Research paper

Taurine promotes cognitive function in prenatally stressed juvenile rats *via* activating the Akt-CREB-PGC1α pathway

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ABSTRACT

Substantial evidence has shown that the oxidative damage to hippocampal neurons is associated with the cognitive impairment induced by adverse stimuli during gestation named prenatal stress (PS). Taurine, a conditionally essential amino acid, possesses multiple roles in the brain as a neuromodulator or antioxidant. In this study, to explore the roles of taurine in PS-induced learning and memory impairment, prenatal restraint stress was set up and Morris water maze (MWM) was employed for testing the cognitive function in the one-month-old rat offspring. The mitochondrial reactive oxygen species (ROS) level , mitochondrial membrane potential (MMP), ATP and cytochrome c oxidase (CcO) activity and apoptosis-related proteins in the hippocampus were detected. The activity of the Akt-cyclic AMP response element-binding protein (CREB)-peroxisome proliferator-activated receptor−γ coactivator-1α (PGC1α) pathway in the hippocampus was measured. The results showed that high dosage of taurine administration in the early postnatal period attenuated impairment of spatial learning and memory induced by PS. Meanwhile, taurine administration diminished the increase in mitochondrial ROS, and recovered the reduction of MMP, ATP level and the activities of CcO, superoxide dismutase 2 (SOD2) and catalase induced by PS in the hippocampus. In addition, taurine administration recovered PS-suppressed SOD2 expression level. Taurine administration blocked PS-induced decrease in the ratio of Bcl-2/Bax and increase in the ratio of cleaved caspase-3/full-length caspase-3. Notably, taurine inhibited PS-decreased phosphorylation of Akt (pAkt) and phosphorylation of CREB (pCREB), which consequently enhanced the mRNA and protein levels of PGC1α. Taken together, these results suggest that high dosage of taurine administration during the early postnatal period can significantly improve the cognitive function in prenatally stressed juvenile rats *via* activating the Akt-CREB-PGC1α pathway. Therefore, taurine has therapeutic potential for prenatal stressed offspring rats in future.

1. Introduction

Over half a century, substantial evidence has shown that prenatal stress (PS), adverse stimuli during gestation, could cause mental and emotional disorders in both juvenile and adult offspring [1–4]. Further studies have indicated that hippocampal damage is involved in mental and emotional disorders induced by PS [5–8], but the underlying mechanism is still unclear. Hence, up to now, there has been no effective measure to improve the ability of learning and memory in the prenatally stressed offspring. In previous studies, we found that prenatal stress resulted in loss of neurons, accumulation of glutamate in the hippocampus and excessive production of intracellular reactive oxygen species (ROS) of pyramidal neurons in the hippocampal CA3 area accompanied by spatial learning and memory damage in one-month-old juvenile rat offspring [5,6,9,10]. These results implied that PS caused oxidative damage to hippocampal neurons in offspring, which reminded us that reducing the ROS generation of the hippocampus would probably help to prevent neuronal damage, and consequently promoted the ability of learning and memory in prenatally stressed offspring.

As one of the major intracellular free β-amino acids in most mammalian tissues, taurine, has received considerable attention with regard to its versatile roles in the brain as a neuromodulator and antioxidant. Studies showed that taurine did not only protect neurons against glutamate-induced cytotoxicity [11], but also prevented arsenite-treated SH-SY5Y cells *via* maintaining mitochondrial membrane

potential (MMP) and reducing the intracellular ROS level. Excitingly, supplementation of taurine benefited neuronal proliferation and synaptogenesis *in vivo*, which suggested its effects on enhancing synaptic plasticity and improving learning and memory [12]. Furthermore, taurine treatments for 5 weeks significantly increased the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α) [13], which was a cold-inducible transcriptional coactivator of peroxisome proliferator-activated receptor-γ (PPAR-γ) [14]. A study reported that PGC1α played a central role in a regulatory network governing the transcriptional control of mitochondrial biogenesis and respiratory function [15]. Moreover, taurine reversed doxorubicin-induced decrease of the expression of superoxide dismutase-2 (SOD2), one of the ROS detoxification enzymes in mitochondria [16]. A recent study showed that DETC-MeSO (an N-methyl-D-aspartate (NMDA) receptor partial antagonist) combined with taurine, not DETC-MeSO alone, obviously reduced the infarct size and attenuated apoptosis in ischemic infarct *via* increasing the level of phosphorylated Akt (pAkt), a pro-survival marker [17]. In addition, anti-depressant-like effect of taurine was associated with the activation of Akt-CREB pathway in the hippocampus [18]. Another study showed that the CREB signaling pathway played a predominant role in regulating PGC1α expression [19]. More interestingly, PGC1α could increase the expression of SOD2 in muscle cells [20]. Based on above data, there are adequate reasons to believe that Akt-CREB-PGC1α pathway is involved in the protection of taurine.

Therefore, in the study presented here, we wonder whether taurine attenuated mitochondrial dysfunction and cognitive impairment induced by PS. The prenatal restraint stress in rats was employed as an animal model and the Morris water maze (MWM) was used for testing the cognitive function. To explore the mechanism of neuroprotection of taurine, the level of mitochondrial ROS, MMP and ATP, the activity of CcO, the mRNA and protein levels of SOD2 and the expression levels of apoptosis-related proteins were determined. Furthermore, we also investigated whether the Akt-CREB-PGC1α pathway was involved in the protection of taurine against prenatal stress.

2. Materials and methods

2.1. Animals and groups

Specific pathogen-free (SPF) Sprague-Dawley rats were purchased from Medical Experimental Animal Center, Xi'an Jiaotong University and maintained at constant temperature (25 ℃) and humidity (60%) on a 12 h light/dark cycle (light on 08:00−20:00 on each day), freely accessing to food and water throughout the experiment. All procedures employed in this study were according to the institutional guidelines of Animals Care and Use Committee of Xi'an Jiaotong University. Measurements were proceeded to minimize pain or discomfort in accordance with the National Institutes of Health Guide for Care and Use of Laboratory. All efforts were made to minimize the number of animals used in each experiment.

Nulliparous female rats weighing 250−270 g were housed with a sexually experienced male rat (280−350 g) for mating (3:1), and the vaginal smear was examined on the following morning. The day on which the vaginal smear was positive was defined as day 0 of gestation. Each pregnant rat was then housed individually. All pregnant rats were randomly assigned to two groups. Members of one group were not disturbed in the whole gestation, and their offspring constituted the control (CON) group in later experiments. Members of another group were exposed to the restraint stress during the late term of pregnancy (days 14−20 of gestation), and their offspring were randomly assigned to the prenatal stress group (PS) and the PS+TAU group. The PS+TAU group was composed by the offspring rats feed by 0.5% taurine solution from postnatal day 21 (P21) to postnatal day 30 (P30).

2.2. Processing of prenatal stress

The restraint stress device was a transparent cylinder (6.8 cm in diameter) and the length could be adjusted to accommodate the size of the animals. Air holes of the cylinder were for breathing. Restraint stress was performed 3 times per day (at approximately 8:00 AM; 12:00 PM; and 4:00 PM), for 45 min per session from embryonic day 14−20 [5,21]. After each restraint stress session, the animals were placed back into their home cage. To avoid rats to being habituated to the daily program, phases were randomly shifted within the below time periods (08:00−11:00, 11:00−14:00 and 16:00−19:00). After childbirth, each mother and her offspring rats were placed in the same plastic cage until weaning on the postnatal day 21. The litters containing 8−14 pups with a suitable number of males and females were chosen for the experiments. After weaning, male and female pups of every litter were separated and housed in two cages. On postnatal day 31, one or two female and male pups were taken from each litter for following experiments.

2.3. Oral taurine administration

The taurine powder (Sigma, purity > 99%) was purchased from Sigma. From postnatal day 21 to postnatal day 30, the offspring rats were treated with oral taurine *via* their drinking water to achieve a daily intake of 2000 mg/kg body weight per day as the high dose group (PS+TAU-H) [22,23]. Meanwhile, a lower daily intake dose of 200 mg/kg body weight per day was conducted as the low dose group (PS+TAU-L). Fresh solutions were prepared every day.

