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Hyperphosphorylation of protein Tau in hippocampus may cause cognitive dysfunction of propofol-anesthetized rats

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Abstract. – OBJECTIVE: We aimed to explore the effect of hyperphosphorylation of Tau on cognitive function of propofol-anesthetized rats.

MATERIALS AND METHODS: Thirty 2-month-old male Wistar rats weighing 180-220 g were randomly divided into 3 groups (n=10): group of treating with saline (C group), group of treating with propofol for 1 hour (P1) and 24 h (P24 group). The cognitive function of rats was tested by Morris water maze before and 1 h or 24 h after drug administration. The rats were then sacrificed. The protein and mRNA expression levels of GSK-3β, total and phosphorylated Tau, cyclin D1, p27kip1 and c-caspase 3 in hippocampus were determined by Western blot and reverse transcriptase-polymerase chain reaction (RT-PCR), respectively.

RESULTS: Compared with group C, the incubation period of P1 group and P24 group was prolonged, and the target quadrant retention time was shortened ($p<0.05$). There was no statistical difference between P1 and P24 group ($p>0.05$). Immunohistochemistry showed that compared with group C, p-Tau in hippocampus of P1 group and P24 group was highly expressed, with statistical difference ($p<0.05$). Western blot and RT-PCR showed that protein and mRNA expressions of GSK-3β, phosphorylated Tau, cyclin D1 and c-caspase 3 in hippocampus of P1 and P24 groups were up-regulated ($p<0.05$).

CONCLUSIONS: Propofol-induced cognitive dysfunction in rats may be related to the hyperphosphorylation of Tau that causes neuronal cells to re-enter the cell cycle, thus leading to apoptosis.

Key Words:
Propofol, Anesthesia, Cognitive function, Tau protein.

Introduction

Postoperative cognitive dysfunction (POCD) refers to cognitive decline that occurs during anesthesia or several weeks or months after surgery. It is one of the postoperative complications of central nervous system and belongs to mild neurocognition disturbance, which is often manifested as decreased cognitive abilities, impaired memory, personality changes, and mental disorders. In severe conditions patients may even experience dementia[1,2]. Most patients can self-recover from POCD and generally return to normal within 3 months after surgery. However, there are still some patients faced with long-term or permanent cognitive impairment. Therefore, POCD has a great destructive effect on patients' postoperative recovery and quality of life[3,4]. At present, it is generally believed that POCD is induced by surgery or anesthesia and formed by a variety of factors. It may be related to many factors such as age, type of surgery, anesthetic methods, drugs, hypoxia, hypotension, and education level[5]. Researches[6,7] have demonstrated that elderly patients over 60 are more likely to suffer from cognitive impairment after receiving surgery, while a higher level of education plays a protective role in postoperative cognitive function. The relationship between anesthetic methods and POCD remains controversial so far. Literature review[8] shows that spinal anesthesia does not reduce the prevalence of POCD compared to general anesthesia. In recent years, numerous papers[9-11] have shown that general anesthetics are one of the definitive factors that affect postoperative cognitive function.

Tau protein is a highly soluble microtubule-associated protein which is mainly found in central nervous cells. Tau mainly combines with tubulin to promote its polymerization to form microtubules, which are impor-

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tant structures that maintain the integrity of the neural cytoskeleton and the transport of substances in axons[17]. When Tau protein is abnormally phosphorylated, glycosylated or ubiquitinated, it loses its stabilizing effect on the microtubules, while can make the nerve fibers lose their normal biological functions. Neurofibrillary tangles caused by abnormally phosphorylated Tau protein are considered to be one of the major pathogenesis of Alzheimer's disease (AD) or protein-related diseases. Investigations[18-20] have shown that general anesthetics can cause abnormal phosphorylation of Tau protein in the hippocampus, which is closely related to degenerative changes in central nervous system[21,22], suggesting that the abnormal phosphorylation of Tau protein caused by general anesthetics is one of the major causes of POCD. Nykanen et al[23] suggested that activation of $GABA_A$ receptor could induce the activation of cyclin-dependent kinase 5 (Cdk5) and reduce the binding of Tau protein to protein phosphatases-2A (PP2A), which may change the degree of phosphorylation of Tau protein. However, whether the high level of Tau protein phosphorylation after activation of $GABA_A$ receptors can be reversed or reduced by its antagonists or inverse agonists, has not been elucidated yet.

