Role of IGF-1 in Neuroinflammation and Cogniton Deficits Induced by Sleep Deprivation

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Research

Keywords: Sleep deprivation, IGF-1, Inflammation, Cognitive impairment, Apoptosis

Posted Date: July 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-680306/v1

Abstract

Background: Sleep deprivation negatively influences cognition, although inflammatory and immune responses participate in sleep deprivation,the molecular regulators of neuroinflammation after sleep deprivation remain to be defined. IGF-1 have shown anti-inflammatory and neuroprotective properties in models of CNS injury. This study investigated the role of IGF-1 on neuroinflammation and cognition deficits after sleep deprivation.

Methods: Human periphery blood were obtained from chronic insomia(CI) patients to evaluated the levels of IGF-1. Mouse model of modified multiple platforms method (MMPM) was used to examine the role of IGF-1 in sleep deprivation. Behavior was evaluated by water maze tests.Inflammatory markers, and neuroinflammation were assessed by western blot and quantitative real-time PCR.

Results: We found down-regulation of IGF-1 in human peripheral blood and in mice subjected to sleep deprivation for 5 days, and reduced activation of downstream the PI3K/AKT/GSK-3β pathway in mice brain. In conjunction, we found reduced levels of anti-apoptosis enzyme Bcl-2 and increased levels of pro-apoptosis enzyme Caspase-9, together with increased pro-inflammatory factors. Administration of IGF-1 induced activation of the PI3K/AKT/GSK-3β pathway, reversed changes in Bcl-2, Caspase-9 and pro-inflammatory factors, and prevented cognitive decline.

Conclusion: These findings indicate that the IGF-1 reduces neuroinflammation and cogniton deficits after sleep deprivation. The effects may be mediated by the interaction among IGF-1 and PI3K/AKT/GSK-3β signaling. IGF-1 may be a viable therapeutic target that requires further investigations in sleep deprivation.

1. Introduction

Sleep deprivation is a common phenomenon with a high incidence, which can lead to increased risk of cardiovascular and cerebrovascular diseases, mental diseases and cognitive impairment. Recent research has focused on the mechanisms by which sleep deprivation causes cognitive impairment,such as BDNF and CaMKII expression were decreased after sleep deprivation[1] and sleep deprivation can inhibit the regeneration and proliferation of neurons in the hippocampus[2]. However,emerging evidence suggests that inflammation plays a key role in the progression of sleep-induced cognitive impairment.Microglia are the primary immune cells of the central nervous system (CNS), and have found to be activated after sleep deprivation[3]. Subsequently, the activated microglia then augment the local production of proinflammatory cytokines, and also recruit other immune cells which can eventually lead to damaged neurons and cognitive impairment[4–6].

Insulin-like growth factor 1 (IGF-1) is a 7.5kDa peptide hormone which plays an important role in promoting cell growth, inhibiting cell apoptosis, inhibiting inflammatory response and promoting synaptic formation[7]. IGF-1 also plays an important role in nerve formation and development. IGF-1 is mainly

produced in the liver and partial IGF-1 is also produced in the CNS [8]. IGF-1 receptors are abundantly expressed in the CNS, especially in the hippocampus[9].

The binding of IGF-1 with receptors causes the recruitment and phosphorylation of intracellular substrates, activating the downstream MAPK/ERK and PI3K/AKT signaling pathways, which are critical for promoting cell growth, inhibiting apoptosis, and inhibiting inflammatory response[10, 11]. This neuroprotective effect can occur in a variety of cells, and IGF-1 is critically involved in several CNS disorders, including cerebral hemorrhage, vascular dementia, Alzheimer's Disease (AD), Parkinson's Disease (PD), and obstructive sleep apnea hypopnea syndrome (OSAHS) [12–16]. For example, animal studies have shown that the injection of IGF-1 in amyloid-beta precursor protein and presenilin 2 (APP/PS2) mutant transgenic mice results in the transport of β-amylase into the serum, thereby reducing its cerebral level [17]. IGF-1 inhibits the activation of GSK-3β, which in turn inhibits Tau phosphorylation and promotes the survival of neurons exposed to β-amylase[18]. As a neurotrophic factor, IGF-1 modulates adult neurogenesis and cognitive function by regulating synaptic plasticity, synaptic density, and signal transduction[2]. Despite its widespread role in cognitive function, the effects of sleep deprivation on IGF-1 and its downstream signaling pathways have not been previously reported.

In this study, we first investigated the change of IGF-1 in periphery blood of patients with chronic insomnia and the effect of sleep deprivation on the expression of IGF-1.Then its downstream PI3K/AKT/GSK-3β pathway in mouse hippocampus were researched. In addition, we analyzed the inflammatory response and apoptosis induced by sleep deprivation, to investigate the role of the IGF-1/PI3K/AKT/GSK-3β pathway in cognitive impairment after following deprivation.

2. Materials And Methods

2.1 Human periphery blood

Human periphery blood were obtained from chronic insomia(CI) patients from 8 am to 10 am and then centrifuged at 2,000 g for 10min at 4◦C.Plasma cytokines were stored at 80◦C for further analysis.Levels of IGF-1 were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits from Abcam (Elabscience, China).The protocols were approved by the institutional review boards in Tianjin Medical University General Hospital. Participants were recruited from Tianjin Medical University General Hospital. The chronic insomia patients were diagnosed according to the standards described in the Internationalclassification of sleep disorders (ICSD-3) criteria [19]. The control subjects had no history of sleep disorders.

2.2Animals

This study was carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Ethics Committee of Animal Experiments of Tianjin Medical University. The protocol was approved by the Ethics Committee of Animal Experiments of Tianjin Medical University. Male C57BL/6 mice, 8 to 10 weeks old, were used. The mice were randomly assigned to each experimental group. All

mice were housed in pathogen-free conditions in the vivarium facilities and all surgeries were performed with animals under anesthesia. Reporting of this study complies with the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines.

