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Research Article
Effect of Low Doses of Ketamine in Mitigating Cognitive Impairment Induced by Propofol Anesthesia in Rats

[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: Propofol anesthesia induces certain neurotoxic effects, leading to apoptosis of hippocampal neurons during the developmental stage of the brain, thereby triggering postoperative cognitive dysfunction (POCD). Ketamine combined with propofol anesthesia is safer and promotes postoperative cognitive recovery, but the specific mechanism of action remains to be further elucidated. This study aims to elucidate the efficacy and underlying mechanism of low doses of ketamine in ameliorating cognitive impairment engendered by propofol anesthesia in a rat model. **Materials and Methods:** Thirty-two, 20-day-old SPF rats were randomly assigned to group C (100 mg/kg fat emulsion), group P (50 mg/kg propofol), group EP (10 mg/kg ketamine, 50 mg/kg propofol) and group LYEP (25 μg LY294002, followed by ketamine-propofol after 30 min), n = 8 each. The hippocampal neuronal apoptosis, expression of PI3K/Akt signaling pathway-related and synaptic plasticity-related proteins and pro- and anti-inflammatory factor levels were evaluated. **Results:** Compared to group P, groups EP and LYEP exhibited elevated P-Akt/Akt, IL-4, IL-10, GAP-43, caveolin-1, NCAM, hippocampal neuron counts and Y-maze correct response rate, decreased Bax, cleaved caspase-3, IL-1β, IL-2 and neuronal apoptosis on days 1 and 3 post-administration, reduced escape latency on days 1, 3, 5 and 7 ($p<0.05$) and diminished neuropathological damage in the hippocampal CA1 area. **Conclusion:** Administering low doses of ketamine can mitigate cognitive dysfunction induced by propofol anesthesia, enhance learning and memory capabilities, inhibit neuronal apoptosis, reduce inflammatory response and alter synaptic plasticity in rats. Its mechanism of action is linked to the activation of the PI3K/Akt signaling pathway.

Key words: Ketamine, propofol, cognitive dysfunction, PI3K/Akt signaling pathway, synaptic plasticity

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Corresponding Author: Jiaping Zhao, Department of Anesthesiology, Beijing Ditan Hospital Capital Medical University, Beijing 100015, China

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

Propofol, a preeminent short-acting intravenous anesthetic, is extensively utilized in clinical settings, distinguished by its rapid onset and recovery, lack of significant accumulation and primarily employed in the induction and maintenance of general anesthesia[1,2]. Notwithstanding its widespread use, animal studies have illuminated the neurotoxic effects of propofol anesthesia. These studies indicate that propofol can precipitate apoptosis in hippocampal neurons within the developing brain, consequently instigating postoperative cognitive dysfunction (POCD)[3]. Cognitive function, encompassing diverse domains such as memory, attention, orientation and computation, is critically impacted. Clinically, POCD is characterized by symptoms including anxiety, memory impairment, disrupted cognitive functioning, confusion, personality alterations and in severe cases, an elevated risk of dementia[4].

Ketamine, functioning as a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor ion channel, selectively impedes nociceptive transmission and inhibits the thalamo-neocortical system[5]. A domestic study revealed that a ketamine-propofol anesthesia induction regimen enhances safety, abbreviates anesthesia duration, mitigates inflammatory stress response, improves hemodynamics and fosters postoperative cognitive recovery. However, the precise mechanisms underlying these effects warrant further exploration[6]. The PI3K/Akt signaling pathway, a quintessential pro-survival and anti-apoptotic cascade modulates an array of cellular processes including survival, motility, invasion, neovascularization, growth and proliferation[7]. Activation of this pathway has been implicated in the inhibition of neuroinflammation and neuronal apoptosis, reducing the extent of neuronal damage[8]. Consequently, stimulating the PI3K/Akt pathway may represent a prospective therapeutic approach to forestall POCD. Chen *et al.*[9] demonstrated in a murine model that ketamine attenuates neuroinflammation and oxidative stress while diminishing autophagy in hippocampal neurons by activating the PI3K/AKT signaling pathway. Building upon these findings, the current study scrutinizes the efficacy and mechanism of low-dose ketamine in mitigating cognitive dysfunction induced by propofol anesthesia in rats, endeavoring to furnish insights for the prevention and treatment of POCD during the perioperative period.

MATERIALS AND METHODS

Study area: The animal experiment of this study was implemented during November, 2021 and April, 2022 in Beijing Daxing District Hospital of Integrated Chinese and Western Medicine.

