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Role of the hippocampal 5-HT1A receptor-mediated cAMP/PKA signalling pathway in sevoflurane-induced cognitive dysfunction in aged rats

Yi Qiu[1,*], Ying Wang[2], Xiaodong Wang[2], Caixia Wang[2] and Zhong-yuan Xia[1,*]

Abstract

Objective: This study aimed to evaluate the role of the hippocampal 5-hydroxytryptamine-1A (5-HT1A)-mediated cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) signalling pathway in sevoflurane-induced cognitive dysfunction in aged rats.

Methods: Sixty 18-month-old Sprague–Dawley rats were divided into the control (n = 30) and experimental (Sev, n = 30) groups. The experimental group inhaled 50% air/oxygen mixture (2 L/min) and 2% sevoflurane for 4 hours. The control group inhaled 50% air/oxygen mixture (2 L/min) for 4 hours. The Morris water maze test was performed The mRNA expression of 5-HT1A receptor, and cAMP PKA, cAMP response element-binding protein (CREB), and phosphorylated CREB (p-CREB) protein expression were determined.

Results: The escape latency and swimming distance were greater, and the number of crossings of the platform location and time spent in the platform quadrant were less in the Sev group compared with the control group. cAMP, PKA, CREB, and p-CREB protein expression was downregulated in the Sev group 1 day after anaesthesia compared with the control group. Hippocampal 5-HT1A receptor mRNA expression was higher 7 days after anaesthesia compared with the control group.

[1]Department of Anesthesiology, Renmin Hospitai of Wuhan University, Wuhan, Hubei Province, China
[2]Department of Anesthesiology, The Second Affiliated Hospital of Inner Mongolia Medical University, Huhhot, Inner Mongolia, China

*These authors contributed equally to this work.

Corresponding author:
Zhong-yuan Xia, Department of Anesthesiology, Renmin Hospital of Wuhan University, No. 238 Jiefang Road, Wuchang District, Wuhan 430060, China.
Email: xiazhongyuanwuhan@126.com

Conclusion: Sevoflurane-induced cognitive dysfunction in aged rats may be related to inhibited expression of the hippocampal 5-HT1A receptor-mediated cAMP/PKA signalling pathway.

Keywords
Cyclic adenosine monophosphate, 5-hydroxytryptamine-1A receptor, protein kinase A, sevoflurane, cognitive dysfunction, cerebral hippocampus

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Introduction

Postoperative cognitive dysfunction (POCD) is a common central nervous system complication of anaesthesia and surgery. The most commonly reported clinical manifestations are an abnormal mental state, change in personality, and impaired memory. Ageing and anaesthesia are major risk factors for POCD. Inhaled anaesthetics are more likely to cause cognitive dysfunction than intravenous anaesthetics.[1] The mechanism of cognitive dysfunction induced by sevoflurane, the most commonly used anaesthetic, has gained an increasing amount attention from anaesthesiologists. At present, studies on the mechanism of sevoflurane-induced cognitive dysfunction have mostly focussed on neurotransmitters and their receptors. Tian et al.[2] found that sevoflurane could induce cognitive dysfunction by inducing amyloid-β precursor protein expression in the rat hippocampus. Yu et al.[3] suggested that sevoflurane could lead to decreased cognitive function by inhibiting cyclic adenosine monophosphate response element-binding protein (CREB) expression in the rat hippocampus.

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter involved in emotional regulation, cognitive function (learning and memory), appetite, sleep, and other biological activities. This compound is mainly distributed in the hippocampus. The 5-HT1A receptor subtype has the highest level in the hippocampus. The 5-HTIA receptor regulates electrical activity of 5-HTergic neurons, and is involved in learning and memory formation and consolidation through similar signal transduction pathways.[4] Additionally, the 5-HTR1A receptor is a G protein coupled receptor. The 5-HTR1A receptor regulates expression of the second messenger cyclic adenosine monophosphate (cAMP) by activating adenylate cyclase, which in turn affects the activity of protein kinase A (PKA). Furthermore, phosphorylation of CREB protein at the corresponding site S133 by activated PKA activates the transcriptional activity of CREB. This induces expression of downstream genes and triggers a range of physiological and biochemical reactions.[5,6] CREB is an important intranuclear transcription factor regulating the central nervous system, and has a variety of biological functions, including involvement in regulating learning, and memory.