In this study, rats were anesthetized with propofol to observe the expression levels of phosphorylated Tau (p-Tau) and total protein Tau (Tau-5) in the hippocampus. We aimed at exploring the effects of Tau hyperphosphorylation on cognitive function of propofol-anesthetized rats.

Materials and Methods

Experimental Animals and Grouping

30 healthy male adult Wistar rats of specific-pathogen-free (SPF) grade, with a weight of 180-220 g, were selected in our experiment. 5 rats were put in one cage, with quiet breeding environment, 12 h lighting/12 h darkness, 22-24°C of room temperature, 55% of humidity, free drinking water, and daily replacement litter. Using a random number table method, they were randomly divided into 3 groups (n = 10): saline group (C group), 1 h of propofol-anesthetized group (P1 group), and 24 h of propofol-anesthetized group (P24 group).

Morris Water Maze Test

Positioning Navigation Training (Acquired Training)

Rats were put into the water pool with their heads toward the water in a random position (east, west, south and north). The time each rat used to find the platform was recorded as the time to escape from the incubation period. If a rat failed to find a platform within 60 s, it could to be guided to the platform where it was kept for 10 s, and the time (escape latency) was recorded as 60 s. Each rat was trained four times a day (four quadrants in each of the east, west, south, and north) and trained for 5 consecutive days. This study was approved by the Animal Ethics Committee of Jilin University Animal Center..

Space Exploration Training

After the positioning navigation training, the platform was removed, and the rats were placed into the water from the target quadrant. The time that the rat stayed in the target quadrant within 60 s and the number of times it entered the platform area were recorded.

Dosage Administration

C Group: tail vein injection of normal saline (3.0 mL/kg); P1 Group: tail vein injection of propofol (30 mg/kg); P24 group: tail vein injection of propofol (30 mg/kg). Propofol was injected slowly at 15 mg/kg *via* the tail vein until the rat's righting reflex disappeared, followed by injection for 30 min at a speed of 30 mg/kg/h. The total amount was 30 mg/kg. Rats were kept with their spontaneous breathing when administered with drug. After awaking from the anesthesia, rats were placed in the cage alone and allowed to free drink.

Quadratic Behavioral Testing (Morris Water Maze Test)

In group C, the secondary water maze was trained 1 h after injection of saline. In the P1 group, the second water maze training was performed 1 h after the injection of propofol. In the P24 group, the second water maze training was performed 24 hours after the injection of propofol.

Immunohistochemical Staining

The tissue slides were subjected to normal gradients of ethanol, washed with phosphate-buffered saline (PBS) for 3 times, and incubated with phosphor-Thr231 polyclonal antibody

and Tau-5 monoclonal antibody in a 4°C freezer for 24 hours. On the next day, the slices were washed 3 times with PBS and incubated with the secondary antibody for 10 min at room temperature. After that, the slices underwent a series of staining such as diaminobenzidine (DAB) coloration, hematoxylin counterstaining and sealed with gum. Under the microscope, the positive cells stained by the phosphor-Thr231 antibody showed brownish yellow cell nucleus. Three slices were randomly selected from each specimen. 6 random fields with non-overlapped fields in the hippocampal CAI region in each slice were selected for capturing images under an inverted microscope.

