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Effects of progesterone on glucose uptake in neurons of Alzheimer's disease animals and cell models

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ABSTRACT

Aims: : Alzheimer's disease (AD) is closely related to abnormal glucose metabolism in the central nervous system. Progesterone has been shown to have obvious neuroprotective effects in the pathogenesis of AD, but the specific mechanism has not been fully elucidated. Therefore, the purpose of this study was to investigate the effect of progesterone on the glucose metabolism of neurons in amyloid precursor protein (APP)/presenilin 1 (PS1) mice and Aβ-induced AD cell model.

Materials and methods: :APP/PS1 mice were treated with 40 mg/kg progesterone for 40 days and primary cultured cortical neurons were treated with 1 μM progesterone for 48 h.Then behavior tests,2-NBDG glucose uptake tests and the protein levels of glucose transporter 3 (GLUT3), GLUT4, cAMP-response element binding protein (CREB) and proliferator-activated receptor γ (PPARγ) were examined.

Key findings: :Progesterone increased the expression levels of GLUT3 and GLUT4 in the cortex of APP/PS1 mice, accompanied by an improvement in learning and memory. Progesterone increased the levels of CREB and PPARγ in the cerebral cortex of APP/PS1 mice. In vitro, progesterone increased glucose uptake in primary cultured cortical neurons, this effect was blocked by the progesterone receptor membrane component 1 (PGRMC1)-specific blocker AG205 but not by the progesterone receptor (PR)-specific blocker RU486. Meanwhile, progesterone increased the expression of GLUT3, GLUT4, CREB and PPARγ, and AG205 blocked this effect.

Significance: These results confirm that progesterone significantly improves the glucose metabolism of neurons.One of the mechanisms of this effect is that progesterone upregulates protein expression of GLUT3 and GLUT4 through pathways PGRMC1/CREB/GLUT3 and PGRMC1/PPARγ/GLUT4.

1. Introduction

Alzheimer's disease (AD), the most common type of senile dementia, is a degenerative disease of the central nervous system characterized by progressive cognitive and memory impairment [1]. Studies have shown a close relationship between AD and glucose metabolism [2]. All types of AD are accompanied by decreased neuron glucose utilization, insulin pathway abnormalities and energy utilization disorder, resulting in insufficient neuron function, decreased neuron number, and apoptosis and necrosis, thereby aggravating AD progress [3–5]. Therefore, research on the relationship between abnormal glucose metabolism and the pathogenesis of AD has attracted much attention in recent years.

Glucose requirements for the brain are completely derived from blood circulation and transported by glucose transporters (GLUTs) across the blood-brain barrier to the central nervous system [6]. The two most important types of GLUTs in central nervous system neurons are GLUT3 and GLUT4. Among the brains of patients with AD, the expression levels of GLUT3 and GLUT4 are significantly reduced, which leads to a decrease in the glucose transport rate, abnormal neuron glucose metabolism, and neuron energy metabolism disorder, resulting in neuron degeneration and the corresponding symptoms of AD [7,8]. Studies have confirmed that decreased GLUT3 and GLUT4 expression causes neuron degeneration rather than neuron degeneration decreasing GLUT3 and GLUT4 expression [9]. Therefore, upregulating

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GLUT3 and GLUT4 expression and increasing neuronal glucose transport, thus improving neuronal energy metabolism, may be an important way to prevent the progression of AD.

Progesterone, as an important neurosteroid, is an endogenous neuromodulator produced by the central nervous system and is widely involved in various physiological and pathological processes of the central nervous system [10,11]. Progesterone has a significant neuroprotective effect, improving learning and memory and the survival rate of newborn hippocampal neurons in rats [12,13]. More importantly, progesterone is closely associated with the development of AD. Epidemiological and clinical investigations of AD have confirmed that the level of progesterone in the brain of AD patients is significantly lower than that of their normal peers [14,15]. The above results suggest that progesterone may be a new target to delay the progression of AD.

Previous studies in our laboratory have confirmed that progesterone can inhibit neuronal apoptosis, reduce oxidative stress and induce neural stem cells to differentiate into neurons in an AD cell model [16,17]. However, whether progesterone can promote the glucose uptake ability of neurons in animal and cell models of AD has not been reported. Therefore, this study used the amyloid precursor protein (APP)/presenilin 1 (PS1) transgenic mouse AD animal model and the Aβ25-35-induced primary cultured rat cortical neurons AD cell model to investigate the effect of progesterone on the glucose uptake capacity of AD neurons and the specific molecular mechanism.

2. Materials and methods

2.1. Animals

Six months male APP/PS1 transgenic c57-bl mice and wild-type c57-bl mice of the same age were purchased from Beijing HFK bioscience company and kept in laboratory animal center of Bethune international peace hospital.36 male c57-bl mice were divided into three groups: control group (wild-type mice), model group (APP/PS1 mice), and progesterone group (APP/PS1 + PROG).The progesterone group was given progesterone by gavage on the first day, once a day with a dose of 40 mg/kg.The other two groups were given the same volume of dissolved oil by gavage.After 40 days of continuous administration, morris water maze experiment were conducted on days 41–46.In the morris water maze experiment, the escape platform was placed 2 cm below the water surface. The ambient temperature is 27 °C, and the water temperature is 24°C– 26 °C. The position of platform, water maze and surrounding environment remained unchanged during the whole experiment. The animals were killed on days 47 and their brains were taken for immunohistochemical experiments.The ethical standards of all research are consistent with the National Institutes of Health guide for the care and use of Laboratory animals. Ethical protocol of the current research was approved by Bethune International Peace Hospital of Chinese PLA, China. (Ethics Committee Issue Number:2019-KY-47,26th March 2019).