2.4. Spatial learning and memory test with the morris water maze (MWM)

The Morris Water Maze was conducted in a black circular pool (180 cm diameter, 50 cm high), filled with water (21−23 ℃), mounted with four different visual cues at four directions. The water surface was divided into 4 quadrants by the two vertically crossed lines. The 4 quadrants were defined as southeast (SE), northeast (NE), northwest (NW) and southwest (SW) quadrants, receptively. A platform 10 cm in diameter was placed 2 cm below the water surface 45 cm away from the center of the circle pool in the NE quadrant. The latency to escape onto the hidden platform was recorded by a computerized video imaging analysis system (Morris Water maze Tracking System 100, Chengdu Taimeng software Co. Ltd.). The whole test consist two portions, the training time and the probe time. The day before the first training, each rat was allowed to swim freely for 120 sec and then taken back to its home-cage. During the training time, all rats received three trials per day for five consecutive days, and the starting quadrant was varied randomly over the trials. For each trial, the time of swimming and climbing the platform was counted after the rat was put into the pool. And then the rats were allowed to stay on the platform for 30 sec. Any rat that failed to reach the platform within 120 sec was guided to find the platform and allowed to stay on it for 3 sec. On the 6th day, after the last training for 24 hr, the probe test was conducted without the hidden platform in the pool. Each rat was placed in the water at the farthest point from the former location of the platform and was allowed to swim for 120 sec. The number of platform-crossing times was recorded as the target cross number.

2.5. Measurement of mitochondrial superoxide in the hippocampus

To measure the mitochondrial ROS level *in vivo*, all rats of each group were anesthetized with ether and then were decapitated. The brain was removed rapidly and the hippocampus was dissected and put onto a box chilled with ice. The hippocampus was cut into slices of 300 μm thick and each slice was loaded with MitoSOX (5 μm, Invitrogen) for 30 min. The fluorescence of the oxidized dye in the

slice or the cell was detected with a laser confocal microscope (TCS-SP2). The excitation and emission wavelength was 498 and 522 nm, respectively. The ROS fluorescence values were analyzed using the Leica SP2 software.

2.6. Mitochondria isolation and measurement of the mitochondrial membrane potential (MMP)

To isolate relatively high-purity mitochondria, each rat from each group was anesthetized with ether and then was decapitated. The brain was removed rapidly and homogenized in an ice-cold glass homogenizer with mitochondrial isolation buffer (225 mM mannitol, 75 mM sucrose, 4 mM HEPES and 0.5 mM EGTA, pH 7.4). The mitochondria were kept on ice and the protein concentration was determined by using Commassie Blue Staining Method.

To determine the mitochondrial membrane potential, a fluorescent dye, rhodamine 123 was employed. In brief, 10 μm of rhodamine 123 was added to the mitochondrial solution. The fluorescence signals were recorded by a fluorometer (Bio-rad) at the excitation and emission wavelength of 490 nm and 535 nm, respectively.

2.7. Determination of ATP level in the hippocampus

The ATP level was determined by using the ATP Determination Kit (BioVision) according to the manufacturer's instruction. Briefly, all rats of each group were anesthetized with ether and then were decapitated. The brain was removed rapidly and the hippocampus was dissected and put onto a box chilled with ice. The hippocampal tissue was homogenized with the cold lysis buffer for 15 min. The mixture was centrifuged at 14,000×g for 15 min at 4 °C. Then the supernatant was collected and chemiluminescence was measured by using a Beckman Coulter DTX880 (Beckman) with an integration time of 10 sec.

2.8. Assessment of enzymatic activity of cytochrome c oxidase (CcO)

Cytochrome c oxidase activity was measured by using the cytochrome c oxidase kit (Sigma). All rats of each group were anesthetized with ether and then were decapitated. The brain was removed rapidly and the hippocampus was dissected and put onto a box chilled with ice. After the hippocampal tissue was rinsed with PBS buffer 3 times, the hippocampal tissue was homogenized at 1: 5 (wt/vol) in an ice-cold lysis buffer. A total volume of 100 μL mixtures of freshly hippocampal tissue lysis buffer and enzyme solution was added into 950 μL assay buffer. The reaction was initiated by adding 50 μL ferro-cytochrome c substrate solution. The fluorescence of the final mixture at 550 nm was recorded with a SmartSpec Plus spectrophotometer (Bio-rad).

2.9. SOD2 activity assay

According to manufacturer instructions, SOD2 enzymatic activity was assayed using a SOD Assay Kit (Beyotime Company) with a water-soluble tetrazolium salt (WST)-8 as a substrate. Briefly, by adding SOD1 inhibitor (potassium cyanide) to inactivate Cu/ZnSOD, SOD2 activity was measured by inhibition of the rate of WST-1 reduction. The absorption at 450 nm was measured using a Bio-rad Microplate Reader.

2.10. Catalase activity assay

The Catalase activity was determined by using a catalase activity kit (Beyotime). Briefly, the ice-cold homogenate from hippocampal tissue was mixed with chromogenic substrate and hydrogen peroxide. To monitor the (N-(4-antipyryl)-3-chloro-5-sulfonate-p-benzoquinone-monoimine) generation, the absorbance at 520 nm was recorded by using a spectrophotometer.

Table 1
Primer sequences.

Target genes	Primer sequence (5′–3′)		Amplicon size (bp)
PGC1α	Forward	ATGAATGCAGCGGTCTTAGC	171
	Reverse	TGGTCAGATACTTGAGAAGC	
SOD2	Forward:	GCTGGAGCCGCACATTAAC	109
	Reverse	CAGCGCCTCGTGGTACTTCT	
β-actin	Forward:	CACCCGCGAGTACAACCTTC	207
	Reverse:	CCCATACCCACCATCACACC	

Table 2
The bodyweight gain in ten days after weaning.

Group (n=10)	Body weight (g)		Bodyweight gain/P21 (%)
	P21	P30	
CON	female		
	36.1 ± 0.4	69.4 ± 0.8	92.24
	male		
	37.1 ± 0.3	72.8 ± 0.7	96.23
PS	female		
	31.2 ± 0.5	57.3 ± 0.8	83.65[*]
	male		
	32.3 ± 0.4	59.6 ± 0.9	84.52[*]
PS+TAU-L	female		
	30.9 ± 0.4	58.6 ± 0.6	89.64
	male		
	31.9 ± 0.4	60.7 ± 0.7	90.28
PS+TAU-H	female		
	30.9 ± 0.3	67.9 ± 0.6	119.74[#]
	male		
	31.8 ± 0.5	70.4 ± 0.6	121.38[#]

[*] $P < 0.01$ *vs* CON,
[#] $P < 0.0.01$ *vs* PS. n=10.

Table 3
The volume of daily fluid intake.

Group (n=10)		Fluid intake (mL)			
		P21	P24	P27	P30
CON	female	19.1 ± 0.9	19.8 ± 1.8	25.9 ± 0.9	30.5 ± 1.5
	male	20.4 ± 1.2	22.3 ± 1.6	27.8 ± 1.2	31.7 ± 0.9
PS	female	18.6 ± 1.5	20.1 ± 1.5	25.8 ± 1.1	30.5 ± 1.7
	male	19.8 ± 1.2	21.9 ± 1.5	26.8 ± 1.5	30.7 ± 1.6
PS+TAU-L	female	18.8 ± 1.8	19.4 ± 1.2	25.5 ± 1.7	30.7 ± 1.8
	male	19.3 ± 1.5	22.0 ± 1.4	27.7 ± 1.6	31.6 ± 1.1
PS+TAU-H	female	18.8 ± 1.2	19.5 ± 1.8	25.6 ± 1.8	30.8 ± 1.2
	male	19.2 ± 0.9	22.1 ± 1.7	27.8 ± 1.1	31.6 ± 1.3