Studies have shown that a reduction in cAMP hydrolysation by inhibiting phosphodiesterase can increase intracellular cAMP concentrations. This in turn can increase phosphorylation of CREB, which is beneficial for formation of hippocampal long-term potentiation (LTP) and enhancement of memory.[7,8]

This study aimed to investigate whether sevoflurane anaesthesia-induced cognitive dysfunction in aged rats is related to changes in hippocampal 5-HT1A receptor expression. We hoped to further clarify the mechanism of action of the 5-HT1A

receptor-mediated cAMP/PKA signalling pathway in sevoflurane-induced cognitive dysfunction in aged rats.

Materials and methods

Animal selection and grouping

Sixty 18-month-old, clean-grade, healthy, male, Sprague–Dawley (SD) rats, weighing 600–750 g, were provided by the Animal Experimental Center of Inner Mongolia Medical University (license number: SCXK (Mongolia) 2015-001). The rats were raised at a room temperature of 18–25°C and environmental humidity of 40–60% in natural day/night lighting conditions. Rats were randomly allocated, using a random number table, to a control group (n = 30) and an experimental group (Sev group, n = 30). The study was approved by the ethics committee of Inner Mongolia Medical University (no: 20160234).

Morris water maze test

The Morris water maze consisted of a circular pool measuring 120 cm in diameter and 50 cm in height. A cylindrical platform (10 cm in diameter and 30 cm high) was 2 cm under the water. Room and water temperature were kept constant at 24–25°C. Rats were allowed to swim in the pool without a platform for 1 day before training to accommodate to the environment. Rats that were floating and did not move were excluded. In the place navigation test, the Morris water maze test began 6 days before modelling. This test was performed between 8:30 and 11:30 am every day, four times daily in the first 4 days. Rats were introduced into the water maze facing the wall at north, south, east, and west directions. Swimming images were captured by a camera that was connected to a path tracking system. During training, the rats were allowed to swim until they found the platform or for a maximum of 90 s. The escape latency was taken as the average of four measurements of the time the rats spent to find the platform. Any rats that failed to find the platform within 90 s were guided to platform, and the escape latency was recorded as 90 s. On day 5, the rats were allowed to swim twice in succession from each of the starting positions. The preoperative escape latency was taken as the average of the measurements. In the space exploration test, on day 6 of swimming training, the number of crossings of the platform location and time spent in the platform quadrant were measured. The water maze test was performed again 1 day after modelling. Rats without cognitive dysfunction were excluded according to the experimental results.

Development of a rat model of cognitive dysfunction

Thirty rats in the Sev group were placed in a self-made transparent anaesthesia box ($50 \times 40 \times 40$ cm in size; made of transparent glass). The temperature inside the box was maintained at 37°C using a heating pad. The rats received 2% sevoflurane (16120131, Shanghai Hengrui Pharmaceutical Co., Ltd., Shanghai Shi, China) in 50% air/oxygen mixture for 4 hours. Rats were allowed to breathe spontaneously and were observed for the colour of the skin and mucous membranes. The concentrations of anaesthetic gas, oxygen, and end-tidal CO_2 in the anaesthesia box were measured using a gas monitor (Datex Cardiocap II; Datex-Ohmeda, Madison, WI, USA). Anaesthesia was finished at the same time. The rats were then returned to cages after naturally waking up. Rats in the control group received 50% air/oxygen mixture alone (2 L/min) for 4 hours.

Collection of hippocampal tissue

Ten rats were randomly selected from the Sev group, as well as from the control group, at 1, 3, and 7 days after modelling, respectively. The rats were intraperitoneally injected with 10% chloral hydrate (0.4 ml/100 g). After anaesthesia, each rat was quickly placed on ice and decapitated. The brain was exposed by removing the skull with bone nibbling forceps. The hippocampus was then collected, weighted, divided into two parts, transferred into labelled cryogenic vials, and stored in a freezer at −80°C.