2.2. Reagent

The glucose uptake kit was purchased from Biovisio.Membrane protein and cytoplasmic protein extraction kit and BCA protein quantitative kit were purchased from Beyotime company.ECL glow kit from Santa Cruz.Progesterone, AG205 and RU486 were purchased from Sigma,666–15 and GW9662 were purchased from Abmole.The GLUT3, GLUT4, polyclonal rabbit antibody purchased from Zenbio biological company. Polyclonal rabbit antibody to proliferator-activated receptor γ(PPARγ), cAMP-response element binding protein (CREB), and horseradish peroxidase(HRP) were purchased from Abways.Polyclonal mouse antibodies to neuronal nuclear antigens(NEUN)were purchased from Abcam.Alex flour 488 goat anti mouse IgG and Alex flour 594 goat anti rabbit IgG were purchased from Abways.

2.3. Primary cortical neuron cultures

New SD suckling rats were born within 24 h. The head was cut off, and the brain was removed under aseptic conditions. The soft meninges, hippocampus and other tissues were removed, and only the cortex was retained. The tissue was then washed with D-hank's solution, cut into pieces, and digested with 1.25 mg/ml trypsin solution for 13 min. After the digestion was terminated by the addition of DMEM complete culture medium, the cells were inoculated in a 200-mesh sieve and incubated in a cell culture plate at 37 °C with 5% CO_2 at a constant temperature for 8 h. The medium was replaced with neurobasal medium (2% B27) after the cells attached to the wall and was changed every three days. Neurons were cultured for 7 days for experiments. Primary cultured neurons were randomly divided into a control group, $A\beta_{25\text{-}35}$ treatment group (25 μM), progesterone treatment group (25 μM $A\beta_{25\text{-}35}$ + 1 μM progesterone), AG205 group (25 μM $A\beta_{25\text{-}35}$ + 1 μM progesterone + 10 μM AG205), RU486 group (25 μM $A\beta_{25\text{-}35}$ + 1 μM progesterone + 10 μM RU486),666-15 group (25 μM $A\beta_{25\text{-}35}$ + 1 μM progesterone + 0.1 μM 666-15) and GW9662 group (25 μM $A\beta_{25\text{-}35}$ + 1 μM progesterone + 0.1 μM GW9662).AG205,RU486,666–15 and GW9662 were added 1 h in advance. The cells were cultured for 48 h after treatment and then used for the various experiments.

2.4. Behavior tests

Morris water maze experiment was performed on day 41st after 40 consecutive days of progesterone administration. Positioning navigation experiments were trained twice at 9:00 a.m. and twice at 3:00 p.m., four times a day for five days and record the time it takes to find the escape platform.The escape platform was placed 2 cm below the water surface. The ambient temperature is 27 °C, and the water temperature is 24°C– 26 °C. The position of platform, water maze and surrounding environment remained unchanged during the whole experiment.The space exploration experiment was conducted on the 46th day, the platform was removed, and The Times of crossing the platform in 90s and the percentage of time spent in the quadrant of the platform were recorded.

2.5. Immunohistochemistry

After the mice were anaesthetized and sacrificed, the brain tissue was fixed in 4% paraformaldehyde for 24 h, dehydrated and embedded with wax blocks, and then cut into thin slices of 5 μm. After the antigen was repaired, the tissue was sealed with goat serum for 2 h, washed with PBS and then incubated with GLUT3, GLUT4,CREB,PPARγ primary antibodies (dilution times were 1:200) overnight at 4 °C. After the tissue was rinsed with PBS, the tissue was incubated in secondary antibody (dilution times were 1:500) at room temperature for 1 h. After the tissue was rinsed with PBS, a DAB kit was used for colour rendering.

2.6. Glucose uptake tests

More than 2000 cells were inoculated per hole in the 96-well plates, glucose uptake was tested after culture for 7 days.The specific steps of the glucose uptake experiment were carried out according to the instructions of Glucose Uptake Colorimetric Assay Kit. The main reagents in the kit included Neutralization Buffer, 2-Deoxyglucose (2-DG, 10 mM), Assay Buffer, Enzyme Mix, Recycling Mix, 2-DG6P Standard, Glutathione Reductase,Substrate-DTNB.PBS was used for washed and incubated overnight with 100 μl serum-free medium, followed by starvation incubation with KRPH buffer containing 2% fetal bovine serum for 40min, 10 μM/ml 2-dg of 10 μl was added to cells in each well and incubated for 20min.PBS was used to wash the cells for 3 times, cell lysis solution was added to lyse the cells, heated at 85 °C for 40min, frozen at -20 °C for 5min, and 10 μl neutralization buffer was added to

each well.Add Assay buffer to 50 μl. Add 10 μl mixA(Assay buffer 8 μl, Enzyme mix 2 μl) to each well. Then incubated at 37 °C for 1 h, 90 μl Extraction buffer was added to each hole, heated at 90 °C for 40min, and 38 μl mixB(Glututhione Reductase 20 μl, DTNB 16 μl, Recycling mix 2 μl) was added to each well.The absorbance value was measured with the wavelength of 412 nm, and the glucose uptake rate was calculated according to the standard curve.

2.7. Western blot analysis

After cell culture, cells were collected, cytoplasmic protein and membrane protein were extracted using a protein extraction kit, protein was quantified using a BCA kit, protein samples were normalized to 5 μg/μl, and SDS-PAGE electrophoresis was carried out. After electrophoresis, the separating gel was cut to the appropriate size according to the position of the marker, and the proteins were transferred to the membrane. The membrane was sealed with protein sealant for 2 h, rinsed with TTBS, incubated with primary antibody overnight at 4 °C (GLUT3 and GLUT4 primary antibody dilution times were 1:2000, CREB and PPARγ primary antibody dilution times were 1:1000 β-actin primary antibody dilution times were 1:5000)and then rinsed with TTBS three times. The membrane was then incubated with the secondary antibody(dilution times were 1:5000) at 37 °C for 2 h. The ECL kit was then used for colour rendering, and the image was exposed in the dark room.

2.8. Immunofluorescence

Mature neurons cultured for 7 days were used for immunofluorescence assay. Washing neurons with PBS 3 times. Then the cells were fixed with 4% paraformaldehyde for 30 min at 4 °C. After 3 rinses with PBS, use 1% TritonX-100 incubate 20 min.Then with rabbit polyclonal anti-GLUT3 or anti-GLUT4 (1:200) and mouse polyclonal anti-NEUN (1:200) overnight at 4 °C. After washing with PBS, the cells were incubated with Alex flour 488 goat anti mouse IgG and Alex flour594 goat anti rabbit IgG (1:500) for 1 h at room temperature (25 ± 2 °C). After final washing with PBS, the neurons were observed using Inverted fluorescence Microscope.