2.11. Measurements of PGC1α mRNA and SOD2 mRNA by Real time PCR (RT- PCR)

Total RNA was isolated from the hippocampus by using TRIzol reagent (Invitrogen) following the manufacturer's protocol. Reverse transcription was performed using Revert Aid™ First Strand cDNA Synthesis Kit (K1621, Formentas) followed by polymerase chain reaction (PCR) (PTC-200, Bio-Rad) with specific primers (shown in Table 1). The thermal cycling programmer consisted of 3 min at 94 °C, followed by 35 cycles of 30 sec at 94 °C, 30 sec at 55 °C (for PGC1α, Bcl-2 and β-actin) or 60 °C (for SOD2) and 30 sec at 72 °C, and then 10 min at 72 °C and cooled down to 4 °C. PCR products were analyzed by electrophoresis ethidium bromide-stained 2.0% agarose gel and the bands corresponding to PGC1α, SOD2, and β-actin were quantified by densitometry. The lanes were calculated and analyzed by the Quantity One software (Bio-Rad).
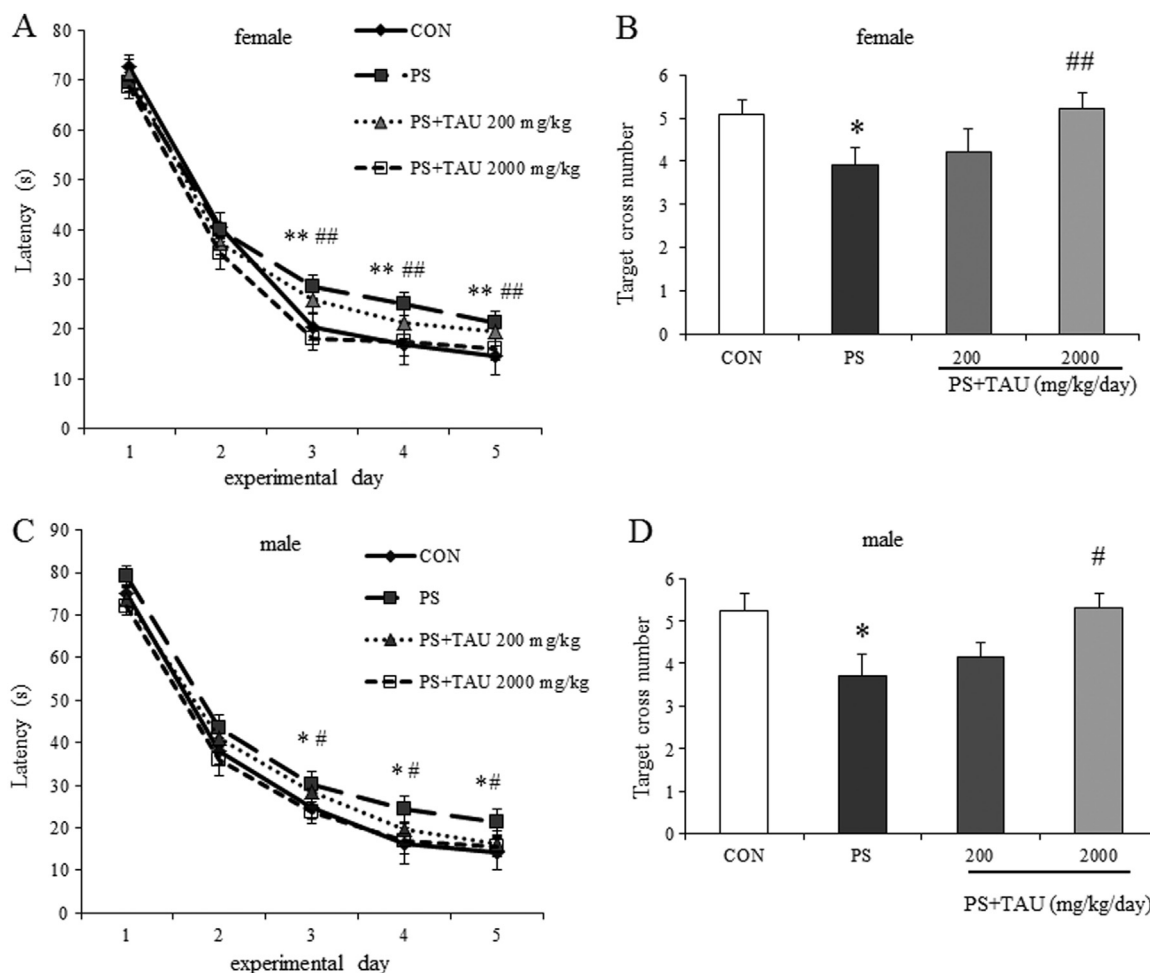
Fig. 1. Taurine counteracted the cognitive impairment induced by PS. After administration of taurine, the offspring rats were subjected to Morris water maze for testing the ability of spatial learning and memory. In the female offspring, PS increased the escape latency (A) on the 3rd, 4th and 5th experimental days and decreased the target cross number (B) in Morris water maze. Administration of high dosage of taurine significantly attenuated the increase in escape latency on day 3, 4 and 5 (A) and the decrease of the target cross number (B) induced by PS. (C) & (D) showed the similar results in the male offspring in Morris water maze. $^*P < 0.05$, $^{**}P < 0.01$ vs CON; $^#P < 0.05$, $^{##}P < 0.01 vs$ PS, n=10.

2.12. Protein extraction and western blot analysis

All rats of each group were anesthetized with ether and then were decapitated. The brain was removed rapidly and the hippocampus was dissected and put onto a box chilled with ice. After the hippocampal tissue was rinsed with PBS buffer 3 times, the hippocampal tissue was homogenized at 1: 5 (wt/vol) in an ice-cold lysis buffer with protease inhibitor cocktail and phosphatase inhibitor cocktail.

The samples were subjected to SDS-PAGE and were transferred to Polyvinylidene Fluoride (PVDF) membranes. The blots were probed with the following primary antibodies: polyclonal mouse anti β-actin (Sigma), rabbit anti-Akt (Cell Signaling), mouse anti-pAkt (Cell Signaling), rabbit anti-CREB (Cell Signaling), mouse anti-pCREB (Cell Signaling), rabbit anti-PGC1α (Cell Signaling) and rabbit anti-SOD2 (Cell Signaling), monoclonal mouse anti-Bax (Cell Signaling), monoclonal mouse anti-Bcl-2 (Cell Signaling), polyclonal rabbit anti-caspase-3 (Santa Cruz), followed by incubation with species-matched horseradish peroxidase-conjugated secondary antibodies. The blots were developed with a chemiluminescence substrate solution (Pierce) and exposed to X-ray film. The optical density of immunoreactive bands was quantified using the Quantity One software (Bio-Rad).

3. Statistics analysis

All values were represented as mean ± SEM, and data analyses were performed using the software of SPSS 11.0. One-way ANOVA was used

to examine differences among the groups. A difference was considered significant at $P < 0.05$ level.

4. Results

4.1. Taurine promoted physical development without affecting on the amount of daily fluid intake

To investigate the effect of taurine on physical development, the body weight of each rat was recorded on postnatal day 21 and postnatal day 30. Within the 10 days, the bodyweight gain represented physical development after weaning. In the CON group, the bodyweight gain increased by 92.24% in female and 96.23% in male. In the PS group, the bodyweight gain increased by 83.65% in female and 84.52% in male. In the PS+TAU-H group, the bodyweight gain increased by was 119.74% in female and 121.38% in male. Both in female and male rats, the bodyweight gain in the PS group was significantly less than that in the CON group ($P < 0.01$). Intake of high dosage of taurine completely reversed the decline of bodyweight gain induced by PS ($P < 0.01$), while low dosage of taurine administration did not significantly increase the bodyweight gain (shown in Table 2).

During the period of taurine administration, the amount of daily fluid intake of each rat was recorded, and the volume of daily fluid intake of postnatal day 21, 24, 27, 30 was listed out in Table 3. The data showed that, both in two genders, there was no significant difference between any two groups of the four groups (CON, PS, PS+TAU-L and
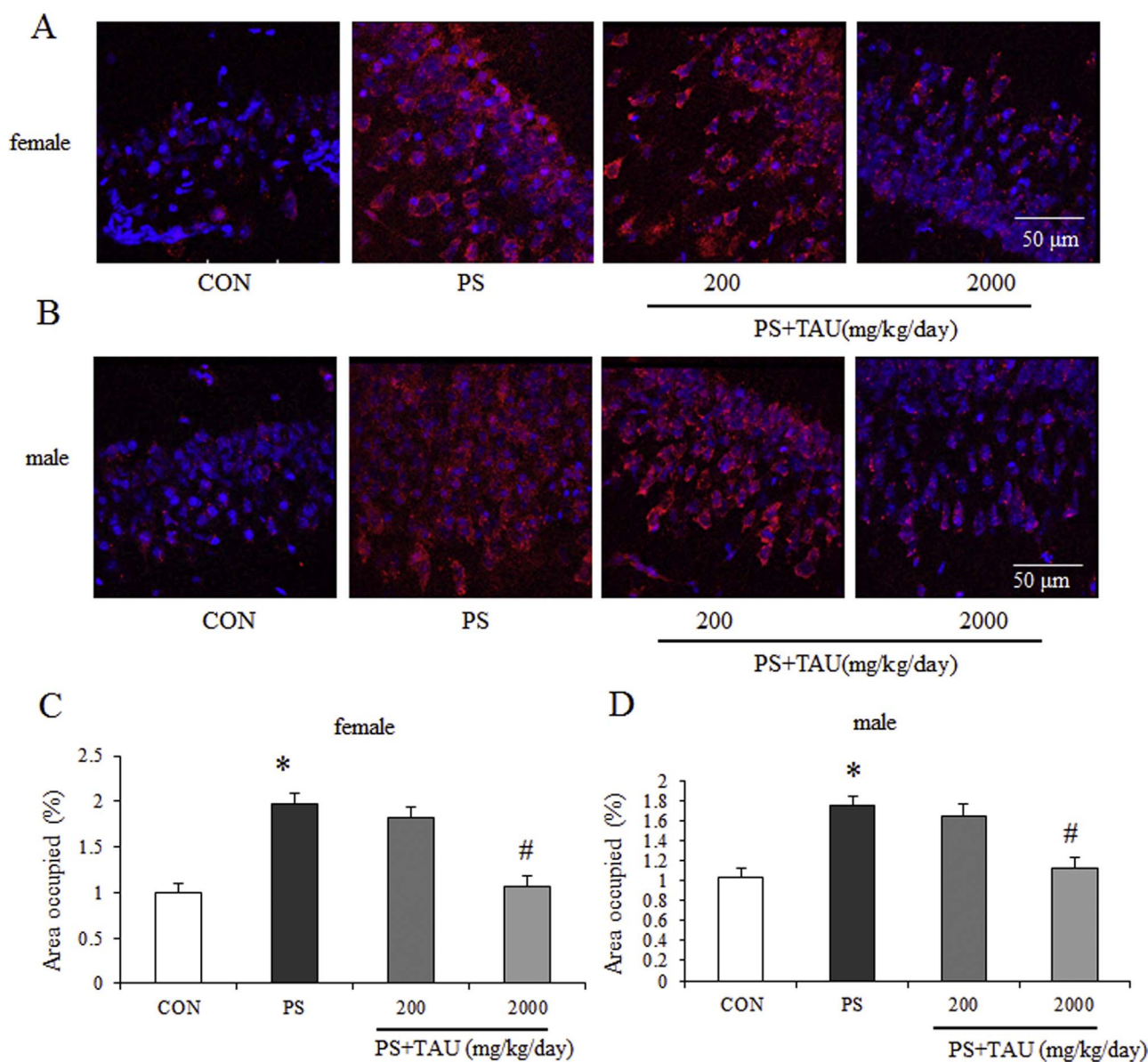
Fig. 2. Taurine reduced mitochondrial ROS generation in the hippocampus induced by PS. Fluorescent Photographs double-stained by MitoSOX (red) and DAPI (blue) in the hippocampus of both the female (A) male (B) offspring, scale bar =50 μm; The quantification analysis showed high dose administration of taurine reduced PS-increased mitochondrial ROS generation in both female (C) and male (D) offspring. $^{*}P < 0.01$ *vs* CON; $^{#}P < 0.01$ *vs* PS, n=6.

