Mol Cell Biochem (2012) 361:229–233
DOI 10.1007/s11010-011-1107-8

The effect of sevoflurane on the expression of M_1 acetylcholine receptor in the hippocampus and cognitive function of aged rats

Sheng Peng · Yan Zhang · Guo-Jun Li · Deng-Xin Zhang · Da-Peng Sun · Qiang Fang

Received: 17 May 2011 / Accepted: 28 September 2011 / Published online: 14 October 2011

Abstract Our aim is to investigate the effect of 1.5 and 3.0% sevoflurane on the expression of M_1 acetylcholine receptor (mAChR M_1) in the hippocampus and the cognitive function of aged rats. Forty Sprague–Dawley (SD) rats of 12-month old were randomly divided into five groups. All SD rats received 1.5 or 3.0% sevoflurane in a special glass anesthesia box for 2 h, respectively, except for the normal control group. Y-maze was used to test the ability of learning and memory after being received sevoflurane for 1 or 7 days at the same moment portion. The expression of mAChR M_1 in the hippocampus of rats was tested by RT-PCR. The results showed that 3% sevoflurane induced the decline of cognitive function and significantly decreased the mAChR M_1 expression at mRNA levels at 1 day in the 3.0% sevoflurane I group when compared with the normal control group. However, there was no significant difference among the other groups when compared with normal control group. Therefore, administration of sevoflurane might temporally affect the ability of cognitive function of rats through suppressing the mAChR M_1 expression at mRNA levels in hippocampus.

Keywords Sevoflurane · Cognition · mAChR M_1 · Hippocampus

This work was supported by The National Natural Science Foundation of China (81000469) and Scientific Foundation from Health Office of Jiangsu (201070).

S. Peng · Y. Zhang (✉) · G.-J. Li · D.-X. Zhang · D.-P. Sun · Q. Fang
Department of Anesthesiology, Affiliated No.4 Hospital of Soochow University, Wuxi 214062, China
e-mail: zhang-yanzy@live.cn

Introduction

Several studies have given strong evidence to the hypothesis that brain cholinergic systems were involved in the process of memory and cognition [1–3]. Meanwhile, cholinergic stimulation facilitates learning and memory consolidation, while its blockade produces amnesia. Therefore, administration of anticholinergic drugs could induce learning deficits in a wide variety of tasks [2, 3]; while treatment with enhanced cholinergic transmission could improve memory [4–6]. The differential distribution of muscarinic receptor subtypes in the brain suggests that the M1 subtype may be particularly important in the process of memory and cognition, since this receptor is rich in forebrain areas [7–9].

Recent studies indicate that doses of volatile anesthetic agents around 0.3 minimum alveolar concentration (MAC) inhibit learning and cause amnesia [10–15]. However, some researchers demonstrated that a low-dose halothane exposure (i.e., 0.1 MAC) during learning significantly enhanced 24-h retention performance [17]. There were a host of paradigms available for studying the effects of anesthetics on cognitive function in both animals and humans [13, 16, 18, 19]. All of these studies suggesting that ketamine might have stronger effect on learning and memory. However, the mechanism of the influence of anesthetics on the neurological functions has not been completely understood. However, we have not known that whether or not sevoflurane have also an effect on learning and memory.

In the present study, we selected the attempt to investigate the effects of sevoflurane on cognitive function in aged SD rats by training with Y-maze task. Since mAChR M_1 played a crucial role in the cognitive function, we also assessed the effects of sevoflurane on mAChR M_1 expression in the

hippocampus. mRNA levels of mAChR M_1 were measured in the hippocampus.

Materials and methods

Animals

Forty SD rats, 12-month old and weighing from 500 to 650 g, were provided by the Laboratory Animal Center of Hangzhou. The housing and treatment of the animals were in accordance with institutional guidelines and approved by the Institutional Animal Care and Use Committee. They were randomly divided into five groups: the normal control group ($n = 8$); 1.5% sevoflurane I group (be tested after received 1.5% sevoflurane for 1 day) ($n = 8$); 1.5% sevoflurane II group (be tested after received 1.5% sevoflurane for 7 day) ($n = 8$); 3.0% sevoflurane I (be tested after received 3.0% sevoflurane for 1 day) ($n = 8$) and 3.0% sevoflurane II group (be tested after received 3.0% sevoflurane for 7 day) ($n = 8$). All rats received 1.5 or 3.0% sevoflurane in a special glass anesthesia box for 2 h, respectively, except for the normal control group. Y-maze was used to test the cognitive function of SD rats in different groups. mAChR M_1 was tested by semi-quantitative RT-PCR at mRNA level.