Western Blot Analysis

Expression level of GSK-3β, total Tau (Tau-5), phosphorylated Tau (phosphorylated-Thr231 site, phosphor-Thr231), cyclin D1, p27kip1, and cleaved caspase 3 (c-Caspase 3) were detected in hippocampus of rats in each group after anesthesia.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Detection of p-Tau Related mRNA Expression in Hippocampus

Primer 5.0 software was used to design specific primers of GSK-3β, phosphor-Thr231, Cyclin D1, and c-caspase 3. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal reference. PCR reaction solution was prepared according to the instructions. The statistical analysis was performed based on the CT value (threshold cycle number) of each sample calculated by the fluorescence quantitative PCR instrument. GAPDH: Forward: 5'-CCTGCACCACCAACTGCTTAGC-3', Reverse: 5'-CCAGTGAGCTTCCCGTTCAGC-3'; GSK-3β: Forward: 5'-TTCAGGCCGCTGCTTCACCG-3', Reverse: 5'-GTGCTGGTCTTTCCCGCGCA-3', Phosphorylated-Tau: Forward: 5'-CGGCGTAAGCAAAGACA-3', Reverse: 5'-TGTAGCCGCTTCGTTCT-3', Cyclin D1: Forward: 5'-CCTGACTGCCGAGAAGTTGT-3', Reverse: 5'-TCATCCGCCTCTGGCATTTT-3', Caspase-3: Forward: 5'-AGAGCTGGACTGCGGTATTGAG-3', Reverse: 5'-GAACCATGACCCGTCCCTTG-3'

Statistical Analysis

Data analysis was performed using software Statistical Product and Service Solutions (SPSS) 17 (SPSS Inc., Chicago, IL, USA). Measured data were expressed as mean±standard deviation. Paired *t*-test was used to compare behavioral test data within the group. Differences between groups were analyzed using factorial variance analysis. $p<0.05$ was considered statistically significant.

Results

Behavioral Tests

There was no statistically significant difference in the number of incubation periods and the number of access to the original platform in each group, (Figure 1A-1C). After drug administration, both of P1 and P24 group showed prolonged incubation period and shortened target quadrant retention time compared with those before treatment ($p<0.05$), (Figure 1B). Compared with group C, the latency in the P1 and P24 group was prolonged (Figure 1D), the target quadrant retention time was shortened (Figure 1F), while the difference of the number of times to enter the platform area was not significant (Figure 1E).

Expression of Phosphorylated Tau (p-Tau) in Hippocampus

The expression of p-Tau in hippocampus of each group was compared by immunohistochemistry. Compared with group C, the expression of p-Tau in hippocampus of P1 and P24 groups was up-regulated, and the number of positive cells was increased ($p<0.05$, Figure 2A-2D). However, no significant difference between P1 and P24 group was observed (Figure 2B-2D, $p>0.05$).

Expression of p-Tau and Related Proteins in Hippocampus

Compared with group C, the expressions of GSK-3β, p-Tau, cyclin D1, and caspase 3 were increased in P1 and P24 group $p<0.05$) (Figure 3A). No significant changes in the expression of total Tau (Tau-5) and p27kip1 were observed ($p>0.05$, Figure 3A, 3C, 3D, 3F). P1 and P24 group showed no significant differences in expression of above proteins ($p>0.05$).

The mRNA Expression of p-Tau Related Gene in Hippocampus

RT-PCR showed that the mRNA expressions of GSK-3β, p-Tau, cyclin D1, and caspase 3 in hippocampus of group P1 and P24 were increa-

