




Taurine inhibits neuron apoptosis in hippocampus of diabetic rats and high glucose exposed HT-22 cells via the NGF-Akt/Bad pathway

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Abstract

Type 2 Diabetes causes learning and memory deficits that might be mediated by hippocampus neuron apoptosis. Studies found that taurine might improve cognitive deficits under diabetic condition because of its ability to prevent hippocampus neuron apoptosis. However, the effect and mechanism is not clear. In this study, we explore the effect and mechanism of taurine on inhibiting hippocampus neuron apoptosis. Sixty male Sprague–Dawley rats were randomly divided into control, T2D, taurine treatment (giving 0.5%, 1%, and 2% taurine in drinking water) groups. Streptozotocin was used to establish the diabetes model. HT-22 cell (hippocampus neurons line) was used for in vitro experiments. Morris Water Maze test was used to check the learning and memory ability, TUNEL assay was used to measure apoptosis and nerve growth factor (NGF); Akt/Bad pathway relevant protein was detected by western blot. Taurine improved learning and memory ability and significantly decreased apoptosis of the hippocampus neurons in T2D rats. Moreover, taurine supplement also inhibited high glucose-induced apoptosis in HT-22 cell in vitro. Mechanistically, taurine increased the expression of NGF, phosphorylation of Trka, Akt, and Bad, as well as reduced cytochrome c release from mitochondria to cytosol. However, beneficial effects of taurine were blocked in the presence of anti-NGF antibody or Akt inhibitor. Taurine could inhibit hippocampus neuron apoptosis via NGF-Akt/Bad pathway. These results provide some clues that taurine might be efficient and feasible candidate for improvement of learning and memory ability in T2D rats.

Keywords Type 2 diabetes (T2D) · Hippocampal apoptosis · Taurine · Nerve growth factor (NGF) · Akt/Bad pathway

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Pingan Wu and Xiaoxia Shi have contributed equally.

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Introduction

Diabetes mellitus (DM) is complicated chronic metabolism disorder, which get more and more public attention. According to the report, there are about 400 million people with diabetes in the world, and the number of patients is still growing (Alam et al. 2014). However, about 90% diabetic patients are Type 2 diabetes (T2D) that is associated with a wide range of complications (Chatterjee et al. 2017). One

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common of complications is brain damage. The clinical studies have showed that people who are T2D patient performed poorly on cognitive tasks examining memory (Gao et al. 2015), and some severe T2D cases would progress into dementia. In experimental T2D model, it was also reported that there were learning and memory deficits in T2D rats (Palleria et al. 2017). These studies indicated that T2D induces cognitive impairment.

Public was known that the characteristic pathogenesis of learning and memory deficits is hippocampus damage. A research has shown that the learning and memory deficits would occur when hippocampal tissue was damaged (Schliebs and Arendt 2006). Additionally, dysfunction of hippocampus was recorded in memory impairment of normal aging and Alzheimer's disease patients (Moheet et al. 2015); similarly, hippocampal damage was also found in rats with memory deficits after ischemia (Wang et al. 2016a, b). These studies implied that deficits of learning and memory were related to hippocampus damage. Hippocampal tissue is composed of hippocampal neurons. Recently, a study found that neuron apoptosis in the hippocampus leads to learning and memory deficits in aging rats (Yu et al. 2017). Moreover, increasing evidence showed that excessive apoptosis in hippocampal neurons leads to memory impairment in diabetic rats (Wang et al. 2016a, b). Owing to these studies, it was thought that neuronal apoptosis in the hippocampus neuron may contribute towards the cognitive deficits. In other words, inhibiting apoptosis in the hippocampal neurons by some way is a novel strategy for ameliorating learning and memory deficits.

The endogenous amino acid taurine (2-aminoethanesulphonic acid), which exists widely in animal tissues (Murakami 2015), has a great diversity of function, like neuromodulator, involving in the formation of bile acids, antioxidant, anti-inflammation, and regulating the osmotic pressure (De la Puerta et al. 2010). It was found that taurine had the effect on improving several disorders because of its ability to prevent apoptosis in cardiomyocytes, liver, and kidney (Das et al. 2011; Das and Sil 2012; Rashid et al. 2013). And other reports suggested that taurine inhibited the apoptosis of hippocampus in aged rats (Zhang et al. 2016); moreover, our previous study showed taurine ameliorated arsenic-induced apoptosis in the hippocampus (Li et al. 2017a, b). However, there are fewer reports on studying protection and underlying mechanism of taurine against apoptosis of hippocampal neurons in T2D-induced memory impairment.

Therefore, in the current study, we tried to investigate the protective effect of taurine against apoptosis of hippocampal neurons in STZ-treated T2D rats and its anti-apoptotic mechanism. First, a model of taurine against STZ-treated T2D in rats was constructed and HT-22 cells (a hippocampal neurons line) were exposed to high glucose as in vitro model. Learning and memory ability of rats were checked

by Morris Water Maze test, and apoptosis of hippocampal neuron was measuring by TUNEL assay. Furthermore, nerve growth factor (NGF) and Akt/Bad pathway relevant protein were detected by western blot. Our study aimed at investigating the underlying effect and mechanism of taurine protecting hippocampal neuron against apoptosis in the brain of diabetic rats, which may provide some clues that taurine might be effective and feasible candidate for improvement of diabetic cognitive deficits.

Materials and methods

Establishment of animal model

Sixty SD (Sprague–Dawley) male rats (weight 180–200 g) were acquired from animal center at Dalian Medical University. Acclimatization was done in the animal room at 22 °C and 50% humidity and 12 h (h) light–dark cycle for 2 weeks. Animals were grouped into two major groups, i.e., control group ($n = 12$) and experimental group ($n = 48$). Control group was given normal diet and normal water for whole-study period, while experimental group was given high fat diet (25% sucrose + 15% oil + 1.5% cholesterol + 1% bile acid + 57.55% normal diet). After 4 week period, they were intravenous injected with streptozotocin (STZ) at 25 mg/Kg of body weight (Lin et al. 2010). Blood glucose was checked after 72 h, to confirm it above ≥ 16.7 mmol/L considered diabetes (Lin et al. 2013). Diabetic rats were kept on normal diet and provided normal water and then divided into four groups (Li et al. 2005; Rahmeier et al. 2016), i.e., T2D = Type 2 Diabetic rats given normal water, T1 = given 0.5% taurine solution, T2 = given 1% taurine solution, and T3 = given 2% taurine solution for a period of 8 weeks, while normal diet was provided to all groups. After 8 weeks, animals were sacrificed and organs preserved for future studies. A schematic illustration of the timeline and the experimental procedures is shown in Fig. 1.

Morris Water Maze test

Rats from every group were subjected to Morris Water Maze (MWM) test to check their spatial learning and memory ability, which included on 1-week training. Before test, all rats were provided with 2-day adaptive training. The hidden platform test was carried out for consecutive 4 days. While during training period, each rat was released randomly at one of the four different points and allowed for 90 s to swim freely. The video recorded the time taken by the rats to find the platform as acquisition latency. If rats could not find the platform within the set time, the latency time was recorded as 90 s. After that, each rat was allowed 10 s to rest, and then continued to the next point training. The average of

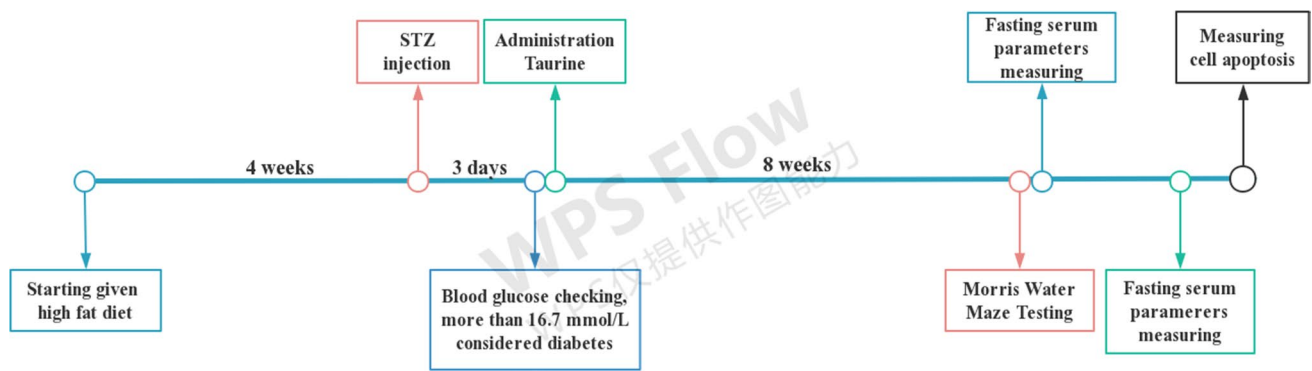


Fig. 1 Diagram illustrating experimental design

every day latency time during the hidden platform test was regarded as the ability of learning. In last day, the platform was removed, one rat was dropped into the water gently at an optional point, and the rest of rats were released at the same point. Every rat had to swim for 90 s. The percentage of time that rats spent in the target quadrant, times across the area that platform formerly was be, and times found original platform were recorded.

