International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.43.53

Research Article
Mechanism of Cognitive Dysfunction Alleviation After Sevoflurane Anesthesia in Aged Rats Through Low-Dose Ketamine Based on the PI3K/Akt Pathway

[1]Misha Hou and [2]Jiaping Zhao

[1]Beijing Daxing District Hospital of Integrated Chinese and Western Medicine, Beijing 100076, China
[2]Department of Anesthesiology, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China

Abstract

Background and Objective: The neurotoxicity of propofol induces hippocampal neuron apoptosis, triggering cognitive dysfunction, emphasizing crucial clinical significance in preventing and treating propofol-related POCD. To explore the mechanism by which low-dose ketamine attenuates cognitive dysfunction after sevoflurane anesthesia in aged rats based on the Phosphatidylinositol 3-Kinase (PI3K)/serine-threonine Protein Kinase (Akt) pathway. **Materials and Methods:** Sixty aged rats were divided into control group, cognitive dysfunction group and low-dose ketamine group according to the random number table method, with 20 in each and the cognitive functions of the rats in the three groups were compared. **Results:** Compared with the cognitive dysfunction group, the low-dose ketamine group exhibited a lower programmed necrosis rate of hippocampus neurons and a decreased cytoplasmic Ca^{2+} concentration of hippocampal neurons ($p<0.05$). Compared with the cognitive dysfunction group, the low-dose ketamine group exhibited lower levels of IL-6, TNF-α, s-100β and NOS, while displaying higher levels of p-PI3K, p-Akt, synapsin I, synaptic proteins, synaptic vesicle proteins, dendritic spine density, neuronal density, SY38 and PSD-95 ($p<0.05$). **Conclusion:** Pre-infusion of low-dose ketamine may alleviate cognitive dysfunction after sevoflurane anesthesia in aged rats and the mechanism may be related to the inhibition of programmed necrosis of hippocampal neurons, activation of the hippocampal PI3K/Akt pathway and improvement of neuronal synaptic plasticity.

Key words: PI3K/Akt pathway, low-dose ketamine, sevoflurane, cognitive dysfunction, programmed necrosis, synaptic plasticity

Citation: Hou, M. and J. Zhao, 2024. Mechanism of cognitive dysfunction alleviation after sevoflurane anesthesia in aged rats through low-dose ketamine based on the PI3K/Akt pathway. Int. J. Pharmacol., 20: 43-53.

Corresponding Author: Jiaping Zhao, Department of Anesthesiology, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China
Tel: +86-010-84322000

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sevoflurane is a commonly employed inhalation anesthetic in clinical practice, with good analgesia, sedation and partial muscle relaxation, which has the characteristics of rapid onset and good controllability and is widely used in clinical practice[1]. However, prolonged sevoflurane exposure may elevate cytoplasmic Ca^{2+} concentration in CA1 vertebral neurons, disrupt calcium homeostasis, decrease CA1 neuron excitability, impair hippocampus-dependent learning and memory impairment and affect cognitive function[2,3]. Cognitive dysfunction is one of the common complications after sevoflurane anesthesia in elderly patients, with amnesia, trance, inability to accurately judge the location of oneself and objects, decreased language ability, anxiety, frustration, etc. as the main clinical manifestations, affecting the daily work and life of patients and even shortening the life span of the patients in severe cases[4,5]. Therefore, how to reduce cognitive dysfunction after sevoflurane anesthesia in elderly patients is currently a hot research topic at major medical institutions.

Ketamine is an intravenous anesthetic that acts on glutamate receptors. Relevant studies have pointed out that low doses of ketamine have a role in ameliorating cognitive dysfunction after anesthesia in elderly patients[6]. Ketamine cannot be used for anesthesia alone because of its psychoactive side effects. With basic anesthesia requirements met low doses of ketamine can avoid its psychoactive effects and mitigate learning and memory impairments[7]. The mechanism of anti-cognitive impairment of ketamine has garnered extensive attention and become a focal point of research. Based on this premise, this study investigated the mechanism through which low-dose ketamine alleviated cognitive dysfunction in aged rats after sevoflurane anesthesia based on the PI3K/Akt pathway, with the aim of furnishing an experimental basis for the clinical application of this medication.

MATERIALS AND METHODS

Study area: This animal experiment was conducted in Beijing Daxing District Hospital of integrated Chinese and Western Medicine from December, 2021 to May, 2022.

Materials: Sixty specific pathogens-free (SPF) grade aged SD rats, male, 20-22 months old, body mass 550-600 g, were provided by Henan Laboratory Animal Center, production license No. SCXK (Yu) 2015-0005. Rats were acclimated for a week under simulated natural light in a quiet environment and had free access to food and water. According to the random number table method, rats were divided into the control group, cognitive dysfunction group and low-dose ketamine group, with 20 in each. The study was approved by the Animal Care and Use Committee of Beijing Daxing District Hospital of Integrated Chinese and Western Medicine and the experimental process strictly adhered to animal protection regulations.