2.3 Sleep deprivation

Sleep deprivation was induced using the modified multiple platforms method as in our previous studies[20,21]. A water box was used which is 50 cm long, 30 cm wide, and 30 cm high. Eight rounded platforms were evenly placed in the water box, which each had a diameter of 3 cm and a height of 6 cm, with intervals between the platforms of 7 cm. Only 6 mice were in the containers at a time, so that they could move without restriction. The water level was controlled to be 1 cm below the platform height and the water was maintained at a temperature of 22 ± 2°C. Food and water were placed in the top of the box where the mice could get to it without difficulty.

Before the sleep deprivation sessions, mice were placed in the modified multiple platform water box for 2 hours every day for 3 consecutive days to adapt to the environment. Sleep deprivation sessions began at 9:00 am, after which the mice were taken out of the deprivation boxes from 17:00 to 21:00 every day and placed in cages where they had free access to food and water for 4 hours. Sleep deprivation sessions were repeated for a total of five days, and the water in the box was replaced every day.

2.4 Experimental groups and drug administration

Animals were randomly assigned to one of four groups: Cage control Group (CC group); sleep deprivation for 5 days (SD group); sleep deprivation with intraperitoneal (i.p.) administration of PBS for 5 days (SD + PBS group); and sleep deprivation with i.p. administration of rhIGF-1 for 5 days (SD + IGF-1 group). Based on previous studies (21,22), 50 ug/kg IGF-1 was administrated by i.p. injection at 17:00 following each sleep deprivation and session. The PBS was similarly administrated at 50ug/kg by i.p. injection in the SD + PBS group. All animals were operated on at 9 am.

2.5 Behavioral testing

Spatial learning and memory were assessed using a morris water maze (MWM) test [22]. Briefly, the experimental apparatus consisted of a round water tank (150 cm wide and 50 cm high) filled with water (at 25°C) and surrounded by visual cues around the tank. An invisible platform (15 cm wide and 35 cm high) was placed 1 cm below the surface of the water in one of four target quadrants. The spatial learning and memory ability of mice were evaluated by the number of times finding the platform, time in target quadrant and average swimming speed.

Mice were tested in different quadrants four times a day. In each trial, the mice were released into the water randomly from one of the four quadrants with their faces toward the wall of the maze. The location of the platform remained fixed during the acquisition phase and the rats were allowed to swim for 60s to find the invisible platform. After the animal found the platform, it was allowed to remain there for 20–30s and then returned to the cage to wait another 20–30s before the start of the next trial. The time taken to

find the invisible platform was recorded. The mice trained at 8:30 am daily after sleep deprivation. After the fourth trial, the animals were kept warm and then put back in the sleep deprivation box. After 5 days of sleep deprivation, a probe phase was performed to assess spatial memory retention. In the probe test, the platform was removed and each mouse was allowed to swim for 60s. The time and distance spent in the target quadrant and the frequency of crossing the target quadrant were recorded as measures of spatial memory retention. Data collection was automated using a video image motion analyzer.

2.6 Elisa

Hippocampal tissues were isolated from brain tissue on ice and homogenized with iced normal saline to a 10% (w/v) homogenate, and then centrifuged (1500 rpm for 10 min) and the supernatants were collected. IGF-1 levels in supernatants from hippocampal tissue cultures were measured with a quantitative ELISA by using a ELISArray Kit from Abcam (Cambridge, United Kingdom). We applied an ELISA protocol under uniform conditions, according to the manufacturer's instructions. Measurements were taken in duplicate on individual mouse brains (n =6 mice per group), and the results are expressed as mean optical density.

2.7 Quantitative real-time PCR

Total RNA was extracted from hippocampal tissues with Trizol. The concentration of RNA was quantified by ultraviolet spectrophotometry at 260/280 nm. Total RNA was reverse-transcribed into complementary DNA (cDNA) by using the SuperScript First-Strand Synthesis System for qPCR (Invitrogen).

The primers used to measure gene expression are the following: IGF-1 (F:GCTGGACCAGAGACCCTTTTG; R:AGGTGCCC TCCGAATGCT), TNF-α (F:CGGGCAGGTCTACTTTGGAG; R:ACCCTGAGCCATAATCCCCT), IL-1β (F: TGCCACCTTTTGACAGTGATG; R:TGATGTGCTGCTGCGAGATT), IL-6 (F:AGCCAGAGTCCTTCAGAGAGAT; R:AGGAGAGCATTGGAAATT GGGG), and GAPDH (F:GCCAAGGCTGTGGGCAAGGT; R:TCTCCAGGCGGCACGCAGA), (F = forward, R = reverse). All procedures were strictly performed as per instructions. The PCR program was run at the following cycling conditions: 44 cycles of 10 sec at 95°C, 30 sec at 58°C, and 20 sec at 72°C. Specificity of the PCR product was confirmed by examination of dissociation reaction plots. A distinct single peak indicated that a single DNA sequence was amplified during PCR. The expression levels of mRNAs were analyzed by the method of 2△△Ct. The result was calculated as levels of target mRNAs relative to GAPDH.

