doi.org/10.36721/PJPS.2022.35.1.REG.059-067.1

Role of RGS2 in sevoflurane-induced cognitive dysfunction in aged rats

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Abstract: After undergoing inhalation anesthesia, some patients, especially elderly patients, experience postoperative cognitive dysfunction, such as personality changes and memory impairment. In the present study, 20-month-old rats were randomly allocated to sevoflurane (Sevo group) and control groups (Con group), and they inhaled 3% sevoflurane or 40% oxygen for 8 hours, respectively. The Morris water maze test found that the cognitive function of rats in the Sevo group were significantly different on 1d and 3d after anesthesia than that of rats in the Con group. The expression of RGS2 mRNA and protein in hippocampus of Sevo group was lower compared to the Con group, while Ca^{2+} was higher than con group. The expression of CaM and CaMK II in Sevo group was higher compared to the Con group. We found that Bcl-2 reduced, but the expression of Bax and Caspase-3 increased, indicating that apoptosis of hippocampal neurons was increased after sevoflurane inhalation. Both the expression of NGF and BDNF was depressed in the Sevo group. After continuous inhalation of 3% sevoflurane for 8h, the expression of RGS2 in the hippocampi of aged rats is down regulated. RGS2 may be an important factor that leads to cognitive dysfunction in rats.

Keywords: Sevoflurane, cognitive dysfunction, RGS2, hippocampus.

INTRODUCTION

With advances in medical technology, the average life expectancy of humans is continuously increasing, along with the probability of elderly patients receiving surgery. Postoperative cognitive dysfunction (POCD) becomes a common complication post surgery in geriatric patients (Kim *et al*. 2020). Sevoflurane is a commonly used drug in surgeries that require anesthesia. Several studies have found that different concentrations, duration and frequencies of sevoflurane inhalation can cause different levels of damage to the cognitive function of rodents (Liu *et al*. 2017). It has also been reported that a single inhalation of sevoflurane in adult mice can lead to the reversible phosphorylation of concentration-dependent hippocampal proteins and even induce apoptosis of the hippocampal neurons (Chen *et al*. 2013). The neurotoxic effects of sevoflurane involve a complex process comprising multiple targets, neurotransmitters, proteins, and molecular changes in the brain (Borgstedt *et al*. 2020; Peng *et al*. 2012). Due to CNS complexity and our limited understanding in regard to the action mechanism of inhaled anesthetics, the mechanism underlying neurological damage caused by these anesthetics is not yet completely understood.

Regulators of G-protein signaling (RGS) are recently discovered intracellular GTPase activating proteins. The domains of RGS accelerate GTP hydrolysis and attenuate or terminate G protein cell information transmission by binding to its α subunit (Zhang *et al*. 2016). RGS2 gene rapidly up regulates RGS2 protein levels in neurons distributed in multiple regions of brain, such as the hippocampus, through excitatory stimulation, thereby moderating synaptic output and behavioral changes (Gerber *et al*. 2016; Kanai *et al*. 2017). Several studies have found that a lack of the RGS2 gene can affect synaptic transmission in the hippocampus of mice, causing anxiety and behavioral changes (Iacono *et al*. 2013).

Therefore, in this study, we selected 20-month-old Wistar rats as the research model to examine neurobehavioral changes after sevoflurane inhalation and selected RGS2 in these changes. After the inhalation of anesthesia, the correlation between changes in the expression of RGS2 and Ca^{2+}/CaM/CaMK II in the hippocampus of rats and cognitive dysfunction was observed.

MATERIALS AND METHODS

Experimental animals and their grouping

In the experiment, 20-month-old Wistar rats, weighing between 600 and 700g, were selected regardless of sex. The rats were ordered from the Animal Experimental Center of Inner Mongolia University (license No: 8806R011) and were housed in a clean animal room under a constant temperature of 25°C. In total, 72 aged rats were

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randomly divided into sevoflurane (Sevo) and Control (Con) group. These rats continuously inhaled 3% sevoflurane with an oxygen concentration of 40% oxygen alone for 8 h, respectively. All animal experiments were approved by the Animal Ethical Committee of Inner Mongolia University.

Sevoflurane anesthesia

Rats in the Sevo group were placed in a PMMA box (35×20×15cm) prepared in-house for administering sevoflurane anesthesia. The head side of the box was connected to a Drager anesthesia machine to administer sevoflurane. Different concentrations of sevoflurane were propelled into the box by the anesthesia machine to adjust the oxygen concentration to 40% and the total gas flow in the breathing circuit was adjusted to 2 L/min. After the sevoflurane concentration in the box stabilized at 3.0%, rats in the Sevo group were placed in the closed anesthesia box and anesthetized for 8h. The sevoflurane concentration was monitored using Datex-Ohmeda S/5 (Wisconsin, United States). Rats in the Con group were placed in the same box and made to inhale 40% oxygen for 8 h.

Behavioral assessment

One day before sevoflurane anesthesia, the Morris water maze test was performed for both the Sevo and Con groups, which was repeated in both groups 1 and 3d after sevoflurane anesthesia, respectively. Morris water maze tests were carried out in a water tank with a diameter of 120cm and a depth of 50 cm, filled with turbid water at 25°C, equipped with a platform with a diameter of 10cm, and kept 1cm below water surface. A positioning navigation test was conducted for the first 4 consecutive days. Rats were put into the water, and the time when each rat arrived at the platform was recorded as the escape latency time. If the rat did not find the platform within 60 sec, it was guided to the platform and allowed to stay on it for 15 sec. In that case, the escape latency was recorded as 60 sec. On day 5, the platform was taken away for the space exploration experiment. Rats were placed in the water in the quadrant furthest from the platform. The swimming time of rats in the original quadrant of the platform in a 60-sec period was recorded as the memory time.