Materials: The 32 specific pathogen-free (SPF) rats were procured from the Department of Laboratory Animal Science, Peking University Health Science Center (Beijing, China), comprising both male and female subjects, aged 7 days, with a body mass of 12.5±2.0 g. The animals were housed under controlled conditions: Room temperature set at (25±1°C), humidity maintained between 20-30% and subjected to a 12 hrs day/night cycle of lighting. The rats had unrestricted access to food and water and were acclimatized for a period of 7 days.

Ethical consideration: All procedures involving animal experimentation received approval from the Animal Ethics Committee of Beijing Daxing District Hospital of Integrated Chinese and Western Medicine.

Main reagents and instruments

Main reagents and instruments utilized in this study include: Ketamine injection (Jiangsu Hengrui Pharmaceutical Co. Ltd., National Drug License H20193336); Medium/long-chain fatty milk injection (Guangzhou Baxter Qiaoguang Medical Supplies Co. Ltd., National Drug License H20010214); Propofol emulsion injection (Jiangsu Yingke Bio-Pharmaceutical Co. Ltd., National Drug License H20223914); LY294002 (Shanghai Yuanye Bio-technology Co. Ltd., Stock No. S43088-500 mg); TUNEL Apoptosis Detection Kit (Wuhan Doctor Bioengineering Co. Ltd.); Y Maze (Zhangjiagang Biomedical Instrument Factory); Optical Microscope (Shenzhen Zike Biotechnology Co. Ltd.); Western blot electrophoresis apparatus (Bio-Rad, USA); Horseradish peroxidase-labeled goat-anti-rabbit IgG (Solabao Technology Co. Ltd., China); Antibodies for P-Akt/Akt, GAP-43, Caveolin-1, GAPDH, etc. (CST Biotechnology, USA); TUNEL kit (Nanjing Novozymes Bio-technology Co. Ltd., China); Nysted Staining Solution (Shanghai Biyuntian Biotech, Co. Ltd., China); ELISA kit (Shanghai Fanshi Biotechnology Co. Ltd.), etc.

Grouping and experimental design: The 32 SPF-grade rats were randomly allocated into four groups, each consisting of eight rats:

- **Fat emulsion group (group C):** This group received an intraperitoneal injection of 100 mg/kg medium/long-chain fat emulsion
- **Propofol group (group P):** Rats in this group were administered an intraperitoneal injection of 50 mg/kg propofol, followed by an additional 50 mg/kg dose upon recovery of the flip-flopping reflex
- **Ketamine+propofol group (group EP):** This group was given an intraperitoneal injection of 10 mg/kg ketamine, followed by the propofol administration regimen identical to that of group P
- **PI3K inhibitor ly294002+ketamine+propofol group (LYEP group):** Rats were initially anesthetized with an intraperitoneal injection of chloral hydrate. Subsequently, 25 μg of LY294002 was administered through the lateral ventricle, with the needle retained in place for 5 min to prevent drug extravasation. Post needle withdrawal and scalp suturing, the rats were injected with ketamine and propofol after 30 min, following the same protocol as the EP group

Post-administration of each drug, the rats were placed in a thermostat with a continuous oxygen supply at a rate of 2 L/min to ensure optimal recovery conditions.

Histological staining and analysis: The 2 hrs after emerging from anesthesia, four rats from each group were randomly selected and anesthetized with an intraperitoneal injection of 50 mg/kg 2% sodium pentobarbital. The brains were then swiftly excised, with a particular focus on isolating the hippocampal tissues, which were conducted on an ice table to preserve tissue integrity. Subsequently, hippocampal tissue blocks not exceeding 0.5 cm in thickness were fixed in 10% formalin. The samples then underwent protein denaturation and coagulation as per the Hematoxylin and Eosin (H&E) staining kit instructions. The neuronal pathology within the hippocampal CA1 area was meticulously examined under a light microscope (Shenzhen ZiKer Biological Technology Co. Ltd., Shenzhen, China) at 400× magnification.

Neuronal apoptosis was quantified following the protocol provided in the TUNEL kit (Vazyme Biotech Co. Ltd., Nanjing, China).

The proportion of neuronal apoptosis was calculated using the formula[10]:

$$\frac{\text{Number of apoptotic cells}}{\text{Total number of cells}} \times 100\%$$

Thereby determining the extent of apoptotic incidence in the hippocampal tissue.

Nissl staining: Hippocampal tissue from the rats was initially fixed using 4% paraformaldehyde and then embedded in paraffin. The tissue was sectioned to a thickness of 5 μm and subjected to a series of treatments including deparaffinization, rehydration through a graded ethanol series (anhydrous ethanol for 5 min, 90% ethanol for 2 min and 70% ethanol for 2 min) and washing with double-distilled water for 2 min. Subsequently, the sections were cleared in xylene for 5 min and mounted using a neutral resin. The neuronal population in the hippocampal regions was assessed under a microscope.