Anesthesia methods: The rats were placed in a homemade transparent organic glass box with a volume of 25×25×40 cm, paved with sodium lime at the bottom. They were continuously exposed to a gas mixture of 3.6% sevoflurane (Shanghai Hengrui Pharmaceutical Co., Ltd., H20173007, specification: 10 mL) and 1 L min^{-1} of air for 3 hrs, maintaining the temperature within the box at 35.5-36.5°C. Blood oxygen saturation of rats was monitored using an infant pulse oximetry probe and respiratory rate, amplitude and lip/limb color were observed. The tail-clamp reflex was tested at 15 min intervals to confirm the disappearance of the tail-clamp reflex and the absence of respiratory depression. At the end of anesthesia, the rats were sent to the rat house for rearing after the righting reflex was restored. Before anesthesia, the low-dose ketamine group was given an intraperitoneal injection of 2.5 mg kg^{-1} ketamine (Zhejiang Jiuxu Pharmaceutical Co., Ltd., H20023609, specification 10 mL/0.1 g). The cognitive dysfunction group was injected intraperitoneally with an equal amount of 0.9% sodium chloride injection before anesthesia. The control group was placed in the same glass box but not given sevoflurane anesthesia treatment and an equal amount of 0.9% sodium chloride injection was given intraperitoneally.

Observation indicators and methods

Open field test: The rats were placed in the middle area of the open field with a volume of 80×80×50 cm and an automatic camera system (EthoVision XT7.0) was used to record the average speed of movement, the residence time in the central area and the total distance of movement of the rats. During the test, gentle handling was performed, the environment was kept quiet and the test was performed continuously for 3d, once daily, each lasting 15 min and the average value was taken.

Morris water maze test: The rats were placed in a water maze with a volume of 150×50×30 cm and the water temperature was controlled at 20-24°C. A video camera was placed directly above the water maze and connected to a data processor.

Rats were placed into the pool from each of the four quadrants, facing the pool wall and the escape latency, average swimming speed and number of times the rats crossed the original platform position were recorded. If the rats did not find the platform within 60 sec, they were guided to board the platform to rest for 15 sec and the test was conducted continuously for 3d, 2 times/d, with an interval of 2 hrs each time. The average value was taken.

At the end of the open field test and Morris water maze test, 10 rats were randomly selected from each group and the rats were executed by decapitation. The hippocampal tissues were taken and made into 1×10^6 cells/mL cell suspension. Then, 5 μL of 7-ADD (TONBO, USA, Lot No. D6993090820136) and 5 μL of annexin (TONBO, USA, Lot No. C6409100218503) were added to the suspension. After mixing well in darkness for 15 min, the fluorescence intensity was measured using a flow cytometer (FACSAria, BD, USA) and the programmed necrosis rate of the hippocampal neurons was calculated. Then 1×10^6 cells/mL cell suspension was added with 3 μL of pre-prepared concentration of Fluo-3 AM (Beijing Solabio Biotechnology, Lot No. 1120A021) calcium ion fluorescent probe. The mixture was incubated at 37°C for 30 min, followed by centrifugation at 12000 r min^{-1} for 5 min (centrifugation radius = 10 cm). The supernatant was discarded and 500 μL of DMEM culture medium was added to resuspend the cells. Subsequently, the cells were incubated at 37°C for 15 min and cytoplasmic Ca^{2+} concentration of hippocampal neurons was measured using a flow cytometer. About 1×10^6 cells/mL cell suspension was taken, centrifuged a t 12000 r min^{-1} for 10 min and the supernatant was taken. The levels of Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α) and central nervous specific Protein (s-100β) were measured by Enzyme-Linked Immunosorbent Assay (ELISA) in hippocampal CA1 region and the activity of nitric oxide synthase (NOS) was determined using fluorescence microplate (GMbH Company, Australia).

Western blot: After the completion of the open field test and Morris water maze test, 8 rats were randomly selected from each group and anesthetized by intraperitoneal injection of a chloral at a concentration of 7% of 2 mL kg^{-1} and then hippocampal tissues were separated on ice. Next, 100 mg of hippocampal tissues were taken and added to the lysis buffer to extract the proteins. Polyacrylamide gel electrophoresis was utilized to separate the target proteins. The proteins were transferred onto membrane in an ice bath, followed by blocking the membrane for 60 min. Subsequently, a mixture containing 5% defatted milk powder TRS diluted with rabbit anti-p-PI3K monoclonal antibody (Wuhan Guge Biological Technology Co., Ltd., 1:1000), rabbit anti-p-Akt monoclonal antibody (Wuhan Guge Biological Technology Co., Ltd., 1:1000), rabbit polyclonal antibody to synapsin I (Abcam, USA, 1:1000), rabbit polyclonal antibody to synaptic protein (Abcam, USA, 1:1000) and rabbit polyclonal antibody to synaptophysin (Cell Signaling Technology, USA, 1:1000) was added and then incubated overnight at 4°C, followed by a 30 min re-warming on the second day. The membrane was washed with TBS three times, 5 min/time and subsequently incubated with horseradish peroxidase-labeled secondary antibodies (Wuhan Guge Biological Technology Co., Ltd., 1:3000) at room temperature for 30 min. After that, the membrane was washed again with TBS three times, 5 min/time. Subsequently, exposure to the chromogenic luminescent solution was conducted in a dark room, followed by scanning. Image analysis software (Adobe) was used to analyze the expression levels of p-PI3K, p-Akt, synapsin I, synaptic proteins and synaptic vesicle proteins as reflected by the ratio of the gray value of the target protein bands to the gray value of the internal reference GAPDH band.