PS+TAU-H groups).

### 4.2. Taurine attenuated the cognitive function of the prenatally stressed juvenile offspring rats

To address the protection of taurine on spatial learning and memory ability, 1-month-old rat offspring were subjected to the Morris water maze. The escape latency of female rats in the PS group was significantly much longer than that in the CON group on the 3rd, 4th and 5th experiment day respectively ($P < 0.01$) (Fig. 1A). The probe test results showed that the target cross number of the rats from the PS group was decreased than that of the rats from the CON group ($P < 0.05$) (Fig. 1B). Administration of high dosage of taurine almost entirely counteracted the effect of PS on the escape latency and the target cross number of female offspring ($P < 0.01$). On the other hand, low dosage of taurine treatment was not displayed significant difference than that of the PS group (shown in Fig. 1A & B).

Similarly, as shown in the Fig. 1C, the escape latency of male rats in the PS group was significantly longer than that in the CON group on the

3rd, 4th and 5th experiment day respectively ($P < 0.05$). The target cross number of the rats from the PS group was decreased than that of the rats from the CON group ($P < 0.05$) (Fig. 1D). High dosage of taurine treatment significantly attenuated PS-induced longer escape latency and more target cross numbers ($P < 0.05$), while low dosage of taurine treatment did not show obviously difference (shown in Fig. 1C & D).

4.3. Taurine significantly decreased the accumulation of mitochondrial superoxide induced by PS

The level of mitochondrial superoxide is not only a sign of mitochondrial dysfunction, but also a marker of oxidative damage in cells. As illustrated in Fig. 2A, in female rats, compared with the CON group, higher level of mitochondrial ROS was detected in the hippocampal pyramidal neurons in the CA3 area of the PS group ($P < 0.01$). Administration of high dosage of taurine significantly decreased the generation of the mitochondrial ROS ($P < 0.01$) in prenatally stressed offspring rats, while low dosage of taurine treatment did not show
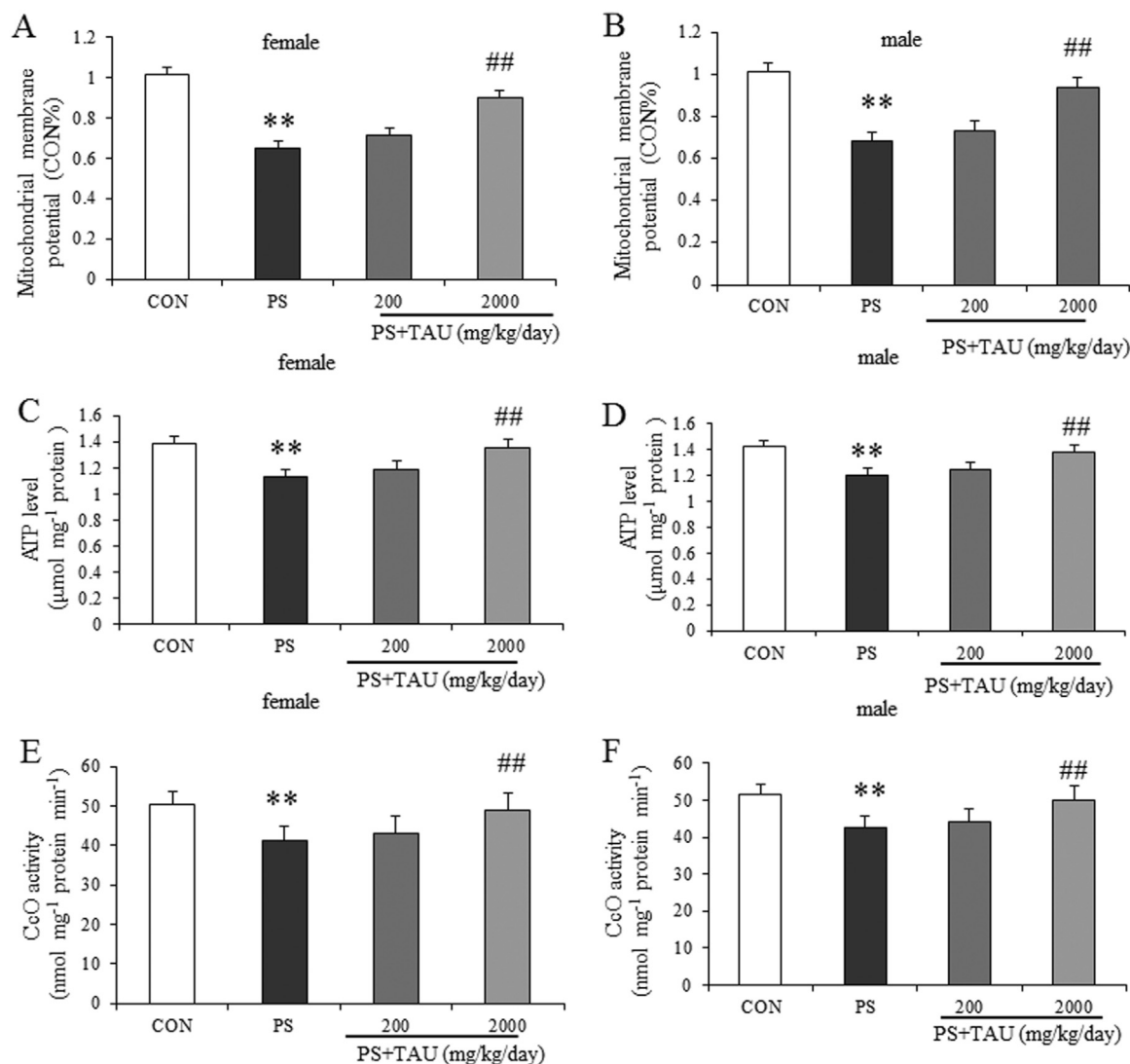
Fig. 3. Taurine recovered mitochondrial membrane potential (MMP), ATP level and CcO activity in the hippocampus. In the female offspring, administration of high dosage of taurine significantly increased PS-induced MMP (A), ATP level (C) and CcO activity (E). In the male offspring, intake of high dosage of taurine significantly increased PS-induced MMP (B), ATP level (D) and CcO activity (F). $^{*}P < 0.01$ *vs* CON; $^{#}P < 0.01$ *vs* PS, n=6.

statistical significance (Fig. 2C). Similarly, PS elevated mitochondrial ROS generation in the hippocampal CA3 region of the male rats, which was attenuated by high dosage of taurine treatment ($P < 0.01$) but not the low dosage of taurine treatment ($P > 0.05$) (Fig. 2B & D).

The MMP is an important indicator to evaluate the function of mitochondria in health or injury cells. As shown in Fig. 3A & B, in both female and male rats, the MMP of hippocampus from the PS group was significantly less than that from the CON group ($P < 0.01$). Intake of high dosage of taurine significantly reversed the decline of MMP induced by PS ($P < 0.01$), while the low dosage did not show statistical significance ($P > 0.05$).