Determination of hippocampal 5-HT1A receptor expression using real-time PCR

Total RNA was extracted from the hippocampus in an ice bath using an RNAprep Pure Tissue Kit according to the protocol of the manufacturer (centrifugal column; Tiangen Biotech, Beijing, China). A volume of 1 µl of total RNA was diluted five times with RNase Free dH_2O. An Evolution™ 300 UV–Vis spectrophotometer (Thermo Fisher Scientific, Rockford, IL, USA) was zeroed with RNase-free dH_2O. The dilution was assayed for RNA concentration and purity. A 260-nm/280-nm ratio of 1.8–2.1 was indicative of a high-quality DNA sample. The final concentration (ng/µl) was calculated as (optical density: 260 nm) × 5 × 40. A 20-µl reaction mixture was prepared according to the instructions of the Takara PrimeScript RT Reagent Kit with gDNA Eraser (Perfect Real Time; Takara, Shiga, Japan) to synthesize cDNA. The resulting cDNA was serially diluted for standard curve determination and for setting up the real-time PCR reaction for each cDNA sample.

The PAGE grade synthesis primer sequences were as follows: 5-HT1A forward, 5′-CACGGCTACACCATCTAC-3′ and 5-HT1A reverse, 5′-CTCCCTTCTTTTCCA CCT-3′; and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward, 5′-GTT ACCAGGGCTGCCTTCTC-3′ and GAP DH reverse, 5′-GGGTTTCCCGTTGATG ACC-3′. The PCR reaction mixture was prepared according to the instructions of the Takara SYBR Premix Ex Taq II (Tli RNaseH Plus) (Takara). The mixture was gently tapped and placed in an Applied Biosystems 7500 Quantitative PCR System (ABI, Foster City, CA, USA). The amplification consisted of initial denaturation for 30 s at 95°C, followed by 40 cycles of 5 s at 95°C, and 30–34 s at 60°C.

GAPDH was used as an internal control. The expression of target mRNA relative to GAPDH mRNA was calculated using crossing point values and scaled relative to control samples set at a value of 1. Hippocampal 5-HT1A receptor mRNA expression in aged rats treated with sevoflurane was measured by the relative quantitation method ($2^{-\triangle\triangle Ct}$).

Determination of cAMP expression in hippocampal neurons by enzyme-linked immunosorbent assay

Hippocampal tissue was homogenized in 0.1 N HCL (1:5 w/v) in an ice bath, centrifuged at 10,000 rpm at 4°C for 10 minutes, and neutralized with 1 N NaOH. The supernatant was then diluted with RD5-55 (R&D Systems, Minneapolis, MN, USA) to twice the volume. Sample loading was performed according to the instructions of the Rat cAMP ELISA Kit (R&D). Optical density was measured at 450 nm using an enzyme immunoassay reader (Thermo Fisher Scientific). The cAMP content of hippocampal neurons was calculated according to the standard curve.

Determination of PKA, CREB, and phosphorylated CREB expression in hippocampal neurons by western blotting

Hippocampal tissue was homogenized in lysate buffer (Beyotime Institute of

Biotechnology, China) in an ice bath, and then centrifuged. The supernatant was then denatured at 95°C for 5 minutes, divided into tubes, and stored at −20°C. The protein in the supernatant was quantified using a BCA protein quantification kit (Beyotime Institute of Biotechnology, China). Samples were then loaded according to the measured protein concentration, followed by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (Solarbio Science and Technology Co., Ltd., Beijing, China) for 150 minutes, wet transfer for 90 minutes, and blocking with 5% skim milk powder at room temperature for 60 minutes. Membranes were then incubated at 4°C overnight with rabbit monoclonal anti-PKA primary antibody (1: 1000; Abcam, Cambridge, UK), rabbit monoclonal anti-CREB primary antibody (1: 1000; Abcam), and rabbit monoclonal anti-P-CREB primary antibody (1:1000; Abcam), and washed with Tris-buffered saline three times for 10 minutes. Membranes were incubated at 4°C overnight with rabbit anti-rabbit secondary antibody (1:1500; LI-COR Biosciences, Lincoln, NE, USA) and washed with Tris-buffered saline three times for 10 minutes. Finally, the membranes were exposed and scanned with an Odyssey CLX infrared laser imaging system (Gen Company Limited, Hong Kong, China). GAPDH (1:2000; Proteintech Group, Inc., Chicago, IL, USA) was used as an internal control. Band intensities were measured using Quantity One software (Bio-Rad, Hercules, CA, USA). Expression of the target protein was determined by the ratio of the target protein band intensity to the GAPDH band intensity.

