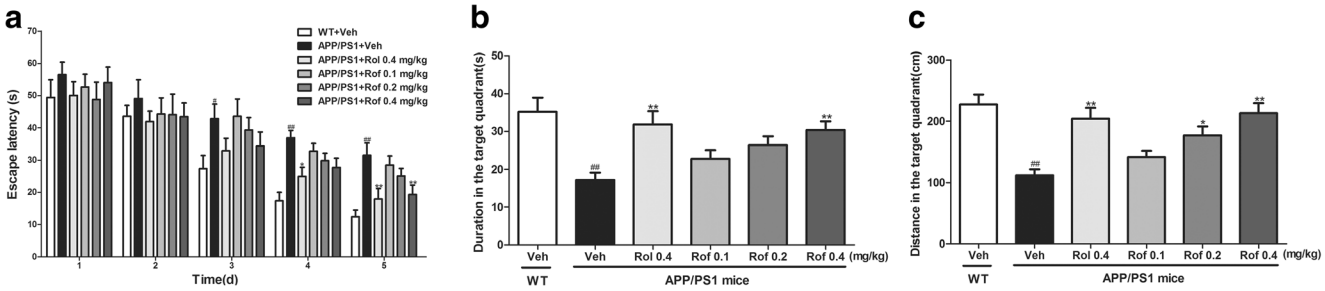



**Fig. 2** Effects of roflumilast on Morris water maze test in APP/PS1 mice. **a** Escape latency. **b** Duration in the target quadrant. **c** Exploratory distance in the target quadrant. The results are presented as the mean ± SEM ($n = 12$). $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with the WT mice; $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the vehicle-treated APP/PS1 mice. *Rof roflumilast, Rol rolipram*

0.4 mg/kg reversed the decline of p-CREB [$F(5, 12) = 9.747$, $P < 0.01$] (Fig. 4b&c), without altering the total CREB level. To determine whether roflumilast's effect on memory performance is related to neuroprotective effect, we also investigated the effect of roflumilast on hippocampal BDNF expression of APP/PS1 mice. The results showed that roflumilast increased both mRNA [$F(5, 12) = 7.242$, $P < 0.01$] (Fig. 4d) and protein level of BDNF [$F(5, 12) = 7.941$, $P < 0.01$] (Fig. 4b&e) in the hippocampus of APP/PS1 mice.

## Effect of roflumilast on nuclear translocation of NF-κB p65 and production of proinflammatory cytokines in the hippocampus of APP/PS1 mice

To verify the possible effect of roflumilast on inflammatory in the hippocampus, we determined the amount of the nuclear translocation of NF-κB p65 in the hippocampus of APP/PS1 mice. Figure 5a&b showed that the level of NF-κB p65 in the nuclei of vehicle-treated APP/PS1 mice were significantly higher than WT mice [$F(5, 12) = 7.771$, $P < 0.01$]. This high level of NF-κB p65 were reversed by chronic treatment with roflumilast.

Given that the transcription factor NF-κB is a crucial regulator of proinflammatory cytokines, we further examined the expression of IL-6, TNF-a and IL-1β in the hippocampus

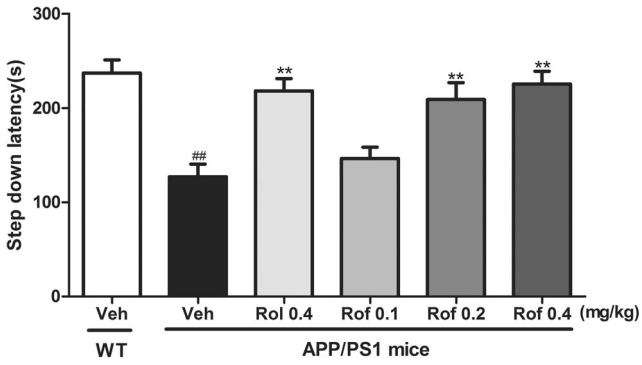



**Fig. 3** Effects of roflumilast on step-down passive avoidance task in APP/PS1 mice. The results are presented as the mean ± SEM (n = 12). $^{\#\#}P < 0.01$ compared with the WT mice; $^{**}P < 0.01$ compared with the vehicle-treated APP/PS1 mice. *Rof roflumilast, Rol rolipram*

(Fig. 5c-e). Consistent with the result of NF-κB p65, the IL-6 [$F(5, 12) = 12.12$, $P < 0.01$], TNF-a [$F(5, 12) = 12.52$, $P < 0.01$] and IL-1β [$F(5, 12) = 10.75$, $P < 0.01$] levels in vehicle-treated APP/PS1 mice were significantly higher than WT mice, and roflumilast (0.2, 0.4 mg/kg) can reversed these changes. These findings suggested that roflumilast may possess the anti-inflammatory properties in APP/PS1 mice.