Measurement of fasting serum parameters

At the end of experiments, after overnight fasting, the animals were anesthetized. The blood was harvested. The fasting blood glucose and serum insulin of rats were measured by the automatic biochemical analyzer (GA-3 type, SANNUO, China) and Rat INS ELISA KIT (Shanghai Lengton, China) according to instructions from the manufacturers, respectively.

Cell culture

The HT-22 cell, the hippocampal neurons, was obtained from BeiNa Culture Collection (Kunshan city, Jiangsu Province, China) and grown in Dulbecco's modified Eagle's medium (containing 25 mM glucose, DMEM, HyClone, USA) with 10% Fetal Bovine Serum (FBS) and 1% penicillin–streptomycin (100 U/ml penicillin and 100 µg/ml streptomycin). The culture temperature was kept 37 °C all the time and incubator atmosphere was filled with 5% CO₂.

Cells grouping and treatment

The cells were seeded in 100 mm culture dish at density of 2×10^6 . The cells were divided into five groups, Control (Con) group: cells were treated with normal medium, high glucose (HG) group: cells were exposed to the normal medium with 150 mM glucose, taurine treatment groups: 10 mM, 20 mM, and 40 mM taurine were added into the

normal medium with 150 mM glucose as T1, T2, and T3 groups. Following 48 h treatment, the cell and medium were collected to next experiment. For inhibitor or antagonist studies, cells were treated with 10 ng/ml NGF-β, or pre-incubated with NGF-neutralizing antibody (10 ng/ml, Abcam, USA) and 5 µM perifosine (Beyotime, China) for 30 min prior to the administration of 150 mM high glucose and 40 mM taurine, respectively. Cells with different treatment were cultured for 48 h at 37 °C.

Cell viability assay

The cell viability was detected by MTT. The cells were plated in 96-microplate well at density of 8000 per well. Following 24 h of incubation, each group was given the corresponding treatment for 48 h, then medium of every group was changed by 0.5 mg/ml MTT (using the normal medium to dilute the 5 mg/ml MTT), after 4 h treatment, the medium was removed, and every well was added with 100 µl DMSO for 1 h. The value of OD was measured by ELISA reader at 490 nm.

LDH assay

Lactate dehydrogenase (LDH), acting as a cytoplasmic enzyme, is not usually released from cells, but leaks out when the cell membrane's integrity is disturbed. The LDH test is a colorimetric assay for the quantification of cell death. The medium in each group was collected after treatment, and then, the activity of LDH was tested by LDH assay kit (NanJing Jiancheng Biology Engineering Institute, NanJing, China) as per the manufacturer's instructions. The value of OD was measured at 450 nm by an ELISA reader.

TUNEL assay

The apoptosis of hippocampal neurons was assessed using TUNEL assay. Briefly, paraformaldehyde fixed brain tissue

sample of each group; sample was cut into 6 μm slices. The apoptosis in hippocampal neurons was checked using TUNEL assay as per the standard manufacturer's protocol, and the nuclei were stained with DAPI and neuron nuclei were stained with NeuN (Neuron Nuclei) (Millipore, USA). The images were taken by the fluorescence microscope. The green fluorescence-stained cells represented positive apoptotic cells. The number of apoptosis further quantified to have statistical analysis in vivo, the apoptosis index showed in vitro (the number of apoptotic cells was counted in every 100 cells as n , apoptosis index = $n/100 \times 100\%$).

Caspase-3 activity

Activity of caspase-3 in each group was checked using caspase-3 activity assay kit (Beyotime, China). In brief, the HT-22 cells were treated by trypsin, and then centrifuged at $600\times g$, at 4°C for 5 min, the cells were homogenized in lysis buffer and extracted the protein, and in vivo hippocampus tissue was extracted the protein. Then caspase-3 activity was checked at the protein concentration of 1–3 mg/ml as per the manufacturer's instructions.

The mitochondria isolation

Mitochondria as well as cytosol were isolated using kit for tissue and cell mitochondria isolation (Beyotime, China) which used to check the cytochrome C (cyt-C) protein. All steps were followed by the manufacturer's instruction. In a briefly, the hippocampus and HT-22 cell were collected to homogenate, then centrifuged at $600\times g$, for 10 min at 4°C , and transferred the supernatant to a new tube continue to centrifuged at $11,000\times g$, 4°C for 10 min, sediment was mitochondria, and supernatant was the cytosol. Then, we quantify the concentration of mitochondria and cytosol for western blot.

ELISA measurement of nerve growth factor (NGF) release

Culture supernatant of HT-22 cells was collected. According to the ELISA kit's instructions, culture supernatant of HT-22 cells was measured by ELISA to detect the concentration of released NGF (Fankew, China).

Western blot analysis

Following behavioral tests, rats were killed and the hippocampus tissues would be acquired. The tissue and HT-22 cell were homogenized in ice cold RIPA Tissue Protein Extraction Reagent (Beyotime, China), which mix the 1% proteinase inhibitor and phosphatase inhibitors. And incubated for 30 min at 4°C , the mixture would be blended

every 10 min. After that, the homogenate was centrifuged at $14,000\times g$ for 15 min at 4°C prior to collect the supernatants. Supernatants were stored at -80°C until using. Protein concentration was quantified using a BCA protein assay kit (Beyotime, China). 50 μg of the sample proteins were isolated by electrophoresis in 12% SDS-PAGE transferred to PVDF. The membrane was blocked by 5% skimmed milk in TBS-T, and then incubated the primary antibody, including NGF (1:500, Santa, USA), TrkA (1:1000, Abcam, USA), p-TrkA (1:1000, Wanleibio, China), Akt/p-Akt (1:1000, Proteintech, USA), Bad (1:1000, Proteintech, USA), p-Bad (1:1000, Beyotime, China), cyt-C (1:1000, Beyotime, China), β -actin (1:1000, Beyotime, China), and VDAC1 (1:1000, Beyotime, China) at 4°C overnight. Next day, the membranes were risen by TBS-T three times, 10 min each time, and then 2 h incubated with HRP-conjugated secondary antibody. The immuno-labeling were detected using the UVP Bio-spectrum Imaging System.

Statistical analysis

All data were analyzed using one-way ANOVA, follow by least significant difference (LSD) test using the Graph-Pad Prism software 5.0. The results were presented by mean \pm SEM, and the difference was regarded as statistically significant when p values of 0.05 or less.

Results

Taurine improved the blood glucose, insulin level, and learning and memory impairment in T2D rats

Insulin resistance is an important pathophysiological change in T2DM, which showed more insulin and higher blood sugar levels in diabetic animal model (Scheen et al. 1995). In this study, after rats being treated with taurine for 8 weeks, body weight, blood glucose, and insulin level were measured. Results showed low body weight, high blood glucose level, and high serum insulin level in diabetic rats. However, taurine administration improved these indicators in diabetic rats ($p < 0.05$) (data shown in Table 1), which suggested that taurine improved insulin resistant of diabetic rats.

The Morris Water Maze (MWM) test was used to check the effect of taurine on cognitive deficits in T2D rats. As shown in Fig. 2, during 4 days of training test, swimming speed of the rats was not significantly different among the groups ($p > 0.05$). On the other hand, from the second day of the training test, the rats in T2D group took more time to locate the platform compared to control ($p < 0.05$) and the distance of swimming significantly increased ($p < 0.05$). However, taurine treatment (T2, T3) significantly reduced the escape time and swimming distance of rats compared to

Table 1 The measurement of blood glucose and insulin of rats

Group	Body weight (g)	Blood glucose (mmol/L)	Insulin (μ U/ml)
Control	568.8 \pm 9.6	4.98 \pm 0.6	16.47 \pm 1.4
DM	349.6 \pm 7.7 ^a	24.5 \pm 0.3 ^a	29.91 \pm 1.0 ^a
0.5%Tau	373.8 \pm 5.8	23.3 \pm 0.8	28.64 \pm 7.3
1%Tau	384.7 \pm 6.9	22.05 \pm 0.4 ^b	26.65 \pm 1.3 ^b
2%Tau	407.2 \pm 9.1 ^b	20.5 \pm 0.1 ^{bc}	21.9 \pm 2.0 ^{bc}

Data are expressed as mean \pm SD, $n = 10$ per group

^aCompared with control group, $p < 0.05$

^bCompared with DM group, $p < 0.05$

^cCompared with 0.5%Tau group, $p < 0.05$

T2D group ($p < 0.05$). After 4-day training, the platform was removed. In this situation, the T2D rats took more time to cross the platform area for the first time compared to control rats ($p < 0.05$), while the number of crossings over platform was less than that in control ($p < 0.05$). On the contrary, taurine supplementation reduced the time to cross the platform area for the first time and increased the number of crossing platform in T2D rats. Especially, there was significant difference between T3 group and T2D group ($p < 0.05$). The percentage of time spent in target quadrant during the 90 s test was less in T2D rats than that in control group ($p < 0.05$), while the taurine groups showed more percentage of time spent in target quadrant than that in T2D group ($p < 0.05$).