2.9. Statistical analysis

The above quantitative data were expressed as the mean ± SD. One-way ANOVA was used for statistical analysis of the data of each group, and Student-Newman-Keuls(SNK) method was selected for pairwise comparison between groups. $P < 0.05$ indicated a statistically significant difference.

3. Results

3.1. Effects of progesterone on learning and memory in APP/PS1 mice

As shown in Fig. 1, the average swimming speed in the Morris water maze did not differ among mouse groups(Table 1). The APP/PS1 mice had a significantly longer escape latency than the control mice ($P < 0.05$), confirming that the learning and memory ability of APP/PS1 mice was significantly impaired. However, compared with the APP/PS1 group, the progesterone treatment group exhibited a significantly shorter escape latency beginning at the third day of training ($P < 0.05$) (Fig. 1A, B). In the space exploration experiment, the APP/PS1 group had a lower number of platform-area crossings and percentage of time spent in the platform quadrant than the control group ($P < 0.05$). However, progesterone treatment increased the number of platform-area crossings in APP/PS1 mice by 4.0 times ($P < 0.05$) and the percentage of time spent in the platform quadrant by 2.23 times ($P < 0.05$) (Fig. 1C, D). These results confirm that progesterone significantly improved the learning and memory ability of APP/PS1 mice.

3.2. Effects of progesterone on GLUT3 and GLUT4 expression levels in the cerebral cortex of APP/PS1 mice

As shown in Fig. 2,immunohistochemistry analysis showed that GLUT3 and GLUT4 expression in the cortex of the normal control group mice was higher than that in the cortex of the APP/PS1 mice ($P < 0.01$). After 40 days of treatment with progesterone, the GLUT3 expression level in the cerebral cortex of APP/PS1 mice was 2.1 times higher ($P < 0.01$), and the GLUT4 expression level was 1.8 times higher ($P < 0.05$) than that in the APP/PS1 group (Fig. 2A–C).

After homogenization of mouse cortical tissue, the protein was extracted, and Western blot was used to detect the protein expression levels of GLUT3 and GLUT4. Compared with normal control group, the APP/PS1 group exhibited a 52.0% reduction in GLUT3 protein expression ($P < 0.01$) and a 76.5% reduction in GLUT4 protein expression ($P < 0.01$). Progesterone significantly improved GLUT3 and GLUT4 protein expression, with GLUT3 expression 1.8 times ($P < 0.01$) that in the untreated APP/PS1 group and GLUT4 expression 2.9 times ($P < 0.01$) that in the untreated APP/PS1 group (Fig. 2D–F).

3.3. Effects of progesterone on cAMP-response element binding protein (CREB) and proliferator-activated receptor γ (PPARγ) expression in the cerebral cortex of APP/PS1 mice

As shown in Fig. 3, the expression levels of CREB and PPARγ in the cerebral cortex of mice in the normal control group were higher than those in the APP/PS1 group. In the cerebral cortex of APP/PS1 mice, CREB protein expression was decreased by 54.0% ($P < 0.01$), and PPARγ expression was decreased by 59.3% ($P < 0.01$). Progesterone treatment significantly increased the expression levels of CREB and PPARγ in the cerebral cortex 1.9 ($P < 0.01$) and 1.8 ($P < 0.01$) times, respectively (Fig. 3A–C).

Western blotting was used to detect the protein expression levels of CREB and PPARγ in the cortical tissues of mice. Compared to the normal control group, APP/PS1 mice displayed a 44.9% decrease in CREB protein expression and a 59.1% decrease in PPARγ protein expression in the cortex ($P < 0.01$). After treatment with progesterone, CREB protein levels in the cortex increased 1.5 times those in the untreated APP/PS1 group ($P < 0.01$), and PPARγ protein levels increased 2.2 times ($P < 0.01$). These results confirm that progesterone increased the expression levels of CREB and PPARγ in the cerebral cortex of APP/PS1 mice (Fig. 3D–F).

3.4. Effects of progesterone on glucose uptake capacity of cortical neurons and expression of GLUT3 and GLUT4

In vitro, glucose uptake was assessed to detect the glucose uptake capacity of primary cultured cortical neurons. As shown in Fig. 4, compared with the glucose uptake capacity in the normal control group, the Aβ-induced glucose uptake capacity of neurons was 51.2% lower ($P < 0.01$). However, progesterone treatment increased the glucose uptake capacity of neurons 1.7 times ($P < 0.01$). AG205, a specific blocker of progesterone receptor membrane component 1 (PGRMC1), significantly inhibited the increase in glucose uptake of neurons caused by progesterone. Compared with the glucose uptake ability in the progesterone treatment group, the glucose uptake ability decreased by 37.2% with the applications of AG205 ($P < 0.05$), while the specific progesterone receptor (PR) blocker RU486 had no significant effect on the glucose uptake ability of neurons (Fig. 4A).

Western blotting was used to detect GLUT3 and GLUT4 protein expression in primary cultured cortical neurons. As shown in Fig. 4B–D, GLUT3 and GLUT4 were highly expressed in the total proteins of neurons in the normal control group. After Aβ induction, GLUT3 and GLUT4 protein expression levels were significantly reduced, with the GLUT3 expression level 68.8% lower ($P < 0.01$) and the GLUT4 expression level 77.1% lower ($P < 0.01$) than that in the normal control