2.8 Western blot

Hippocampal tissue was removed from each group and was homogenized by sonication in a ristocetin-induced platelet aggregation buffer containing protease and phosphatase inhibitors (Complete Protease Inhibitor Cocktail and PhosStop Phosphatase Inhibitor Cocktail; both from Roche Diagnostics). Protein concentration was assessed with BCA Assay Kit (Solarbio Life Science, Beijing, China), and 10-20 ug protein was loaded per lane. Equal amounts of proteins were deposited on 10% Tris-glycine gradient gels (Bio-Rad) at 80 V and the voltage was then raised to 120 V. The proteins were transferred onto PVDF

membranes for 1.5 h at 4°C with a current of 100 V. The membranes were blocked for 2 h at room temperature in 5% nonfat dry milk powder dissolved in buffer and incubated with primary antibody overnight at 4°C. The primary antibodies used were anti- p-AKT (1:1000, Cell Signal Technology, USA), rabbit anti-AKT (1:1000, Cell Signal Technology, USA), rabbit anti-p-GSK3β (1:1000, Cell Signal technology, USA), rabbit anti-GSK (1:1000, Cell Signal Technology, USA), rabbit anti-Caspase-9 (1:2000), rabbit anti-Bcl-2 (1:1000), mouse anti-GAPDH (1:2000, TransGen Biotech Co., Beijing, China), and mouse anti-β-action (1:5000, TransGen Biotech Co., Beijing, China). The day following incubation, the membranes were washed 3 times for 5 min each at room temperature, incubated with a horseradish peroxidase coupled secondary antibody (1:6000, TransGen Biotech Co., Beijing, China) for 1 h, and washed 3 times for 5 min each at room temperature. The membrane was scanned on an Odyssey Infrared Imaging System (Bio-Rad). The optical densities of target protein bands were measured and normalized to the corresponding β-actin bands. Samples were run in triplicate or quadruplicate.

2.9 Statistical analysis

Data were presented as mean ± SEM. One-way analysis of variance (ANOVA) followed by post hoc tests was used to compare among the four groups. Data with a nonparametric distribution were analyzed using Mann-Whitney tests. A P value < 0.05 was considered significant. SPSS for Windows version 17.0 software (SPSS, Inc., Chicago, IL, USA) was used for analyses.

3. Results

3.1 Reduced expression of IGF-1 in human periphery blood and in mouse after sleep deprivation

Sleep deprivation was induced using the modified multiple platforms method (Fig 1A). Elisa was used to test the level of IGF-1 in periphery blood of patients with chronic insomnia and mouse after 5 days of sleep deprivation. qPCR were used to determine the mRNA level of IGF-1 after 5 days of sleep deprivation. We found a marked decrease in IGF-1 in chronic insomnia patients(Fig 1B).The expression of IGF-1 proteins and mRNA in the hippocampus were also decreased in the SD group compared to the CC group (Fig 1C,D).

3.2 Suppressed PI3K/AKT/GSK-3β pathway in mouse hippocampus after sleep deprivation

PI3K/AKT/GSK-3β is an important downstream pathway of IGF-1 in the hippocampus. To assess whether IGF-1 regulates the PI3K/AKT/GSK-3β pathway after sleep deprivation, we examined the expression of p-AKT and p-GSK-3β after chronic sleep deprivation with Western blot. Sleep deprivation for 5 days induced decreased expression of the protein p-AKT and increased expression of the protein p-GSK-3β in the hippocampus. However, the expression of AKT and GSK-3β did not change (Fig 2A-D). These results suggest that sleep deprivation for 5 days inhibits the PI3K/AKT/GSK-3β pathway.

3.3 IGF-1 treatment prevented cognitive impairment after sleep deprivation

To assess whether the IGF-1, affects cognition after sleep deprivation, we examined neurodeficits by hippocampal-dependent memory function using the MWM. Exogenous IGF-1 (50 ug/kg) or PBS (50 ug/kg) was administered by i.p. injection for 5 consecutive days at 17:00 (Fig 3A). Results indicated that sleep deprivation caused memory impairment, with escape latency and time in the target quadrant longer for the SD group than the CC group. And SD group find the platform less frequently than CC group. Conversely, IGF-1 treatment ameliorated memory impairment (Fig 3B-G).

3.4 IGF-1 treatment induced activation of PI3K/AKT/GSK-3β pathway in hippocampus

We assessed whether IGF-1 affected the expression of the PI3K/AKT/GSK-3β pathway in hippocampus. After IGF-1 treatment for 5 consecutive days during sleep deprivation (Fig 3A), the expression of proteins p-AKT and p-GSK-3β were increased (Fig 4).

3.5 IGF-1 treatment alleviated neuroinflammation and apoptosis after sleep deprivation

Bcl-2 and Caspase-9 are the major enzymes involved in apoptosis. Bcl-2 mainly plays an anti-apoptotic role, while Caspase-9 mainly plays a pro-apoptotic role. Sleep deprivation activates glial cells, which release proinflammatory cytokines. To assess whether IGF-1 regulates the apoptosis and inflammatory response via the PI3K/AKT/GSK-3β pathway, the expression of Bcl-2, Caspase-9, and inflammatory cytokines were assessed. We found a significant decrease in the expression of Bcl-2 and increase in the expression of Caspase-9 after sleep deprivation. However, after treatment with IGF-1, the expression of Bcl-2 increased and Caspase-9 decreased (Fig 5A,B). In addition, sleep deprivation promoted the release of pro-inflammatory cytokines. As Fig 5C-E show, IGF-1 treatment attenuated the release of TNF-a, IL-1β and IL-6. This result suggests that IGF-1 regulates inflammatory and apoptosis responses via the PI3K/AKT/GSK-3β pathway in sleep deprivation, such that treatment with IGF-1 reduces inflammatory cytokines and has an inhibitory effect on apoptosis.

4. Discussion

This study provides novel evidence that stimulation of IGF-1 alleviates neuroinflammation, apoptosis response and cognitive decline following sleep deprivation. As documented here, the levels of IGF-1 in periphery blood from chronic insomia patients were decreased. Sleep deprivation for 5 days induced downregulation of IGF-1 and inhibition of the PI3K/AKT/GSK-3β pathway. IGF-1 treatment induced the activation of PI3K/AKT/GSK-3β. The hippocampal inflammation, apoptosis response and behavioral declines were ameliorated following IGF-1 treatment. These results suggest IGF-1 as a relevant hippocampal biomarker predicting inflammation and apoptosis following sleep deprivation, and that IGF-1 supplementation may protect against the adverse effects induced by sleep deprivation.