Measurement of Ca^{2+} in the hippocampus

Hippocampal tissues were separated and digested with 0.25% trypsin (Sigma, MO, USA) for 15 min, and then the reaction was stopped with DMEM (Invitrogen, CA, USA) containing 10% of fetal bovine serum (Invitrogen, CA, USA) to prepare a single cell suspension. The concentration of cell suspension was adjusted to a 1×10^5/mL. A total of 1mL of the single cell suspension was centrifuged for 5 min at 1600 r/min and 1mL of 5μM Fluo-3/AM (Beyotime Biotechnology, Shanghai China) working solution was added after the cell precipitation to make the cell dispersed. In the working solution, the cell concentration was adjusted to 1×10^5/mL. Then the cell suspension was incubated in the dark for 30 min at 37ºC. A flow cytometer (BD, NJ, USA) was used to determine the relative content of Ca^{2+} in 1×10^5 cells with a 488-nm excitation wavelength and 526-nm emission wavelength. Each group of experiments was repeated twice under the same conditions and the experimental results were recorded.

Real-time PCR analysis

Rats in the Sevo and Con groups were euthanized. Then, the rat brains were taken out and placed on ice and their hippocampi were separated and washed three times with 4°C DEPC-treated water to remove blood for RNA extraction experiments. Primers were designed using primer premier 5.0 based on GenBank data to identify the mRNA sequence of the related genes and the primers were synthesized by Huada Gene Technology Co., Ltd. The primer sequences are shown in table 1. The conditions of PCR reaction were: 95°C pre-denaturation for 30s, 95°C denaturation for 5s, annealing and 60°C extension for 30 s, for a total of 40 cycles. The relative expression of sample mRNA relative to the β-actin (as an internal reference) was calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blotting

Rats in the Sevo and Con groups were euthanized by decapitation, the brains were excised and put onto ice and the hippocampus was separated and stored in liquid nitrogen. The RIPA lysis buffer, protease inhibitor and phosphatase inhibitor PMSF were added to 40mg of tissue sample to perform homogenization, lysis, centrifugation, and heat denaturation with loading buffer to prepare the protein sample. The BCA method was used for protein quantification before the samples underwent SDS-PAGE electrophoresis (50μg/well). The proteins were transferred onto PVDF membrane, then blocked with 5% skimmed milk and washed with TBST. Then, the membrane was incubated with the primary antibodies overnight at 4°C, washed with TBST and incubated with the fluorescent secondary antibodies for 1 h. The primary antibodies were rabbit anti- brain derived neurotrophic factor (BDNF) (all 1: 1000, Abcam, MA, USA), rabbit anti-CaM and mouse anti-CaMKII; and rabbit anti- nerve growth factor (NGF) (1: 500) & rabbit anti-RGS2 (1: 1000, SAB, MD, USA). The secondary antibodies were goat anti-mouse IgG labeled with horseradish peroxidase (1: 10000 Zhongshan Golden Bridge Bio-technology, Beijing, China) and goat anti-rabbit IgG labeled with horseradish peroxidase (1: 10000 Zhongshan Golden Bridge Bio-technology, Beijing, China). ImageJ software was used to measure the grayscale of the protein bands, and the relative gray values of the target protein/GAPDH were calculated.

Immunofluorescence histochemistry

Rats in the Sevo and Con groups were euthanized following intraperitoneal injection of 3.6% chloral hydrate (20 ml/kg) and were then immediately perfused with 0.1 M PBS solution through the heart and then with 4% paraformaldehyde. Hippocampal tissues were isolated and fixed for 24 h with 4% paraformaldehyde. Then, the tissues were embedded in paraffin and cut into 5 μm of sections for immunofluorescent staining. The primary antibodies were rabbit anti-BDNF, rabbit anti-CaM and mouse anti-CaMKII (all 1: 500, Abcam, MA, USA); and rabbit anti-NGF (1: 300) & rabbit anti-RGS-2 (1: 500, SAB, MD, USA). FITC-KPL Cy^{TM}3 anti-rabbit IgG (H + L) (1: 1000, Milford, MA, USA) and Dy Light 549 Affini Pure Donkey anti-mouse IgG (H + L) (1: 1000, Jackson Immuno Research, PA, USA) were used as the secondary antibodies. The samples were incubated in the dark at 37℃ for 1h. After incubation, the sections were washed thrice with washing solution for 10 min. Next, DAPI (Beyotime Biotechnology, Shanghai, China) was used to stain the nucleus.

STATISTICAL ANALYSIS

One-way and Mann Whitney post hoc analysis were used to determine statistical comparison between protein expression, fluorescence intensity and neural activity, and Graph Pad Prism (version 7.0) was used. All results were presented as bar charts with error bars describing the mean and standard error. *P*<0.05 was considered to be statistically significant.

RESULTS

The findings of Morris water maze test

There was no difference between the Sevo and Con groups in either the positioning navigation test or space exploration experiment. On day 1 and day 3 after sevoflurane anesthesia, the times spent in the positioning navigation experiments in the Sevo group were 57.34±5.36 s and 54.61±6.14 s, respectively. Compared to that of the Con group, the escape latency in the Sevo group was much longer (p<0.05) (fig. 1A). Compared to the control group, in the space exploration experiment, the percentage of time in the NE quadrant was much lower on both day 1 and 3 (p<0.05) (fig. 1B).