To further determine the effect of taurine on learning and memory impairment in T2D rats, taurine content in the brain of rats was checked using a rat taurine enzyme-linked immunosorbent assay kit (mlbio, China). Results found taurine content in the brain was significantly increased after diabetic rats given more taurine in drinking water (Data in Supplemental Fig. 1), which revealed that taurine was taken in the brain of rats and the learning and memory impairment in T2D rats could be mediated by taurine supplement.

Taurine ameliorated apoptosis in hippocampal neurons in T2D rats and HT-22 cells exposed to HG

In this study, we checked the apoptosis of hippocampus in CA1 area, because clinical neuropathologic studies indicated the selective vulnerability of hippocampal CA1 pyramidal neurons playing an important role in cognitive deficits (Counts et al. 2014). After the hippocampal tissue in T2D rats was double stained by DAPI and NeuN, hippocampus neuron apoptosis was checked by TUNEL assay kit. The results indicated that the number of apoptotic cells in the hippocampal CA1 area increased significantly in T2D group as compared to control ($p < 0.05$) (Fig. 3a, b). Nevertheless, taurine treatment mitigated the increased neuron apoptosis in T2D rats ($p < 0.05$). Caspase-3 is

an effector of cell apoptosis, which its activation plays a vital role in the start of apoptosis (Choudhary et al. 2015). Hence, caspase-3 activity in the hippocampus of rats was tested by specific kit. As shown in Fig. 3c, T2D group showed significant higher activity of caspase-3 as compared to control group ($p < 0.05$). However, the activity of caspase-3 in taurine groups was lower significantly than that in T2D group ($p < 0.05$), and dose-dependent decrease (Fig. 3c), supporting the above apoptotic results.

To further demonstrate the anti-apoptotic effect of taurine in vitro, caspase-3 activity and apoptosis were also checked in the HG-exposed HT-22 cells with and without taurine. As given in Fig. 3d–f, apoptosis and caspase-3 activity in HG group was evidently higher than that in control group with significant difference ($p < 0.05$). However, these indicators in T2D group were significantly mitigated by taurine ($p < 0.05$), being accordant with the results in vivo.

Taurine up-regulated the expression level of NGF in hippocampus of T2D rats and HT-22 cells exposed to HG

Nerve growth factor (NGF) performs an important role in neuronal survival and is important in the anti-apoptotic functioning of neuroprotective agents (Li et al. 2017a, b). Tyrosine kinase receptor type A (TrkA) is specific receptor of NGF (Marlin and Li 2015). NGF triggers downstream signaling reaction by activating the receptor. To examine effect of taurine on NGF, expression levels of NGF, TrkA and phosphorylated TrkA (p-TrkA) were detected in the hippocampal tissue of rats. As shown in Fig. 4, the expression levels of NGF and p-TrkA in T2D group showed significantly lower expression than that in control ($p < 0.05$). However, the expression level of NGF and p-TrkA in taurine groups was significantly higher than that of T2D group ($p < 0.05$) and dose-dependent increase was seen (Fig. 4a, b). To further certify the beneficial effect of taurine on NGF in vitro, the expression of these proteins was also determined in HT-22 cells. HG caused a significant decreased expression of NGF, the release of NGF in the culture medium and phosphorylation of TrkA in HT-22 cells compared to control ($p < 0.05$). However, treatment of 20 mM and 40 mM taurine reversed the decreased expression level and release of NGF and phosphorylation of TrkA in the HG group ($p < 0.05$) (Fig. 4c–e). Moreover, effect of taurine in the HG-exposed HT-22 cells was blocked in the presence of an NGF-neutralizing antibody ab16161 (ab) ($p < 0.05$) (Fig. 4f), indicating that taurine up-regulated the expression level of NGF and activate TrkA.

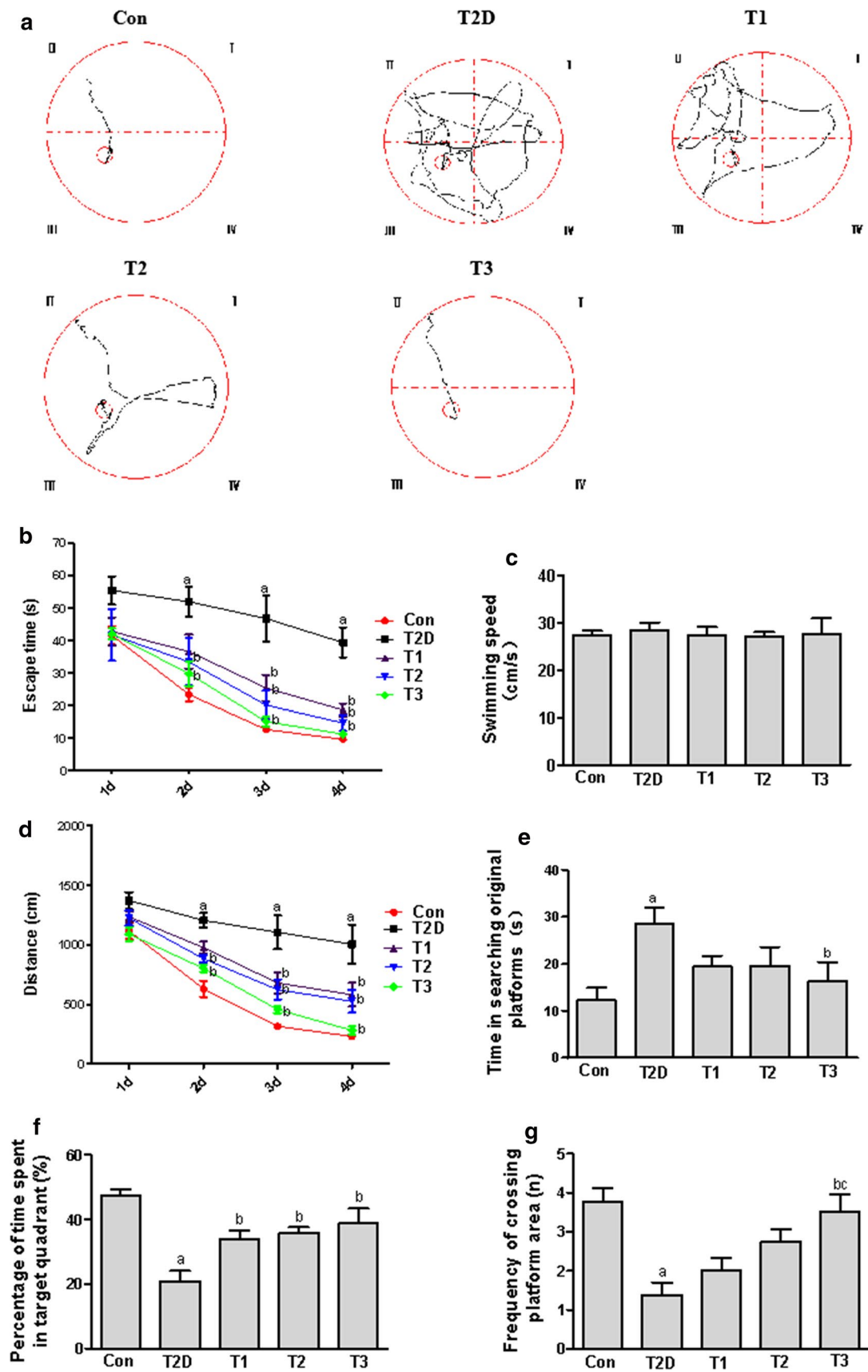


Fig. 2 The effect of taurine on learning and memory in T2D rats. **a** The swimming routes showed by a representative rat in each group during the 4-day training period. **b** Escape latency to find the platform during the navigation test. **c** Mean swimming speed in different group during the training test. **d** The total distance that each group rats swimming. **e** The time that rats crossed the original platform for the first time in the last day of test. **f** Percentage of time spent in target quadrant during 90-s test. **g** The number of crossing the platform area in the last day of test. Values are mean \pm SEM. ^a $p < 0.05$, compared to the Con group. ^b $p < 0.05$, compared to the T2D group. ^c $p < 0.05$, compared to the T1 group. ^d $p < 0.05$, compared to the T2 group