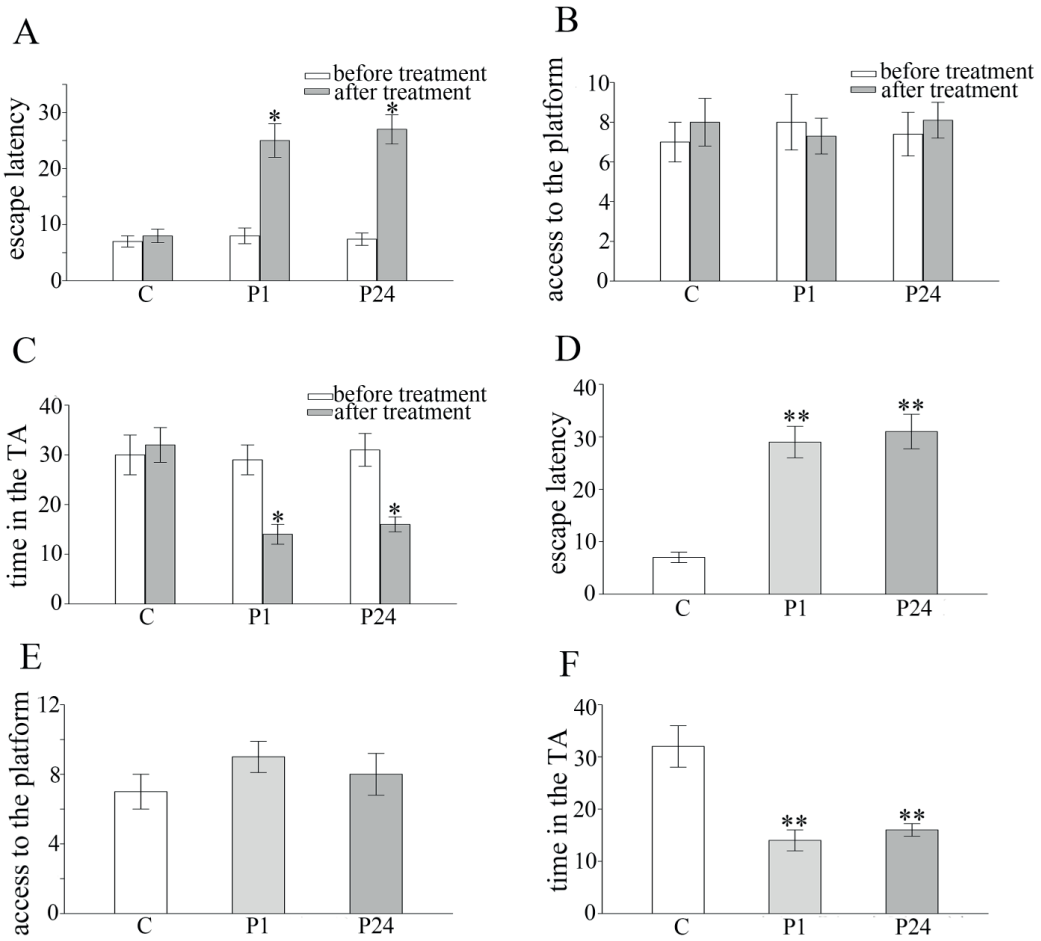

Figure 1. Latency, number of accesses to the platform area, and target quadrant retention times of the rats in each group. ***A,*** Comparison of the latency before and after administration in each group. ***B,*** Comparison of the number of times of entry into the plateau before and after administration in each group. ***C,*** Comparison of the retention time of target quadrants before and after administration in groups. ***D,*** Comparison of post-latency of rats in groups. ***E,*** Comparison of the number of times of platform entry after drug administration in each group. ***F,*** Comparison of the target quadrant retention time of rats in each group after treatment. *compared with that before treatment, $p<0.05$; **compared with group C, $p<0.05$.

sed ($p<0.05$). However, there was no significant difference between P1 and P24 group ($p>0.05$, Figure 4A-4D).