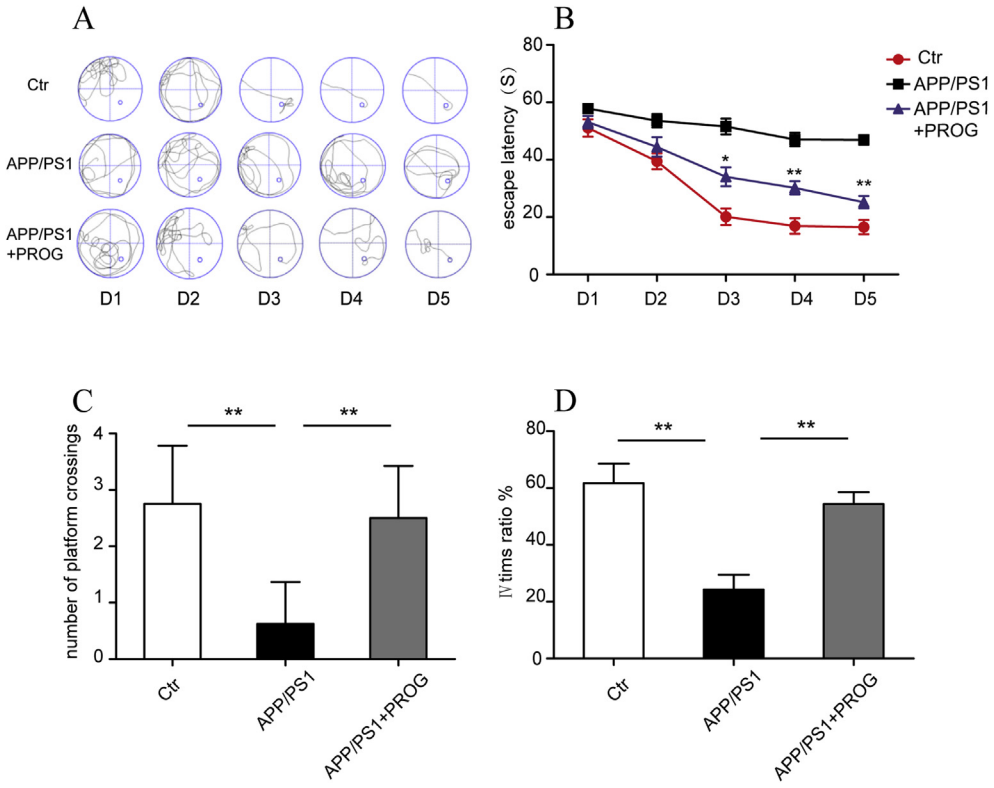

Fig. 1. Progesterone improves learning and memory in APP/PS1 mice. (A) The swimming pathway of mice in each group. (B) Effects of progesterone on escape latency in APP/PS1 mice. (C) Effect of PROG on numbers of platform crossings. (D) Effect of PROG on IVtime ratio. The data were presented as mean ± SEM, n = 12 in each group.*P < 0.05, **P < 0.01.

group. After treatment with progesterone, the expression levels of GLUT3 and GLUT4 in the total protein increased significantly to 1.4 times and 1.3 times those in the Aβ-induced group, respectively (P < 0.01). The increases in GLUT3 and GLUT4 expression induced by progesterone were obviously blocked by AG205, with GLUT3 protein expression 75.4% lower (P < 0.01) and GLUT4 protein expression 70.1% lower (P < 0.01) in the AG205-treated group than in the group treated with progesterone alone.

We extracted membrane proteins from primary cultured cortical neurons and detected GLUT3 and GLUT4 protein expression in the membrane proteins by Western blot. As shown in Fig. 4E–G, GLUT3 and GLUT4 protein expression levels were significantly reduced by Aβ to levels 64.7% and 50.2% lower than those in the normal control group, respectively (P < 0.01). GLUT3 and GLUT4 expression levels in the membrane proteins of neurons were significantly increased by progesterone to levels 1.79 times and 1.85 times those in the Aβ-induced group, respectively (P < 0.01). AG205 significantly blocked this effect of progesterone; compared with the progesterone treatment group, the group also treated with AG205 showed a 29.2% reduction in GLUT3 membrane protein expression (P < 0.01) and 47.7% reduction in GLUT4 membrane protein expression (P < 0.01).Immunofluorescence results also confirmed that progesterone could up-regulate the expression of GLUT3 and GLUT4, and this effect could be blocked by AG205(Fig. 4H–K).

3.5. Effects of progesterone on CREB and PPARγ expression in primary cortical neurons

Western blotting was used to detect the protein expression levels of CREB and PPARγ in primary cultured cortical neurons. As shown in Fig. 5A and B, Aβ significantly reduced the protein expression level of CREB in neurons by 67.8% compared with no Aβ induction (P < 0.01). Progesterone significantly increased the protein expression of CREB to levels 2.2 times higher than those in the Aβ-induced cells (P < 0.01). AG205 blocked the upregulation of CREB expression caused by progesterone; the expression level of CREB in AG205-treated cells was 59.9% lower than that in the progesterone group (P < 0.01). Figure 5A and 5Cshows the effect of progesterone on PPARγ expression in primary cultured cortical neurons. Aβ significantly inhibited the expression of PPARγ, decreasing its expression by 34.3% compared with no Aβ induction (P < 0.01). PPARγ expression was significantly increased by progesterone to levels 1.5 times higher than those in the Aβ-induced cells (P < 0.01). AG205 blocked the upregulation of PPARγ expression induced by progesterone; the expression level of PPARγ in AG205-treated cells was 22.4% lower than that in the progesterone group (P < 0.01).Fig. 5D and E show the effect of CREB inhibitor 666-15 on GLUT3 expression. The results show that 666-15 can significantly inhibit the up-regulation of GLUT3 expression induced by progesterone.Fig. 5F and G show the effect of PPARγ inhibitor GW9662 on GLUT4 expression. The results show that GW9662 inhibit the up-regulation of GLUT4 expression induced by progesterone significantly.

Table 1
Effect of PROG A on APP/PS1 mice mean swim speed in the Morris water maze on different days (mean ± SD, n = 12).