IGF-1 is a peptide neurotrophic factor similar to insulin, which regulates the growth and differentiation of nerve cells, and has a protective effect on the nervous system. In the CNS[23], IGF-1 is widely distributed in cortex, hippocampus and other brain regions closely related to cognitive function[24]. Previous studies have shown that mice exposed to sevoflurane will present with spatial learning and memory ability

impairment, but the addition of exogenous IGF-1 can improve this cognitive impairment [25]. In an animal model of traumatic brain injury, continuous lateral ventricle injection of IGF-1 for seven days after trauma led to neural restructuring and the movement function of mice and cognitive function were improved[26]. In our study, we found that exogenous supplementation of IGF-1 improved the spatial learning and memory ability of mice who had been sleep deprived, suggesting that their cognitive decline was related to decreased hippocampal IGF-1. To investigate the mechanisms by which IGF-1 deficits affect cognition functions we examined the function of the PI3K/AKT/GSK-3β signaling pathway. The results demonstrated that sleep deprivation inhibited the PI3K/AKT/GSK-3β signaling pathway, and that this effect was due to changes in IGF-1 levels. Taken together these findings suggest that sleep deprivation leads to cognition deficits by negatively impacting the IGF-1/PI3K/AKT/GSK-3β signaling pathway in the hippocampus.

The PI3K/AKT/GSK-3β pathway is critically involved in several CNS disorders including stroke, AD, and PD[27–30]. In an animal model of permanent occlusion of common carotid arteries, Zhang et al found that nitrate tetramethyl pyrazine (TBN) activated the PI3K/Akt signaling pathway, raising Bcl-2 expression and downregulating Caspase 3 expression; this modulation promoted the growth of hippocampal neurons and inhibited neuronal apoptosis, ultimately improving the cognitive impairment caused by chronic hypoperfusion[28]. In a model of PD, the activated PI3K/AKT/GSK-3β signaling pathway promotes the growth of endothelial cells and suppresses neural apoptosis, resulting in improved cognitive function[29]. A growing number of studies suggest that stimulation of the PI3K/Akt/GSK-3β pathway plays a protective role mainly by reducing neuronal apoptosis, reducing neuroinflammation and regulating synaptic plasticity. However, how the IGF-1/PI3K/Akt/GSK-3β pathway in the CNS is modulated by sleep deprivation is still unclear.

Bcl-2 and Caspase-9 are two important downstream effector molecules of the PI3K/Akt pathway. Bcl-2 plays a negative role in the regulation of apoptosis while Caspase-9 is the main pro-apoptotic protease in mitochondria-mediated apoptosis pathways[31–33]. Consistent with other studies, we found that Bcl-2 expression decreased and Caspase-9 expression increased after sleep deprivation, while IGF-1 treatment downregulated the expression of Caspase-9 and upregulated the expression of Bcl-2. The MWM data demonstrated impaired cognition after sleep deprivation, while IGF-1 treatment prevented this cognitive decline, raising the possibility that the IGF-1/PI3K/AKT/GSK-3β pathway is involved in the development of cognitive decline after sleep deprivation because its disruption interferes with the resolution of the apoptotic response in the hippocampus.

Our study also showed that IGF-1 treatment significantly reduced the production of pro-inflammatory factors such as TNF-α, IL-1β, and IL-6 following sleep deprivation. Previous research has reported that stimulation of the PI3K/Akt pathway protects against neuroinflammation and improves hippocampal dependent memory dysfunction through the modulation of NF-kB activation[34]. Animal experiments have founds that the release of inflammatory factors such as IL-1β, IL-6, and TNF-α were increased in the ischemic hemisphere[35]. However, following stroke, IGF-1 treatment significantly inhibited the post-stroke immune response and reduced the expression of pro-inflammatory factors. TNF-α and IL-1β acted on the

target cell related receptors, further promoted the inflammatory response of target cells, expanded the inflammatory environment, and caused neuronal damage in the hippocampus. Further research is needed to fully understand how the increased expression of TNF-α,IL-1β, and IL-6 after sleep deprivation is involved in regulating apoptotic response in the hippocampus.

In summary, this is the first study to report the role of IGF-1 in the modulation of inflammatory response and apoptosis in a sleep deprivation model. Our data reveal that IGF-1 attenuates cognitive decline, neuroinflammation and apoptosis by regulating the PI3/AKT/GSK-3β pathway. These findings broaden our understanding of the neuroinflammatory response to sleep deprivation and can contribute to developing therapies to treat the neural impairment caused by sleep deprivation.

Abbreviations

IGF-1: Insulin-like Growth Factor-1; CNS: Central nervous system; SD:Sleep deprivation; CI:Chronic insomia; MMPM: Modified multiple platforms method; PI3K: Phosphatidylinositide 3-kinases; AKT:Protein kinase B; GSK3β: Glycogen synthase kinase 3 beta; BDNF: Brain derived neurotrophic factor; MAPK: Mitogen-activated protein kinas; ERK: Extracellular signal regulated kinase; TNF-α: Tumor necrosis factor alpha; IL-1β: Interleukin-1 beta; IL-6: Interleukin 6; NFκB:Nuclear factor-kappa B; AD: Alzheimer's disease; PD: Parkinson's disease; OSAHS:Obstructive sleep apnea hypopnea syndrome; APP/PS2:amyloid-beta precursor protein and presenilin 2; PCR: Polymerase chain reaction; ELISA:Enzyme-linked immunosorbent assay.

Declarations

Acknowledgements

Not applicable

Funding

This work was supported in part by Tianjin Science and Technology Planning Project (17ZXMFSY00180) and Tianjin Binhai New Area Health Commission Science and technology project (2019BWKY015).