Taurine promoted the phosphorylation of Akt and Bad in hippocampus of T2D rats and HT-22 cells exposed to HG

Akt/Bad pathway was downstream molecules of NGF and its phosphorylation was associated with induction of apoptosis (Pierucci et al. 2001). To investigate effect of taurine on Akt/Bad pathway, the phosphorylation of Akt and Bad was measured in the hippocampal tissue of rats. As given in Fig. 5, Akt and Bad were not significantly different among the groups. But the contrary, the expression levels of p-Akt and p-Bad in T2D group were significantly low as compared to control ($p < 0.05$). However, expression of these two phosphorylated proteins in taurine groups was significantly as compared to T2D ($p < 0.05$) with a dose-dependent increase. Moreover, data from the HT-22 cells in vitro also showed the same results in vivo, indicating that taurine increases phosphorylation of Akt and Bad.

It was reported that Akt/Bad signaling pathway controlled cytochrome C (cyt-C) release from mitochondria (Abhishek et al. 2018). Therefore, the expression levels of cyt-C in cytosol and mitochondria were checked. The results showed that the expression level of cyt-C in mitochondria was lower in T2D group than that in control ($p < 0.05$), while the expression level of cyt-C in cytosol was higher in T2D group than in control group ($p < 0.05$). However, taurine treatment dose-dependently reversed the results by T2D ($p < 0.05$). Moreover, the beneficial effect of taurine was further confirmed by in vitro HT-22 cell assay, implying inactivation of mitochondria-dependent apoptotic pathway.

Taurine regulated Akt/Bad pathway and mitochondria-dependent apoptotic pathway in HG-exposed HT-22 cells via NGF

To investigate whether NGF was involved in regulating Akt/Bad signaling pathway and mitochondria-dependent apoptotic pathway by taurine, an intervention trial of ab as NGF-neutralizing antibody was performed in vitro (Jiang et al. 2015). As given in Fig. 6, the expression levels of p-Akt and p-Bad were significantly lower and release of cyt-C from mitochondria into cytosol was

higher significantly in HG-exposed HT-22 cells than those in control cells ($p < 0.05$). On the opposite, the decreased phosphorylation levels of Akt and Bad, and the increased release of cyt-C in the HG-treated HT-22 cells were reversed by taurine treatment ($p < 0.05$). However, the protective roles of taurine were blocked in the presence of ab ($p < 0.05$). Moreover, the effect of taurine on p-Bad expression and cyt-C release in the HG-treated HT-22 cells was also blocked by an Akt inhibitor perifosine (P) ($p < 0.05$), indicating that taurine regulated Akt/Bad pathway and mitochondria-dependent apoptotic pathway via NGF.

Taurine protected against the apoptosis of HG-exposed HT-22 cells via NGF/Akt/Bad pathway

To further confirm the association between anti-apoptosis of taurine and activation of NGF/Akt/Bad pathway, an intervention trial of ab and P was performed in vitro. The results were showed in Fig. 7. The HT-22 cell apoptosis in HG-exposed cells was significantly higher than that in control group ($p < 0.05$). However, the indicator in HG group was significantly mitigated by taurine ($p < 0.05$). Nonetheless, the effect of taurine was blocked in the presence of ab or P ($p < 0.05$). Moreover, data of caspase-3 activity in HT-22 cells were also accordant with the results in above, indicating that taurine protected against the cell apoptosis via NGF/Akt/Bad pathway.

Taurine prevented the apoptotic death of HG-treated HT-22 cells via NGF/Akt/Bad pathway

The effect of taurine on HG-induced viability of HT-22 cells was measured by MTT assay. As shown in Fig. 8a, the cells treated with HG showed lower cell viability compared to control group ($p < 0.05$). On the contrary, taurine significantly increased cell viability compared to HG group ($p < 0.05$). To determine if the decreased viability was due to HG-induced cell death, death of HT-22 cells was measured by LDH assay. The results showed that HG significantly increased the release of LDH into the medium of the treated HT-22 cells culture ($p < 0.05$) (Fig. 8b), while taurine significantly decreased the release of LDH compared to HG group ($p < 0.05$), revealing that the increased viability of HT-22 cells may be partly attributed to protection of taurine against HG-induced cell death.

To confirm whether the protection of taurine is associated with activation of NGF/Akt/Bad pathway, an intervention trial of ab and P was performed in HT-22 cells. The results showed that taurine significantly reversed the increased release of LDH in the HG-treated HT-22 cells ($p < 0.05$). However, the protection of taurine was blocked

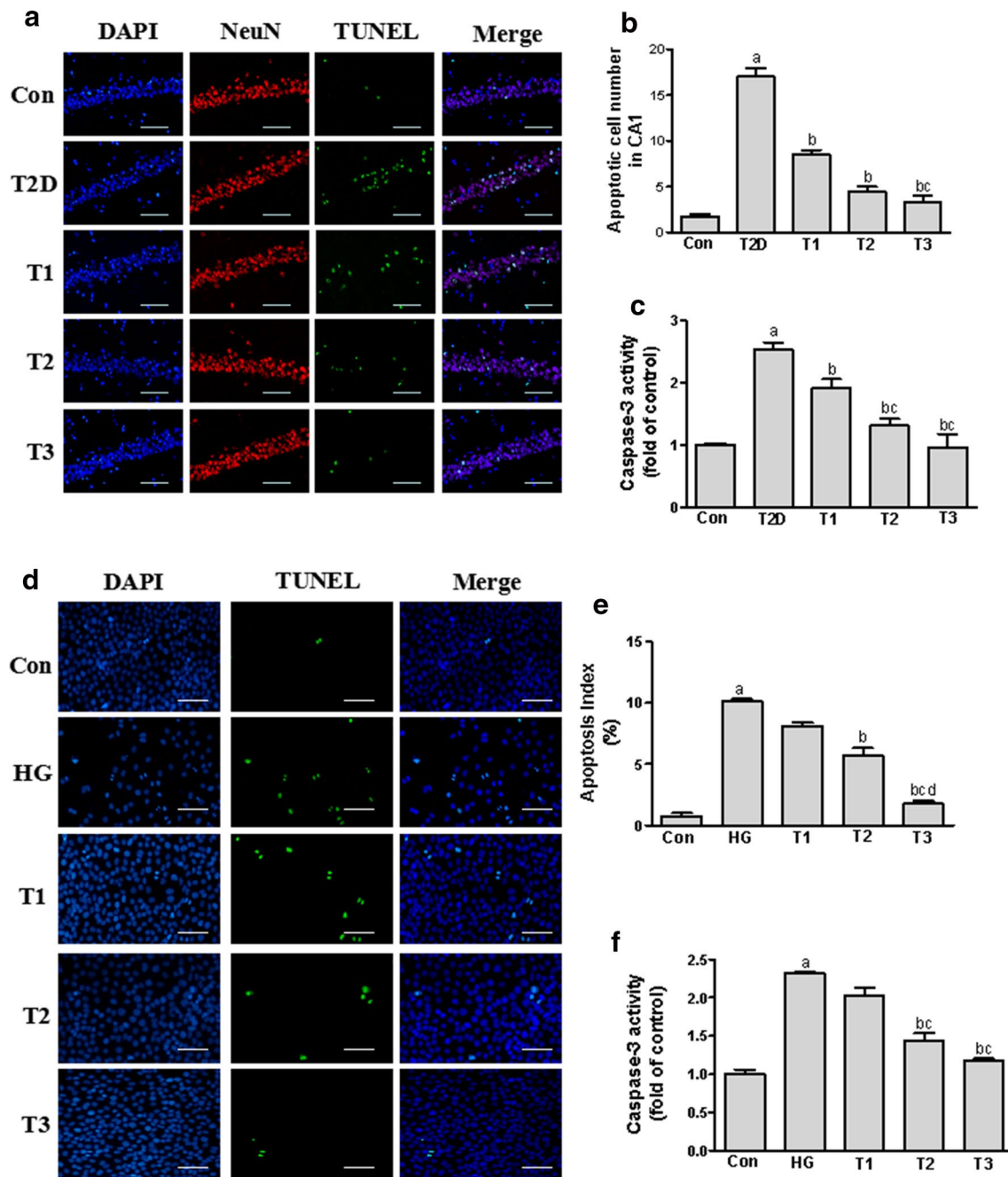


Fig. 3 The effect of taurine on apoptosis of hippocampal neurons in T2D rats and HG-treated HT-22 cells. **a** Representative images of TUNEL-stained cells were shown for the control group (Con), type 2 diabetes group (T2D), and taurine treatment groups (0.5%tau, 1%tau and 2%tau). **b** Quantified on data of the apoptotic cells. **c** Caspase-3 activity was checked by caspase-3 activity assay kit in each group. **d** Representative images of TUNEL-stained HT-22 cells in each group,