Statistical analysis

The data were analysed by IBM SPSS for Windows, version 20.0 (IBM Corp., Armonk, NY, USA). Measurement data are expressed as mean ± standard deviation. Statistical analysis was carried out by repeated measures two-way analysis of variance and the t-test. $P < 0.05$ indicates a statistically significant difference.

Results

General characteristics

Vital signs of the two groups of rats were stable during anaesthesia. Anoxia and CO_2 storage were not observed ($Sp0_2 < 90\%$),

Morris water maze test results

Before anaesthesia, the escape latency was significantly reduced and swimming distance shortened with an increase in training days. There was no significant difference in the escape latency, swimming distance, number of crossings of the platform location, and time spent in the platform quadrant between the control and Sev groups before anaesthesia. The escape latency and swimming distance were significantly greater, and the number of crossings of the platform location and time spent in the platform quadrant were significantly lower in the Sev group 1 day after anaesthesia compared with the control group (all $P < 0.05$, Tables 1–4).

Effect of sevoflurane on changes in hippocampal 5-HT1A receptor mRNA expression in aged rats

Hippocampal 5-HT1A receptor mRNA expression of rats was unchanged in the Sev group 1 and 3 days after anaesthesia compared with the control group. However, hippocampal 5-HT1A receptor mRNA expression was significantly higher in the Sev group 7 days after anaesthesia compared with the control group ($P < 0.05$, Table 5 and Figure 1).

Table 1. Comparison of escape latency between the two groups

	Latency (s)					
Group	Day 1 of training (n = 30)	Day 2 of training (n = 30)	Day 3 of training (n = 30)	Day 4 of training (n = 30)	Day 5 of training (n = 30)	One day after anaesthesia (n = 30)
Control group	83.00 ± 3.12	51.90 ± 5.92	18.60 ± 3.38	15.60 ± 1.88	11.90 ± 1.59	15.80 ± 1.62
Sev group	84.40 ± 2.67	48.10 ± 3.84	22.40 ± 3.62	19.10 ± 3.40	15.30 ± 1.78	26.60 ± 3.18*

Values are expressed as mean ± standard deviation. Note: There was no significant difference in escape latency between the control and Sev groups before anaesthesia. The escape latency was prolonged in the Sev group 1 day after anaesthesia compared with the control group; *$P < 0.05$.

Table 2. Comparison of total swimming distance between the two groups

	Total swimming distance (cm)					
Group	Day 1 of training (n = 30)	Day 2 of training (n = 30)	Day 3 of training (n = 30)	Day 4 of training (n = 30)	Day 5 of training (n = 30)	One day after anaesthesia (n = 30)
Control group	243.07 ± 2.67	49.92 ± 9.19	48.08 ± 9.63	45.19 ± 5.21	20.90 ± 2.68	21.93 ± 2.58
Sev group	205.52 ± 4.52	54.54 ± 9.43	51.51 ± 10.10	46.99 ± 8.19	23.21 ± 4.95	63.53 ± 5.21*

Values are expressed as mean ± standard deviation. Note: There was no significant difference in total swimming distance between the Sev and control groups before anaesthesia. The total swimming distance was longer in the Sev group 1 day after anaesthesia compared with the control group; *$P < 0.05$.

Table 3. Comparison of number of platform location crossings between the two groups

	Number of platform location crossings	
Group	Day 6 of training (n = 30)	One day after anaesthesia (n = 30)
Control group	4.94 ± 0.23	4.83 ± 0.30
Sev group	4.70 ± 0.33	2.25 ± 0.25*

Values are expressed as mean ± standard deviation. Note: There was no significant difference in the number of platform location crossings between the Sev and control groups before anaesthesia. The number of platform location crossings was significantly lower in the Sev group 1 day after anaesthesia compared with the control group; *$P < 0.05$.