Western blot analysis: Hippocampal tissues were dissected into small pieces and placed into an Eppendorf (EP) tube. Lysis buffer was added and the tissue was homogenized for 1 min. The homogenate was then centrifuged for 10 min (centrifugation radius of 10 cm, speed of 12,000 rpm), after which the supernatant containing the protein extract was collected. For each sample, 50 μg of protein was subjected to SDS-PAGE electrophoresis, followed by membrane transfer. The membrane was blocked for 1 hr and subsequently incubated overnight at 4°C with primary antibodies, including rabbit anti-Akt, rabbit anti-Bax and rabbit anti-GAP-43, all diluted at 1:1000. The following day, the membranes were allowed to return to room temperature for 30 min, washed three times with tris-buffered saline (TBS) and then incubated with secondary antibodies diluted at 1:5000. After further washes in TBS, the membranes were developed using an electrochemiluminescence (ECL) luminescent solution. Quantitative analysis of the protein bands was conducted using Image J V1.8.0.112 software. The GAPDH, diluted at 1:1000, served as the internal reference, with the ratio of band intensities indicating the relative content of each target protein.

ELISA: Rat hippocampal tissue was homogenized in a solution containing 0.9% sodium chloride, with the volume of the solution being 9 times that of the tissue sample. The homogenate was then centrifuged for 10 min (centrifugation radius of 10 cm, speed of 12,000 rpm). The supernatant was collected for the assessment of interleukin levels, including IL-1β, IL-2, IL-4 and IL-10, using an Enzyme-Linked Immunosorbent Assay (ELISA).

Statistical analysis: The data acquired in this study were analyzed using SPSS statistical software, version 24.0.

Measurement data, conforming to a normal distribution, were expressed as Mean±Standard deviation ($\bar{x}\pm S$). For comparisons among different groups, One-way Analysis of Variance (ANOVA) was employed. Subsequent pairwise comparisons between groups were conducted using the Least Significant Difference test (LSD-t). A p-value of less than 0.05 was deemed indicative of statistical significance.

RESULTS

Neuronal pathology in the hippocampal CA1 area of rats: The histological examination of the hippocampal CA1 area revealed distinctive variations among the groups:

- **Group C (Control):** Neurons within the CA1 region exhibited a neat arrangement, intact morphology, clearly defined nuclei and uniformly stained cytoplasm
- **Group P (Propofol):** Neurons in the CA1 area appeared structurally disorganized. Notably, nucleoli were observed to be shrunken and segmented and cells often assumed irregular or triangular shapes, indicative of cellular damage
- **Group EP (ketamine+propofol):** While there was some disorganization in the neuronal arrangement within the CA1 area, the extent of pathological damage was markedly less severe compared to group P
- **Group LYEP (PI3K inhibitor LY294002+ketamine +propofol):** Neuronal disorganization in the CA1 area was apparent; however, the degree of pathological damage observed was more pronounced than that in group EP (Fig. 1)

Expression of PI3K/Akt signaling pathway-related proteins in rat hippocampal tissue

In terms of the PI3K/Akt signaling pathway-related proteins: In group P, EP and LYEP, the level of phosphorylated Akt (P-Akt/Akt) was lower (Fig. 2a-b), while Bax (Fig. 2a and c) and cleaved caspase-3 (Fig. 2a and d) levels were higher compared to group C ($p<0.05$). Group EP and LYEP exhibited higher levels of P-Akt/Akt (Fig. 2a and b) and lower levels of Bax (Fig. 2a and c) and cleaved caspase-3 (Fig. 2a and d) in comparison to group P ($p<0.05$). The hippocampal tissue of the EP group showed a higher expression of P-Akt/Akt (Fig. 2a and b) and lower levels of Bax (Fig. 2a and c) and cleaved caspase-3 (Fig. 2a and d) when compared to the LYEP group ($p<0.05$) (Fig. 2).

Y-maze experiment outcomes in rats: In the Y-maze experiment, the correct response rate in the propofol (P), ketamine+propofol (EP) and PI3K Inhibitor LY294002+ ketamine+propofol (LYEP) groups was found to be lower than that in the Control (C) group. Correspondingly, the number of trials required for learning in these groups exceeded that of the C group ($p<0.05$). Conversely, both the EP and LYEP groups demonstrated a higher correct response rate and required fewer learning trials compared to the P group ($p<0.05$). Notably, the EP group exhibited a superior correct response rate and necessitated fewer trials for learning than the LYEP group ($p<0.05$). These results were tabulated in Table 1.