Discussion

Propofol is a short-acting, novel intravenous anesthetic drug with rapid onset, rapid recovery, and fewer adverse reactions. However, researches[24,25] have showed that propofol can affect memory function in a dose-dependent manner, which may produce anterograde or retrograde amnesia. Currently, propofol is commonly used with an induction dose of 2-2.5 mg/kg and maintenance dose of 4-12 mg/kg/h. In this research, the induction and maintenance dose were set at 15 mg/kg and 30 mg/kg/h respectively by tail vein injection, which is equivalent to doses used for human. It was found that propofol prolonged the escape latency of rat in Morris water maze and shortened the target quadrant retention time. Besides, it increased the expression of Tau-pSer356 in hippocampus and impaired hippocampal neurons and NISSL body. However, it did not significantly affect the number of rats entering the plate in the water maze. This may be related to the difference of the retention time of the target quadrant and the number of rats entering the plate regions, which reflected the sensitivity of spatial memory ability of rats[26,27].

Tau protein is a glycoprotein mainly distributed in central nervous cells. There are 79 se-

rine and threonine phosphorylation sites in the longest isoforms of human Tau protein, of which 30 have been found to be phosphorylated in humans[28]. Scholars[29-33] have confirmed that phosphorylation of Tau protein serine or threonine sites is mainly regulated by protein kinase-catalyzed phosphorylation or protein phosphatase (catalyzed de-phosphorylation). Among those protein kinases and protein phosphatases, glycogen synthase kinase-3β (GSK-3β) and protein phosphatase 2A (PP2A) play the most important roles in the abnormal phosphorylation of Tau protein. Protein phosphatase dysfunction or its decreased activity is one of the important causes of Tau protein aggregation in neurons[34]. Whittington et al[19] showed that the activity and expression of PP2A in the cerebral cortex were significantly decreased in mice 30 min after propofol anesthesia. At the same time, different sites of Tau protein in the hippocampus were significantly phosphorylated. In addition, abnormal phosphorylation in the AT8 site continued for 2 hours after propofol anesthesia and returned to normal condition until 6 hours after anesthesia. This work showed that decreased PP2A activity or expression may be the key reason for abnormal phosphorylation at different sites of Tau protein in the hippocampus.

Some researches have showed that cell cycle markers are abnormally expressed in neurons with deposition of fibroid Tau. These cell cycle markers include cyclin D, a protein associated with cell G0/G1 transition, and p27kip, a G2/M transition regulator[35]. Consistent with this, in Tau mice (transgenic mice lacking mouse Tau gene but expressing human Tau gene), Tau-induced