Table 4. Comparison of time spent in the platform quadrant between the two groups

	Time spent in the platform quadrant (s)	
Group	Day 6 of training (n = 30)	One day after anaesthesia (n = 30)
Control group	24.1 ± 0.9	22.3 ± 0.7
Sev group	22.8 ± 0.8	13.4 ± 0.7*

Values are expressed as mean ± standard deviation. Note: There was no significant difference in time spent in the platform quadrant between the Sev and control groups before anaesthesia. The time spent in the platform quadrant was significantly shorter in the Sev group 1 day after anaesthesia compared with the control group; *$P < 0.05$.

Table 5. Relative quantities of hippocampal 5-HT1A in aged rats of the two groups

Group	Relative quantities
Control group (n = 10)	1.00 ± 0.01
Sev group – Day 1 (n = 10)	0.89 ± 0.03
Sev group – Day 3 (n = 10)	1.11 ± 0.01
Sev group – Day 7 (n = 10)	1.74 ± 0.04*

Note: Expression of hippocampal 5-HT1A receptor mRNA of rats appeared to be lower in the Sev group 1 day after anaesthesia compared with the control group ($P > 0.05$). However, 5-HT1A receptor mRNA expression gradually increased and was significantly higher than that of the control group 7 days (*$P < 0.05$) after anaesthesia. 5-HT1A, 5-hydroxytryptamine-1A.

Effect of sevoflurane on changes in hippocampal cAMP protein expression in aged rats

Expression of cAMP protein in hippocampal neurons was significantly lower in the Sev group 1 and 3 days after anaesthesia compared with the control group (both $P < 0.05$, Table 6). However, there was no significant difference in cAMP protein expression at 7 days after anaesthesia between the Sev and control groups (Figure 2).

Effect of sevoflurane on changes in hippocampal PKA protein expression in aged rats

PKA protein expression in hippocampal neurons was significantly lower in the Sev group 1 and 3 days after anaesthesia compared with the control group (both $P < 0.05$). However, there was no significant difference in PKA protein expression at 7 days after anaesthesia between the Sev and control groups (Figure 2).

Effect of sevoflurane on changes in hippocampal CREB protein expression in aged rats

CREB protein expression in hippocampal neurons was significantly lower in the Sev group 1 day after anaesthesia compared with the control group ($P < 0.05$, Table 8). However, there was no significant difference in CREB protein expression at 3 and 7 days after anaesthesia between the control and Sev groups (Figure 2).

Effect of sevoflurane on changes in hippocampal phosphorylated CREB expression in aged rats

*P*hosphorylated CREB (p-CREB) protein expression in hippocampal neurons was significantly lower in the Sev group 1 day after anaesthesia ($P < 0.05$, Table 8). However, there was no significant difference in p-CREB protein expression at 3 and 7 days after anaesthesia between the control and Sev groups (Figure 2).

Discussion

China has become an aging society. Song et al.[9] suggested that the proportion of the population aged older than 65 years is expected to be >20% by 2040 in China. According to statistics, half of this older population will undergo at least one surgery in their remaining years. POCD is a common complication of anaesthesia and surgery in older patients. Sevoflurane is widely used in various surgeries involving a wide range of age and sites because of a low blood/gas distribution coefficient, rapid induction of anaesthesia, and rapid waking up. Previous studies have shown that in rats exposed to sevoflurane of 0.5%, 1%, and 2%, 2% of the rats had memory impairment.[10,11] Sevoflurane can lead to POCD by affecting the release of neurotransmitters, increasing the expression of inflammatory factors, promoting neuronal apoptosis, and inhibiting LTP.[12,13,14,15] Roberts et al.[16] showed that inhaled sevoflurane enhances related oxidation of 5-HT and thus affects cognitive function.