Detection of neuronal density by NISSL staining: After the open field test and Morris water maze test, 5 rats were randomly selected from each group and anesthetized with an intraperitoneal injection of 2 mL 7% chloral. Next, a rapid infusion of 200 mL of physiological saline solution was administered through the left ventricle, followed by fixation in 4% paraformaldehyde. Brain tissues were taken, fixed in formaldehyde for 24 hrs, dehydrated by gradient alcohol and embedded in paraffin wax. Referring to The Rat Brain in Stereotaxic Coordinates of Paxinos and Watson, coronal plane consecutive sections were performed at a distance of -5.3 to -7.8 mm from the bregma with a thickness of 5 μm and an interval of 30 μm, comprising 8-10 sections per sample. After dewaxing, samples were washed with double-distilled water for 5 min and dyed with a 0.3% concentration of cresyl violet acetate for 120 min at 37°C, rinsed with water for 3 min, differentiated for 15 sec, dehydrated, transparent and sealed. Three fields of view in layers II-III of the entorhinal cortex were randomly selected under a 40× microscope (Leica, Germany) and the number of neurons in the three fields of view was counted using Image J1.8.0.345 software (National Institute of Health, USA). The average value was taken as the neuronal density.

Determination of neuronal dendritic spine density by Golgi silver staining: Rat brain tissues were taken. The rapid staining kit (FD Neuro Technologies, USA) was used to mix solutions A and B in a 1:1 ratio and the tissues were left to

stand for 24 hrs away from light. Brain tissue specimens were washed with double-distilled water, placed in a mixture of A and B and transferred to a 2nd portion of mixture A and B after 24 hrs. After 2 weeks, specimens were transferred to solution C, which was replaced after 24 hrs. They were placed for 4 d and then embedded in agarose. According to "The Rat Brain in Stereotaxic Coordinates of Paxinos and Watson", coronal plane consecutive sections were performed at a distance of -5.3 to -7.8 mm from bregma with a thickness of 200 μm, moderately flattened, air-dried and stained and processed in strict accordance with the instructions of the kit. After successful preparation of the sections, the dendrites of neurons in layers II-III of the entorhinal cortex were observed under low magnification, the number of dendritic spines was analyzed and counted using ImageJ software and the dendritic spine density was calculated, $\geq$20 segments of terminal dendrites were selected for each sample and the average value was taken as the dendritic spine density.

The prepared rat brain tissue sections were taken, dewaxed into double-distilled water for 5 min, washed twice with PBS, 5 min/time and blocked with 0.3% bovine serum and 10% goat serum at room temperature for 2 hrs. After adding SY38 primary antibody (Abcam, UK, 1:1000) and PSD-95 primary antibody (Millipore, USA, 1:1000) overnight, the tissue sections were washed three times with PBST, 5 min/time and added with fluorescent secondary antibody (1:1000) for incubation at the greenhouse for 60 min and the slices were sealed. Pictures of the same parts of layers II-III of the entorhinal cortex were taken using a microscope and SY38 and PSD-95 protein integral absorbance values reflecting their expression levels were analyzed using ImageJ software.

Statistical analysis: The SPSS 23.0 statistical analysis software was used. Shapiro-Wilk was used to determine whether the data were normally distributed and the measurements that conformed to normal distribution were expressed as $\overline{\chi} \pm S$. Comparisons between multiple groups were conducted using one-way ANOVA and pairwise comparisons were made using the LSD t-test for further examination, with $p<0.05$ as the statistically significant difference.

RESULTS

Comparison of the open field test results of the three groups of rats: Compared with the control group, the cognitive dysfunction group had a lower average speed of movement, a shorter residence time in the central area and a shorter total distance of movement ($p<0.05$). The low-dose ketamine group also exhibited a shorter residence time in the central area in comparison to the control group ($p<0.05$). Compared with the cognitive dysfunction group, the low-dose ketamine group had a faster average speed of movement, a longer residence time in the central area and a longer total distance of movement ($p<0.05$) (Table 1).

Comparison of Morris water maze test results of the three groups of rats: Compared with the control group, the cognitive dysfunction group had a prolonged escape latency (Fig. 1a), a slower average swimming speed (Fig. 1b) and a reduced number of times the rats across the platform position (Fig. 1c) ($p<0.05$). The low-dose ketamine group showed a prolonged escape latency (Fig. 1a) and a reduced number of times the rats across the platform position (Fig. 1c) in comparison to the control group ($p<0.05$). Compared with the cognitive dysfunction group, the low-dose ketamine group exhibited a shorter escape latency (Fig. 1a), a faster average swimming speed (Fig. 1b) and a higher number of times the rats across the platform position (Fig. 1c) ($p<0.05$).

Comparison of programmed necrosis rate of hippocampal neurons and cytoplasmic Ca^{2+} concentration of hippocampal neurons in the three groups of rats: Compared with the control group, the programmed necrosis rate of hippocampal neurons and the cytoplasmic Ca^{2+} concentration of hippocampal neurons was elevated in the cognitive dysfunction group and the programmed necrosis rate of hippocampal neurons (Fig. 2a) and the cytoplasmic Ca^{2+} concentration of hippocampal neurons (Fig. 2b) were elevated in the low-dose ketamine group ($p<0.05$). Compared with the cognitive dysfunction group, the low-dose ketamine group exhibited a lower programmed necrosis rate of hippocampus neurons (Fig. 2a) and a decreased cytoplasmic Ca^{2+} concentration of hippocampal neurons (Fig. 2b) ($p<0.05$).

Comparison of IL-6, TNF-α, s-100β and NOS of the three groups of rats: Compared with the control group, IL-6 (Fig. 3a), TNF-α (Fig. 3b), s-100β (Fig. 3c) and NOS (Fig. 3d) were elevated in the cognitive dysfunction group and IL-6 and TNF-α were elevated in the low-dose ketamine group ($p<0.05$). Compared with the cognitive dysfunction group, the low-dose ketamine group exhibited lower levels of IL-6, TNF-α, s-100β and NOS ($p<0.05$).