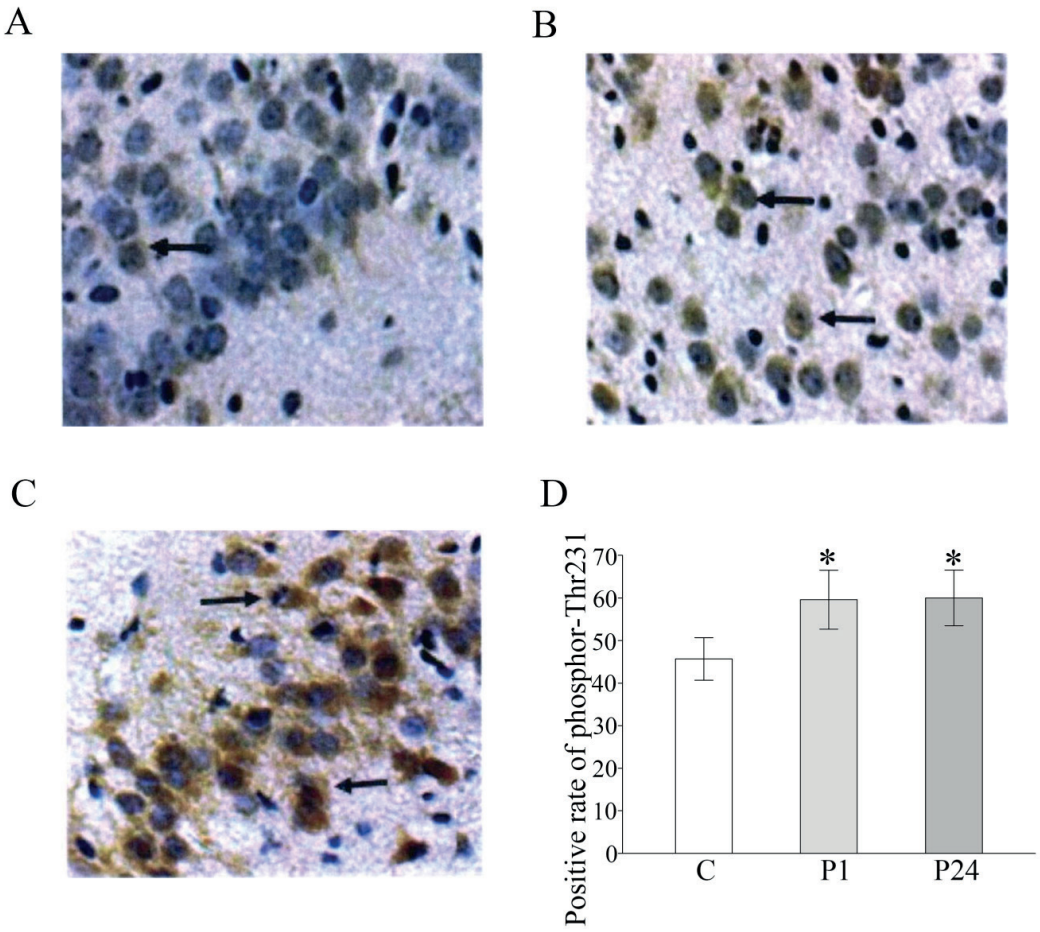

Figure 2. Expression of p-Tau in hippocampus in each group (immunohistochemical staining, ×400). ***A,*** The expression of p-Tau in rat hippocampus of group C. ***B,*** Expression of p-Tau in hippocampus of rats in group P1. ***C,*** Expression of p-Tau in hippocampus of rats in group P 24. ***D,*** Comparison of p-Tau positive cells in hippocampus of rats in each group. *: compared with group C, $p<0.05$.