Rat avoidance latency periods: Analysis of avoidance latency periods revealed that on the 1, 3, 5 and 7th days, these periods were longer in the P, EP and LYEP groups compared to the C group ($p<0.05$). However, the EP and LYEP groups displayed shorter avoidance latency periods on these days relative to the P group ($p<0.05$). Additionally, the EP group showed shorter latency periods on these days when compared to the LYEP group ($p<0.05$). These findings were detailed in Table 2.

Target quadrant percentage in rats: In the evaluation of the percentage of time spent in the target quadrant, the P, EP and LYEP groups showed lower percentages on the 1, 3, 5

Table 1: Comparison of the results of Y-maze test ($\bar{\chi}\pm s$)

Group	Correct response rate (%)	Number of training sessions required for learning (times)
C group	84.63±4.84	12.85±3.62
P group	59.63±2.74***	25.85±2.63***
EP group	78.05±2.88******^^^	20.05±3.32******^
LYEP group	73.69±3.65****###	22.84±2.15***#

Compared to group C, ***p<0.001, Compared to group P, #p<0.05, ###p<0.001, Compared to group LYEP, ^p<0.05 and^^^p<0.001

Table 2: Comparison of escape latency periods in 4 groups of rats ($\bar{\chi}\pm s$, s)

Group	1st day	3rd day	5th day	7th day
C group	17.32±3.26	15.68±2.26	16.86±3.62	16.95±2.07
P group	60.32±6.65***	51.85±5.52***	40.25±5.25***	30.02±4.69***
EP group	35.62±5.52***###^	30.16±4.62***###^	24.02±3.69***###^^	21.68±3.69***###^
LYEP group	41.65±4.98****###	36.25±5.25****###	30.33±3.84***###	25.69±3.32***#

Compared to group C, ***p<0.001, Compared to group P, #p<0.05, ###p<0.001 and Compared to group LYEP, ^p<0.05 and ^^p<0.01

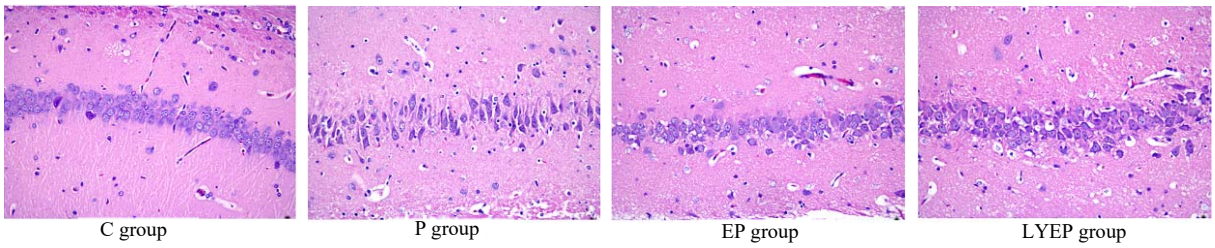

Fig. 1: Neuronal pathology in the hippocampal CA1 region of rats

Figure delineates the neuronal pathology within the CA1 area of the hippocampus across the different groups. In the propofol (P) group, it is evident that the neurons in the hippocampal CA1 area were structurally disorganized, with nucleoli that were shrunken and cleaved, assuming irregular or triangular shapes, indicative of cellular damage. In contrast, the ketamine+propofol (EP) group exhibited a less severe disorganization of neurons within the CA1 area of the hippocampus, with a correspondingly lesser degree of pathological damage compared to the P group