	D1	D2	D3	D4	D5
Ctr	199.8 ± 10.2	199.0 ± 11.2	199.2 ± 9.9	195.1 ± 11.6	198.1 ± 10.1
APP/PS1	199.1 ± 11.1	197.1 ± 9.2	195.6 ± 9.3	199.0 ± 11.1	195.3 ± 13.5
APP/PS1 + PROG	198.1 ± 8.4	197.9 ± 9.5	197.5 ± 10.7	201.4 ± 15.4	200.1 ± 11.5

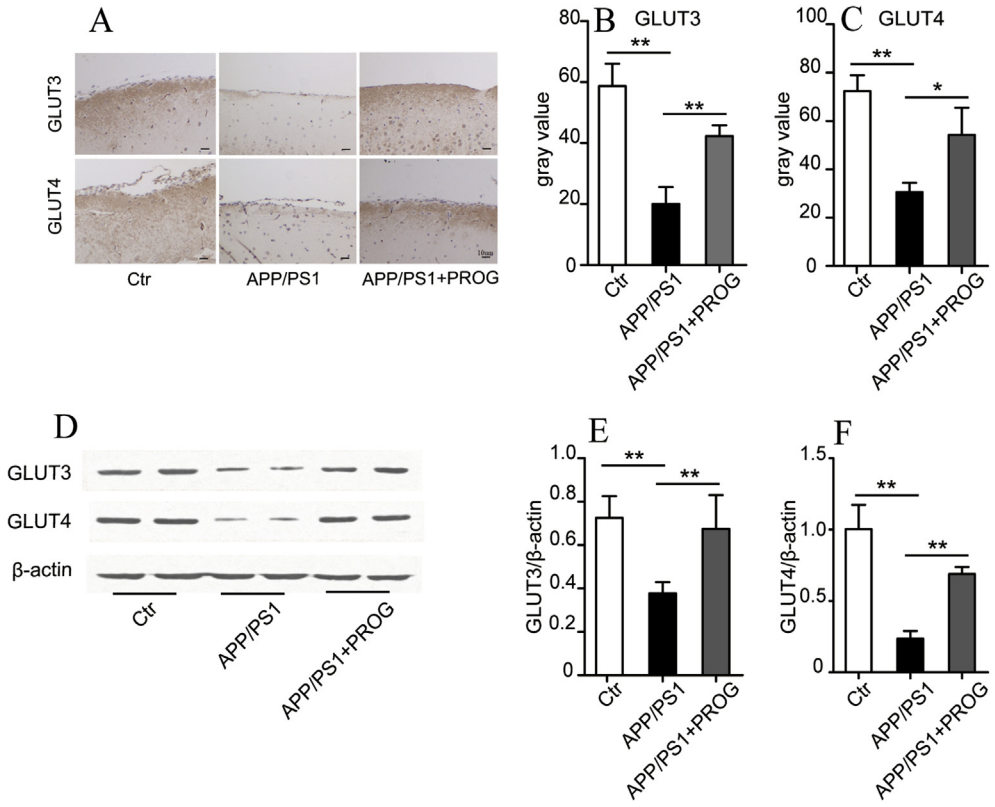

Fig. 2. Effects of progesterone on GLUT3 and GLUT4 expression levels in the cerebral cortex of APP/PS1 mice. (A) GLUT3 and GLUT4 were detected by immunohistochemistry. (B) and (C) Immunohistochemistry were quantitated by densitometry, the relative level of GLUT3 and GLUT4 is presented as mean ± SD. (D) Western blots of GLUT3 and GLUT4 in the cortex. The immunoreactivity of protein was normalized to β-actin. (E) and (F)Western blots were quantitated by densitometry, the relative level of GLUT3 and GLUT4 is presented as mean ± SD. *P < 0.05, **P < 0.01.

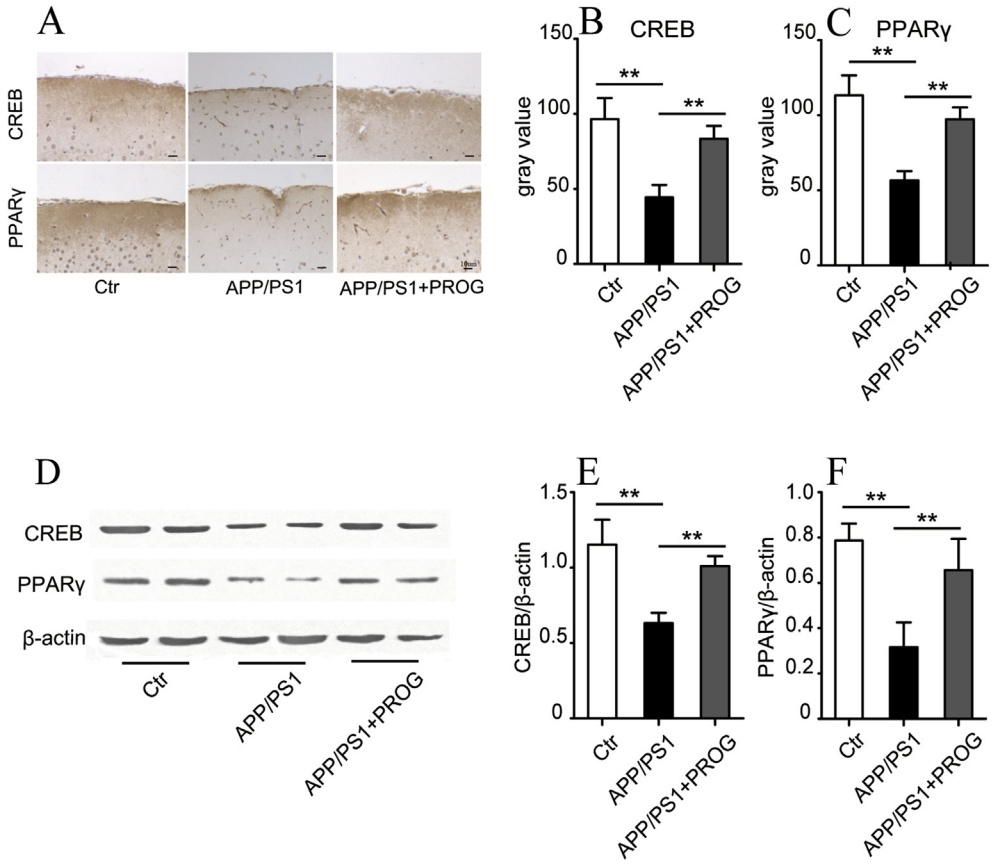

Fig. 3. Effects of progesterone on CREB and PPARγ expression levels in the cerebral cortex of APP/PS1 mice. (A) CREB and PPARγ were detected by immunohistochemistry. (B) and (C) Immunohistochemistry were quantitated by densitometry, the relative level of CREB and PPARγ is presented as mean ± SD. (D) Western blots of CREB and PPARγ in the cortex. The immunoreactivity of protein was normalized to β-actin. (E) and (F)Western blots were quantitated by densitometry, the relative level of CREB and PPARγ is presented as mean ± SD. *P < 0.05, **P < 0.01.