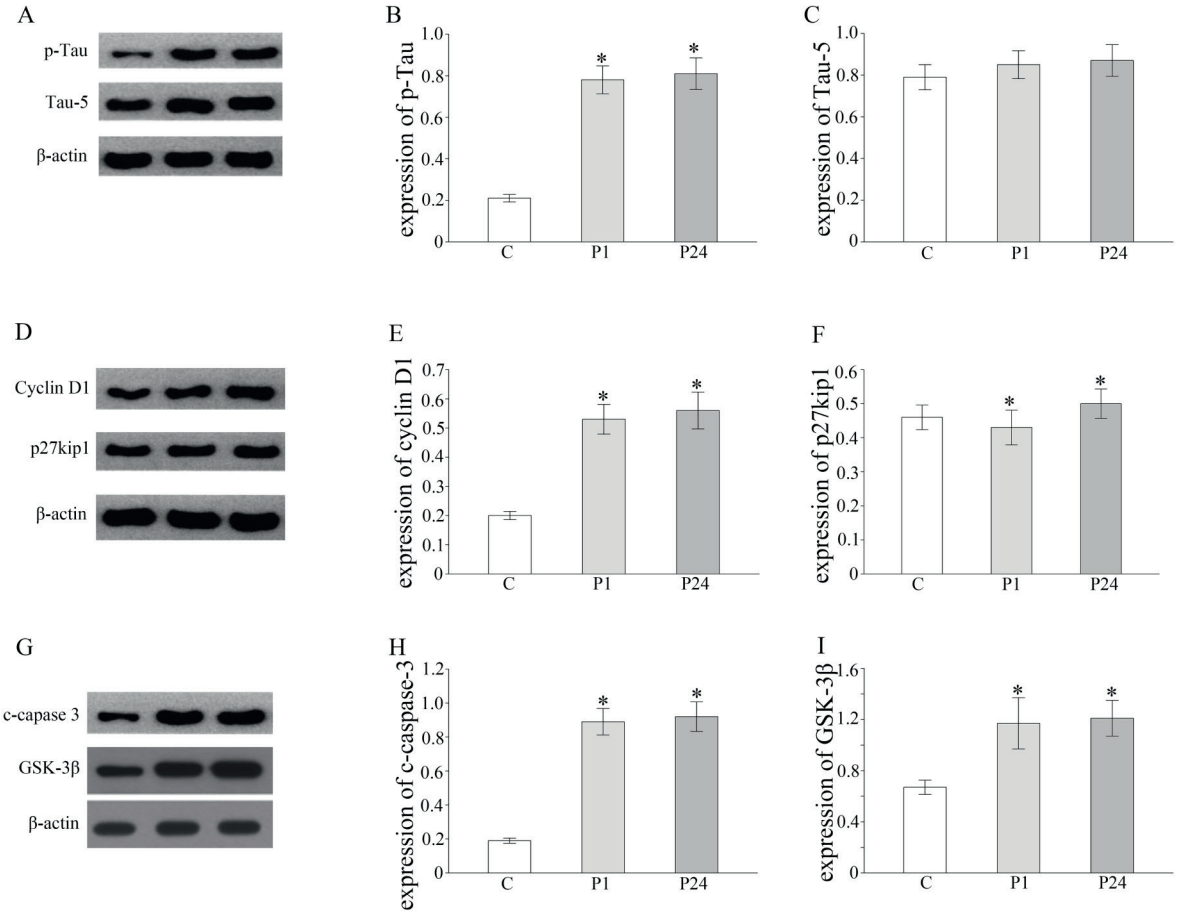

Figure 3. Western blot analysis of Tau-related protein expression in hippocampus of rats in each group. ***A,*** Expression of p-Tau and Tau-5 in hippocampus of rats in each group. ***B,*** Comparison of p-Tau expression in hippocampus of rats in each group. ***C,*** Comparison of expression levels of Tau-5 in hippocampus of rats in each group. ***D,*** Expression of Cyclin D1 and p27kip1 in hippocampus of rats in each group. ***E,*** Comparison of Cyclin D1 expression in hippocampus of rats in each group. ***F,*** Comparison of expression level of p27kip1 in hippocampus of rats in each group. ***G,*** Expression of c-caspase 3 and GSK-3β in hippocampus of rats in each group. ***H,*** Compared c-caspase 3 expression levels in hippocampus of rats in each group. ***I,*** Comparison of GSK-3β expression in hippocampus of rats in each group. *: Compared with group C, the difference was statistically significant. (p<0.05).

cell death is accompanied by high expression of cell cycle regulatory proteins[36]. This means that phosphorylation of Tau protein can cause mature neuronal cells to re-enter the cell cycle[28]. *In vivo* and *in vitro* studies[29-34] have confirmed that fully differentiated axoneure does not normally enter the cell cycle in mammals. This will lead to their death if they enter the cell cycle again due to external factors or natural processes. Therefore, the abnormal phosphorylation of Tau can cause the completely differentiated neuronal cells to re-enter the cell cycle and then lead to their death[37,38].

In this study, we found that compared with the control group, rats with propofol-anesthesia for 1 hour and 24 hours showed prolonged incubation period and shortened target quadrant retention time. In addition, cognitive function of the rates did not recover after 24 hours, indicating that the effect of propofol-anesthesia on cognitive function of rats lasted a long time. At the same time, the results of immunohistochemistry showed that the levels of p-Tau in the rat hippocampus remained high at both 1 and 24 hours after anesthesia. The protein and mRNA expression of the phosphorylation of Tau protein (p-Tau), cyclin D1, and cleaved caspase 3 (c-Caspase 3) showed the same trend. As an upstream molecule of Tau, GSK-3β also showed high expression in the hippocampal