Data Avalability Statement

All datasets generated for this study are included in the article or supplementary material.

Ethics Statement

This study was carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Ethics Committee of Animal Experiments of Tianjin Medical University. The protocol was approved by the Ethics Committee of Animal Experiments of Tianjin Medical University and institutional review boards in Tianjin Medical University General Hospital.

Author Contributions

YW and WG designed the experiment, acquired and analyzed the data, and drafted the manuscript; KZ ,PZ and XL participated in the data acquisition; RX and YW revised the manuscript, formulated the study concept, designed the study, and acquired funding for the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Fig.1

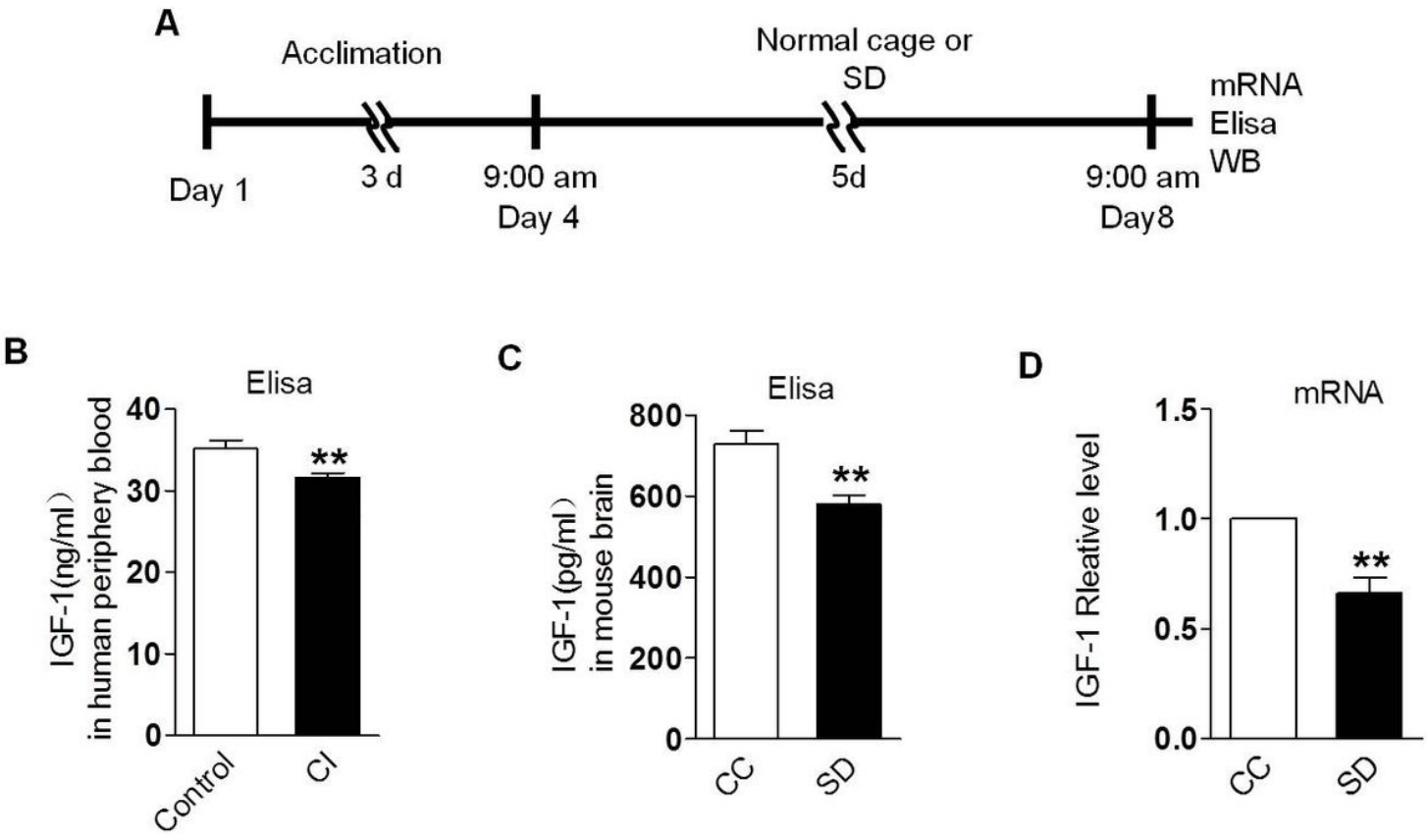

Figure 1

Reduced expression of IGF-1 in human periphery blood and in mouse after sleep deprivation. A.Schematic diagram depicting the experimental procedures. Elisa and qPCR were used to examined the protein and mRNA level of IGF-1,while Western blot was used to examined the protein p-AKT, AKT, p-GSK-3β, GSK-3β.B Elisa showed that the IGF-1 was decreased in chronic insomnia patients.C,D Sleep deprivation for 5 days significantly decreased the expression of IGF-1 in hippocampus.Data are expressed as means ± SEM. *P <0.05, **P <0.01.