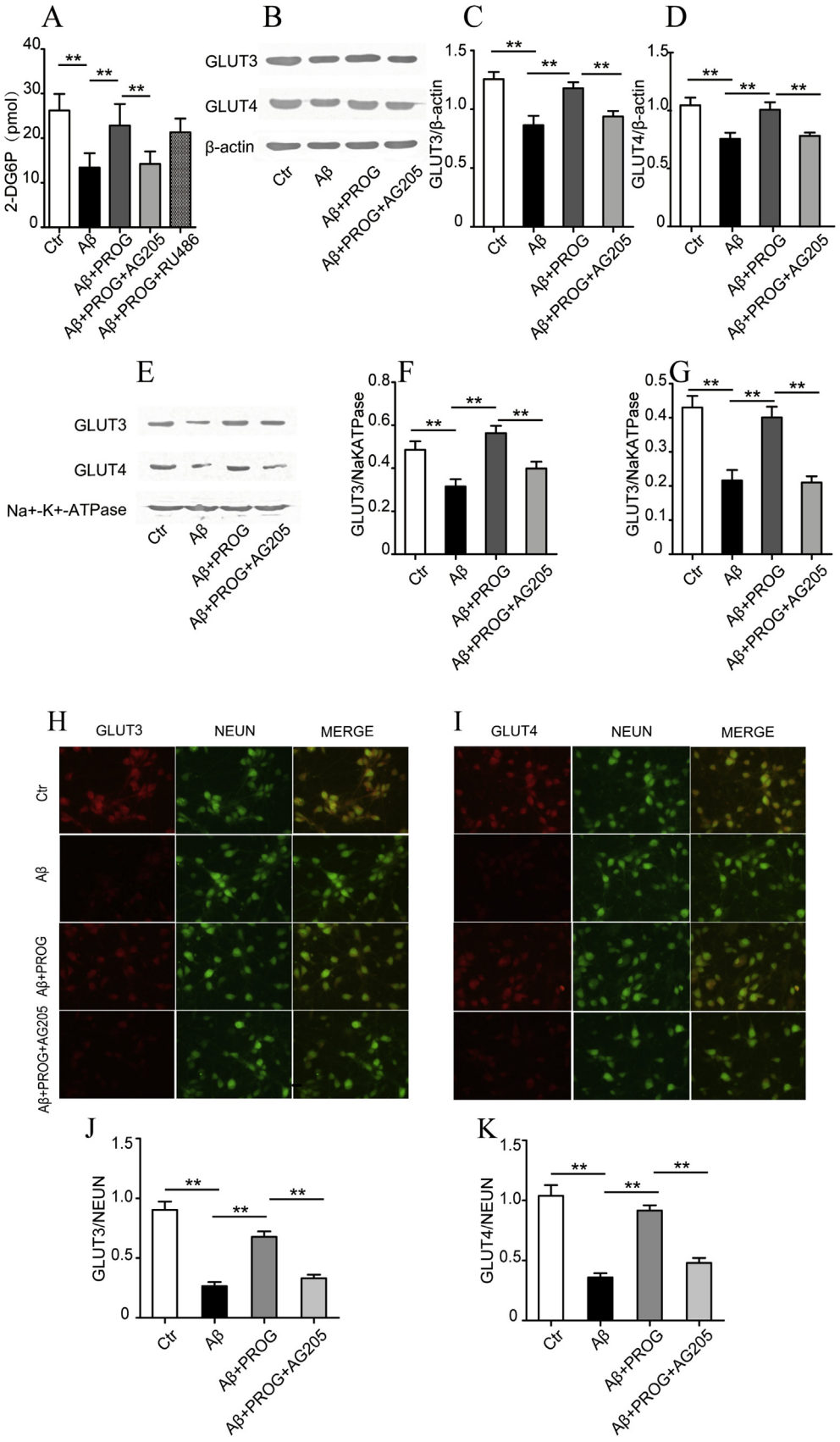

Fig. 4. Progesterone increases the glucose uptake capacity of cortical neurons by promoting the expression of GLUT3 and GLUT4. (A)Effects of progesterone against Aβ25-35 induced glucose uptake defection in primary cultured cortical neurons.(B) Western blots of total GLUT3 and GLUT4 in primary cultured cortical neurons. The immunoreactivity of protein was normalized to β-actin. (C) and (D) Western blots were quantitated by densitometry, the relative level of total GLUT3 and GLUT4 is presented as mean ± SD. (E)Western blots of membrane GLUT3 and GLUT4 in primary cultured cortical neurons. The immunoreactivity of protein was normalized to Na + -K + -ATPase. (F) and (G) Western blots were quantitated by densitometry, the relative level of membrane GLUT3 and GLUT4 is presented as mean ± SD.(H) The co-expression of GLUT3 and NEUN in neurons. GLUT3-positive cells are red. NeuN-positive cells are green.(I) The co-expression of GLUT4 and NEUN in neurons. GLUT4-positive cells are red. NeuN-positive cells are green. (J) and (K) Immunofluorescence were quantitated by densitometry, the relative level of total GLUT3 and GLUT4 is presented as mean ± SD.*P < 0.05, **P < 0.01. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