Expression of RGS2 in the hippocampus

After continuous inhalation of sevoflurane, the mRNA expression of RGS2 in the hippocampal tissues in the Sevo group was lower compared to the Con group (p< 0.05) (fig. 2). Western blotting and immunofluorescence histochemistry revealed that RGS2 protein was expressed in the cytoplasm of hippocampal neurons and its expression in the Sevo group was down regulated in contrast with the Con group (p<0.05) (fig. 3).

Expression of Ca^{2+}/CaM/CaMK II in the hippocampus

Flow cytometry showed that the Ca^{2+} content was higher in the Sevo group compared to Con group (95.49±4.02% vs. 84.75±4.08%, p<0.05) (fig. 4). Western blotting showed that the expression levels of both CaM and CaMK II in the Sevo group were higher than those in the Con group (p<0.05) (fig. 5). Immunofluorescence histochemistry showed that both CaM and CaMK II were expressed in the cytoplasm of hippocampal neurons (fig. 5).

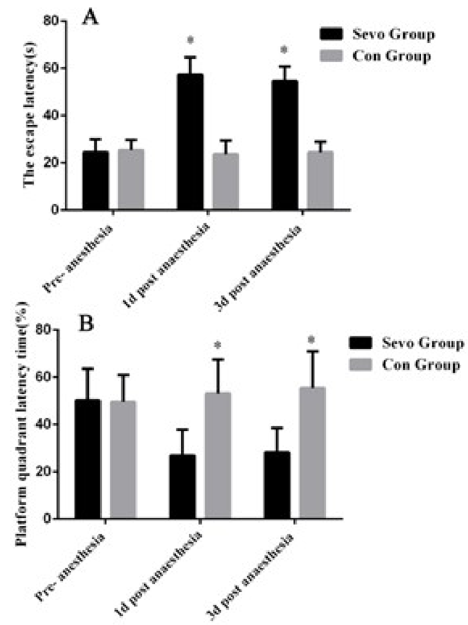

Fig. 1: Cognitive function of rats (n=30, 15 rats in each group) in the two groups. A: Positioning navigation experiment, escape latency measurement, *compared with the Con group, p<0.05. B: Space exploration experiment, percentage of length of stay, *compared with Sevo group, p<0.05.

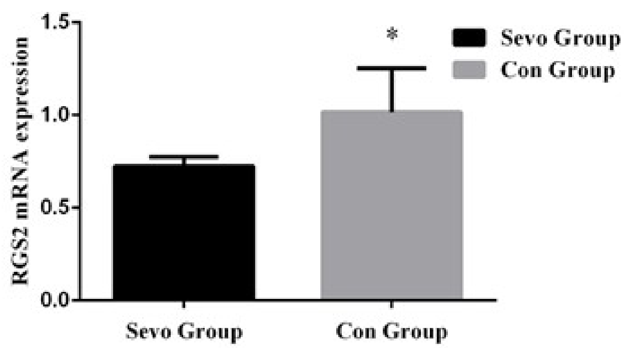

Fig. 2: Relative expression of RGS2 mRNA in the rat hippocampus (n=12, 6 rats in each group). *Compared with the Sevo group, p<0.05.

Expression of Bcl-2, Bax and caspase-3 in the hippocampus

Compared with Con group, Bcl-2 mRNA expression was lower (p<0.05), but the mRNA expressions of both Bax and caspase-3 were higher in the Sevo group (p<0.05) (fig. 6). This indicates that the continuous inhalation of sevoflurane promotes the apoptosis of hippocampal neurons in aged rats.