Fig.2

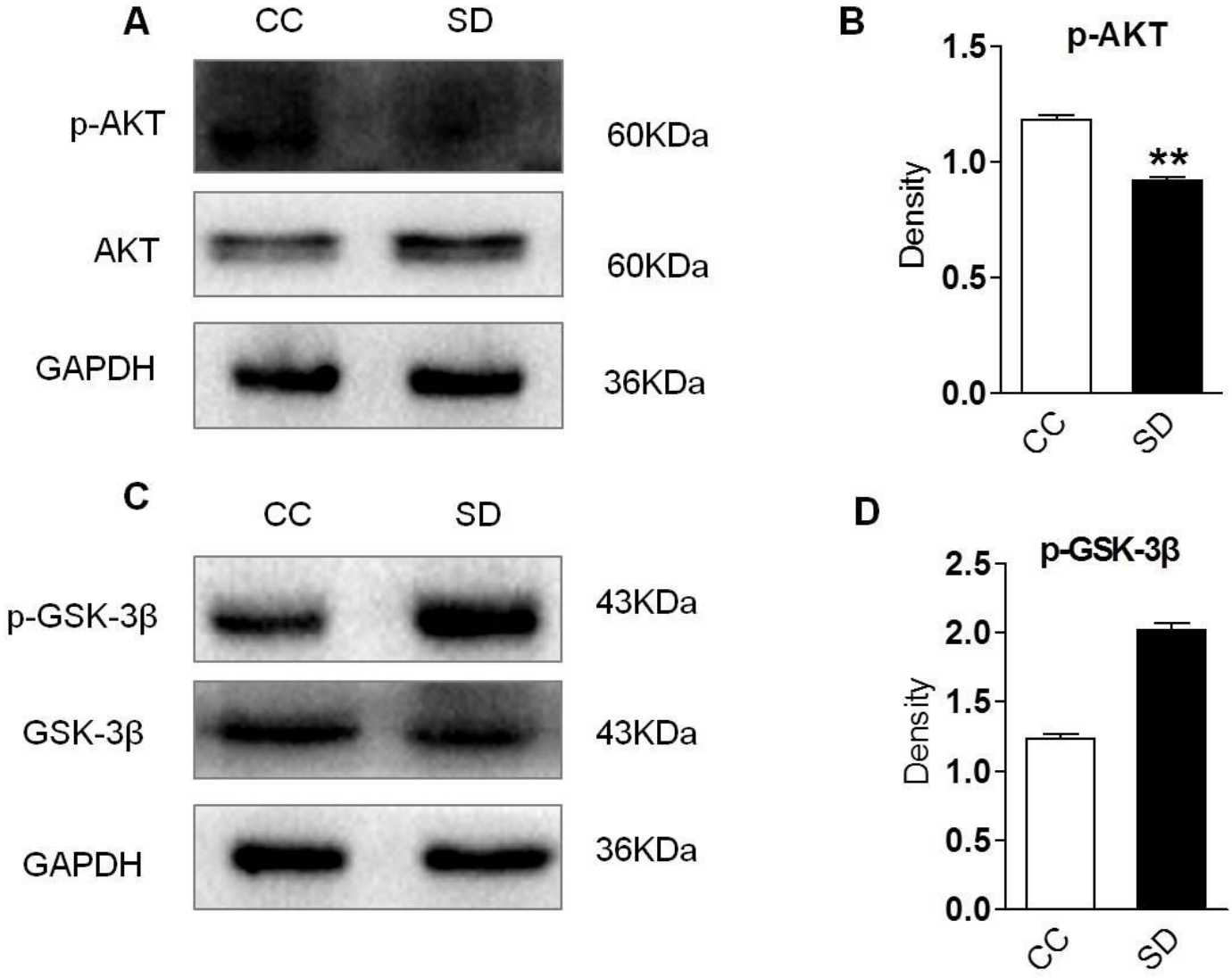

Figure 2

Suppressed PI3K/AKT/GSK-3β pathway in mouse hippocampus after sleep deprivation. A-B. Sleep deprivation for 5 days significantly decreased expression of p-AKT in hippocampus. C-D Sleep deprivation for 5 days increased the expression of p-GSK-3βin hippocampus.Data are expressed as means ± SEM. *P <0.05, **P <0.01.