Comparison of p-PI3K and p-Akt of the three groups of rats: Compared with the control group, p-PI3K (Fig. 4a, b) and p-Akt (Fig. 4a, c) were lower in the cognitive dysfunction group ($p<0.05$); compared with the cognitive dysfunction group, p-PI3K (Fig. 4a, b) and p-Akt (Fig. 4a, c) were higher in the low-dose ketamine group ($p<0.05$).

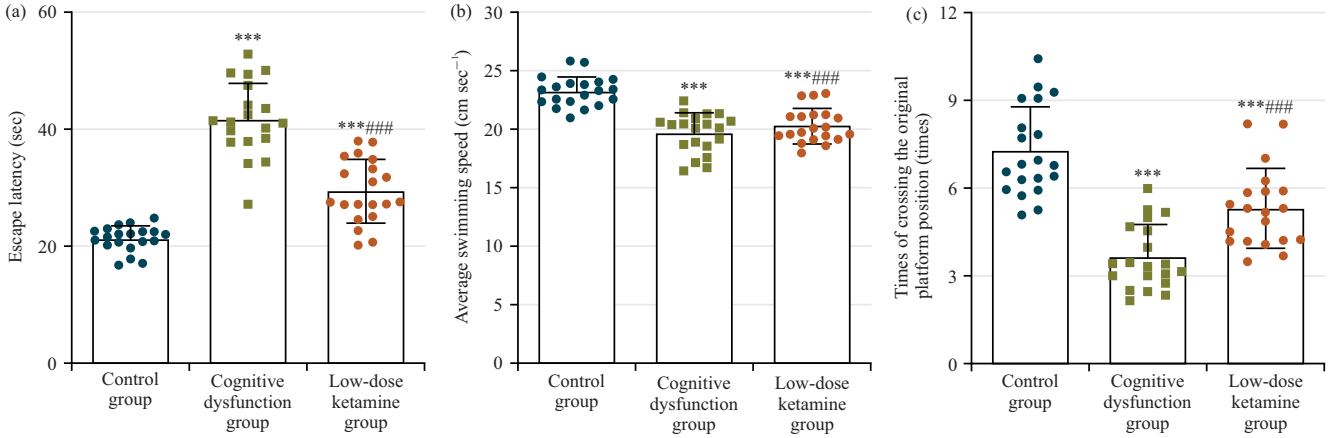

Fig. 1(a-c): Comparison of Morris water maze test results of the three groups of rats, comparison with the cognitively dysfunction group, (a) Low-dose ketamine group had a shorter escape latency, (b) Faster average swimming speed and (c) Higher number of times the rats across the platform position

Compared with the control group, ***p<0.0001, compared with the cognitive dysfunction group and ###p<0.001

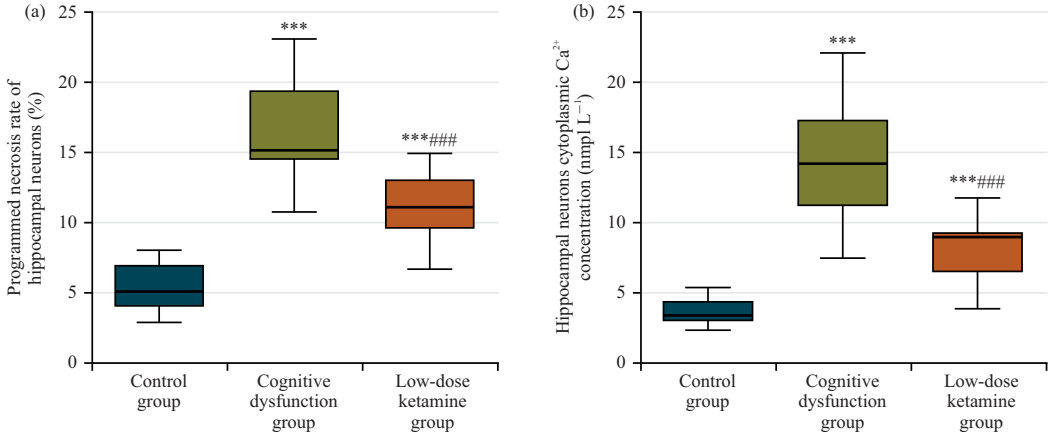

Fig. 2(a-b): Comparison of programmed necrosis rate of hippocampal neurons and cytoplasmic Ca^{2+} concentration of hippocampal neurons in the three groups of rats, comparison with the cognitive dysfunction group, (a) Programmed necrosis rate of hippocampal neuron and (b) Cytoplasmic Ca^{2+} concentration of hippocampal neuron were lower in the low-dose ketamine group

Compared with the control group, ***p<0.001, compared with the cognitive dysfunction group and ###p<0.001

Table 1: Comparison of the open-field test results of the three groups of rats ($\bar{\chi}\pm S$)

Group	Average speed of movement (cm sec^{-1})	Residence time in the central area (sec)	Total distance of movement (cm)
Control group (n = 20)	3.60±0.32	41.36±5.97	3218.42±219.53
Cognitive dysfunction group (n = 20)	3.02±0.28***	23.68±3.12***	2783.25±226.71***
Low-dose ketamine (n = 20)	3.45±0.29###	35.29±5.14**###	3081.39±234.78###

Compared with the control group, **p<0.01, ***p<0.001, compared with the cognitive dysfunction group and ###p<0.001

Comparison of synapsin I, synaptic proteins, synaptic vesicle proteins of the three groups of rats: Compared with the control group, the cognitive dysfunction group exhibited a notable reduction in synapsin I (Fig. 5a, b), synaptic proteins (Fig. 5a, c) and synaptic vesicle proteins (Fig. 5a, d) (p<0.05); compared with the cognitive dysfunction group, the low-dose ketamine group showed higher levels of synapsin I (Fig. 5a, b), synaptic proteins (Fig. 5a, c) and synaptic vesicle proteins (Fig. 5a, d) (p<0.05).