4.4. Taurine recovered the level of ATP and maintained the enzymatic activity of cytochrome c oxidase (CcO)

To further evaluate the function of the mitochondria, the level of the ATP and the enzymatic activity of cytochrome c oxidase (CcO) were detected. As shown in Fig. 3C & D, in both female and male rats, the level of ATP of hippocampal pyramidal neurons from the PS group was significantly less than that from the CON group ($P < 0.01$). Intake of high dosage of taurine significantly reversed the decline in the level of ATP induced by PS ($P < 0.01$), while the low dosage did not show

obviously change ($P > 0.05$).

In addition, as shown in Fig. 3E & F, the enzymatic activity of CcO of hippocampal pyramidal neurons from the PS group was significantly less than that from the CON group ($P < 0.01$). Intake of high dosage of taurine significantly attenuated the effect of PS on the enzymatic activity of CcO ($P < 0.01$), while the low dosage did not display significant difference. Notably, there is no significant difference between the female and male rats (data not shown).

4.5. Taurine preserved the enzymatic activities of SOD2 and catalase

To explore how taurine reduced the level of ROS, the enzymatic activities of SOD2 and catalase in hippocampus were tested. In the present study, in both female (Fig. 4A) and male (Fig. 4B) offspring rats, the results showed that the enzymatic activity of SOD2 in the hippocampus was significantly decreased in the PS group compared with that in the CON group ($P < 0.01$). Intake of high dosage of taurine markedly enhanced the activity of SOD2 ($P < 0.01$), while the low dosage of taurine administration did not show this effect.

Similarly, in both female (Fig. 4C) and male (Fig. 4D) offspring rats, the results showed that the enzymatic activity of catalase in the hippocampus was significantly decreased in the PS group compared
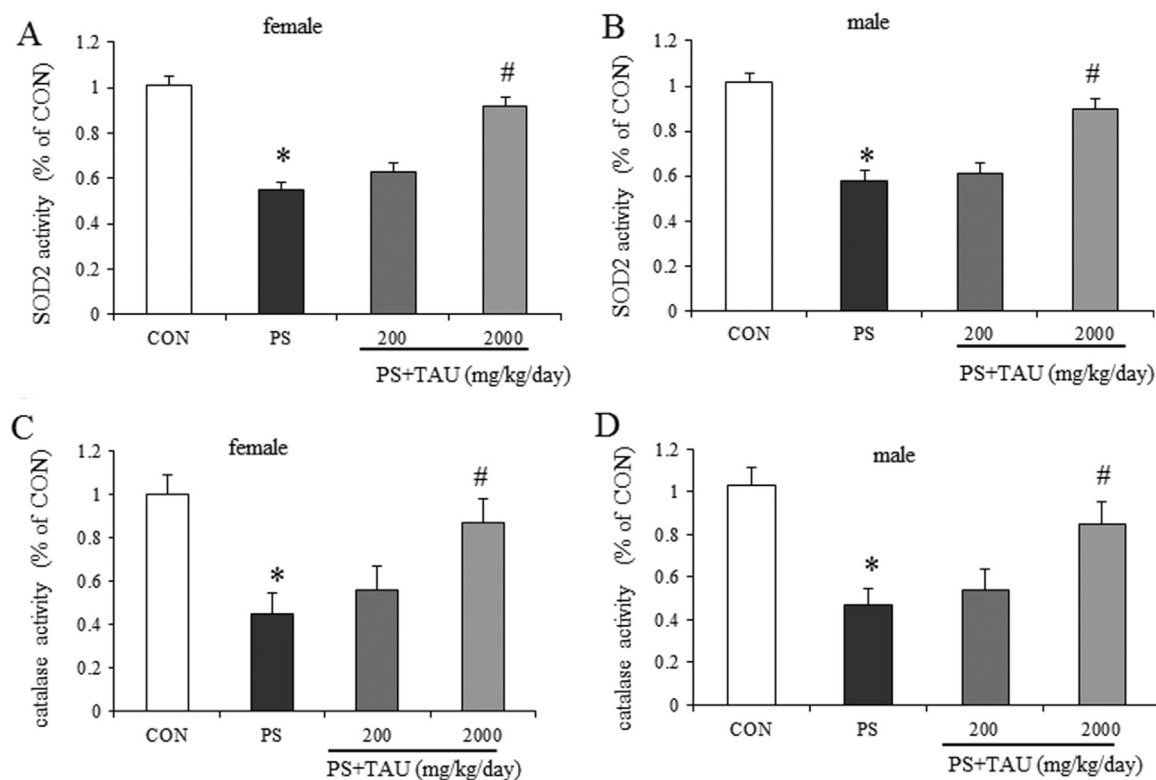
Fig. 4. Taurine preserved the enzymatic activities of SOD2 and catalase in the hippocampus. In female (A, C) or male offspring rats (B, D), PS significantly reduced the SOD activity and the catalase activity, and administration of high dosage of taurine significantly preserved the enzymatic activities of SOD2 and catalase. $^{*}P < 0.01$ *vs* CON; $^{\#}P < 0.01$ *vs* PS, n=6.

with that in the CON group ($P < 0.01$). Intake of high dosage of taurine markedly enhanced the activity of catalase ($P < 0.01$), while the low dosage of taurine administration did not show this effect. There was no significant difference between the female and male rats (data not shown).

4.6. Taurine increased the mRNA and protein level of SOD2 in the hippocampus

As a member of the iron/manganese superoxide dismutase family, SOD2 clears mitochondrial reactive oxygen species (ROS). Here, we detected the mRNA and protein levels of SOD2. As shown in Fig. 5, in both female and male offspring rats, the mRNA and protein levels of SOD2 in the hippocampus of the PS group were significantly less than that of the CON group ($P < 0.01$). Refreshingly, administration of high dosage of taurine recovered the decline in the mRNA and protein levels of SOD2 induced by PS ($P < 0.01$), and administration of the low dosage of taurine did not show statistical significance. Notably, there was no significant difference between the female and male rats (data not shown).

4.7. Taurine attenuated apoptosis induced by PS in the hippocampus

To observe the hippocampal damage induced by PS, the expression levels of cleaved caspase-3, full-length caspase-3, Bcl-2 and Bax were detected in the hippocampus by employing Western blot. As shown in Fig. 6, in both female and male offspring rats, the expression of Bcl-2 was decreased and the expression of Bax was increased in the PS group compared with that in the CON group, respectively ($P < 0.01$). The ratio of Bcl-2/Bax was significantly lower in the PS group than that in the CON group accordingly ($P < 0.01$). Meanwhile, the cleaved caspase-3 was significantly increased, accompanied by a remarkable decrease of full-length caspase-3, in the PS group compared with that in the CON group ($P < 0.01$), And thus the ratio of cleaved caspase-3/full length caspase-3 was significantly increased in the PS group compared with

that in the CON group ($P < 0.01$). Excitingly, intake of high dosage of taurine did not only largely recover the ratio of Bcl-2/Bax, but also significantly attenuated the effect of PS on the ratio of cleaved caspase-3/full length caspase-3 ($P < 0.01$). By contrast, intake of low dosage of taurine did not reverse the effects of PS on the ratios of Bcl-2/Bax and cleaved caspase-3/full length caspase-3. There was no significant difference between the female and male rats (data not shown).

4.8. Taurine activated the Akt-CREB-PGC1α pathway in the hippocampus

To explore the mechanism of the protection of taurine against hippocampal damage induced by PS, we detected the ratios of pAkt/Akt, pCREB/CREB and the mRNA and protein levels of the PGC1α. As shown in Fig. 7, in both female and male offspring rats, the ratios of pAkt/Akt and pCREB/CREB in the hippocampus of the PS group were significantly less than that of the CON group ($P < 0.01$). Intake of high dosage of taurine reversed the decrease of the ratios of pAkt/Akt and pCREB/CREB ($P < 0.01$). Similarly, as shown in Fig. 8, the mRNA and protein levels of PGC1α in the hippocampus of the PS group were significantly less than that of the CON group ($P < 0.01$). Administration of high dosage of taurine recovered the decline in the mRNA and protein levels of PGC1α induced by PS ($P < 0.01$), and administration of the low dosage of taurine did not show statistical significance. Notably, there was no significant difference between the female and male rats (data not shown).