Fig.3

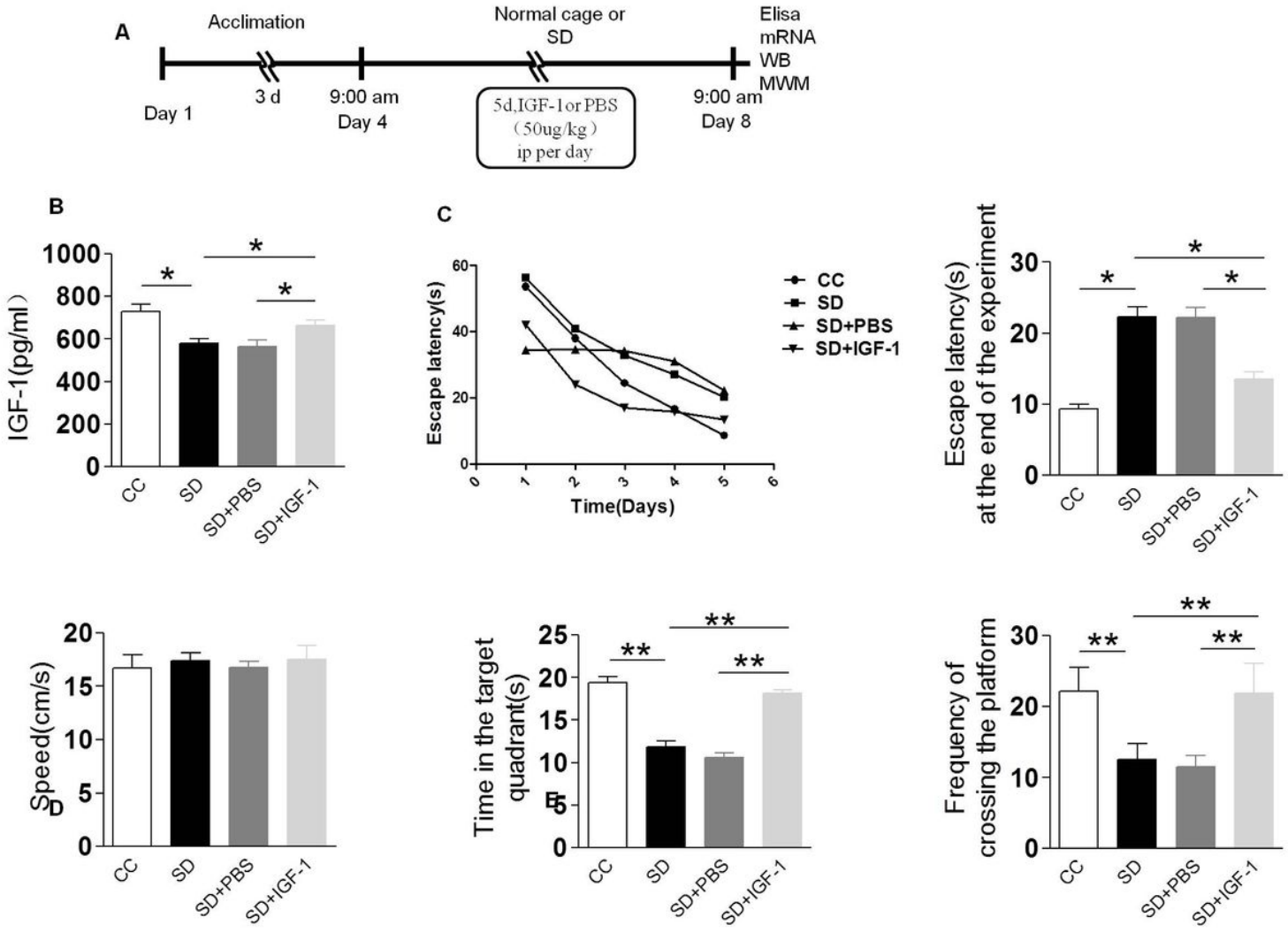

Figure 3

Effects of IGF-1 on memory function following sleep deprivation. Water moris maze was used to test the learning and memory function of mice. A.Schematic diagram depicting the experimental procedures. B. Elisa showed the expression of IGF-1 were increased after IGF-1 treatment. C,D The escape latency of mice in the four groups decreased gradually and compared with CC group, the escape latency significant increased in SD group,the IGF-1 treatment reduced the escape latency at the end of experiment. E-G Compared with the CC group,sleep deprivation decreased the frequency of crossing the platform , and shorten the time in the target quadrant.Following the IGF-1 treatment,the frequency of crossing platforms and the time in the target quadrant was prolonged compared to the SD group.There was no significance in swimming speed among the groups. Data are expressed as means ± SEM. *P <0.05, **P <0.01.