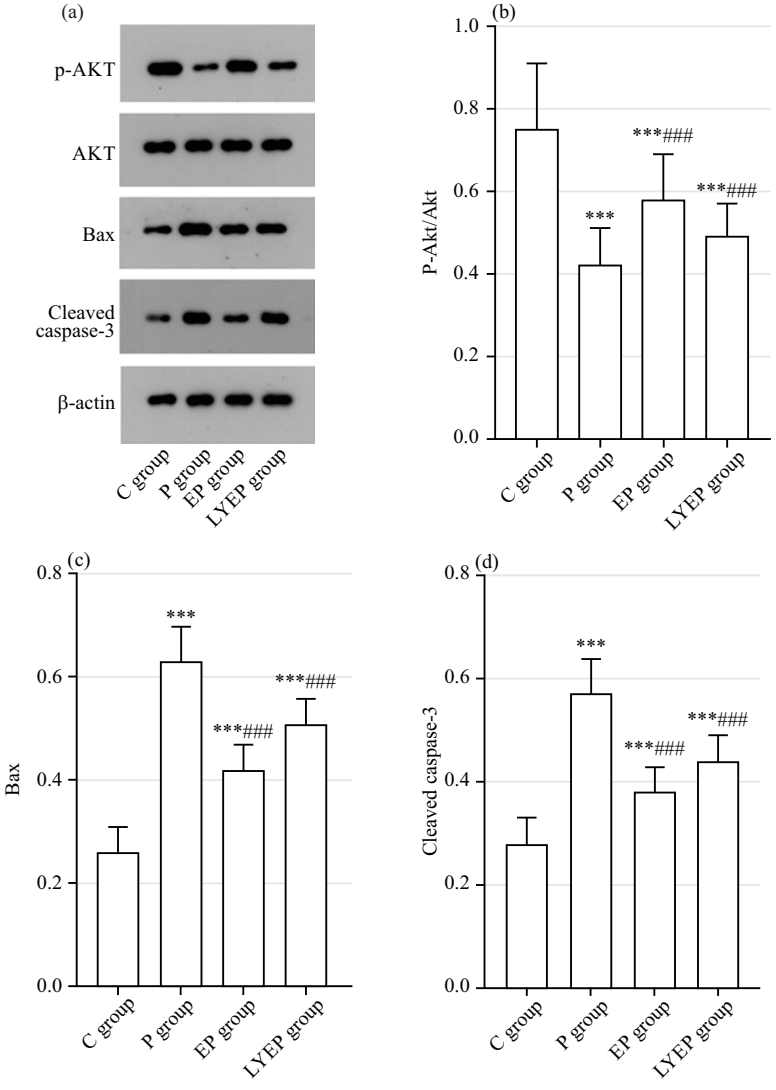

Fig. 2(a-d): Comparison of PI3K/Akt signaling pathway-related protein expression in the hippocampus of 4 groups of rats, (a) Shows the protein banding diagram Compared to group C, (b) p-Akt/Akt was higher than that of LYEP group and (c) Bax and (d) Cleaved caspase-3 were lower than that of LYEP group

Hippocampal tissue of EP group, ***p<0.001, Compared to group P, ###p<0.001

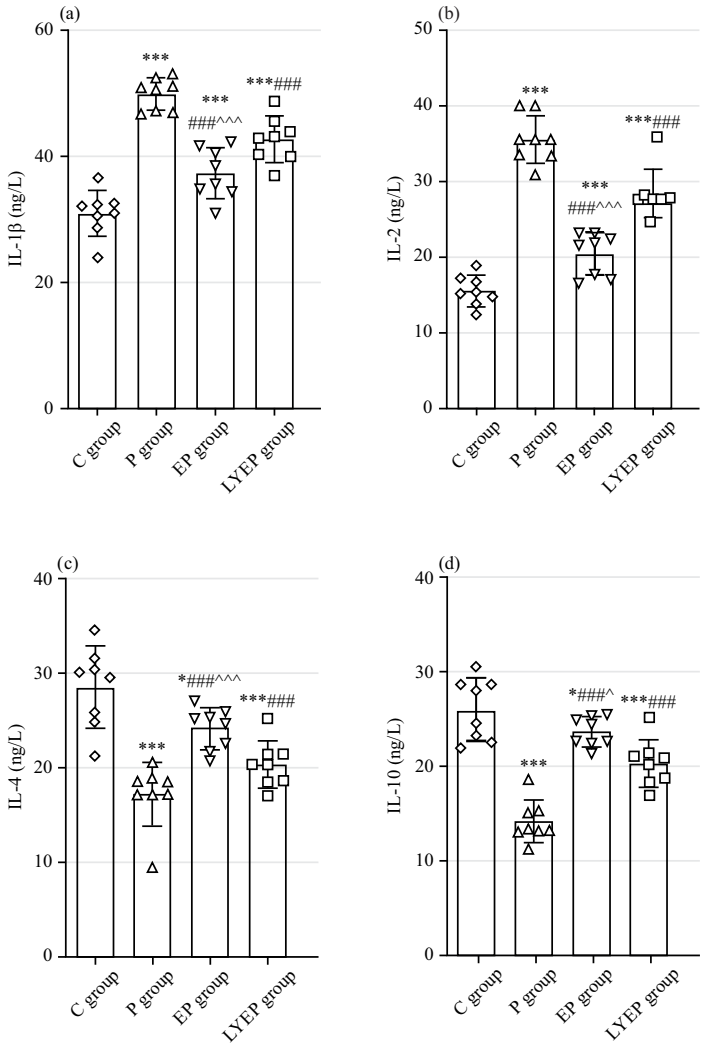

Fig. 3(a-d): Comparison of pro-inflammatory/anti-inflammatory factor levels in the hippocampal tissues of rats in the 4 groups (ng/L), (a) IL-1β and (b) IL-2 in the hippocampal tissues of EP and LYEP groups were lower than those of P group and the levels of (c) IL-4 and (d) IL-10 were higher than those of P group