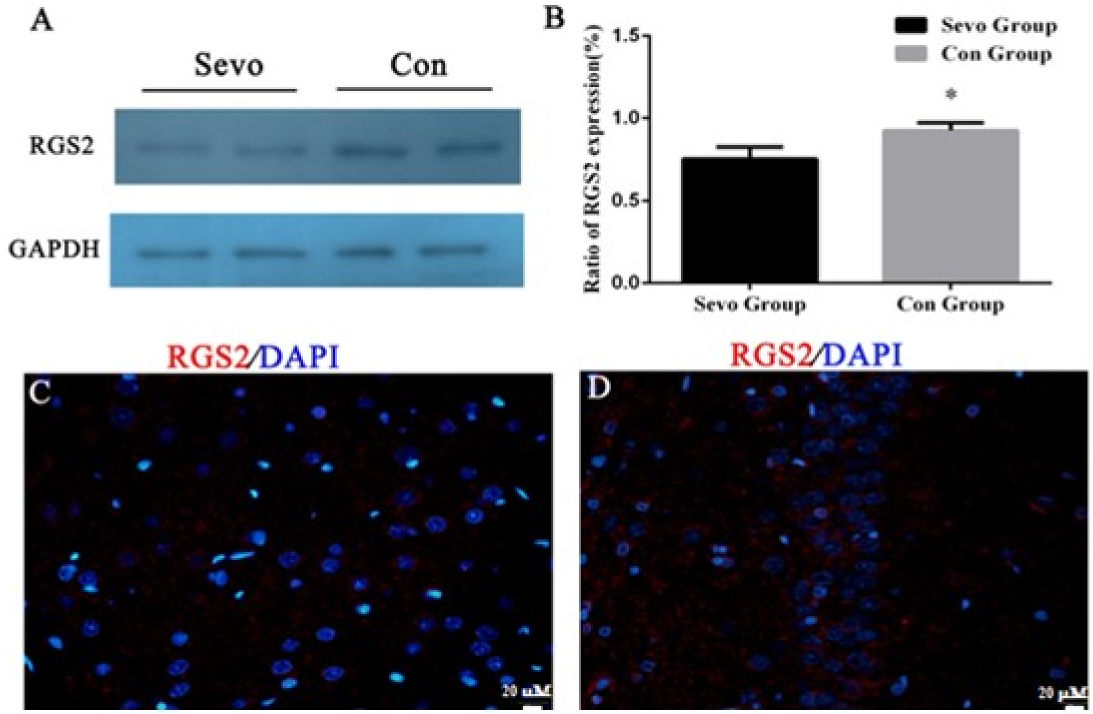

Fig. 3: RGS2 protein expression in the hippocampal tissues (n=12, 6 rats in each group). A and B: relative expression of RGS2 in the hippocampal tissues viewed using western blotting. *Compared to the Sevo group, $p<0.05$. C and D: RGS2 protein expression in the hippocampal neuron cytoplasm viewed using immunofluorescence histochemistry.

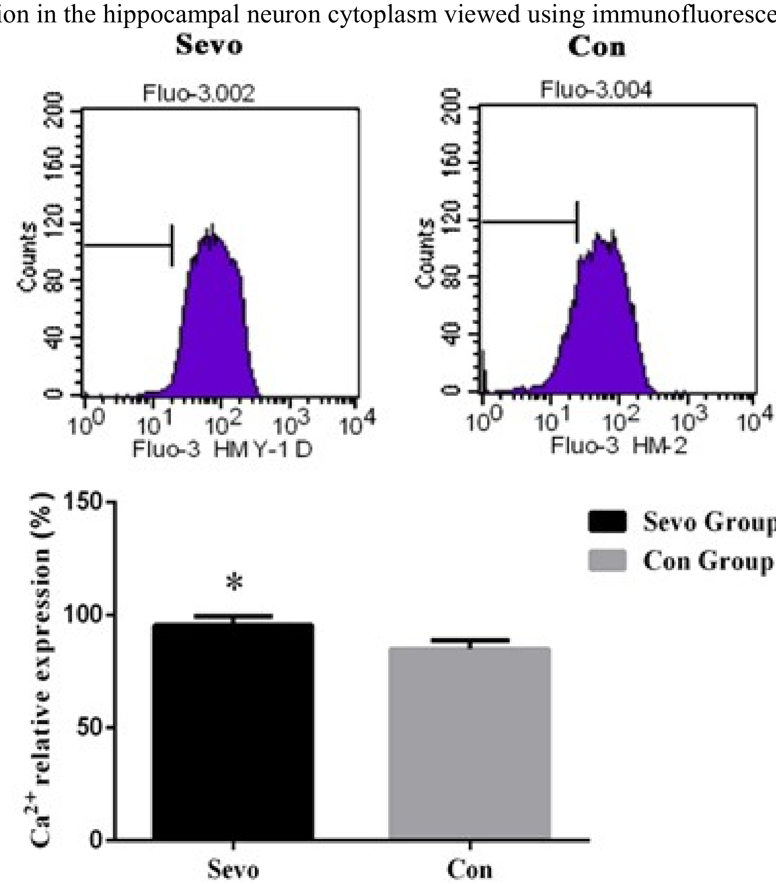

Fig. 4: Expression of Ca^{2+} in the hippocampus (n=12, 6 rats in each group). *Compared with the Con group, $p<0.05$.

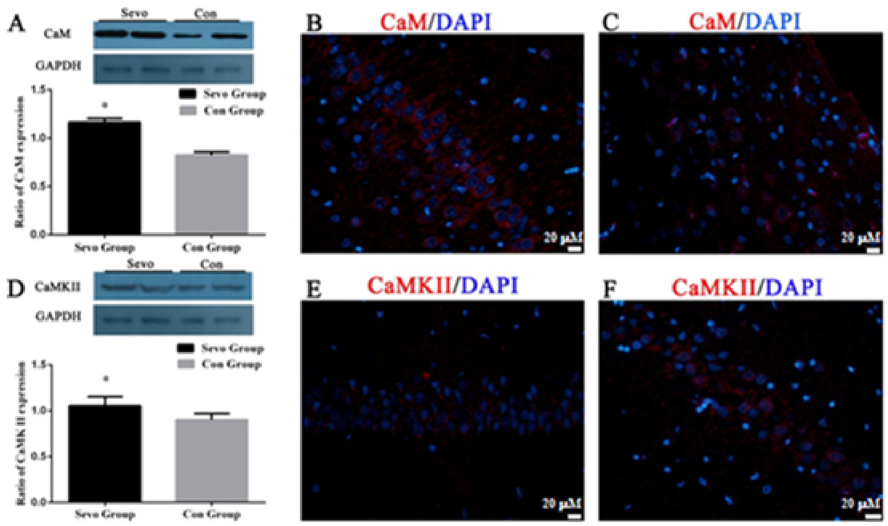

Fig. 5: Expression of CaM and CaMK II proteins in the hippocampus (n=12, 6 rats in each group). A: CaM expression as viewed using western blotting, *Compared with the Con group, p<0.05; B and C: immunofluorescent histochemical detection of CaM in the Sevo and Con group; D: CaMK II expression in hippocampal tissues viewed using western blotting, *Compared with the Con group, p<0.05; E and F: immunofluorescent histochemical detection of CaMKII in the Sevo and Con group.