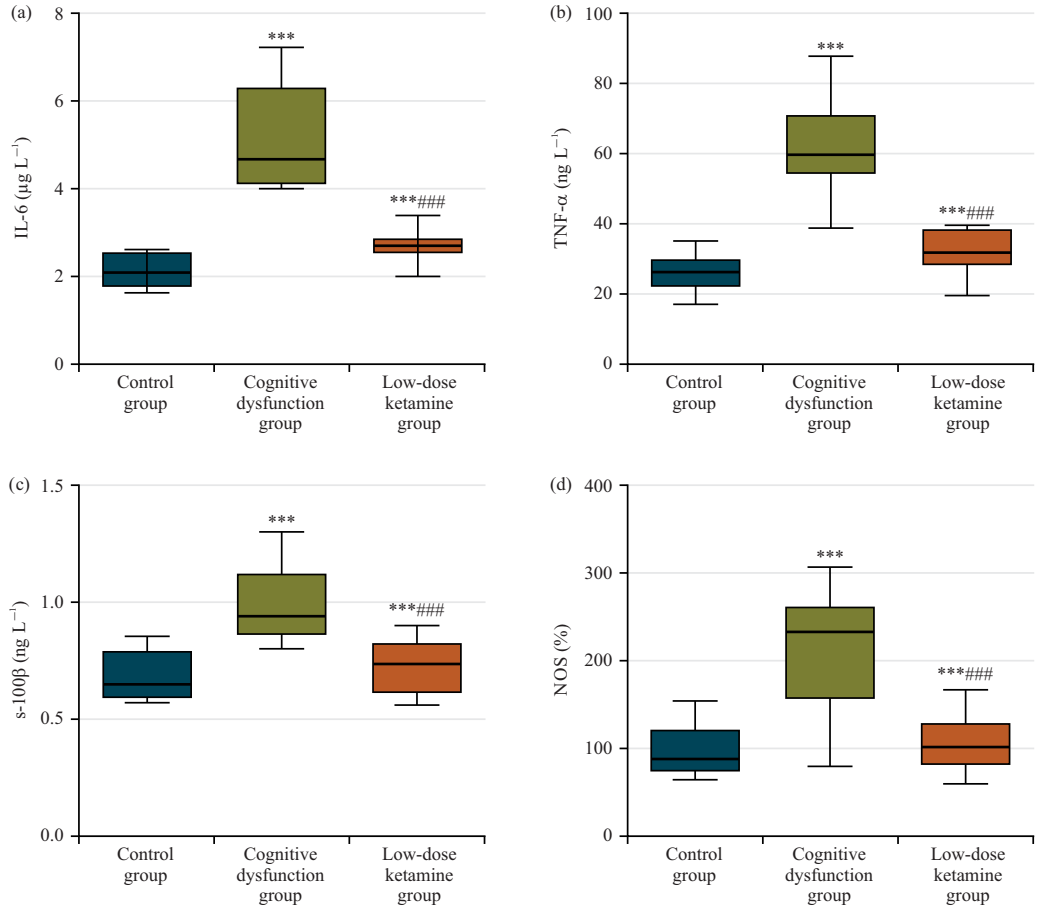

Fig. 3(a-d): Comparison of the three groups of rats, (a) IL-6, (b) TNF-α, (c) s-100β and (d) NOS in the low-dose ketamine group compared with the dysfunction group

Compared with the control group, ***p<0.001, compared with the cognitive dysfunction group and ###p<0.001

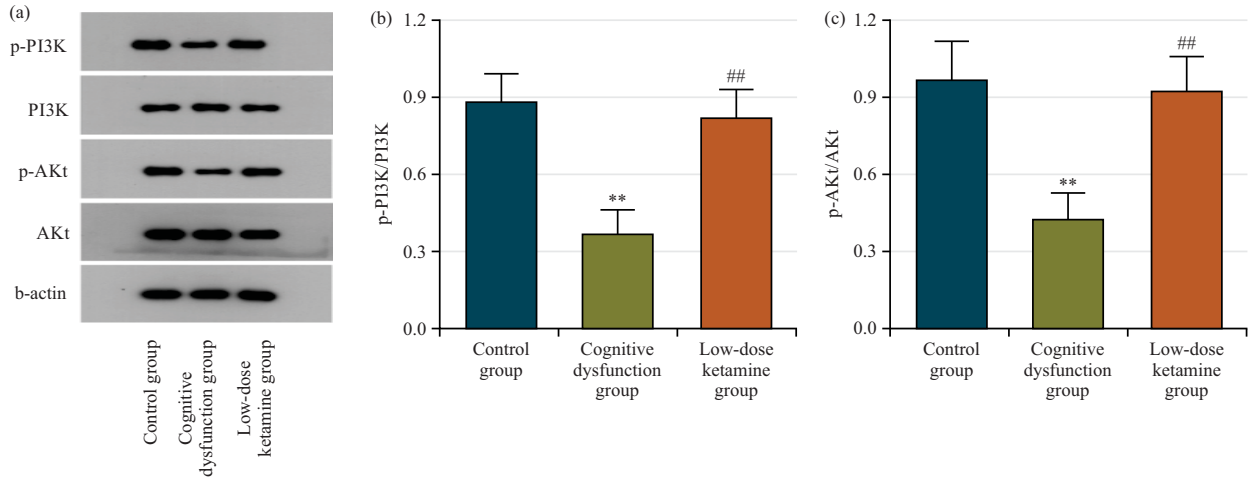

Fig. 4(a-c): Comparison of p-PI3K and p-Akt of the three groups of rats, (a) Control group, (b) p-PI3K and (c) p-Akt were higher in the low-dose ketamine group compared with the cognitive dysfunction group