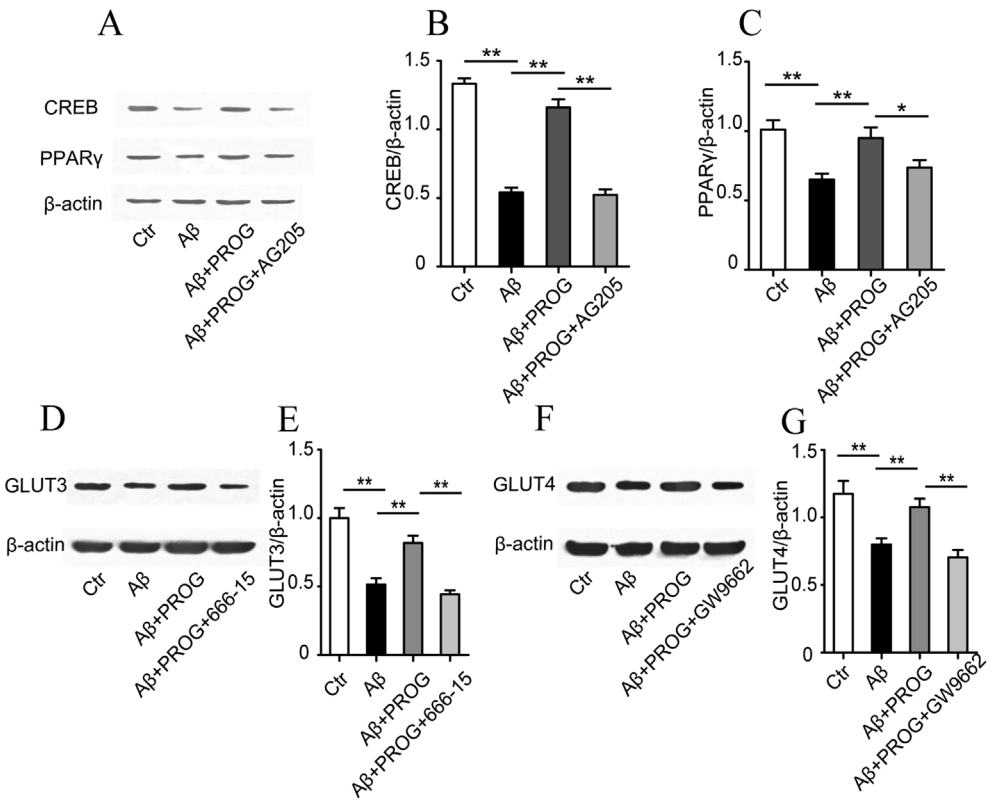

Fig. 5. Effect of progesterone on CREB and PPARγ expression in primary cortical neurons. (A) Western blots of CREB and PPARγ in primary cultured cortical neurons. The immunoreactivity of protein was normalized to β-actin.(B) and (C)Western blots were quantitated by densitometry, the relative level of CREB and PPARγ is presented as mean ± SD. *P < 0.05, **P < 0.01. (D) and (E) Effect of 666-15 on expression of GLUT3.(F)and(G)Effect of GW9662 on expression of GLUT4.

4. Discussion

Previous studies have shown a bilateral decline in the whole-brain glucose metabolism rate of AD patients to levels 30% to 70% of those in normal people [18]. In the early stage of AD, the decrease in the glucose metabolism rate is mainly observed in the parietal cortex and cingulate gyrus [19,20]. As the disease progresses, the glucose metabolism rate in the temporal lobe and part of the frontal cortex also decreases significantly [21]. Once AD has progressed to a later stage, the glucose metabolism rate of the bilateral parietal lobe, frontal lobe and temporal lobe is significantly reduced [22]. Moreover, the degree and range of glucose metabolism rate reduction are positively correlated with AD severity [21]. However, the glucose metabolism rate of the occipital cortex does not significantly change across the development and progression of AD.Memory is a complex process involving multiple brain regions.Although the hippocampus is an important part of the brain responsible for memory, but in addition to the hippocampus, other structures in the brain, such as the frontal cortex, parietal cortex, thalamus and amygdala, also play a key role in learning and memory.Spatial memory is a form of memory used to identify the external geographic environment or direction.Among the animal experiments, morris water maze experiment is a common method to measure the spatial memory ability of animals.The parietal cortex and the hippocampus are the areas of the brain that are most closely associated with spatial memory.In animal experiments, damage to the parietal lobe can lead to damage to spatial memory [23], and in humans, the medial parietal sulcus in the parietal cortex is thought to be closely related to spatial memory [24]. At the early stage of AD, the glucose metabolism rate in the parietal cortex shows a significant downward trend. Therefore, in this study, parietal cortical neurons of APP/PS1 mice and parietal cortex neurons of newborn Sprague-Dawley (SD) rats were selected as the research objects of the in vivo model and in vitro model, respectively.

Previous studies have confirmed that progesterone can improve memory [25] and glucose metabolism [26]. Our results show that the spatial memory function of APP/PS1 mice has been significantly improved after progesterone intervention, which is consistent with previous studies.At the early stage of AD, the glucose metabolism rate in the parietal cortex shows a significant downward trend. Therefore, we speculate that progesterone improves glucose metabolism in parietal cortex may be one of the reasons that progesterone improves memory.

The brain's demand for glucose depends entirely on the transport of glucose from the blood across the blood-brain barrier to neurons, a process that must be facilitated by glucose transporters [27]. Among the cortical neurons, the two most important glucose transporters are GLUT3 and GLUT4 [28]. In the pathogenesis of AD, downregulation of the expression of GLUT3 and GLUT4 has been confirmed to lead to the decline in the glucose uptake rate of neurons, thus causing abnormal glucose metabolism, accelerating the degeneration and apoptosis of neurons and resulting in cognitive impairment [29–35]. Immunohistochemical results showed that the expression levels of GLUT3 and GLUT4 in the cortex of APP/PS1 mice were significantly lower than those in the cortex of control mice, while the expression levels of GLUT3 and GLUT4 were significantly increased by progesterone treatment. In addition, progesterone significantly shortened the escape latency of APP/PS1 mice and increased the number of times APP/PS1 mice crossed the platform area and the percentage of time spent in the platform quadrant. The above results suggest that progesterone upregulates the expression levels of GLUT3 and GLUT4 in cortical neurons, which is one of the mechanisms by which progesterone improves AD.