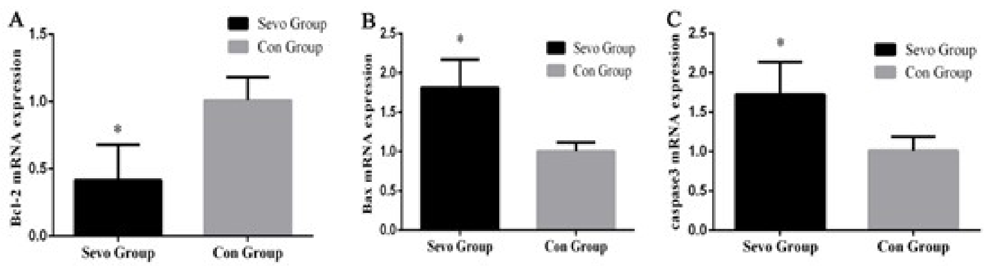

Fig. 6: Bcl-2, Bax and caspase-3 mRNA expression in the hippocampus measured using real-time PCR (n=12, 6 rats in each group). * Compared with the Con group, p<0.05.

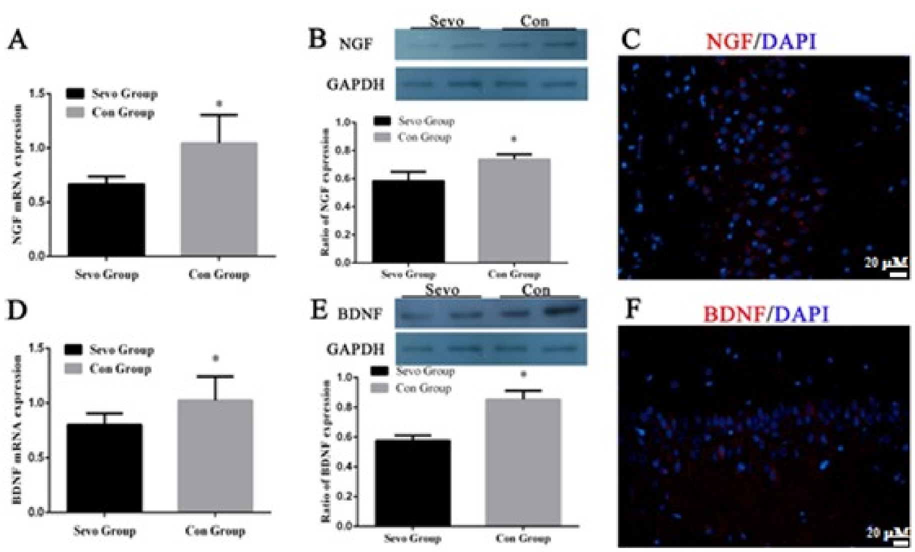

Fig. 7: NGF and BDNF mRNA expression in the hippocampus (n=12, 6 rats in each group). A: relative expression of NGF mRNA measured using real-time PCR, *Compared with the Sevo group, p<0.05; B and E: NGF protein expression measured using western blotting. *Compared with the Sevo group, p<0.05; C and F: immunofluorescent histochemical detection of NGF in the Sevo and Con group, respectively; D: the relative expression of BDNF mRNA measured using real-time PCR, * Compared with the Sevo group, p<0.05.

Table 1: Primer sequences

Gene	Sequence of primer
β-actin-F	ATATCGCTGCGCTGGTCGTC
β-actin- R	AGGATGGCGTGAGGGAGAGC
RGS2-F	GATTGGAAGACCCGTTTGAG
RGS2-R	TTCCTCAGGAGAAGGCTTGA
BDNF-F	ACCAGGAGCGTGACAACAAT
BDNF- R	GCCTTCATGCAACCGAAGTA
NGF-F	TCATCCACCCACCCAGTCTT
NGF- R	TTTGGAGGAGTGCGGATG
Caspase3-F	TTACCCTGAAATGGGCTTGT
Caspase3- R	TGAGGTTAGCTGCATCGACA
Bcl-2-F	AGTACCTGAACCGGCATCTG
Bcl-2- R	CAGCCAGGAGAAATCAAACAG
Bax-F	GACAGGGGCCTTTTTGCTAC
Bax- R	CCAATTCGCCTGAGACACTC

Expression of NGF and BDNF in the hippocampus

Compared with Con group, the expressions of NGF and BDNF mRNA in the Sevo group was reduced ($p<0.05$). Moreover, NGF and BDNF were found to be expressed in the cytoplasm of hippocampal neurons (fig. 7) and their protein expressions were both lower in the Sevo group ($p <0.05$) (fig.7).

DISCUSSION

Sevoflurane, one of the most commonly used inhaled anesthetics in clinical practice, has the characteristics of rapid induction, rapid awakening and little effect on respiratory and circulatory functions. However, some studies have found that inhalation of 3.6% sevoflurane for 6h can destroy blood-brain barrier components in rats, leading to fibrinogen invasion and downregulation of Annexin A1 expression (Hu *et al*. 2016). In some studies, sevoflurane-induced toxicity has been shown to cause cognitive dysfunction (Zheng *et al*. 2017; Yufune *et al*. 2016). In the present study, we administered aged rats with 3.0% sevoflurane for 8h. The Morris water maze test demonstrated that the spatial memory function of the rats in the Sevo group was affected and the escape latency was longer than that in the Con group, suggesting that learning ability decreased after sevoflurane inhalation. In the space exploration experiment, the number of times the aged rats crossed the original platform was obviously decreased and the percentage of time spent in the quadrant of the original platform was also reduced in the Sevo group, indicating that the spatial memory ability of the aged rats decreased. This result shows that after continuous inhalation of 3% sevoflurane for 8h, a certain level of cognitive dysfunction occurred in the aged rats, which is consistent with previous reports (Wang *et al*. 2019; Yan *et al*., 2019).