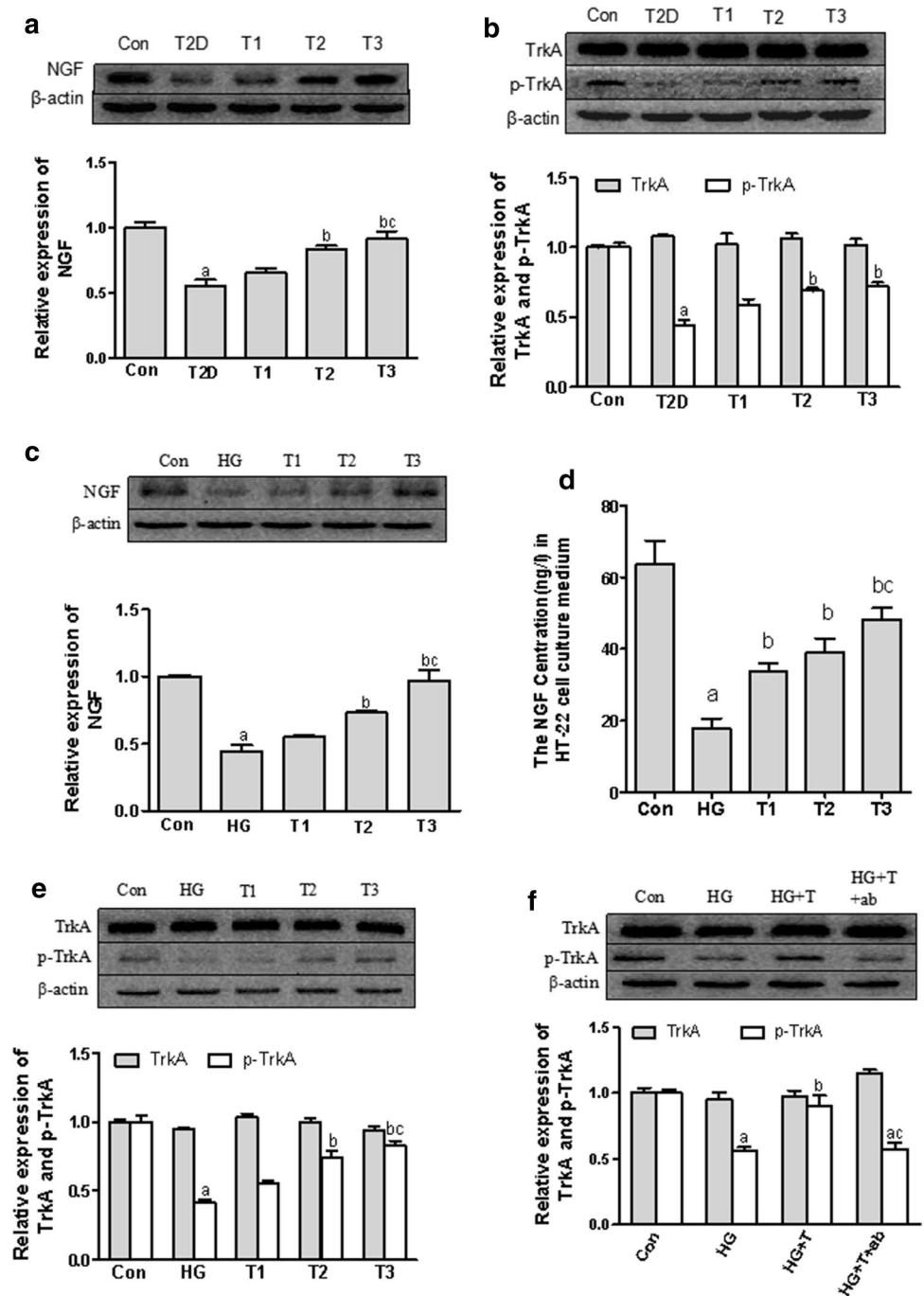
e Apoptosis index in each group. **f** Caspase-3 activity was checked by caspase-3 activity assay kit in each group. Data are shown as the mean \pm SEM ($n=3$ per group). Significant statistical difference was indicated by ^a $p < 0.05$ with respect to the Con group, ^b $p < 0.05$ with respect to the T2D group in vivo, ^c $p < 0.05$ with respect to the HG group in vitro, ^d $p < 0.05$ with respect to the T1 group. Bar = 100 μ m

in the presence of ab or P ($p < 0.05$), implying that taurine prevented the apoptotic death of HG-treated HT-22 cells via NGF/Akt/Bad pathway.

Discussion

T2D has negative effect on cognitive function of patients (Wong et al. 2014) which may lead to dementia. Researchers have reported that T2D rats have been found to undergo

Fig. 4 The effect of taurine on NGF and TrkA in T2D rats and in HG-treated HT-22 cells. **a** The expression of NGF protein in T2D rats. **b** The expression of NGF in HT-22 cell. **c** The expression of TrkA/p-TrkA proteins in T2D rats. **d** The content of NGF in HT-22 cells culture medium. **e** The expression of TrkA/p-TrkA proteins in HT-22 cell with ab. Data are shown as the mean \pm SEM ($n=3$ per group). Significant statistical difference was indicated by ^a $p < 0.05$ with respect to the Con group, ^b $p < 0.05$ with respect to the T2D group in vivo, ^b $p < 0.05$ with respect to the HG group in vitro, ^c $p < 0.05$ with respect to the T1 group. ^d $p < 0.05$ with respect to T2 group