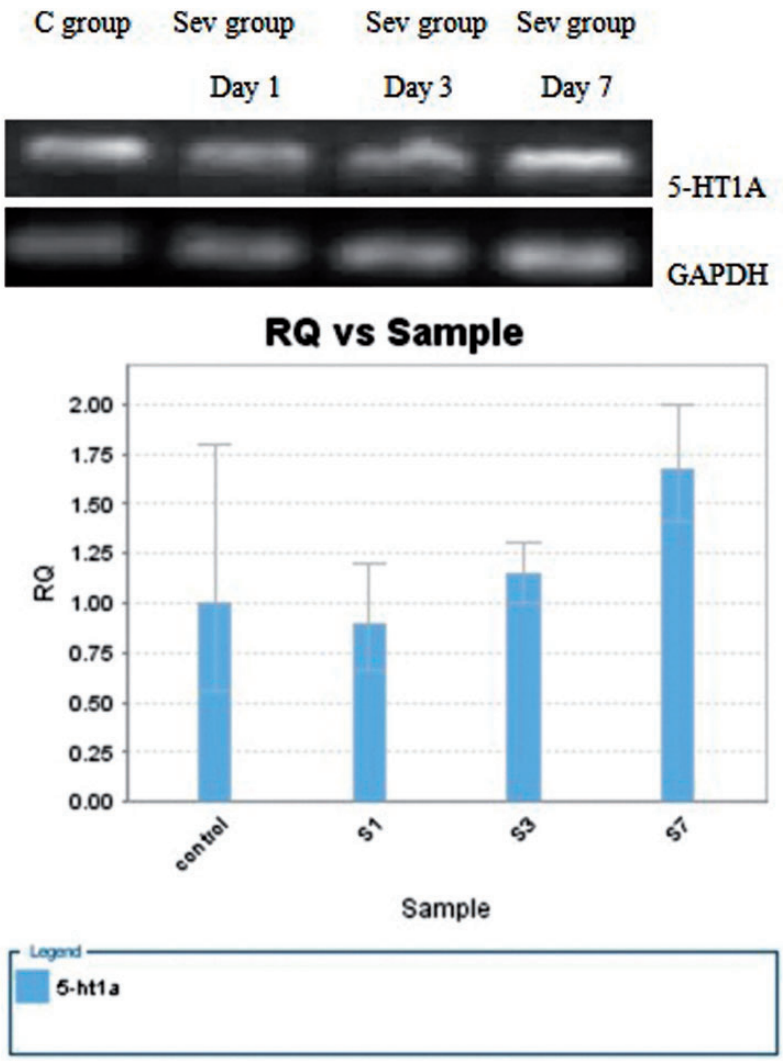

Figure 1. Relative quantities (RQ) of hippocampal 5-hydroxytryptamine-1A in aged rats after sevoflurane anaesthesia

The 5-HT1A receptor, a 5-HT receptor subtype, is mainly distributed in the hippocampus and dorsal raphe nucleus. A human gene mapping study showed a relationship of 5-HT1A receptor distribution and density with spatial memory.[17] The 5-HT1A receptor is mainly involved in cognitive function and emotional activities, and plays an important role in learning, memory maintenance, and short-term and long-term memory.[5] In recent years, the 5-HT1A receptor has been suggested as a target for improving cognitive function. Release of synaptic neurotransmitters induced by stimulating the 5-HT1A receptor can improve memory function of rats with cognitive and emotional dysfunction.[18] Olsen et al.[4] found that treatment with buspirone, a 5-HT1A receptor agonist, could improve the learning and memory abilities

Table 6. Comparison of cAMP expression in hippocampal neurons of the two groups (n = 30)

	cAMP (pmol/ml)		
Group	Day 1	Day 3	Day 7
Control group	189.3 ± 3.6	187.16 ± 3.21	191.72 ± 1.9
Sev group	164.7 ± 7.4*	173.20 ± 9.08*	181.93 ± 10.13

Values are expressed as mean ± standard deviation. Note: Expression of cAMP in hippocampal neurons in the Sev group was significantly lower 1 and 3 days after anaesthesia compared with the control group (*$P < 0.05$), but there was no significant difference 7 days after anaesthesia. cAMP, cyclic adenosine monophosphate.