G protein is the basic intermediate substance for cell information transmission. It is the basis for hormones to moderates metabolism and the cardiovascular system and it is the key substance that maintains normal brain function (Kach *et al*. 2012). RGS is an intracellular GTPase activating protein. The RGS domain binds to the α-subunit of the G protein, accelerates GTP hydrolysis, and attenuates or stops G protein signal transduction (Zhang *et al*. 2016). In the RGS family, RGS2 is a critical molecule in terms of maintaining the normal physiological function of the brain. RGS2 gene have been demonstrated to regulate behavioral changes and the synaptic output of central neurons through changes in the expression level of protein (Gerber *et al*. 2016; Kanai *et al*. 2017). Some studies have found that the lack of the RGS2 gene can affect synaptic transmission in the hippocampi of mice, causing anxiety and behavioral changes (Iacono et. 2013). We found that after continuous inhalation of sevoflurane in aged rats, RGS2 expression in the hippocampal tissues was decreased. It is reported that the synaptic activity of hippocampal CA1 neurons in RGS2-knockout mice may be related to increased anxiety in mice, which was consistent with our findings (Mark *et al*. 2019). We observed Ca^{2+} content in hippocampal neurons was higher in the Sevo group than that in the Con group. RGS2 inhibits the presynaptic Ca^{2+} channel by down regulating the $G_{i/o}$ pathway, affecting the short-term synaptic plasticity of hippocampal neurons (Mochida *et al.* 2019). Their study speculates that when RGS2 expression is low, the $G_{i/o}$ pathway is activated, which attenuates the inhibition of presynaptic Ca^{2+} channels and promotes the flow of presynaptic Ca^{2+} into the cell. RGS2 can specifically and negatively regulate $G_{i/o}$ and $G_{q/11}$ signaling pathways (Evans *et al*. 2019; Gurevich *et al*. 2016; Meleka *et al*. 2019). Low RGS2 expression can activate $G_{i/o}$ and $G_{q/11}$ signaling pathways, which promotes PLC-β to hydrolyze phosphatidylinositol diphosphate (PIP2) on the plasma membrane to generate inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). These two molecules act as the secondary messenger and play a role in intracellular information transmission. The generated IP3 causes the release of Ca^{2+} from the endoplasmic reticulum (Li *et al*. 2020; Hajdu *et al*. 2020). In addition, the DAG that activates PLC-β can specifically activate protein kinases under the synergistic effect of intracellular Ca^{2+}, thereby stimulating ion channel transporters on the cell membrane to cause Ca^{2+} influx (Li *et al*. 2018).

Ca^{2+}-CaM-CaMK II is an important signal transduction pathway in the nervous system. We found that CaM expression was higher in the Sevo group, and CaMK II expression in the hippocampi of the Sevo group was also higher than that in the Con group. Excessive Ca^{2+} influx causes intracellular calcium overload, increases the binding of Ca^{2+} and CaM, activates CaMK II and causes its own phosphorylation, eventually causing the dysregulated phosphorylation of CaMK II. Persistent phosphorylation of CaMK II exacerbates Ca^{2+} influx and cytoplasmic Ca^{2+} overload and further induces the phosphorylation of N-methyl-D-aspartate receptor type 2B (NR2B), thereby forming a vicious cycle and aggravating cell damage and leading to death. However, the persistent over-phosphorylation of CaMK II, which is the main component of postsynaptic densities (PSD), likely activates of the downstream c-jun N-terminal kinase (JNK) signaling pathway, thereby inducing apoptosis. Additionally, the persistent phosphorylation of CaMK II leads to the loss of its role as a molecular switch in learning and memory, leading to the impairment of learning and memory functions (Cai *et al*., 2021; Engin *et al*., 2021).

Bcl-2 can stabilize mitochondria by regulating mitochondrial membrane permeability, thereby preventing mitochondria from releasing apoptosis-related proteins, such as Cyt-c, and inhibiting the occurrence of apoptosis. In contrast, Bax can disrupt the stability of mitochondrial membranes, thereby promoting cell apoptosis (Ghanbarabadi *et al*. 2019). Caspase-3 is the key enzyme and executor of apoptosis (Fan *et al*. 2018). In our study, we found suppressed expression of Bcl-2 in the hippocampus in the Sevo group, with increased expression of Bax and casepase-3. The findings indicate sevoflurane could induce apoptosis in the hippocampal neurons of aged rats. We assume that the continuous inhalation of sevoflurane leads to decreased RGS2 expression in the hippocampus and an overload of intracellular Ca^{2+}, thereby activating the Ca^{2+}-CaM-CaMK II pathway and causing apoptosis of hippocampal neurons. Our finding was not in line with the finding of O'Brien et al (O'Brien *et al*. 2019), who found that upregulated RGS2 expression induced by ischemic stress enhances apoptosis in C6 cells and primary astrocytes. Raab et al reported the role of RGS2 deletion in fear learning and reactivity to stress. Nevertheless, all of the studies revealed that RGS2 is associated with stress and cell apoptosis (Raab *et al*. 2018).