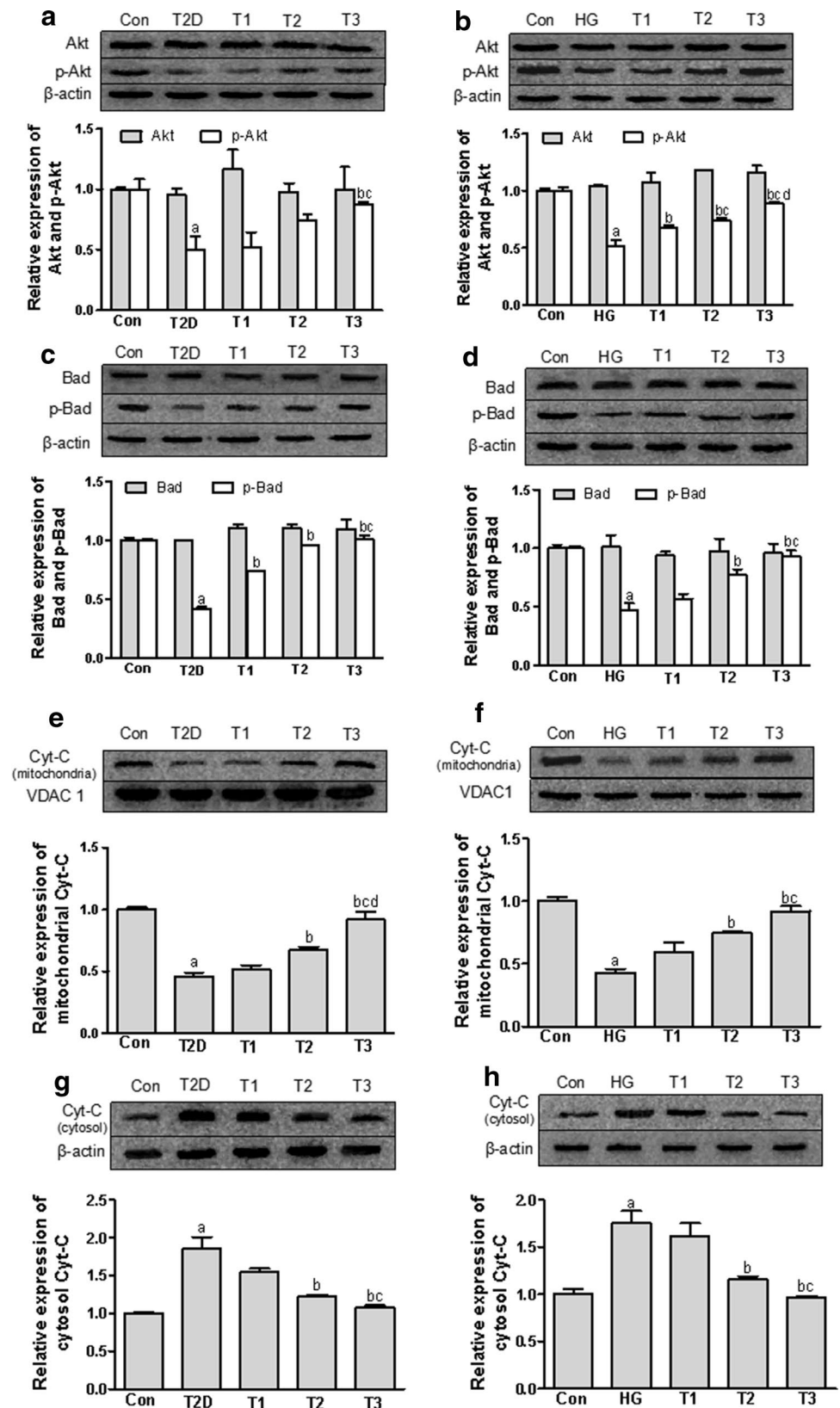


memory loss (Jones et al. 2014). In our MWM test, we also found that T2D rats spent more time in swimming and took long to locate the platform, indicating that T2D really caused a memory deficits in rats. However, this change was attenuated by taurine treatment. Rats given taurine spent less time in swimming as compared to T2D rats reached the platform relatively in less time. These results were supported by the previous studies. Lu et al. (2014) reported that taurine improved the spatial learning and memory impairment in rats caused by manganese exposure, the improvement

effect of taurine on cognitive deficits was also reported by Akande et al. (2014) in model of rats exposed to chlorpyrifos and lead acetate. Moreover, taurine exerts a neuroprotective effect against Alzheimer’s associated cognitive decline (Jang et al. 2017). These studies and our results indicate that taurine improves learning and memory in T2D rats.

Hippocampus is located between the cerebral thalamus and the medial temporal lobes, and belongs to the part of the brain limbic system. It was thought to be an important brain region responsible for memory formation and consolidation

Fig. 5 The effect of taurine on Akt/Bad pathway and mitochondria-dependent apoptotic pathway in T2D rats and in HG-treated HT-22 cells. **a** The expression of Akt/p-Akt proteins in each group rats. **b** The expression of Akt/p-Akt proteins in HT-22 cell. **c** The expression of Bad/p-Bad proteins in each group rats. **d** The expression of Bad/p-Bad proteins in HT-22 cells. **e** The expression of cyt-C protein in mitochondria in each group rats. **f** The expression of cyt-C protein in mitochondria in HT-22 cell. **g** The expression of cyt-C protein in cytosol in each group rats. **h** The expression of cyt-C protein in cytosol in HT-22 cell. Data are shown as the mean \pm SEM ($n = 3$ per group). Significant statistical difference was indicated by ^a $p < 0.05$ with respect to the Con group, ^b $p < 0.05$ with respect to the T2D group in vivo, ^b $p < 0.05$ with respect to the HG group in vitro, ^c $p < 0.05$ with respect to the T1 group, ^d $p < 0.05$ with respect to T2 group