To further study the mechanism by which progesterone upregulates GLUT3 and GLUT4 expression, primary cortical neurons of newborn SD rats were used in in vitro experiments. Progesterone plays a biological role mainly by binding progesterone and its receptor, thus mediating a series of downstream biological effects [36,37]. The receptors for progesterone are mainly divided into two categories: the classical progesterone receptor (PR) and the progesterone receptor membrane component (PGRMC). Classical PRs are located in many regions of the central nervous system, including the cortex, hippocampus, hypothalamus and cerebellum [38,39]. In addition to the classical PR, an

important PGRMC, PGRMC1, has also been found to be expressed in the central nervous system [40,41]. Our previous results confirmed that PGRMC1 is expressed in primary cortical neurons of rats and that PGRMC1 is involved in the neuroprotective effects of progesterone [16,17]. The glucose uptake experiment showed that the glucose uptake capacity of primary neurons was significantly decreased by Aβ injury and that progesterone treatment improved the glucose uptake capacity of the Aβ-injured neurons. AG205, a specific antagonist of progesterone [42], blocked this effect, while RU486, a specific antagonist of the classic PR [43], had no such effect. Western blot analysis and immunofluorescence also confirmed that AG205 blocked the upregulation of GLUT3 and GLUT4 expression induced by progesterone. The above results suggest that progesterone upregulates the expression of GLUT3 and GLUT4 through its membrane receptor PGRMC1, and the classic PR is not involved in this effect.

At present, there is abundant evidence of a trend towards a low GLUT3 expression in the brains of AD patients, which may be an important reason for the reduced brain glucose uptake rate and energy metabolism disturbance, resulting in the degeneration of neurons [30–32,44]. As an important nuclear transcription factor, CREB is obviously associated with many related genes during the occurrence of AD [45,46]. The target genes of CREB play an important role in central nervous system development, synaptic plasticity and neuroprotection [47,48]. CREB binds to the cyclic adenosine phosphate reactive element (CRE) in the promoter and regulates transcription of downstream target genes [49]. In the animal model of spinal cord injury, progesterone increased the expression of BNDF by up-regulating the expression of CREB, so as to alleviate spinal cord injury [50].However, the GLUT3 gene does not contain the typical CRE structure but contains three base sequences similar to the CRE structure, which are called CRE1, CRE2 and CRE3 [51]. The ability of these three sequences to bind CREB is weaker than that of the sequence with the typical CRE structure. When CRE1 is missing, there is no obvious effect on the expression of GLUT3, whereas mutation or deletion of CRE2 and CRE3 significantly inhibits the expression of GLUT3, thus confirming that GLUT3 expression is regulated by CREB [52]. Previous studies have shown that the expression level of CREB is significantly decreased in the brains of patients with AD [53]. Our results show that progesterone upregulates the expression of CREB, and this effect can be blocked by AG205, To further prove the relationship between CREB and GLUT3, in this part, we used a specific inhibitor 666-15 of CREB in vitro to illustrate the relationship between CREB and GLUT3.The results showed that the expression of GLUT3 decreased significantly after adding the specific inhibitor of CREB 666-15, which confirmed that CREB was a decisive transcription factor in the upstream of GLUT3. Our results suggesting that one of the mechanisms by which progesterone upregulates GLUT3 expression is mediated by the upregulation of CREB.

PPARγ belongs to the superfamily of intranuclear nonsteroidal hormone receptors and is a ligand-dependent transcription factor that plays a key role in various biological processes, such as glucose metabolism, lipogenesis, inflammation, immunity, tumour cell differentiation and apoptosis [54–58]. PPARγ is mainly distributed in fat cells, immune cells and some parts of the brain, such as the cerebral cortex, hippocampus, substantia nigra, striatum and midbrain [59]. The high expression of PPARγ in the brain is thought to be closely related to some pathophysiological processes of the central nervous system. Previous studies have shown that the protein expression level of PPARγ in the brain of AD patients is approximately 40% lower than that of normal people, suggesting that PPARγ plays a crucial role in the pathogenesis of AD [60]. PPARγ has the potential to become a therapeutic target for the treatment of neurodegenerative diseases, including AD [61,62]. More importantly, PPARγ plays a critical role in glucose metabolism. PPARγ improves insulin resistance, enhances the insulin signalling pathway and lowers blood glucose [63,64]. PPARγ can directly bind to the PPAR response elements on the GLUT4 promoter and promote the transcription level of GLUT4, thus promoting GLUT4 protein expression and increasing the glucose uptake ability of cells [65–67]. Here, progesterone significantly increased the protein expression level of PPARγ in a cell model of AD, and this effect was blocked by AG205. These observations suggest that one of the mechanisms by which progesterone upregulates GLUT4 expression is achieved by upregulating the expression of PPARγ.To further demonstrate the relationship between PPARγ and GLUT4, we used a specific inhibitor of PPARγ, GW9662, in cell experiments to illustrate the relationship between PPARγ and GLUT4.The results showed that GW9662 could significantly block the up-regulation of GLUT4 expression caused by progesterone.Therefore, one of the mechanisms by which progesterone up-regulates GLUT4 expression is to activate PPARγ expression.

5. Conclusions

Progesterone significantly improves the ability of neurons to uptake glucose in AD models. One of the mechanisms of this effect is that progesterone upregulates the expression of GLUT3 and GLUT4 by activating PGRMC1. Our study also confirmed that progesterone promotes the expression of GLUT3 by upregulating the expression of CREB, while progesterone promotes the expression of GLUT4 by upregulating the expression of PPARγ.

Author contributions

Y.H. was responsible for the conception of the research. H.W.was responsible for the design of the research, most of the experiments, experimental data analysis, and manuscript preparation.Z.W. and W.S.were responsible for a part of experiments. H.G. is responsible for a part of manuscript preparation and the proofreading of manuscripts. HH.W. was responsible for providing experimental sites and equipment. F.B. and P.J. were responsible for animal management.

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Declaration of competing InterestCOI

The authors declare no conflict of interest.

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