Compared with the control group, **p<0.01, compared with the cognitive dysfunction group and ##p<0.01

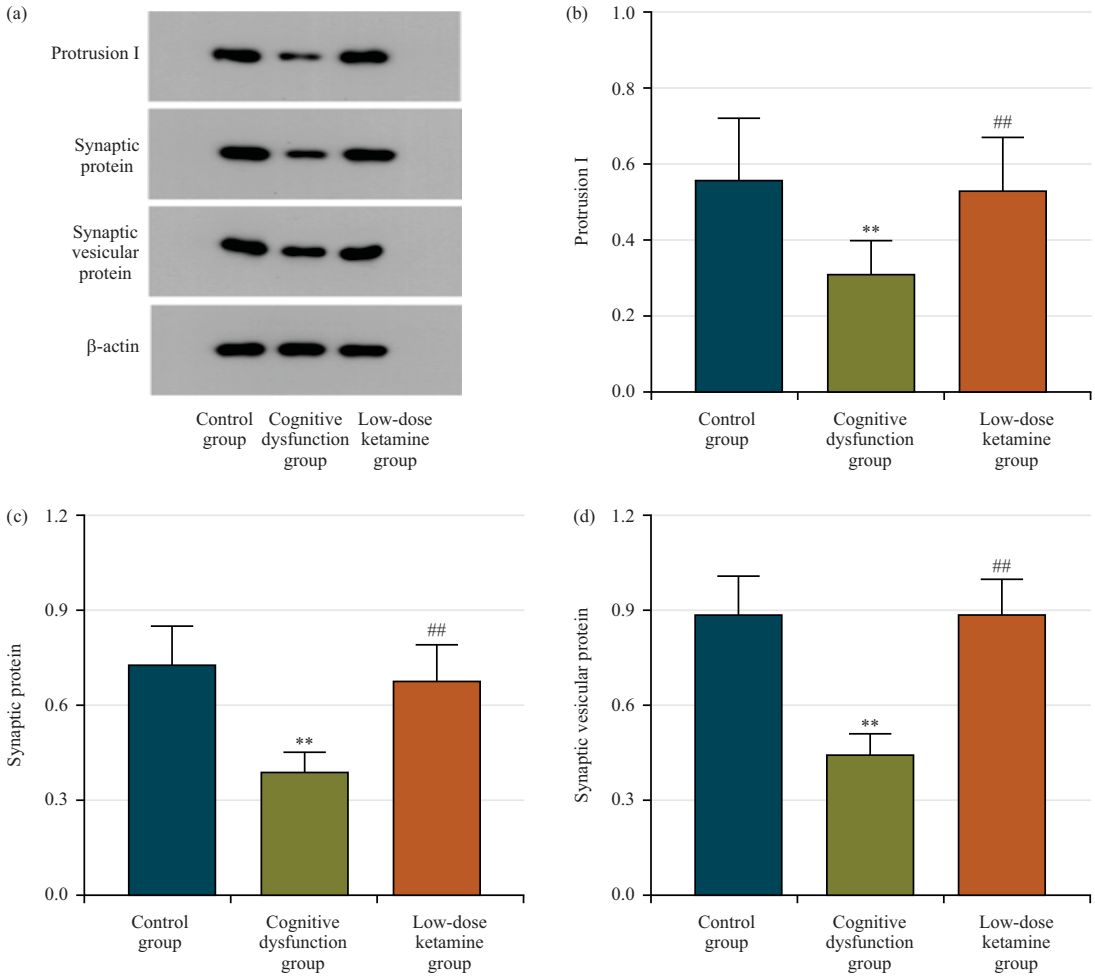

Fig. 5(a-d): Comparison of synapsin I, synaptic proteins, synaptic vesicle proteins of the three groups of rats ($\bar{\chi}\pm S$), (a) Control group, (b) Higher synapsin I, (c) Synaptic proteins and (d) Synaptic vesicle proteins in the low-dose ketamine group compared with the cognitive dysfunction group

Compared with the control group, **p<0.01, compared with the cognitive dysfunction group and $^{\#\#}$p<0.01

Table 2: Comparison of SY38 and PSD-95 of the three groups of rats ($\bar{\chi}\pm S$)

Group	SY38	PSD-95
Control group (n = 8)	176.58±28.47	133.42±19.57
Cognitive dysfunction group (n = 8)	109.56±16.43***	82.91±11.42***
Low-dose ketamine group (n = 8)	143.69±19.54$^{\#\#}$	118.63±16.45$^{\#\#\#}$

Compared with the control group, *p<0.05, ***p<0.001, compared with the cognitive dysfunction group, $^{\#\#}$p<0.01 and $^{\#\#\#}$p<0.001

Comparison of dendritic spine density and neuronal density of the three groups of rats: Compared with the control group, the cognitive dysfunction group showed reduced dendritic spine density (Fig. 6a, b) and neuronal density (Fig. 6a, c) and the low-dose ketamine group exhibited decreased dendritic spine density (p<0.05). In comparison to the cognitive dysfunction group, the low-dose ketamine group demonstrated higher dendritic spine density (Fig. 6a, b) and neuronal density (Fig. 6a, c) (p<0.05).