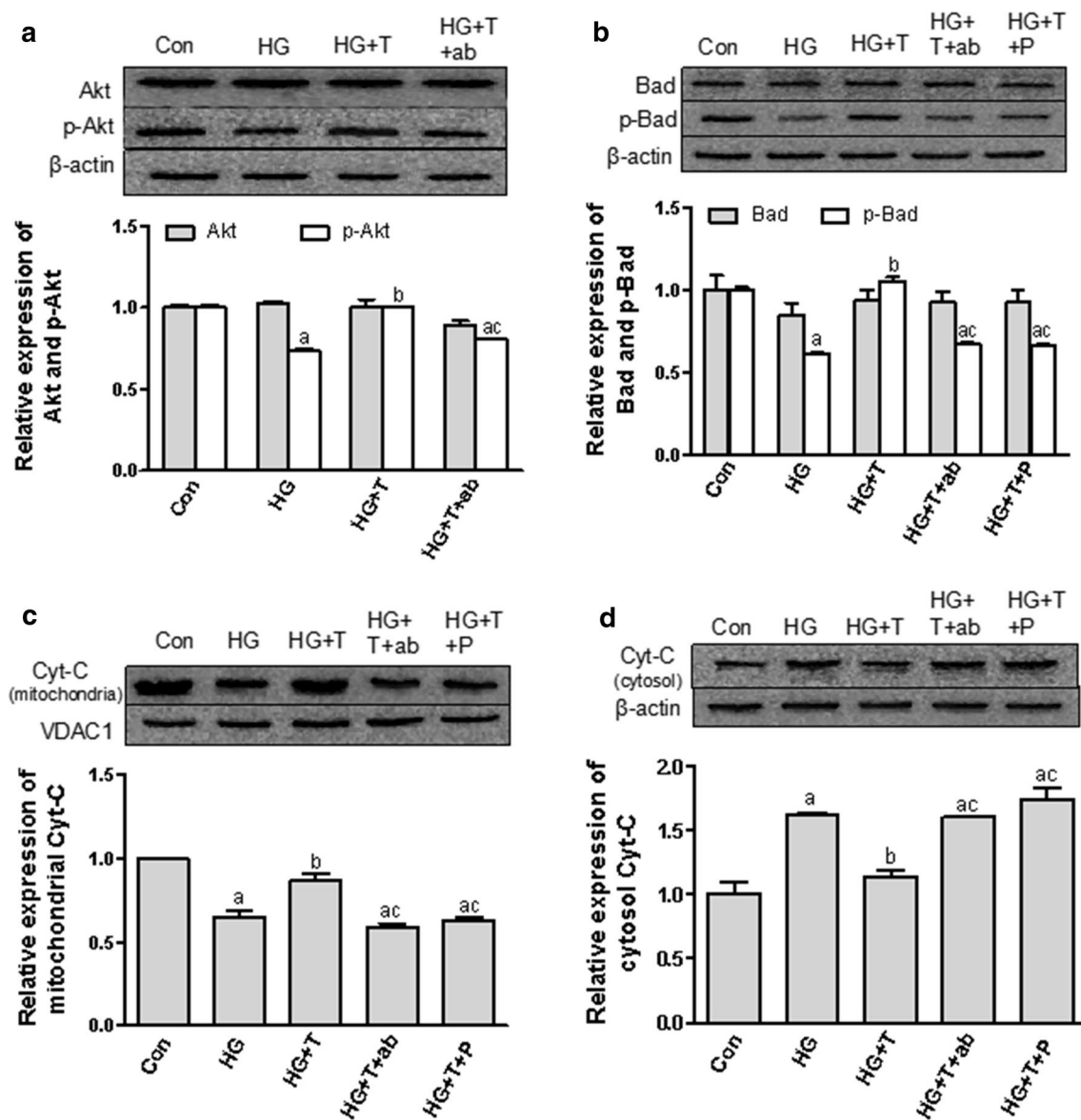


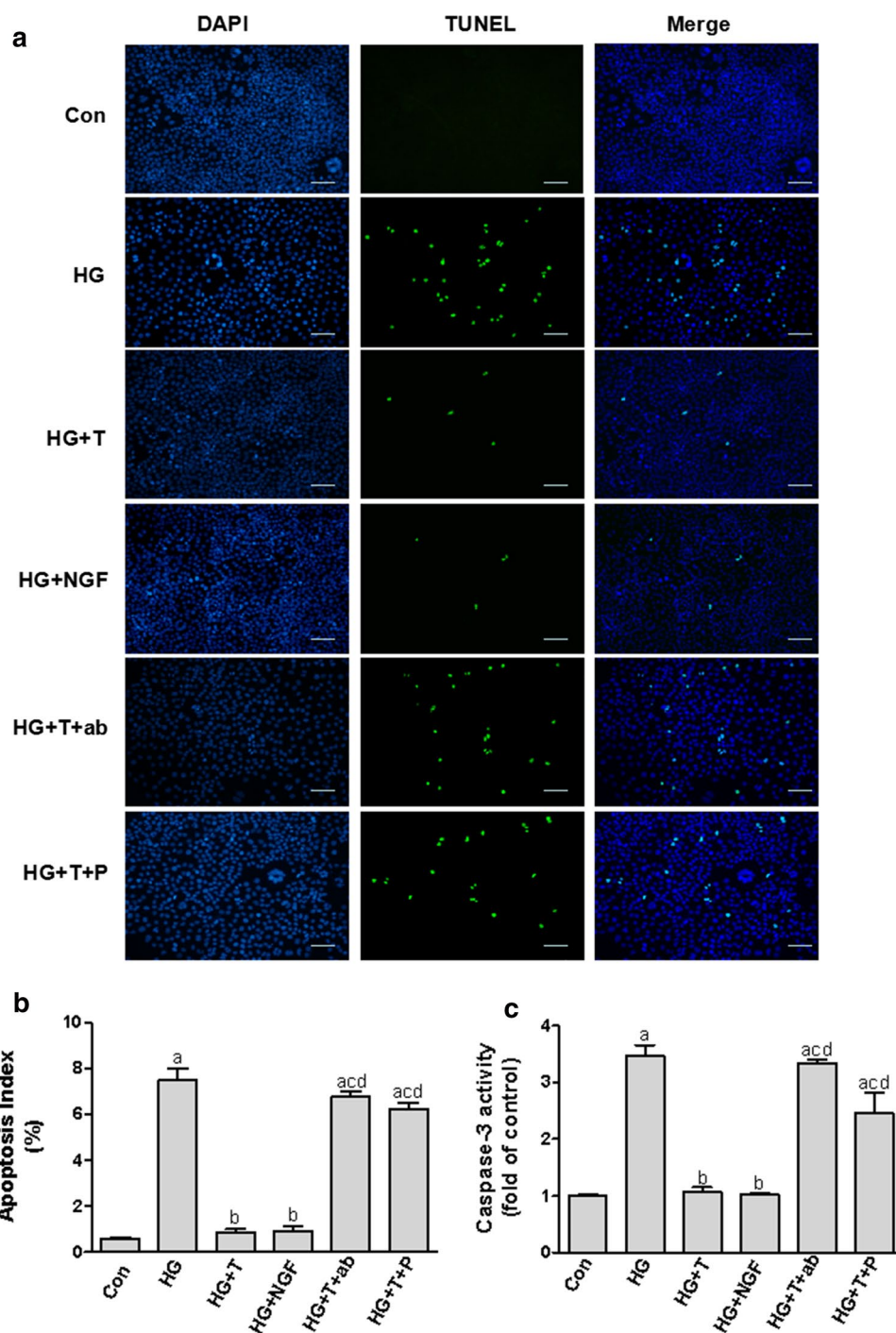
Fig. 6 The effect of taurine on Akt/Bad pathway and mitochondria-dependent apoptotic pathway in HG-treated HT-22 cells in presence of ab or P. **a** The expression of Akt/p-Akt proteins in each group. **b** The expression of Bad/p-Bad proteins in each group. **c** The expression of cyt-C protein in mitochondria. **d** The expression of cyt-C pro-

tein in cytosol. Data are shown as the mean \pm SEM ($n=3$ per group). Significant statistical difference was indicated by ^a $p < 0.05$ with respect to the Con group, ^b $p < 0.05$ with respect to the HG group, ^c $p < 0.05$ with respect to the HG+T group

(Deuker and Bellmund 2016). Extensive animal and human studies indicated that hippocampal neuronal apoptosis could result in functional deficits in learning and memory (Tompkins et al. 2013; Kuang et al. 2014). However, ameliorating apoptosis of hippocampal neuron contributed to restoring function of learning and memory. Recently, studies have reported that taurine played a role in inhibiting the apoptosis of hippocampus exposed to arsenic (Li et al. 2017a, b). These studies suggested that taurine protected against cognitive impairment in the T2D rats via controlling the apoptosis of hippocampus neuron. To confirm the hypothesis

in T2D, in this study, we checked the apoptosis of hippocampal neuron. TUNEL assay results showed that the number of hippocampal neuronal apoptotic cells had a significant increase in T2D group. In vitro, HT-22 cells treated with 150 mM glucose also exhibited an increased apoptosis index, being accordant with our results in vivo. On the other hand, in the current study, 1% and 2% taurine in drinking water significantly decreased the number of apoptotic cells in hippocampal neurons and taurine-exposed HT-22 cells also showed a decline in apoptotic index. Moreover, apoptosis of HT-22 cells were not affected by changing osmotic

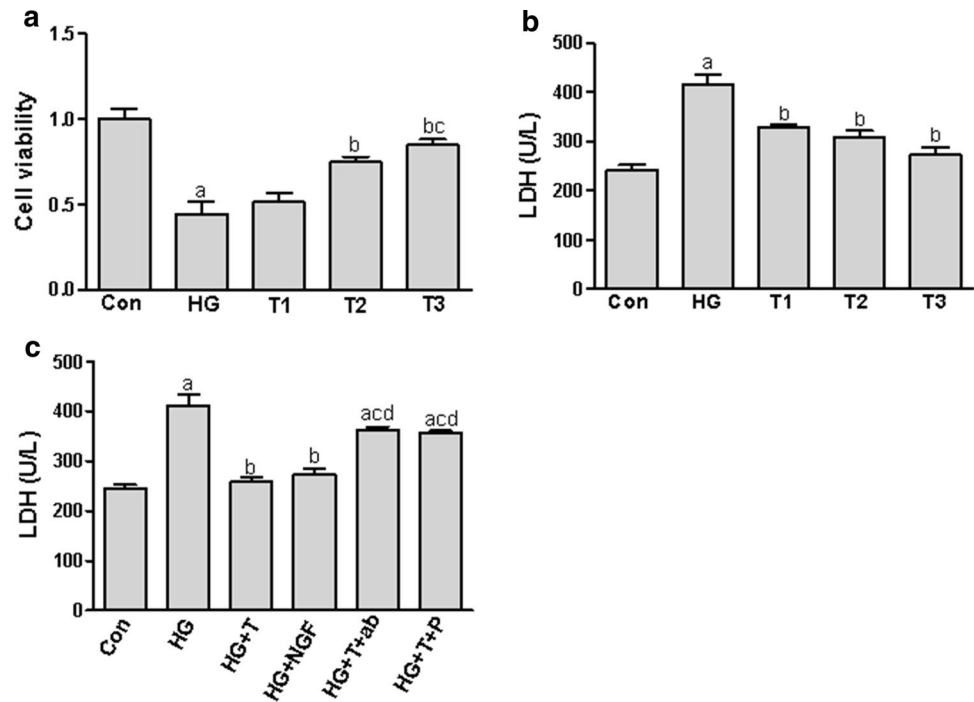
Fig. 7 The effect of taurine on HG-induced apoptosis of HT-22 cell in the presence of ab or P. **a** Representative images of TUNEL-stained cells are shown for the Con group, HG and HG + T, HG + NGF, HG + T + ab, and HG + T + P groups. **b** Quantified on data of the apoptotic index. **c** Caspase-3 activity was checked by caspase-3 activity assay kit in each group. Data are shown as the mean \pm SEM ($n = 3$ per group). Significant statistical difference was indicated by ^a $p < 0.05$ with respect to the Con group, ^b $p < 0.05$ with respect to the HG group, ^c $p < 0.05$ with respect to the HG + T group, ^d $p < 0.05$ with respect to the HG + NGF group. Bar = 100 μ m



stress (Data showed in Supplemental Fig. 2), indicating that the effect of taurine in HT-22 cells is against high glucose, but not hypertonicity. Anti-apoptosis effects of taurine were recorded in lots of studies, including anti-apoptotic activity in cardiomyocytes against homocysteine-induced H9C2 cardiomyocyte apoptosis (Zhang et al. 2017), and decreased hippocampal apoptosis induced by Arsenic (Li et al. 2017a, b), supporting our results. These data suggested that taurine protected against hippocampal neuron apoptosis in the T2D.