5. Discussion

Since the 60's in last century, more and more evidences have shown that prenatal stress can lead to mental and behavioral disorder in offspring at childhood and adulthood. Our previous studies showed that the ability of spatial learning and memory was impaired in the prenatally stressed juvenile offspring rats at the age of 1 month compared with the offspring of the same age in the normal control
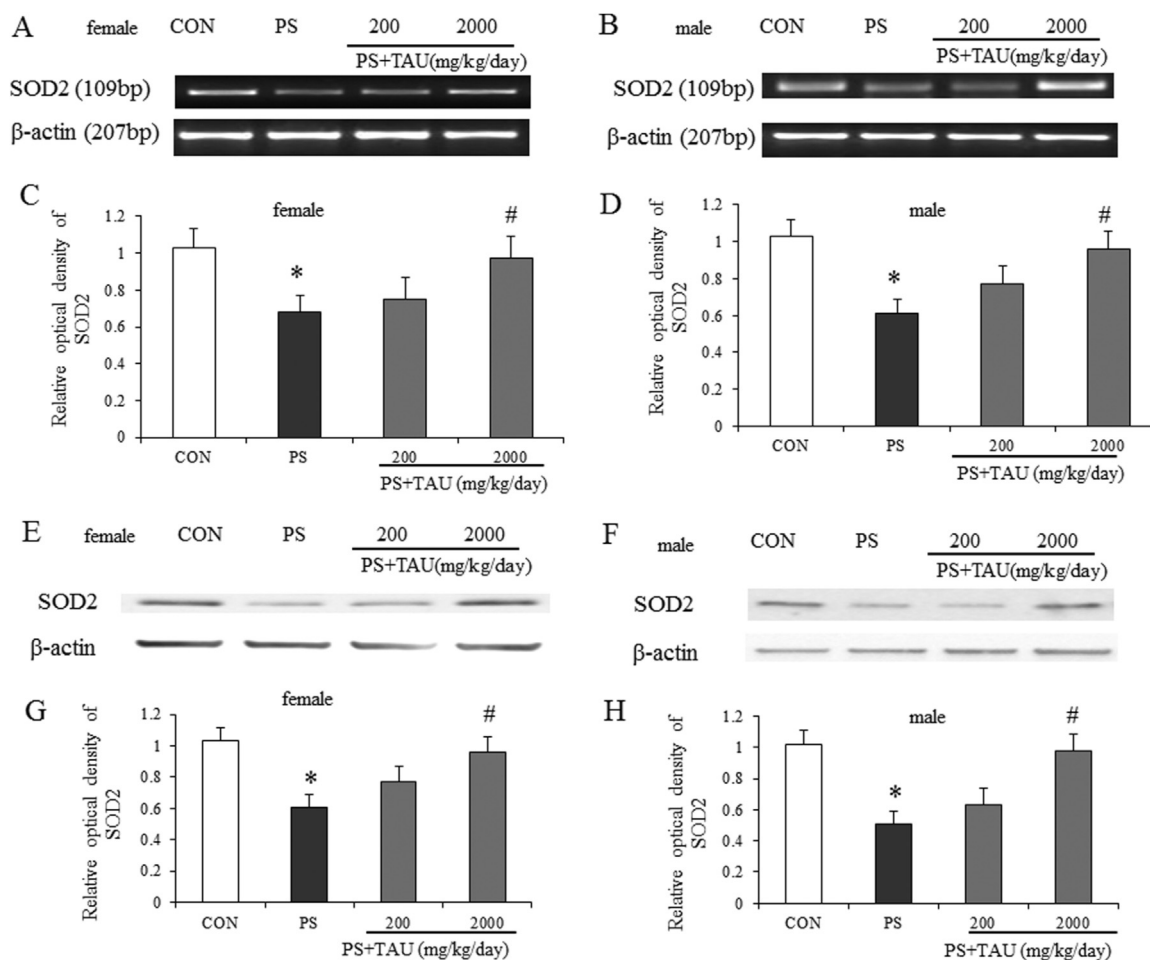
Fig. 5. Taurine attenuated effects of PS on the mRNA and protein levels of SOD2 in the hippocampus. Representative results for the mRNA levels of SOD2 and β-actin by RT-PCR in female (A) or male (B) offspring rats. The quantification analysis of the gel bands showed that, in both female (C) and male (D) offspring rats, PS significantly decreased the level of SOD2 mRNA, and administration of high dosage of taurine significantly enhanced the level of SOD2 mRNA. Representative immunoblot bands for the protein levels of SOD2 and β-actin in female (E) or male (F) offspring rats. The quantification analysis of the immunoblot bands showed that, in both female (G) and male (H) offspring rats, PS significantly decreased the expression of SOD2, and administration of high dosage of taurine significantly enhanced the level of SOD2. The mRNA and protein levels of SOD2 were normalized to β-actin. $^*P < 0.01$ vs CON; $^\#P < 0.01$ vs PS, n=6.

group. Although the underlying mechanism remains elusive, over recent decade, increasing scholars have begun to explore effective medications and measures to improve the cognitive impairment induced by PS. A number of studies showed that impaired learning and memory induced by prenatal stress were recovered by enriched environment treatment on early postnatal periods [24,25]. Exposure to chronic mild prenatal stress led to cognitive deficits in female offspring, which could be improved by treatment with antidepressant drug amitriptyline [26]. Maternal feeding with docosahexaenoic acid (DHA) significantly prevented prenatal stress-induced impairment of learning and memory in offspring [27]. Resveratrol reversed early and late gestational stress-induced cognitive deficits in offspring [28]. Taken together, although there has been not consensus on the results and views, it is possible to improve the impairment of learning and memory induced by PS via taking some measures or medications in maternal pregnancy or early postnatal period in offspring. However, the underlying mechanism remains unknown and the effects of medicating or measures were limited. Up to now, seeking a safe and effective medication or measure is still an urgent issue.

Taurine, one of the free sulphur-containing amino acids, is widely distributed among human body and is one of inhibitory neurotransmitters in the brain. Multiple researches suggest that taurine appears neuroprotective activity in prevention and treatment for neurological disease [29,30]. In the present study, the results showed that PS significantly reduced the body weight gain, from postnatal day 21 (P20)

to postnatal day 30 (P30) in the female offspring rats. And high dosage of taurine administration (daily intake of 2000 mg taurine), not low dosage of taurine (daily intake of 200 mg taurine) from P20 to P30 significantly blocked the decrease of body weight induced by PS. Similar results were to be found in the male offspring. Meanwhile, we detected the volume of daily fluid intake of each rat. The result showed that neither low dosage of taurine nor high dosage of taurine influenced the daily fluid intake both in female and male rats from the PS group. These results implied that taurine administration during the early postnatal period could accelerate physical development in the prenatally stressed offspring rats without obvious toxic side effects. Therefore, it was possible to study on whether taurine could influence brain development and cognition.

Coincidently with previous studies, in this study, in the Morris water maze, both female and male rats of the PS group showed longer escape latency during training period and less target cross numbers in probe test, compared with the rats of the CON group. As expected, high dosage of taurine from P21 to P30 significantly attenuated the effect of PS on the escape latency and the target cross number. These results indicated that high dosage of taurine administration in the early postnatal period reversed the impairment of spatial learning and memory induced by PS.