Fig.4

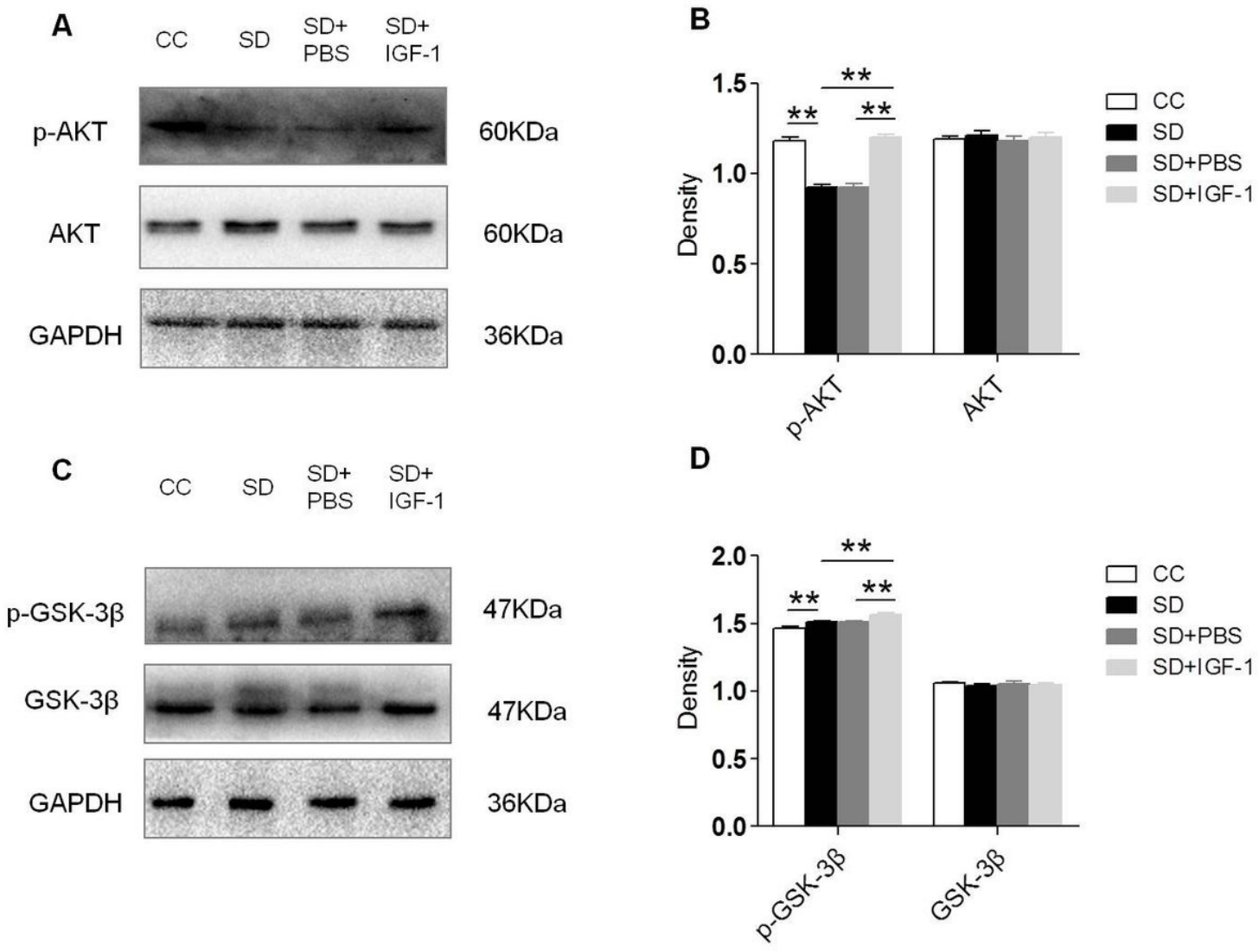

Figure 4

IGF-1 treatment activated PI3K/AKT/GSK-3β in hippocampus after sleep deprivation To investigate how cognition functions are affected by IGF-1, we further examined the state of PI3K/AKT/GSK-3β signaling pathway.Western blot show the expression of p-AKT, p-GSK-3β. Sleep deprivation for 5 days significantly decreased expression of p-AKT and increased the expression of p-GSK-3βin hippocampus. While the expression of p-AKT,p-GSK-3βwere increased after IGF-1 treatment(A-D). Data are expressed as means ± SEM.*P <0.05, **P <0.01.

Fig.5.

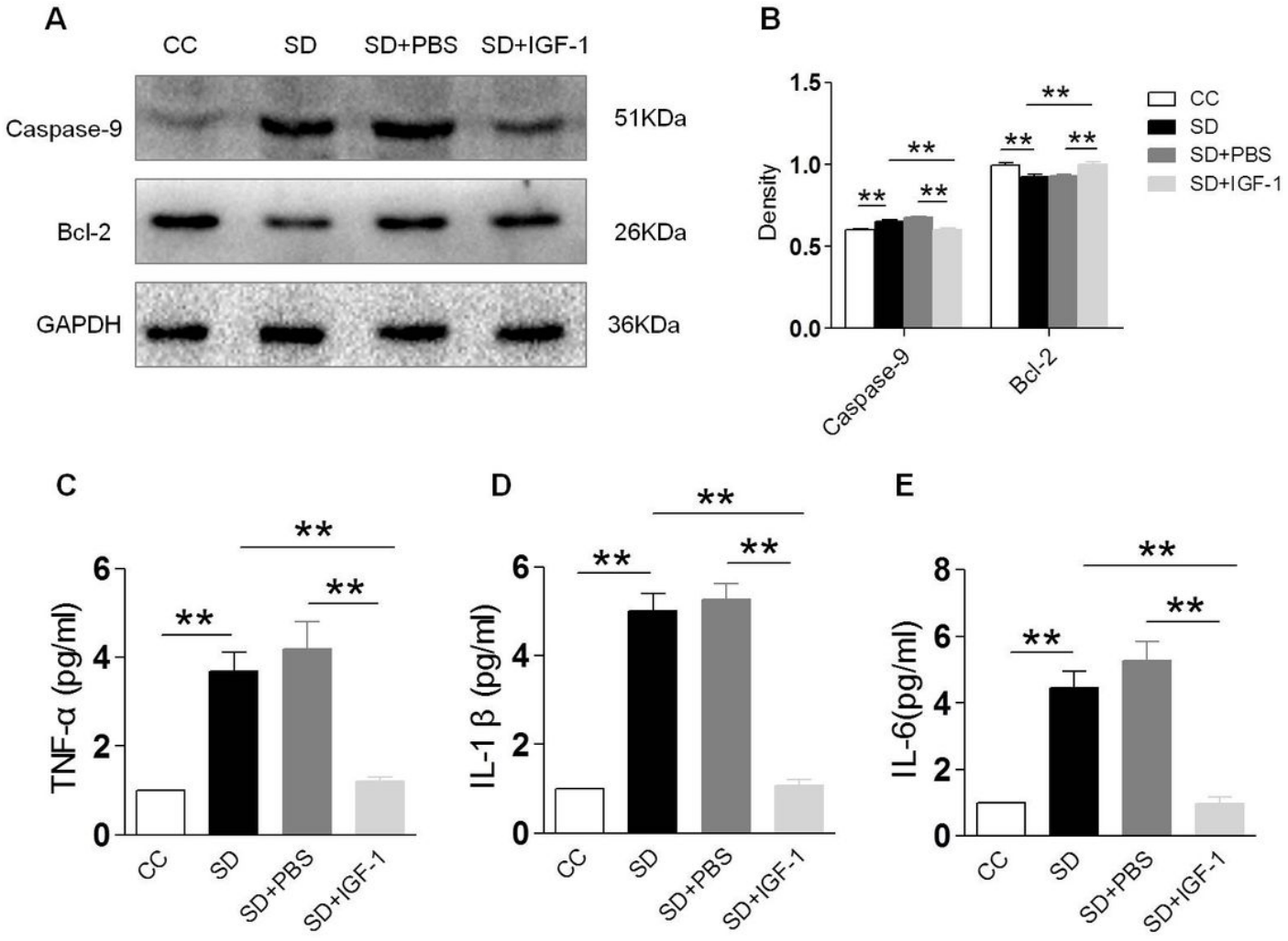

Figure 5

Actived IGF-1/PI3K/AKT/GSK-3βalleviates apoptosis and inflammatory response induced from sleep deprivation A,B Western blot showed expression of Bcl-2,Caspase-9 in the hippocampus after IGF-1 treatment in sleep deprivation. Sleep deprivation for 5 days inhibited the expression of Bcl-2 and promoted the expression of Caspase-9 in the hippocampus and IGF-1 action was just the reverse (A, B) .C-E qPCR was used to reveal the production of proinflammatory cytokines. After sleep deprivation for 5 days,the releases of proinflammatory cytokine were increased. However, after IGF-1 treatment, the release of pro-inflammatory was decreased. Data are expressed as means ± SEM.*P <0.05, **P <0.01.