Levels of Compared with group C, *p<0.05, p<0.001, Compared with group P, ###p<0.001, Compared with group LYEP, ^p<0.05 and ^^^ p<0.001

Table 3: Comparison of the percentage of target quadrant of rats in 4 groups ($\bar{\chi}\pm s$, %)

Group	1st day	3rd day	5th day	7th day
C group	38.56±2.84	42.28±3.35	44.58±4.02	47.36±4.96
P group	20.35±4.52***	26.84±4.11***	33.25±4.15***	37.52±3.25***
EP group	35.63±2.51***#^	38.52±4.02**###^	41.25±2.53***#^	44.02±2.58***#^
LYEP group	31.63±4.11***###	34.20±3.32***###	38.54±3.65***###	41.68±4.10***#

Compared to group C, *p<0.05, **p<0.01, ***p<0.001, Compared to group P, #p< 0.05, ###p<0.001 and Compared to LYEP group, ^p<0.05

and 7th days compared to the C group (p<0.05). However, the percentages in the EP and LYEP groups were higher on these days compared to the P group (p<0.05). Furthermore, the EP group outperformed the LYEP group in terms of target quadrant percentage on these days (p<0.05). These data are presented in Table 3.

Pro/anti-inflammatory factor levels in rat hippocampal tissue: Upon analyzing the levels of pro- and anti-inflammatory cytokines in hippocampal tissues: The concentrations of IL-1β (Fig. 3a) and IL-2 (Fig. 3b) were elevated in the propofol (P), ketamine+propofol (EP) and PI3K inhibitor LY294002+ketamine+propofol (LYEP) groups

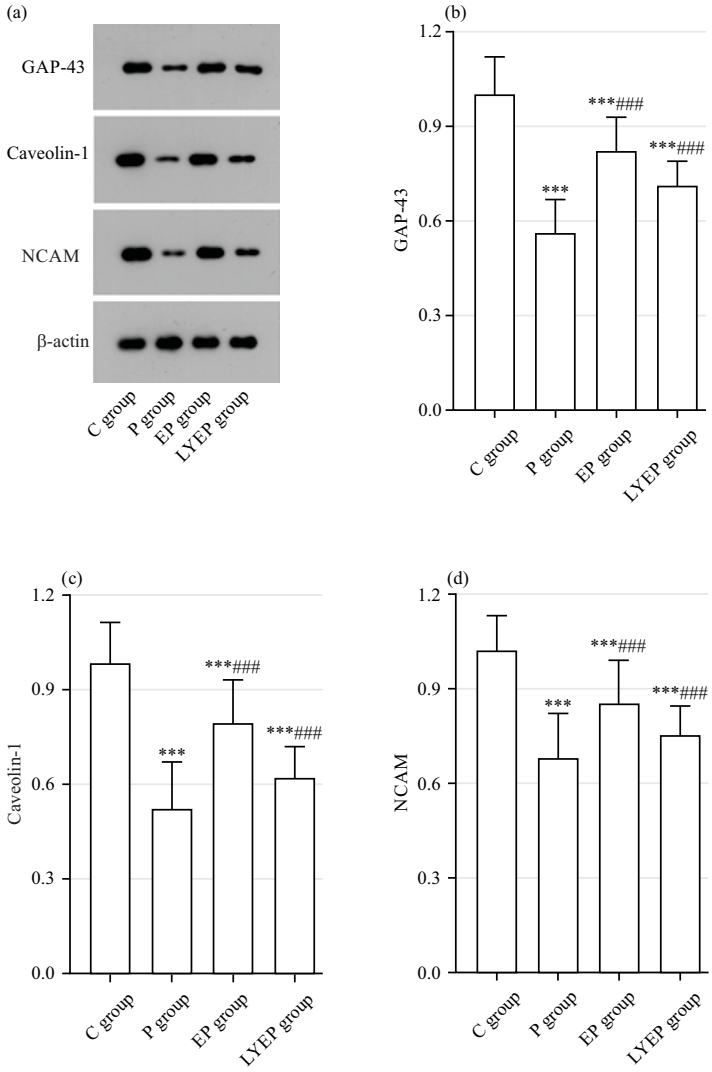

Fig. 4(a-d): Comparison of synaptic plasticity-related protein expression in hippocampal tissues of 4 groups, (a) Western blot image, (b) GAP-43, (c) Caveolin-1 and (d) NCAM were higher in EP and LYEP groups than in P group.
Figure shows that, Compared to group C, ***p<0.001, Compared to group P, ###p<0.001, Compared to group LYEP and ^^^p<0.001