Our previous studies indicated that reactive oxygen species (ROS) accumulation in neurons and neuronal loss in hippocampal CA3 region were involved in the impairment of spatial learning and memory [9].
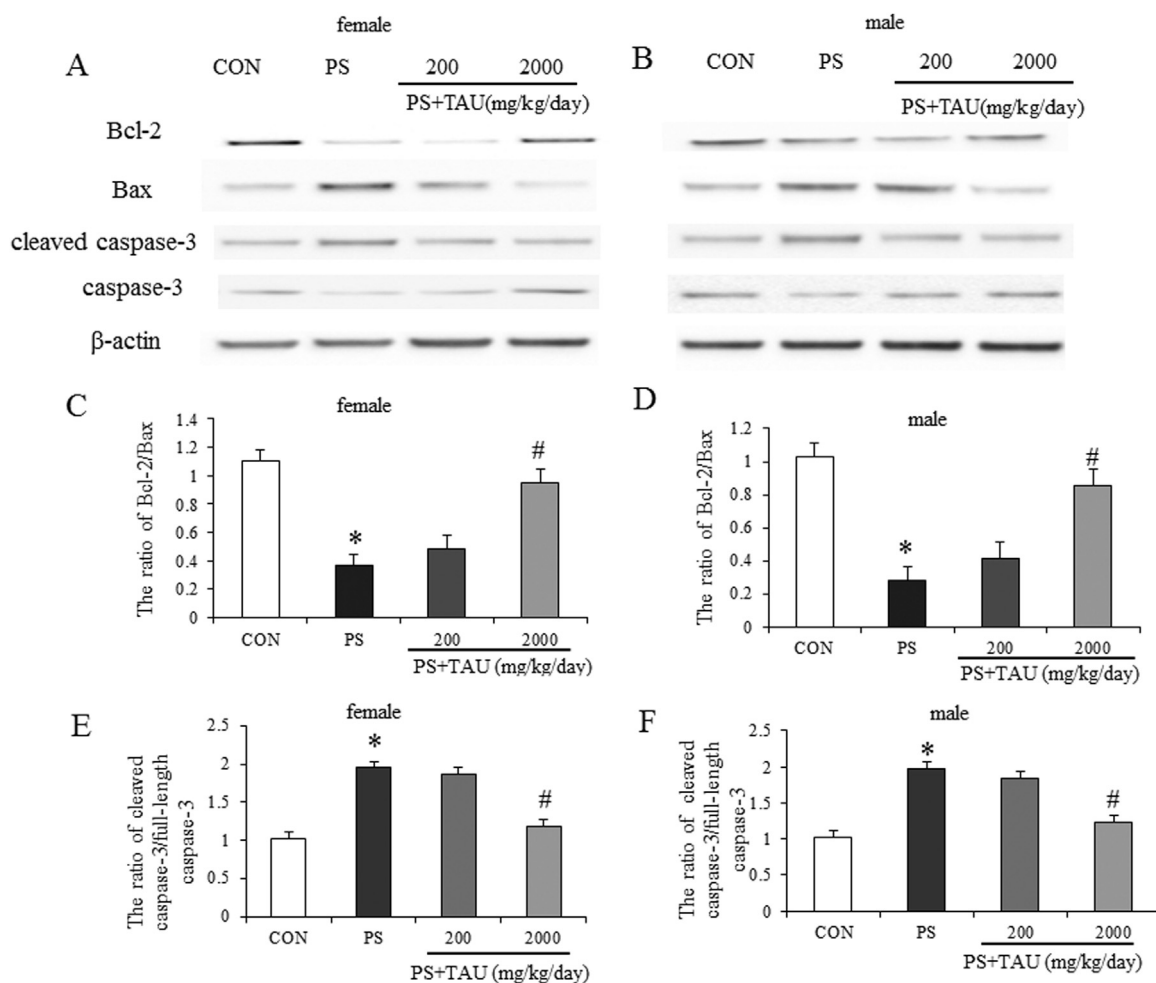
Fig. 6. Taurine reversed PS-induced decline of the ratio of Bcl-2/Bax and increase of ratio of cleaved caspase-3/full-length caspase-3. Representative immunoblot bands for Bcl-2, Bax, full-length caspase-3 and cleaved caspase-3 and β-actin in the hippocampus in female (A) or male (B) offspring rats. The quantification analysis of the immunoblot bands showed that, in both female (C, E) and male (D, F) offspring rats, PS decreased the ratio of Bcl-2/Bax and increased the ratio of cleaved caspase-3/full-length caspase-3, and administration of high dosage of taurine reversed the effects of PS on the aforementioned ratios. Protein expression levels were normalized to β-actin. $^*P < 0.01$ *vs* CON; $^#P < 0.01$ *vs* PS, n=6.

Mitochondria have been considered to play important roles in energy metabolism, free radical production and cell apoptosis. Mitochondrial dysfunction could result in disrupted electron flow in electron transferring chain leading to excessive generation of mitochondrial ROS [31]. In order to explore how taurine improved the spatial learning and memory in the prenatally stressed offspring, the level of mitochondrial ROS, MMP, ATP and CcO activity in the hippocampus were detected. The results showed that PS increased mitochondrial ROS accumulation, reduced the MMP and the ATP level, and decreased the activity of CcO. The accumulation of mitochondrial ROS is believed to be a result of mitochondrial dysfunctions, including the deficit of scavenging enzymes and decrease of enzymatic activity. Just as expected, the results showed that the expression of SOD2 and the enzymatic activities of SOD2 and catalase were significantly less in the PS group compared with the CON group. On the other hand, excessive mitochondrial ROS can lead to further deterioration of mitochondrial dysfunctions. Studies have shown that elevated generation of ROS led to the collapse of the mitochondrial membrane potential (MMP), outer mitochondrial membrane rupture and inactivation of mitochondrial metabolic enzymes [32]. Our previous study also provided evidence that PS increased 8-Hydroxy-20-deoxyguanosine (8-OH-dG) level in hippocampal mitochondrial, which is the major final product of oxidative DNA damage [5]. Thus, a vicious circle of mutually reinforcing would become between excessive mitochondrial ROS and mitochondrial dysfunction, which finally led to neuronal apoptosis and necrosis. In this study, we determined the apoptotic related proteins and found that PS decreased the ratio of Bcl-2/Bax and increased the ratio of cleaved caspase-3/full-length caspase-3. Could taurine administration interrupt this vicious circle? In this study, the results demonstrated that high dosage of taurine administration in the early postnatal period blocked the increase in mitochondrial ROS accumulation, the decline in ATP level, MMP and CcO activity induced by PS. In addition, high dosage of taurine administration attenuated the inhibition of PS on the expression of SOD2 and the enzymatic activities of SOD2 and catalase. Meanwhile, the results demonstrated that high dosage of taurine administration inhibited PS-induced decrease of the ratio of Bcl-2/Bax and increase of the ratio of cleaved caspase-3/full-length caspase-3. Combining with the behavioral results in the Morris water maze, we postulated that taurine administration could improve spatial learning and memory *via* preserving the expression of SOD2 and enzymatic activities of SOD2 and catalase, inhibiting mitochondrial ROS accumulation, maintaining MMP and ATP levels, enhancing the activity of CcO, which might interrupt the chain reaction of prenatal stress-induced hippocampal neuronal apoptosis.

The phosphorylated Akt (pAkt) in the cytosol may cause translocation of Akt, which subsequently induces activations of its downstream proteins [33,34]. CREB, known as one of the downstream effectors of Akt, is an important regulator in the expression of functional proteins associated with learning and memory [35–37]. Notably, the Akt- CREB pathway has been considered mediating the neuroprotection of taurine [17,18], and CREB signaling pathway has been considered as a predominant regulator of PGC1α expression [19]. Therefore, to
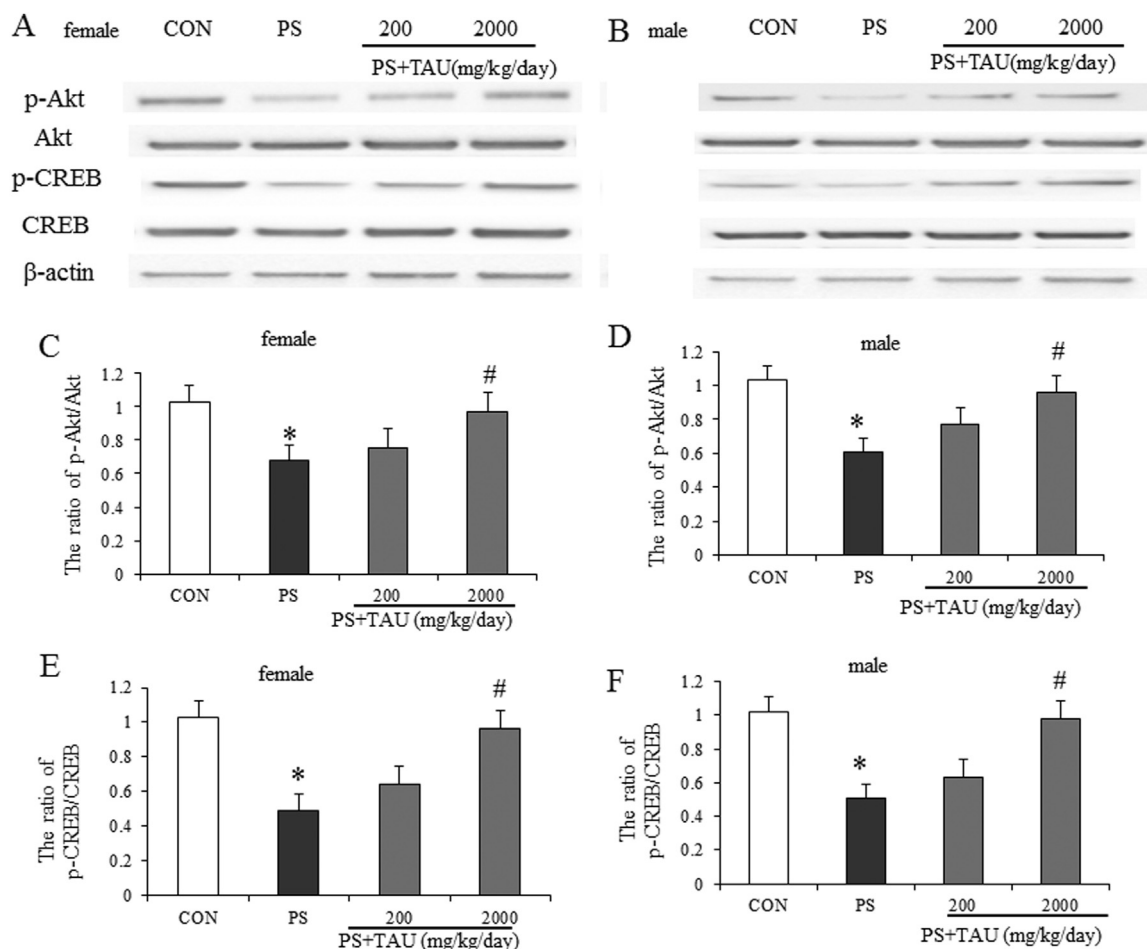
Fig. 7. Taurine inhibited PS-induced decline of ratios of pAkt/Akt, pCREB/CREB. Representative immunoblot bands for pAkt, Akt, pCREB, CREB, PGC1α, SOD2 and β-actin in the hippocampus in female (A) or male (B) offspring rats. The quantification analysis of the immunoblot bands showed that, in both female (C, E) and male (D, F) offspring rats, PS decreased the ratios of pAkt/Akt and pCREB/CREB, and administration of high dosage of taurine reversed the effects of PS on the aforementioned ratios. Protein expression levels were normalized to β-actin. $^*P < 0.01$ vs CON; $^#P < 0.01$ vs PS, n=6.