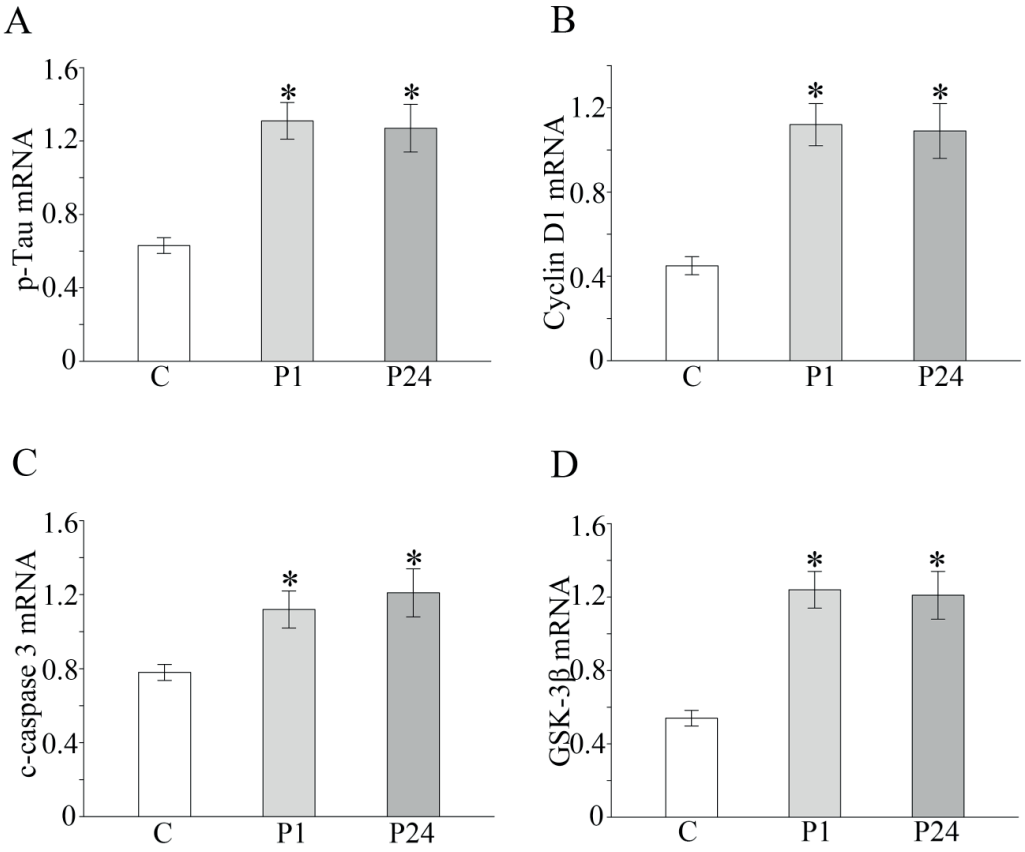

Figure 4. RT-PCR detection of Tau-related mRNA in hippocampus of rats in each group. ***A,*** Comparison of p-Tau mRNA expression in hippocampus of rats in each group. ***B,*** Comparison of expression of Cyclin D1 mRNA in hippocampus of rats in each group. ***C,*** Comparison of c-caspase 3 mRNA expression in hippocampus of rats in each group. ***D,*** Comparison of GSK-3β mRNA expression levels in hippocampus of rats in each group. *Compared with group C, the difference was statistically significant. ($p<0.05$)

tissue of propofol-anesthetized rats. Therefore, combining these results, we believed that the hyperphosphorylation of protein Tau caused by propofol may give rise to cell apoptosis through inducing mature neural cells to re-enter the cell cycle, which could ultimately lead to impairment of cognitive function.

Conclusions

Propofol-induced cognitive dysfunction after surgery in rats may be related to the hyperphosphorylation of Tau, which causes neuronal cells to re-enter the cell cycle, thereby resulting in cell apoptosis.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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