Table 4: Comparison of the number of hippocampal neurons and the proportion of neuronal apoptosis in 4 groups of rats (x̄±sec)

Group	Number of hippocampal neurons (pcs/field of view)	Proportion of neuronal apoptosis (%)
Group C	35.25±4.02	2.36±0.51
P group	12.36±3.65***	15.96±2.65***
EP group	26.52±3.26******^^^	6.21±3.25******^^^
LYEP group	19.63±2.85******	10.65±2.15******

Compared with group C, ***p<0.001, Compared with group P, ###p<0.001 and Compared with group LYEP, ^^^p<0.001

compared to the Control (C) group, whereas the levels of IL-4 (Fig. 3c) and IL-10 (Fig. 3d) were diminished (p<0.05). In the EP and LYEP groups, IL-1β (Fig. 3a) and IL-2 (Fig. 3b) levels were lower and IL-4 (Fig. 3c) and IL-10 (Fig. 3d) levels were higher than in the P group (p<0.05). The EP group exhibited lower levels of IL-1β (Fig. 3a) and IL-2 (Fig. 3b) and higher levels of IL-4 (Fig. 3c) and IL-10 (Fig. 3d) compared to the LYEP group (p<0.05) (Fig. 3).

Hippocampal neuron count and neuronal apoptosis proportion: The count of neurons in the hippocampus was lower in the P, EP and LYEP groups compared to the C group. Correspondingly, these groups also showed a higher proportion of neuronal apoptosis (p<0.05). The EP and LYEP groups had a higher neuron count and a lower proportion of neuronal apoptosis compared to the P group (p<0.05). The EP group demonstrated a higher neuron count and a lower

proportion of neuronal apoptosis than the LYEP group ($p<0.05$) (Table 4).

Expression of synaptic plasticity-related proteins in hippocampal tissue: The expression levels of GAP-43 (Fig. 4a and b), caveolin-1 (Fig. 4a and c) and NCAM (Fig. 4a and d) in the hippocampal tissues of the P, EP and LYEP groups were lower than those in the C group ($p<0.05$). In the EP and LYEP groups, the levels of these synaptic plasticity-related proteins were higher compared to the P group ($p<0.05$). Notably, the EP group exhibited higher levels of GAP-43, caveolin-1 and NCAM compared to the LYEP group ($p<0.05$) (Fig. 4).

DISCUSSION

The impairment of central nervous system functions is a frequent complication following anesthesia and surgical procedures, predominantly manifesting as abnormalities in cognitive function and a decline in learning and memory capabilities. These impairments can persist for several months or even longer. Presently, the pathophysiology of postoperative cognitive dysfunction (POCD) remains incompletely understood. It involves a complex interplay of protein expression and signaling pathway dysregulation, potentially culminating in hippocampal neuronal apoptosis[11]. Propofol, a short-acting intravenous anesthetic known for its rapid onset and prompt recovery properties, when administered repeatedly or at high concentrations, induce developmental damage to central nervous functions. This damage is mediated through the activation of hippocampal neuronal autophagy, leading to long-term POCD and exhibiting a degree of neurotoxicity[12,13]. Consequently, the mitigation of propofol-induced POCD and the protection of neuronal integrity have emerged as critical concerns in perioperative care.

Ketamine, a non-barbiturate, rapid-acting intravenous general anesthetic, blocks cerebral pathways and thalamic projections to the neocortex, exhibiting hypnotic, sedative and anesthetic effects[14]. In a study by Wang and Pu[15], patients receiving 0.5 mg/kg ketamine in conjunction with ultrasound-guided nerve block exhibited reduced postoperative adverse reactions, improved anesthesia success rates and increased Mini-Mental State Examination (MMSE) scores. Additionally, a meta-analysis by Li *et al.*[16] highlighted ketamine's superior efficacy over drugs like lidocaine and dexamethasone in preventing POCD in cardiac surgery. These studies collectively suggest ketamine's potential in preventing POCD. However, consensus is yet to be established regarding its effectiveness in reducing cognitive impairment specifically induced by propofol.

The Phosphoinositide 3-Kinase/Protein Kinase B (PI3K/Akt) signaling pathway is paramount in neural survival signaling and regulates cognitive functions within the brain. The PI3K, a heterodimeric enzyme, can be activated through two distinct mechanisms: Either by the direct binding of its binding proteins, p110 and Ras, resulting in PI3K activation, or through interactions with connexin or growth factor receptors possessing phosphorylated tyrosine residues, leading to a conformational change and subsequent activation of the enzyme[17,18]. The Akt, a downstream effector of PI3K, plays a critical role in the intracellular milieu. Phosphorylated Akt is involved in regulating various cellular activities, including differentiation, proliferation, growth and migration, through the activation of downstream target genes.