NGF and BDNF are the two main nutritional factors in the neurotrophic factor family that perform the functions of nourishing neurons and promoting the growth of processes. NGF and BDNF also regulate synaptic plasticity and contribute to learning and memory (Raab *et al*. 2018). Some studies have found high levels of NGF and BDNF in the pyramidal layer of CA1-CA4 regions of hippocampus, indicating that there is a close relationship between NGF and BDNF in terms of learning and memory. Boskovic *et al* (Boskovic *et al*. 2019) used NGF gene knockout heterozygous mutant mice to study the role of endogenous NGF in obtaining spatial memory in mice, and found that mice had significant decline of learning and memory in the maze test when the levels of NGF mRNA and protein in the hippocampus of mutant mice was decreased. Additionally, when the endogenous BDNF level decreased, the escape latency in the maze test was prolonged, and the performance in the space exploration experiment worsened, indicating impairment of spatial learning and memory ability (Canu *et al*. 2017). We demonstrated that NGF and BDNF in the hippocampus of the Sevo group was significantly lower than that in the Con group. The increased apoptosis of hippocampal neurons after inhalation, might result in the down regulation of NGF and BDNF. Our findings are consistent with previous studies (Wang *et al*. 2012; Tian *et al*. 2015).

CONCLUSION

We propose a mechanism for sevoflurane-induced cognitive dysfunction. After continuous inhalation of 3% sevoflurane for 8h, RGS2 expression in the hippocampi of aged rats is down regulated. Moreover, excessive Ca^{2+} influx causes intracellular calcium overload, increases the binding of Ca^{2+} and CaM, activates CaMK II, and leads to increased apoptosis of hippocampal neurons and decreased secretion of NGF and BDNF, which may be an important factor that leads to cognitive dysfunction in rats.

ACKNOWLEDGEMENT

This work was supported by the Project of Natural Science Foundation of Inner Mongolia Autonomous Region (2020MS08095).

REFERENCES

Borgstedt L, Blobner M, Musiol M, Bratke S, Syryca F, Rammes G, Jungwirth B and Schmid S (2020). Neurotoxicity of different amyloid beta subspecies in mice and their interaction with isoflurane anaesthesia. *PLoS. One.*, **15**(12): e0242989.

Boskovic Z, Meier S, Wang Y, Milne MR, Onraet T, Tedoldi A and Coulson EJ (2019). Regulation of cholinergic basal forebrain development, connectivity, and function by neurotrophin receptors. *Neuronal. Signal.*, **3**(1): NS20180066.

Cai Q, Zeng M, Wu X, Wu H, Zhan Y, Tian R and Zhang M (2021). CaMKIIα-driven, phosphatase-checked postsynaptic plasticity via phase separation. *Cell. Res.*, **31**(1): 37-51.

Canu N, Amadoro G, Triaca V, Latina V, Sposato V, Corsetti V, Severini C, Ciotti MT and Calissano P (2017). The intersection of NGF/TrkA signaling and amyloid precursor protein processing in Alzheimer's disease neuropathology. *Int. J. Mol. Sci.*, **18**(6): 1319.

Chen G, Gong M, Yan M and Zhang X (2013). Sevoflurane induces endoplasmic reticulum stress mediated apoptosis in hippocampal neurons of aging rats. *PLoS. One.,* **8**(2): e57870.

Engin AB and Engin A (2021). Alzheimer’s disease and protein kinases. *Adv. Exp. Med. Biol.*, **1275**: 285-321.

Evans PD (2019). Rapid signaling responses via the G protein-coupled estrogen receptor, GPER, in a hippocampal cell line. *Steroids.*, **152**: 108487.

Fan CH, Peng B and Zhang FC (2018). The postoperative effect of sevoflurane inhalational anesthesia on cognitive function and inflammatory response of pediatric patients. *Eur. Rev. Med. Pharmacol. Sci.*, **22**(12): 3971-3975.

Gerber KJ, Squires KE and Hepler JR (2016). Roles for regulator of G protein signaling proteins in synaptic signaling and plasticity. *Mol. Pharmacol.*, **89**(2): 273-286.

Ghanbarabadi M, Falanji F, Rad A, Chazani Sharahi N, Amoueian S, Amin M, Molavi M and Amin B (2019). Neuroprotective effects of clavulanic acid following permanent bilateral common carotid artery occlusion in rats. *Drug. Dev. Res.*, **80**(8): 1110-1119.

Gurevich EV, Gainetdinov RR and Gurevich VV (2016). G protein-coupled receptor kinases as regulators of dopamine receptor functions. *Pharmacol. Res.*, **111**: 1-16.

Hajdu I, Szilagyi A, Vegh BM, Wacha A, Gyorffy D, Gráczer E, Somogyi M, Gal P and Závodszky P (2020). Ligand-induced conformational rearrangements regulate the switch between membrane-proximal and distal functions of Rho kinase 2. *Commun. Biol.*, **3**(1): 721.

Hu N, Wang C, Zheng Y, Ao J, Zhang C, Xie K, Li Y, Wang H, Yu Y and Wang G (2016). The role of the Wnt/β-catenin-Annexin A1 pathway in the process of sevoflurane-induced cognitive dysfunction. *J. Neurochem.*, **137**(2): 240-252.