Comparison of SY38 and PSD-95 of the three groups of rats: In comparison to the control group, the cognitive dysfunction group showed a decrease in SY38 and PSD-95 (p<0.05). The low-dose ketamine group exhibited a decrease in SY38 compared to the control group (p<0.05). When compared to the cognitive dysfunction group, the low-dose ketamine group demonstrated higher SY38 and PSD-95 levels (p<0.05) (Table 2).

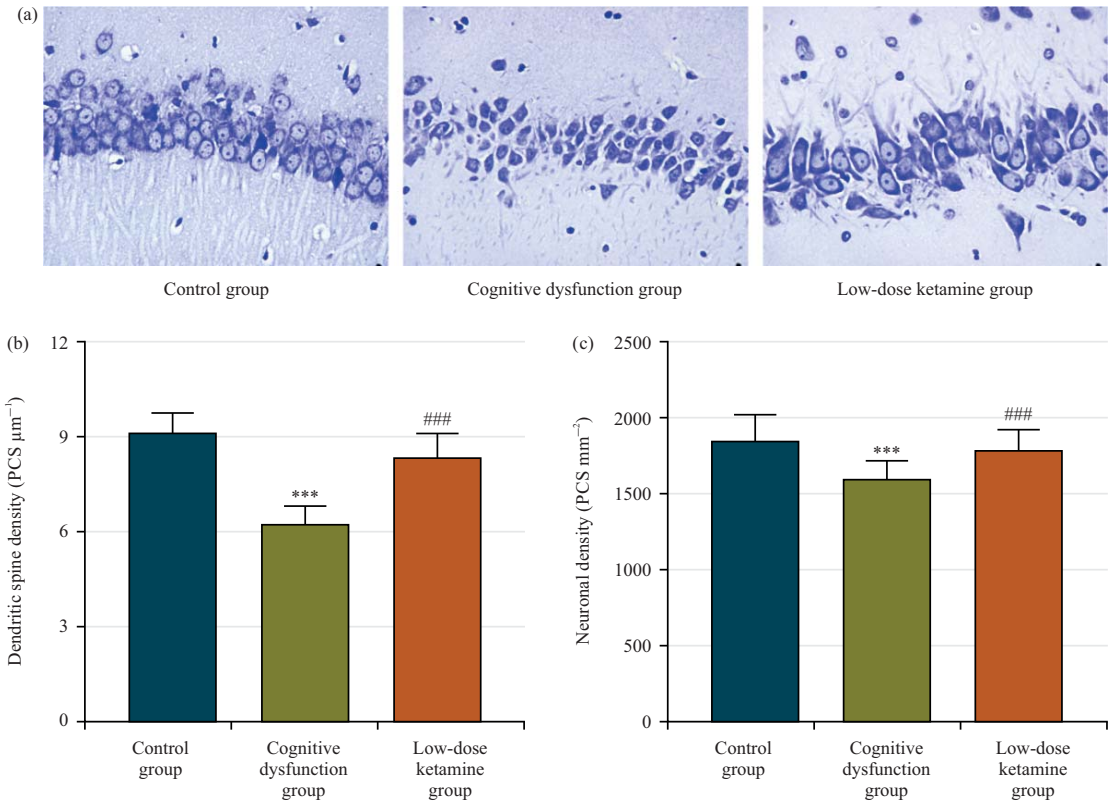

Fig. 6(a-c): Comparison of dendritic spine density and neuronal density of the three groups of rats, (a) Control group, (b) Higher dendritic spine density and (c) Neuronal density in the low-dose ketamine group compared to the cognitive dysfunction group

Compared with the control group, ***p<0.001, compared with the cognitive dysfunction group and ###p<0.001

DISCUSSION

Due to the diminished physical function and relatively poorer psychological resilience of elderly patients, they are susceptible to stress responses during the anesthesia process, leading to a higher incidence of post-anesthesia cognitive dysfunction. Cognitive dysfunction is notably severe, resulting in challenges with abstract thinking, memory impairment and brain dysfunction and may be accompanied by a decline in social function[8,9]. It has been noted that cognitive dysfunction after anesthesia is associated with central nervous system inflammation in patients[10]. However, the mechanism of inflammation has not been clarified, so it is important to explore its occurrence for the prevention and treatment of cognitive dysfunction.

The open field test and the Morris water maze test stand as two contemporary classic experiments in the realm of learning and memory studies, which are widely employed in the cognitive function assessment of rodent animal experiments. In this study, after sevoflurane anesthesia was administered to rats, the results of behavioral experiments showed that compared with the control group, the cognitive dysfunction group had a lower average speed of movement, a shorter residence time in the central area, a shorter total distance of movement, a longer escape latency, a slower average swimming speed and a lower number of times the rats across the platform position, indicating that sevoflurane anesthesia induced cognitive dysfunction model in rats was successfully prepared; compared with the cognitive dysfunction group, the low-dose ketamine group showed a faster average speed of movement, longer residence time in the central area, longer total distance of movement, shorter escape latency, faster average swimming speed and higher number of times the rats across the platform position, suggesting that the low-dose ketamine was able to improve the cognitive function of rats. The N-methyl-D-aspartate receptors are widely distributed in the hippocampal and cortical tissues of the brain and are involved in learning, memory, emotion and mood regulation, which are closely related to cognitive functions[11]. The N-methyl-D-aspartate

receptor activation increases intracellular cytoplasmic Ca^{2+} concentration and mediates neuronal necrosis inducing neurotoxicity and affecting cognitive function through related mechanisms[12]. Ketamine acts as an N-methyl-D-aspartate receptor inhibitor, exhibiting strong affinity for the N-methyl-D-aspartate receptor. Upon entering the organism, this medication binds to the ion channel pore of the N-methyl-D-aspartate receptor, disrupting its coupling with calcium channels, reducing Ca^{2+} influx, modulating cytoplasmic Ca^{2+} concentration, inhibiting the expression of programmed necrosis-related proteins and decreasing the programmed necrosis rate of hippocampal neurons[13,14].