## Discussion

In the present study, we evaluated the possibility of PDE4 inhibitor roflumilast as a therapeutic agent for cognitive dysfunction. We investigated the effects of chronic roflumilast oral administration on the impairment of learning and memory functions as well as the underlying mechanism in the APP/PS1 mouse model of AD. We demonstrated that roflumilast reversed the cognitive deficits of APP/PS1 mice in different behavioral tasks, including the novel object recognition test, step-down passive avoidance test and Morris water maze test. Further molecular biological assays suggested that roflumilast restored the levels of cAMP, p-CREB and BDNF in the hippocampus of APP/PS1 mice. Moreover, we also found that roflumilast exerted an anti-neuroinflammatory effect via suppressing nuclear translocation of NF-κB p65 and production of proinflammatory cytokines, including IL-6, IL-1β and TNF-a. All of these may contribute to cognitive benefits brought by roflumilast.

The APP/PS1 transgenic mouse is one of the most internationally recognized AD animal models that partially reproduces cognitive impairments and other relevant pathological processes that occur in AD patients, such as Aβ deposition and a robust inflammatory response toward senile plaques (Lopez-Gonzalez et al. 2015; Wengenack et al. 2000). These mice began to show Aβ deposition at 2.5 months of age and experience cognitive dysfunction from 7 months of age (Bruce-Keller et al. 2011; Webster et al. 2013; Liu et al. 2017). Considering the peculiar pathogenesis of AD in APP/PS1 mice, we selected 7-month-old APP/PS1 mice for the 3-week roflumilast treatment followed by behavioral evaluation.

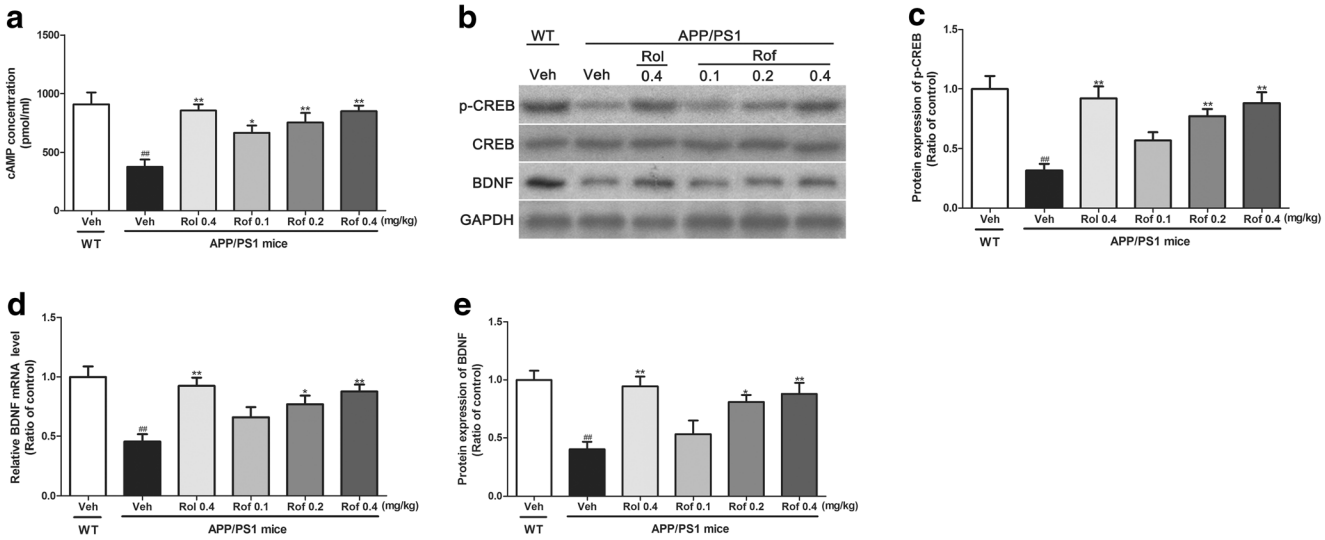



**Fig. 4** Effects of roflumilast on the cAMP/CREB/BDNF pathway in the hippocampus of APP/PS1 mice. **a** cAMP level. **b** Immunoblot bands for p-CREB, CREB, BDNF and GAPDH. **c** Relative protein level of p-CREB. **d** mRNA level of BDNF. **e** Relative protein level of BDNF. The results are presented as the mean ± SEM ($n = 3$). $^{\#\#}P < 0.01$ compared with the WT mice; $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the vehicle-treated APP/PS1 mice. *Rof roflumilast, Rol rolipram*