examine whether the Akt-CREB-PGC1α pathway was involved in the protection of taurine against learning and memory damage and hippocampal neuronal apoptosis induced by PS, we measured both phosphorylated and total protein levels of Akt, CREB and the expression of PGC1α in the hippocampus. The results indicated that PS significantly reduced the levels of phosphorylated Akt (pAkt) and phosphorylated CREB (pCREB), although the total protein levels of Akt and CREB was not obviously changed. It was refreshing that high dosage of taurine administration in the early postnatal period increased the levels of pAkt and pCREB in the prenatally stressed offspring. Meanwhile, the results showed that PS decreased the mRNA and protein levels of PGC1α, which did not exist with administration of high dosage of taurine. Data from other groups have indicated that reduction of PGC1α is associated with cell loss in Huntington's disease (HD), and overexpression of PGC1α protects against alpha-synuclein-mediated mitochondrial dysfunction and subsequent cell loss [38,39]. Furthermore, SOD2 is one of the ROS-detoxifying enzymes regulated by PGC1α [40,41], and the up-regulation of PGC1α induced by taurine might be involved in the increase of the expression of SOD2 as we found in this study.

In addition, in the present study, all of experimental subjects were from both genders, although there was no significant difference between two genders in behavioral and biochemical detections. Is there a gender difference in the effect of PS on offspring? There has been controversy about this point. Richardson et al. [42] have found that female fetuses were more vulnerable to prenatal stress than male fetuses. Data from another group indicated that maternal prenatal stress was more likely to cause anxiety in adult female offspring, whereas learning and memory problems in male offspring [43]. These incompletely consistent outcomes might be due to some factors, including the styles and intensity of prenatal/maternal stress, the species and age of the subjects, the adopted methods and so on. In addition, the 1-month-old rat offspring used for behavioral and biochemical detections were before sexual maturity, so that the results showed no difference between the female and male.

6. Conclusions

In conclusion, these above results suggest the decline in activation of the Akt-CREB-PGC1α pathway is involved in cognitive impairment induced by PS. Excessive mitochondrial ROS generation and mitochondrial dysfunction might participate in the process. Taurine administration during the early postnatal period can significantly improve the cognitive function via activation of the Akt-CREB-PGC1α pathway. Taurine administration also can reduce the mitochondrial ROS level, increase the levels of MMP, ATP and CcO activity as well. The present study provides a theoretical foundation that taurine can be as a supplement to the infant food during the early postnatal period to counteract cognitive impairment induced by PS. In future, there need more work to formulate the appropriate dose and effective measures for infants and babies food.
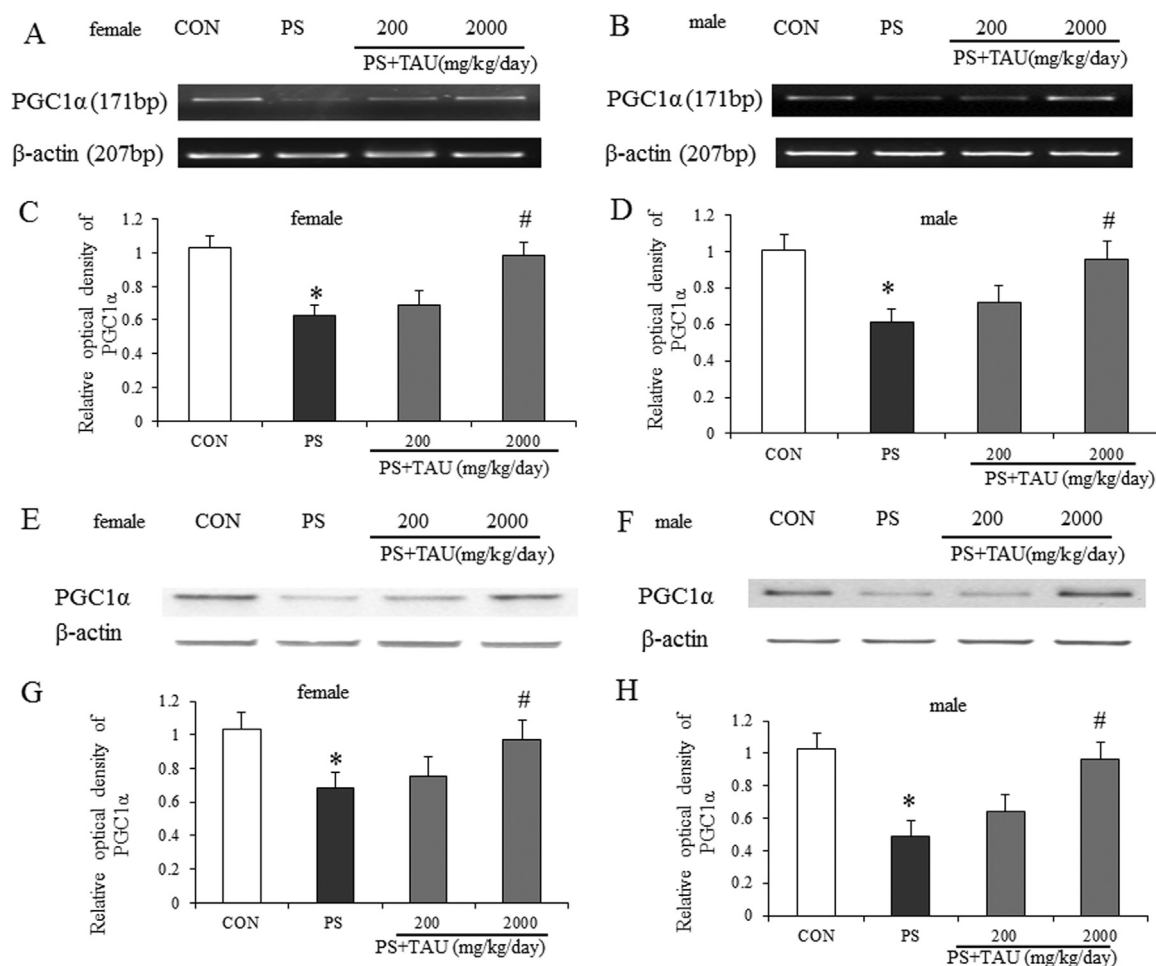
Fig. 8. Taurine inhibited PS-induced decline the mRNA and protein levels of PGC1α in the hippocampus. Representative results for the mRNA levels of PGC1α and β-actin by RT-PCR in female (A) or male (B) offspring rats. The quantification analysis of the gel bands showed that, in both female (C) and male (D) offspring rats, PS significantly decreased the level of PGC1α mRNA, and administration of high dosage of taurine significantly enhanced the level of PGC1α mRNA. Representative immunoblot bands for the protein levels of PGC1α and β-actin in female (E) or male (F) offspring rats. The quantification analysis of the immunoblot bands showed that, in both female (G) and male (H) offspring rats, PS significantly decreased the expression of PGC1α, and administration of high dosage of taurine significantly enhanced the level of PGC1α. The mRNA and protein levels of PGC1α were normalized to β-actin. $^*P$ < 0.01 vs CON; $^\#P$ < 0.01 vs PS, n=6.

Author contributions

Ning Jia and Qinru Sun conceived and designed the experiments. Ning Jia, Qinru Sun, Qian Su, Shaokang Dang and Guomin Chen performed the experiments. Ning Jia and Qinru Sun analyzed the data. Ning Jia and Qinru Sun contributed reagents/materials/analysis tools; Ning Jia wrote the draft, and Qinru Sun checked and revised. All authors approved to submit this version to this publication.

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