Iacono G, Altafini C and Torre V (2013). Early phase of plasticity-related gene regulation and SRF dependent transcription in the hippocampus. *PLoS. One.*, **8**(7): e68078.

Kach J, Sethakorn N and Dulin NO (2012). A finer tuning of G-protein signaling through regulated control of RGS proteins. *Am. J. Physiol. Heart. Circ. Physiol.*, **303**(1): H19-35.

Kanai SM, Edwards AJ, Rurik JG, Osei-Owusu P and Blumer KJ (2017). Proteolytic degradation of regulator of G protein signaling 2 facilitates temporal regulation of $G_{q/11}$ signaling and vascular contraction. *J. Biol. Chem.*, **292**(47): 19266-19278.

Kim JL, Bulthuis NE and Cameron HA (2020). The effects of anesthesia on adult hippocampal neurogenesis. *Front. Neurosci.*, **14**: 588356.

Li B, Wang R, Wang Y, Stief CG and Hennenberg M (2020). Regulation of smooth muscle contraction by monomeric non-RhoA GT Pases. *Br. J. Pharmacol.*, **177**(17): 3865-3877.

Li L, Lai EY, Luo Z, Solis G, Mendonca M, Griendling KK, Wellstein A, Welch WJ and Wilcox CS (2018). High salt enhances reactive oxygen species and angiotensin ii contractions of glomerular afferent arterioles from mice with reduced renal mass. *Hypertension*, **72**(5): 1208-1216.

Liu J, Zhao Y, Yang J, Zhang X, Zhang W and Wang P (2017). Neonatal repeated exposure to isoflurane not sevoflurane in mice reversibly impaired spatial cognition at juvenile-age. *Neurochem. Res.*, **42**(2): 595-605.

Mark MD, Wollenweber P, Gesk A, Kösters K, Batzke K, Janoschka C, Maejima T, Han J, Deneris ES and Herlitze S (2019). RGS2 drives male aggression in mice via the serotonergic system. *Commun. Biol.*, **2**: 373.

Meleka MM, Edwards AJ, Xia J, Dahlen SA, Mohanty I, Medcalf M, Aggarwal S, Moeller KD, Mortensen OV and Osei-Owusu P (2019). Anti-hypertensive mechanisms of cyclic depsipeptide inhibitor ligands for Gq/11 class G proteins. *Pharmacol. Res.*, **141**: 264-275.

Mochida S (2019). Presynaptic calcium channels. *Int. J. Mol. Sci.*, **20**(9): 2217.

O'Brien JB, Wilkinson JC and Roman DL (2019). Regulator of G-protein signaling (RGS) proteins as drug targets: Progress and future potentials. *J. Biol. Chem.*, **294**(49): 18571-18585.

Peng S, Zhang Y, Li GJ, Zhang DX, Sun DP and Fang Q (2012). The effect of sevoflurane on the expression of M1 acetylcholine receptor in the hippocampus and cognitive function of aged rats. *Mol. Cell. Biochem.*, **361**(1-2): 229-233.

Raab A, Popp S, Lesch KP, Lohse MJ, Fischer M, Deckert J and Hommers L (2018). Increased fear learning, spatial learning as well as neophobia in Rgs. 2(-/-) mice. *Genes. Brain. Behav.*, **17**(4): e12420.

Tian XS, Tong YW, Li ZQ, Li LX, Zhang T, Ren TY, Zhou T, Wang HC, Zhan R, Sun Y, Yan Z, Wang QD, Fan DS, Kong FJ, Guo XY, Xiao WZ and Chui DH (2015). Surgical stress induces brain-derived neurotrophic factor reduction and postoperative cognitive dysfunction via glucocorticoid receptor phosphorylation in aged mice. *CNS. Neurosci. Ther.*, **21**(5): 398-409.

Wang SH, Liao XM, Liu D, Hu J, Yin YY, Wang JZ and Zhu LQ (2012). NGF promotes long-term memory formation by activating poly (ADP-ribose) polymerase-1. *Neuropharmacology,* **63**(6): 1085-1092.

Wang X, Dong Y, Zhang Y, Li T and Xie Z (2019). Sevoflurane induces cognitive impairment in young mice via autophagy. *PLoS. One.*, **14**(5): e0216372.

Yan WJ, Wang DB, Ren DQ, Wang LK, Hu ZY, Ma YB, Huang JW and Ding SL (2019). AMPKα1 overexpression improves postoperative cognitive dysfunction in aged rats through AMPK-Sirt1 and autophagy signaling. *J. Cell. Biochem.,* doi: 10.1002/jcb.28443.

Yufune S, Satoh Y, Akai R, Yoshinaga Y, Kobayashi Y, Endo S and Kazama T (2016). Suppression of ERK phosphorylation through oxidative stress is involved in the mechanism underlying sevoflurane-induced toxicity in the developing brain. *Sci. Rep.*, **6**: 21859.

Zhang H, Wheat H, Wang P, Jiang S, Baghdoyan HA, Neubig RR, Shi XY and Lydic R (2016). RGS Proteins and Gαi2 modulate sleep, wakefulness and disruption of sleep/wake states after isoflurane and sevoflurane anesthesia. *Sleep.*, **39**(2): 393-404.

Zheng JW, Meng B, Li XY, Lu B, Wu GR and Chen JP (2017). NF-κB/P65 signaling pathway: A potential therapeutic target in postoperative cognitive dysfunction after sevoflurane anesthesia. *Eur. Rev. Med. Pharmacol. Sci.*, **21**(2): 394-407.