To assess the effectiveness of roflumilast in AD, the classic PDE4 inhibitor rolipram was selected as the positive control drug. Consistent with the results of rolipram, roflumilast significantly reversed the dysfunction of spatial learning and memory abilities in APP/PS1 mice, as reflected by Morris water maze test. The results of the step-down passive avoidance test and novel object recognition test further suggested roflumilast also has a significant ameliorative on the non-spatial memory impairments in APP/PS1 mice. Moreover, we found that roflumilast appears equally potent as rolipram, which implies an excellent cognitive enhancement property of roflumilast.

AD hallmarks encompass intracellular neurofibrillary tangles and extracellular amyloid-b (Aβ) plaques in the brain. According to the amyloid cascade hypothesis (Karran et al. 2011; Barage and Sonawane 2015), accumulation of Aβ in the brain were seems to be an underlying cause of neuronal loss and cognitive impairment. Therefore, it is of interest to investigate whether roflumilast could influence the production of Aβ peptides. Actually, we have carried out the experiment concerning the effect of roflumilast on Aβ production, which showed that roflumilast did not alter the hippocampal Aβ40 and Aβ42 level in APP/PS1 mice (data not shown). This is consistent with the previous studies that PDE4 inhibitors

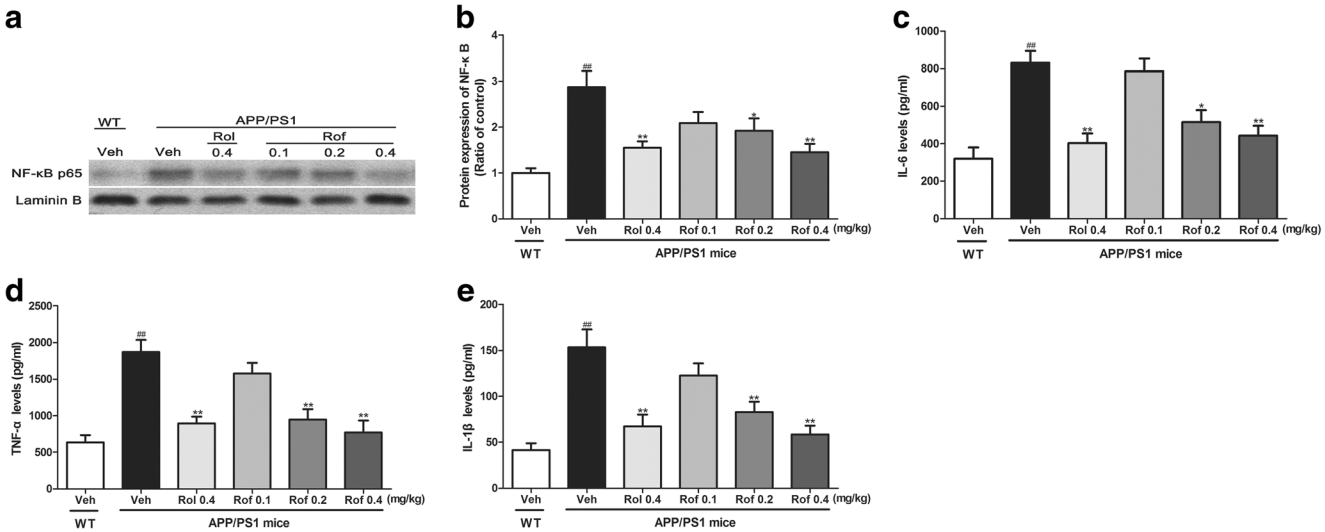



**Fig. 5** Effects of roflumilast on nuclear translocation of NF-κB p65 and production of proinflammatory cytokines in the hippocampus of APP/PS1 mice. **a** Immunoblot bands for nuclear NF-κB p65 and laminin B. **b** Relative protein level of nuclear NF-κB p65. **c** IL-6 level. **d** TNF-a level. **e** IL-1β level. The results are presented as the mean ± SEM ($n = 3$). $^{\#\#}P < 0.01$ compared with the WT mice; $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the vehicle-treated APP/PS1 mice. *Rof roflumilast, Rol rolipram*

appeared to exert cognitive enhancement effect independently of Aβ production (Gong et al. 2004; Sierksma et al. 2014; Guo et al. 2017). Thus, it appears that PDE4 inhibitors can strengthen the synaptic transmission and make neurons more resistant to the detrimental effects of Aβ in a non-amyloid way and, thereby, improving memory function.