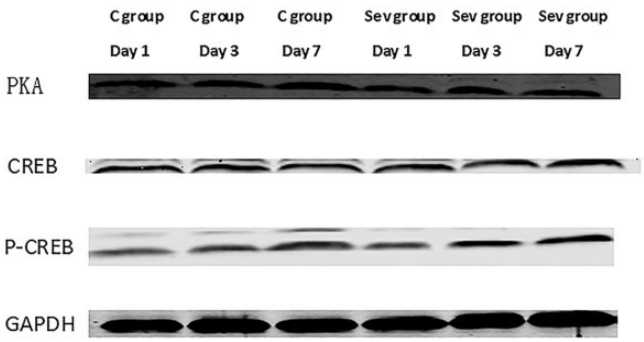

Figure 2. Western blot results of the two groups

Table 7. Comparison of PKA expression in hippocampal neurons of the two groups (n = 30)

	PKA		
Group	Day 1	Day 3	Day 7
Control group	0.084 ± 0.006	0.083 ± 0.006	0.084 ± 0.005
Sev group	0.051 ± 0.008*	0.061 ± 0.006*	0.078 ± 0.007

Values are expressed as mean ± standard deviation. Note: Expression of PKA in hippocampal neurons in the Sev group was significantly lower 1 and 3 days after anaesthesia compared with the control group (*$P < 0.05$), but there was no significant difference 7 days after anaesthesia. PKA, protein kinase A.

in animals with traumatic brain injury. However, the specific mechanism of action of the 5-HT1A receptor is unclear.

cAMP is an important second messenger in intracellular signal transduction. This molecule is produced by activating adenylate cyclase and catalysing hydrolysis of adenosine triphosphate after hormones and neurotransmitters act on specific target cells.[19] cAMP activates gene

Table 8. Comparison of CREB and p-CREB expression in hippocampal neurons of the two groups (n = 30)

	CREB			p-CREB		
Group	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Control group	0.666 ± 0.037	0.623 ± 0.038	0.617 ± 0.036	0.176 ± 0.027	0.199 ± 0.051	0.189 ± 0.037
Sev group	0.291 ± 0.038*	0.546 ± 0.038	0.596 ± 0.042	0.077 ± 0.010*	0.143 ± 0.036	0.174 ± 0.037

Values are expressed as mean ± standard deviation. Note: Expression of CREB and p-CREB in hippocampal neurons in the Sev group was significantly lower 1 day after anaesthesia compared with the control group (*$P < 0.05$), with no differences at 3 and 7 days after anaesthesia. CREB, cyclic adenosine monophosphate response element-binding protein; p-CREB, phosphorylated-CREB.

expression mainly by phosphorylating target enzymes through activation of PKA. cAMP plays an important role in cell life activities, with involvement in regulating cell metabolism, gene expression, and cell growth and division.[19,20] The cAMP/PKA signalling pathway plays an important role in regulation of learning and memory.[21,22,23] The cAMP/PKA signalling pathway regulates LTP formation and late memory. The cyclic guanosine monophosphate/protein kinase G signalling pathway regulates early memory and LTP formation, and requires involvement of the cAMP-PKA signalling pathway.[20,24,25] cAMP can activate PKA and protein kinase C. The activated PKA and protein kinase C enter the nucleus, leading to CREB phosphorylation at Serl33 and consequent activation of CREB. The p-CREB binds to CRE sites on DNA, which leads to expression of downstream genes and proteins related to learning and memory, thereby regulating learning and memory.[25–27] In short, cAMP regulates learning and memory through the cAMP-PKA-CREB signalling pathway.

In summary, sevoflurane can alter the expression of 5-HT1A receptor, which may regulate transcriptional activity, and alter cognitive function by activating or inhibiting the cAMP/PKA signalling pathway. Therefore, in this study, the cognitive function of aged SD rats at 18–20 months old, which corresponds to the age of 65 years in humans, was evaluated using the Morris water maze test. Rats with sevoflurane-induced cognitive dysfunction were identified and included. Furthermore, the molecular mechanism of sevoflurane was investigated by gene expression and protein signalling pathways. We hoped provide effective intervention strategies for cognitive dysfunction and offer new ideas for clinical prevention and treatment of cognitive dysfunction.