The PI3K/Akt is one of the classical signaling pathways regulating apoptosis and plays an important role in the process of many neurodegenerative diseases, including regulating the transcription of brain-derived neurotrophic factors and other neuropeptides, promoting the hyperphosphorylation of tau proteins, influencing synaptic plasticity, affecting mitochondrial function, participating in the progression of neuroinflammation and regulating neuronal growth, differentiation, proliferation and apoptosis[15,16]. Inhibition of the PI3K/Akt signaling pathway in hippocampal and cortical tissues can lead to spatial learning deficits and neurodegenerative pathologies[17]. The results of this study showed that after sevoflurane anesthesia, p-PI3K and p-PI3K were decreased in the cognitive dysfunction group, while p-PI3K and p-PI3K were higher in the low-dose ketamine group than in the cognitive dysfunction group, suggesting that sevoflurane inhibits the PI3K/Akt pathway and impairs cognitive function. In contrast, low doses of ketamine can activate the inhibited PI3K/Akt pathway, exerting a series of beneficial effects such as limiting neuronal cell inflammation, ameliorating neuronal damage, promoting endothelial cell survival and improving cognitive function[18].

Studies have pointed out that inflammation can impair brain function and brain damage is an important mechanism for the occurrence of cognitive dysfunction[19]. The IL-6 and TNF-α are important factors produced by the body to fight against inflammation, which can aggravate neuroinflammation; s-100β protein is a biomarker reflecting the degree of brain damage, which plays an important role in exploration and learning and is associated with neurobehavioral impairments such as responsiveness and memory, which have an important impact on cognitive function; NOS is a key enzyme in the NMDA signaling pathway and its excessive expression may lead to a pathological reaction in organs[20,21]. The results of this study showed that IL-6, TNF-α, s-100β and NOS were lower in the low-dose ketamine group than in the cognitive dysfunction group, suggesting that low-dose ketamine can alleviate neuroinflammatory responses and improve neurobehavior such as responsiveness and memory through the mechanism of modulating inflammation levels. Low doses of ketamine can reverse sevoflurane-induced oxidative stress in the brain, inhibit excitatory neurotoxicity, reduce apoptosis and inhibit the levels of inflammatory factors, such as IL-6 and TNF-α, thereby exerting neuroprotective effects.

The entorhinal cortex serves as a relay station for information transmission in the hippocampal-cortical neural circuitry, which plays a pivotal role in sustaining cognitive functions. Abnormal plasticity of neuronal dendritic spines can impede neural circuitry, triggering cognitive impairments[22,23]. Dendritic spines are primarily composed of postsynaptic density, skelemin, signaling protein molecules and transmitter receptors. The SY38 is a vesicle membrane protein specifically distributed in the presynaptic membrane, involving in the circulation of the vesicular membrane and transmitter release, constituting an important protein molecule in synaptic ultrastructure; PSD-95 is the main postsynaptic density protein, which plays an important role in regulating the cytoskeletal structure and both of them play an crucial part in the process of information transmission[24,25]. In this study, synapsin I, synaptic proteins, synaptic vesicle proteins, dendritic spine density, neuronal density and SY38 and PSD-95 were higher in the low-dose ketamine group than in the cognitive dysfunction group, suggesting that low doses of ketamine can reduce cognitive dysfunction by affecting the expression of vital structural proteins, such as SY38 and PSD-95 within the dendritic spine and attenuating the damage to the dendritic spine of entorhinal cortex neurons. Low doses of ketamine can increase the release of brain-derived trophic factor and glutamate, activate the mTOR signaling pathway and upregulate the expression of synapse-related proteins such as SY38 and PSD-95, which ultimately serve to improve cognitive function.

CONCLUSION

Pre-infusion of low-dose ketamine may alleviate cognitive dysfunction after sevoflurane anesthesia in aged rats and the mechanism may be related to the inhibition of programmed necrosis of hippocampal neurons, activation of the hippocampal PI3K/Akt pathway and improvement of neuronal synaptic plasticity. The findings may provide a theoretical basis for the prevention and treatment of the neurotoxic effects induced by propofol anesthesia. However, since this is only an animal experiment, large-scale clinical studies are still needed to verify the specific clinical effects and mechanisms.

SIGNIFICANCE STATEMENT

The administration of propofol anesthesia can induce neurotoxic effects, leading to long-term cognitive impairments and in severe cases, it may elevate the risk of dementia. In this study, it has been observed that low doses of ketamine can mitigate the extent of cognitive impairments caused by propofol anesthesia in rats, enhance learning and memory capabilities, inhibit neuronal apoptosis, mitigate inflammatory responses, alter synaptic plasticity and its mechanism of action is associated with the activation of the PI3K/Akt signaling pathway. The findings may provide a theoretical basis for the prevention and treatment of the neurotoxic effects induced by propofol anesthesia.

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