Cyclic AMP response element-binding protein (CREB) is a critical transcription factor in the mediation of memory, and activation of CREB by phosphorylation is necessary for its function (Yamashima 2012; Josselyn and Nguyen 2005; Kandel 2012). Aβ-induced deficits of memory and synaptic plasticity are involved by cAMP/CREB signaling, as evidenced by decreased intracellular cAMP levels and lower CREB-mediated gene transcription following Aβ treatment (Saura and Valero 2011; Puzzo et al. 2005; Wang et al. 2016). It has been well established that rolipram can increase activation of brain cAMP/CREB signaling, neuron survival, and synaptic density in various animal models, including APP/PS1 mouse (Gong et al. 2004; Smith et al. 2009; Wang et al. 2012). Similarly, here we showed that roflumilast reversed the cognitive impairment by increasing hippocampal cAMP levels and p-CREB expression in the APP/PS1 mice. Moreover, BDNF, a downstream target gene of CREB, was also increased by roflumilast at both transcriptional and protein levels.

Considerable evidence supports that activation of microglia, indicative of neuroinflammation, is another histological characteristic of AD (Dansokho and Heneka 2017; Bolos et al. 2017). Aβ deposition can stimulate the microglia, leading to an inflammatory status in the brain of patients with AD as well as animal models of AD. It has been reported that Aβ-stimulated transcription of inflammatory genes responsible for cytokines production is dependent on NF-κB pathways (Dewapriya et al. 2013; Pan et al. 2009; Luccarini et al. 2012). Previous studies have also suggested that PDE4 inhibitors might slow down the progression of AD through inhibiting neuroinflammation. Specifically, rolipram increases intracellular cAMP levels and exerted anti-inflammatory effects in Aβ-induced rats by inhibiting NF-κB pathways (Wang et al. 2012). Therefore, to prove and understand the anti-inflammatory effects of roflumilast, we monitored the nuclear translocation of NF-κB p65, a component of the NF-κB pathway, in the hippocampus of APP/PS1 mice. Our results demonstrated that roflumilast reversed not only the deficits of cAMP/CREB signaling, but also the inflammatory response, as indicated by decreased nuclear NF-κB p65 level and releasing cytokines in the hippocampus. Thus, the effects of roflumilast on cognition in APP/PS1 transgenic mice may due to both activate cAMP/CREB pathway and decrease cytokine-dependent signaling.

Despite the beneficial effects of PDE4 inhibitors on memory functions, the clinical development of the PDE4 inhibitors in the treatment of cognitive-related disorders is hampered by its emetic side effect. So far, no PDE4 inhibitors have been approved for application in AD in a clinical setting, and only two PDE4 inhibitors, roflumilast for chronic obstructive pulmonary disease and apremilast for active psoriatic arthritis, were approved by the FDA because of their favorable emetic/clinical profile in humans (Pinner et al. 2012; Poole and Ballantyne 2014). In addition, recent research demonstrated that roflumilast has a good brain penetration after oral administration, and can ameliorate the hypertension-induced memory impairment of the rats (Jabaris et al. 2015). Roflumilast can also enhance cognitive function in normal mice at doses that do not cause emesis-like behaviour, and its emetic potential is more than 10 times lower than that of rolipram (Vanmierlo et al. 2016). Likewise, it has recently been demonstrated that acute administration of roflumilast enhances immediate recall of verbal word memory in healthy young adults (Van Duinen et al. 2018). These results, together with our current study suggest that roflumilast have potential as an effective PDE4 inhibitor for treatment of AD.

In summary, here we investigated the effects of roflumilast on learning and memory impairment and the underlying mechanism in the APP/PS1 mouse model of AD. Our data shows that chronic roflumilast attenuates the cognitive impairment in APP/PS1 mice, which may be due to the upregulation of the cAMP/CREB/BDNF pathway and inhibition of the NF-κB-activated inflammatory pathway. This study provides a new insight into the cognitive enhancement effects of roflumilast, and we consider roflumilast to be a potential therapeutic agent in AD therapy.

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## Compliance with ethical standards

**Conflicts of interest** The authors declare that there are no conflicts of interest.



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