Mechanistically, the most critical question to consider is why taurine after onset of hippocampal neuron apoptosis still displays protection. Nerve growth factor (NGF), as an important neurotrophin, plays a critical role in promoting the growth, development, and differentiation of central and peripheral neurons, such as hippocampal neuron (Zhang et al. 2018; Haque et al. 2018). A number of studies have shown that NGF plays critical roles in anti-apoptosis in high glucose exposed Schwann cell (Li et al. 2017a, b) and in the

Fig. 8 The effect of taurine on the viability and death of HG-treated HT-22 cells. **a** The effect of taurine on cell viability. The cell viability was checked by MTT. **b** The effect of taurine on HG-induced LDH release. Data are shown as the mean \pm SEM ($n=3$ per group). ^a $p < 0.05$ with respect to the Con group, ^b $p < 0.05$ with respect to the HG group, ^c $p < 0.05$ with respect to the T1 group, and ^d $p < 0.05$ with respect to T2 group. **c** The LDH activity in Con group, HG and HG + T, HG + NGF, HG + T + ab, and HG + T + P groups. Significant statistical difference was indicated by ^a $p < 0.05$ with respect to the Con group, ^b $p < 0.05$ with respect to the HG group, ^c $p < 0.05$ with respect to the HG + T group, and ^d $p < 0.05$ with respect to the HG + NGF group



diabetic cornea (Park et al. 2016). It has been demonstrated that NGF promoted neuroprotection through activating of TrkA, which is a receptor of NGF (Marlin and Li 2015). In our study, T2D and HG decreased the expression of NGF and p-TrkA. On the other hand, treatment with taurine significantly increased the level of NGF and p-TrkA in vivo and in vitro. This result is consistent with study of Obrosova et al. (2001) in which taurine up-regulated the level of NGF in early experimental diabetic neuropathy. These results indicated that taurine up-regulated NGF expression and activated its receptor TrkA in the T2D rats, implying that the effect of taurine may be mainly responsible for its anti-apoptosis of hippocampal neurons.

Akt/Bad signaling pathway is the downstream pathway of NGF, which plays an important role in neuronal survival. Akt is a type of cellular protein kinase, whose activation is involved in combating apoptosis and supporting neuronal survival (Wang et al. 2014). Akt activation caused an increase in phosphorylation of downstream target Bad, phosphorylated Bad prevented release of Cyt-C from the mitochondria to the cytosol, which blocked the cell apoptosis (Abhishek et al. 2018; Tang et al. 2016). In the present study, our results showed reduction in expression of p-Akt and p-Bad, and increase in release of Cyt-C from the mitochondria in hippocampal tissue of T2D rats and in HT-22 cells treated with HG, indicating inactivation of Akt/Bad pathway and activation of mitochondria-dependent apoptotic pathway. However, taurine treatment increased the expression of p-Akt, p-Bad, and prevented the Cyt-C release into the cytosol from mitochondria in the T2D rats or the treated

HT-22 cells. Moreover, the effects of taurine were blocked by ab16161 (ab) an anti-NGF antibody. The same results showed in study by Sun et al. (2014) that taurine could activate the Akt/Bad signaling pathway. These results indicated that taurine activated Akt/Bad pathway and inactivation of mitochondria-dependent apoptotic pathway in hippocampal tissue of T2D rats, and the effects of taurine might attribute to the NGF regulation.

To further certify that NGF/Akt/Bad signaling pathway is really involved in the anti-apoptotic effect of taurine, the apoptosis in the HG-treated HT-22 cells with or without taurine was examined in the presence of NGF ab or perifosine (P) an Akt inhibitor (Zhu et al. 2018) by TUNEL staining. Caspase activation was an indispensable event in the initiation of mitochondria-mediated apoptosis (Zou et al. 2013). Especially, caspase 3 activation was thought to be a downstream key step to apoptosis (Roy 2000). The caspase 3 activity was also measured. The results showed significant increase in the number of apoptosis and caspase-3 activity in the HG-treated HT-22 cells compared to the control group. On the other hand, taurine supplementation significantly decreased the number of apoptotic cells and the caspase-3 activity in the treated HT-22 cells. However, the anti-apoptotic effect of taurine was blocked by ab or P, implying that taurine protected against hippocampal neuron apoptosis via the NGF/Akt/Bad signaling pathway.

The studies showed that abnormal apoptosis in nerve tissue was involved in loss of neurons under several pathological conditions (Wang et al. 2017). To investigate the role of taurine against apoptotic death of hippocampal neurons,

its effect on viability and death of HG-induced HT-22 cells was observed in the present study. We found that survival cells decreased and dead cells increased after HG exposure, implying induction of cell death. However, the decreased survival and the increased death of the HT-22 cells exposed to HG were significantly mitigated by taurine, which was blocked in the presence of ab or P. These results indicated that taurine protected against apoptotic death of hippocampal neurons via the NGF/Akt/Bad signaling pathway, which may be involved in the improvement in cognitive deficits in diabetic rats.

Increasing evidence indicates that in addition to the pivotal role of the pancreatic hormone insulin in peripheral glucose regulation, the brain is its major target (Benedict et al. 2007). Its neuroanatomical basis is the localization of insulin receptors, predominantly in the olfactory bulbs, hypothalamus, and hippocampus (Stockhorst et al. 2004). The source of insulin in the brain may originate from circulating insulin that crosses the blood brain barrier via a saturable transporter. It is generally accepted that insulin signaling enhances memory and facilitates synaptic plasticity in the hippocampus, which has an important role in memory and learning (Zilliox et al. 2016). Cumulative evidence indicates that insulin resistance is associated with cognitive decline. For instance, insulin response was markedly decreased in post-mortem brain of individuals in T2D (Li et al. 2015). Enhancing central nervous insulin action has been shown to improve memory functions in animals as well as in humans, benefiting in particular hippocampus-dependent memory, suggesting that impaired brain insulin signaling may play an important role in the loss of memory functions associated with this disease (Benedict et al. 2011). Taurine is involved in β -cell function and insulin action regulation. Several reports have shown that taurine supplementation improved insulin sensitivity and normalized glycemia and insulinemia in T2D experimental models (Ribeiro et al. 2012). In the present study, we found that taurine addressed hyperglycemia and reduced insulin resistance in T2D rats (data not shown), being accordant with the literatures in above. These studies indicated that taurine against insulin resistance may also partly contribute to the improvement in cognitive deficits in T2D rats. Therefore, it needs to study further to clarify the direct effect of taurine to hippocampus through the experiment protocol giving taurine from several weeks after streptozotocin in rats.

Conclusion

In the present study, we found that taurine could ameliorate the cognitive impairment and control the hippocampal neuronal apoptosis in T2D rats. In addition, taurine

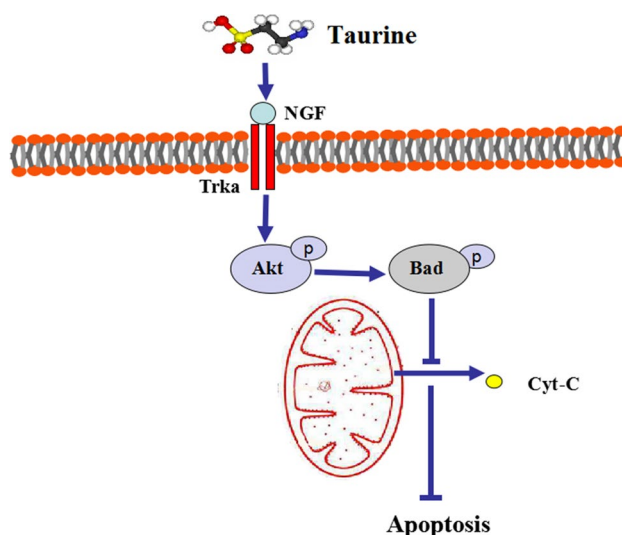


Fig. 9 A schematic diagram of the molecular mechanisms that taurine ameliorates hippocampus apoptosis in type 2 diabetic rats

could improve neuronal cell viability, and LDH release in HT-22 cell exposed to HG. It also found that taurine inhibiting apoptosis in hippocampal neurons is mediated via regulating NGF and phosphorylation of Akt and Bad (Fig. 9). Our findings suggests that taurine can be a potential candidate against memory impairment in T2D via inhibiting hippocampal neuronal apoptosis. However, it needs to study further to clarify the direct effect of taurine to hippocampus through the experiment protocol giving taurine from several weeks after streptozotocin in rats for verifying protective mechanism of taurine on diabetic cognitive deficits.

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Author contributions FP and XC conceived and designed the experiments; PW, XS, IU, KL, MZ, JM, YL and YL and CZ performed the experiments; XC and QL analyzed the data; PW and XS wrote the manuscript; ML, XL and SL supplied the data and revised this manuscript.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Human and animal rights statement This research was conducted in according to animal guideline from Dalian Medical University which was in agreement to ethical committee of Dalian Medical University, (Permit number: SCXK (liao) 2015-2003). The work was conformed to NIH Guide for Care. Every effort was made to minimize the amount of stress and suffering to animals. Adequate and standard measures were taken to reduce pain and discomfort considering human endpoints for animal suffer.

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