Notably, Cao *et al.*[19] reported a reduction in PI3K/Akt activity in the hippocampal tissues of mice with Alzheimer's disease. Conversely, up-regulating protein expression within the PI3K/Akt signaling pathway led to a diminution in neuroinflammatory responses and bolstered synaptic plasticity in these mice. In a similar vein, Zhou *et al.*[20] discovered that activation of the PI3K/Akt pathway diminished cortical injury and neuronal damage and curtailed neuronal apoptosis in rats afflicted with subarachnoid hemorrhage. Liu *et al.*[21] also highlighted that stimulating the PI3K/Akt pathway could inhibit apoptosis in the neural stem or progenitor cells in mice, thus mitigating symptoms of depression and anxiety-like behaviors and alleviating cognitive dysfunction. Therefore, the activation of the PI3K/Akt signaling pathway is crucial in safeguarding brain tissue cells and forestalling brain tissue degradation.

In this study, rats were administered an intraperitoneal injection of 10 mg/kg ketamine followed by propofol. The findings revealed an increase in hippocampal tissue Phosphorylated Akt (P-Akt/Akt), an escalation in the number of hippocampal neurons, an enhanced correct response rate in the Y-maze experiment and a reduction in Bax, cleaved caspase-3 and neuron apoptosis. Furthermore, the evasion latency periods on the 1, 3, 5 and 7th days post-treatment were shortened. Additionally, neurons in the hippocampal CA1 area exhibited reduced pathological damage. These results suggested that a low dose of ketamine can ameliorate cognitive dysfunction induced by propofol, protect neurons and inhibit neuronal apoptosis.

The LY294002, a specific inhibitor of PI3K, was also used in this study. Following the dosage guidelines from the literature of Li *et al.*[22], it was observed that the neuroprotective function of ketamine was attenuated, indicating that the beneficial effects of low-dose ketamine may operate through the activation of the PI3K/Akt signaling pathway. This pathway plays a role in reducing the severity of cognitive

dysfunction induced by propofol anesthesia in rats, enhancing learning and memory abilities and inhibiting neuronal apoptosis.

Synaptic plasticity is the biological foundation for emotions, memory, learning and other cellular functions. Alterations in the structure and number of synapses can profoundly impact brain neurotransmission, potentially leading to hippocampal atrophy and cellular demise[23,24]. Activation of PI3K, which phosphorylates Akt, has been shown to subsequently activate downstream mammalian target proteins of rapamycin (MTOR). This activation promotes the synthesis of proteins associated with synaptic plasticity, thereby mediating the long-term maintenance of synaptic viability[25].

In the current study, it was observed that in the ketamine+propofol (EP) group, there was a reduction in the levels of GAP-43, caveolin-1 and NCAM proteins, as well as pro-inflammatory cytokines IL-1β and IL-2 and an increase in the levels of anti-inflammatory cytokines IL-4 and IL-10 in the hippocampal tissues of rats. Conversely, the magnitude of these improvements was less pronounced in the PI3K Inhibitor LY294002+ketamine+propofol (LYEP) group. This finding suggested that low doses of ketamine can modify synaptic plasticity and decrease the inflammatory response by activating the PI3K/Akt signaling pathway.

CONCLUSION

In summary, low doses of ketamine have the potential to mitigate cognitive dysfunction induced by propofol anesthesia, enhance learning and memory capabilities, inhibit neuronal apoptosis, reduce inflammatory responses and alter synaptic plasticity in rats. The underlying mechanism for these effects appears to be related to the activation of the PI3K/Akt signaling pathway. This revelation underscores the importance of further exploration into the neuroprotective roles of anesthetics and their mechanisms, with a focus on synaptic dynamics and molecular pathways.

SIGNIFICANCE STATEMENT

Propofol anesthesia may induce certain neurotoxic effects, leading to apoptosis of hippocampal neurons during the developmental stage of the brain, thereby triggering postoperative cognitive dysfunction. The combination of ketamine and propofol anesthesia has been reported to have higher safety inducement, promoting postoperative cognitive function recovery. However, the specific mechanisms of action still require further clarification. The findings of current study demonstrated that low-dose ketamine can mitigate cognitive impairment induced by propofol anesthesia, enhance learning and memory abilities, inhibit neuronal apoptosis, reduce inflammatory responses, alter synaptic plasticity and its mechanism of action is associated with the activation of the PI3K/Akt signaling pathway. This may potentially offer a theoretical basis for the pharmacological intervention against neurotoxic effects induced by propofol anesthesia.

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