Learning and memory are the storage and export of knowledge. We found that the escape latency was significantly reduced and the total swimming distance was gradually shortened with an increase in Morris training time before modelling. These findings indicated that the aged SD rats formed a good learning and memory process in the 6-day water maze training. Development of sevoflurane-induced cognitive dysfunction was examined by comparison of escape latency, total swimming distance, the number of crossings of the platform location, and the time spent in the platform quadrant between rats treated with 2% sevoflurane for 4 hours and those treated with 50% air/oxygen mixture alone. The escape latency and swimming distance were greater, and the number of crossings of the platform location and time spent in the platform quadrant were lower 1 day after anaesthesia in rats from the Sev group compared with the control group.

These findings are similar to those of previous studies.[11,12] This suggests that sevoflurane can cause cognitive impairment in aged rats. In our study, hippocampal 5-HT1A receptor mRNA expression in the Sev group appeared to be lower than that of the control group 1 day after anaesthesia. However, 5-HT1A receptor mRNA expression in the Sev group gradually increased, and by 7 days after anaesthesia, it was higher than that of the control group, which may be related to recovery of cognitive function. This coincides with the duration and reversibility of POCD in older patients caused by inhaled anaesthetics. Moreover, ELISA and western blotting showed that protein expression of cAMP, PKA, CREB and p-CREB in the hippocampus of the Sev group appeared to be lower than that of the control group at all three time points after sevoflurane anaesthesia. However, this lower expression was only significant on day 1 for cAMP, PKA, CREB, and p-CREB, while an increase was observed on days 3 and 7. The increase in hippocampal cAMP, PKA, CREB, and p-CREB protein expression over time is consistent with that in hippocampal 5-HT1A receptor mRNA expression after sevoflurane anaesthesia in the Sev group. This finding suggests that sevoflurane can affect cognitive function by inhibiting expression of the cAMP/PKA signalling pathway mediated by the 5-HT 1A receptor in hippocampal neurons. Moreover, sevoflurane-induced POCD in aged rats is a transient and reversible change, which can gradually recover after surgery.

On the 7th day after anaesthesia, 5-HT1A receptor, cAMP, PKA, CREB, and p-CREB in the hippocampus of rats showed elevated expression. These findings suggest that 5-HT1A receptor is associated with memory recall. However, this elevation in cAMP, PKA, CREB, and p-CREB expression was not significant by 7 days after anaesthesia. The reason for this difference between 5-HT1A receptor and the other molecules may be because the cAMP/CREB signalling pathway was affected by various upstream factors. Our experiment only aimed to investigate the upstream factors for 5-HT1A receptor, and could not fully reflect the effect of upstream factors related to the cAMP/CREB signalling pathway of protein expression. Therefore, 5-HT1A receptor expression levels in the hippocampus were slightly different from cAMP, PKA, CREB, and p-CREB expression, but the trend was the same. This issue needs to be further studied in the future.

Conclusions

Our findings on changes in hippocampal 5-HT1A receptor, cAMP, PKA, CREB, and p-CREB expression in aged SD rats with sevoflurane-induced POCD suggest the following conclusions. (1) Inhalation of 2% sevoflurane for 4 hours can lead to cognitive dysfunction in aged SD rats. (2) Lower expression of hippocampal 5-HT1A receptor mRNA, and cAMP, PKA, CREB, and p-CREB protein in aged SD rats after sevoflurane anaesthesia compared with controls shows that the 5-HT1A-mediated cAMP/PKA signalling pathway may be involved in development of sevoflurane-induced cognitive dysfunction in aging rats.

In summary, sevoflurane-induced cognitive dysfunction in aged rats is associated with inhibited expression of the hippocampal 5-HT1A receptor-mediated cAMP/PKA signalling pathway. Moreover, such effects on cognitive function are reversible. These findings can provide guidance for clinical treatment and prevention of POCD.

Limitations

1. We only evaluated short-term cognitive function after inhalation anaesthesia. We did not study the effect of anaesthetic

drugs on cognitive function of geriatric rats in the long term (1 or 3months).
2. We only analysed the receptor upstream of the hippocampal cAMP/PKA signalling pathway, and did not investigate associations of other important upstream factors affecting this pathway. In subsequent studies, we will focus on these deficiencies.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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