The test of cognitive function

As described in our previously published study [20], the experiment of learning and memory was performed using Y-maze in the quiet, low-light situations in the afternoon. The operation for the Y-maze and the assessment for the behavior of rats were conducted by the fixed personnel, which could exclude the interference with the experimental results by the noise and time factors, and so on. The bottom of Y-Maze is the copper grid interval, the end of each arm have lights, the copper grid in the bottom of Y-Maze with lights bright of one arm has no current, while no light source of two-arms and three-arms were electrified. Adapting the maze for 5 min before the test, the test stimulation voltage was regulated to ensure that rats could run to escape within 10 s. It was considered the correct response that the rats ran directly to the security place after the electric shock, otherwise an error response. When the rats ran to a safe place the light bright was continued to 15 s, then turn off the lights for 45 s, across the next test. The direction of lighting appeared in accordance with I → II → III → I sequence of transformations, there shall be to reach the learning criteria until nine times correct response of the 10 consecutive responses. The total number of learning and the total of time were recorded.

mAChR M_1 expression by Semi-quantitative RT-PCR analysis

After anesthetizing with a solution of chloral hydrate (0.4 ml/100 g, i.p.), mice were perfused transcardially with 40 ml of normal saline followed by 30 ml of 4% formaldehyde in PBS (pH 7.4). The brain was fixed in 4% formaldehyde at 4 for 6 h and kept in a 25% sucrose solution overnight. With reference to the former studies [21], the total RNA was isolated using Trizol reagent (Invitrogen). cDNA was synthesized using the QuantiTect Reverse Transcription kit (Qiagen, USA) according to manufacturer's instructions. The primer sequences and the expected sizes of PCR products were as follows: mAChR M_1: (sense) 5′-GCACAGGCACCCACCAAGCAG-3′ and (antisense) 5-AGAGCAGCAGCAGGCGGAACG-3 (373 bp); β-actin: (sense) 5′-TGGTGGGTATGGGTCAGAAGGAC TC-3′ and (antisense) 5-CATGGCTGGGGTGTTGAAGGT CTCA-3′ (265 bp). Total RNA was extracted using Trizol reagent (Invitrogen, USA) and RT-PCR was performed with conditions as follows: reverse transcription at 48°C for 30 min and denaturation at 94°C for 2 min; then amplification for 30 cycles at 94°C for 0.5 min, annealing at 60°C for 0.5 min, and extension at 72°C for 0.5 min; then terminal elongation step at 72°C for 10 min and a final holding stage at 4°C.

Statistical analysis

All statistical analyses were done by the computer program SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± SD. P values <0.05 were considered as statistically significant.

Results

Cognitive function

To evaluate cognitive function in the subjects, a Y-maze test was conducted. In this task, rats had to learn and remember an association between the light and escape. As is shown in Table 1, the effect of different doses of sevoflurane on cognitive function of rats showed that the time of learning and number of training of rats in 3% sevoflurane I group (be tested after received 3.0% sevoflurane for 1 day) was significantly increased compared with the normal saline group ($P < 0.05$). However, there was no significant difference in 1.5% sevoflurane I group (be tested after received 1.5% sevoflurane for 1 day), 1.5% sevoflurane II group (be tested after received 1.5% sevoflurane for 7 days) and 3.0% sevoflurane II group (be tested after received 3.0% sevoflurane for 7 days) when compared with the cognitive function of rats in normal saline group ($P > 0.05$).

Table 1 The test results of cognitive function in each group of SD rats

Group	Cases	Time of training (min)	Number of training
Normal control group	8	38.24 ± 0.68	33.50 ± 4.80
1.5% sevoflurane I group	8	39.55 ± 1.40	34.67 ± 3.84
1.5% sevoflurane II group	8	39.43 ± 0.87	34.50 ± 3.58
3.0% sevoflurane I group	8	48.76 ± 1.24*	41.83 ± 4.30*
3.0% sevoflurane II group	8	38.49 ± 0.73	34.46 ± 3.83

Compared with normal control group, *$P < 0.05$

Analysis of mAChR M_1 expression by RT-PCR

Next, we determined whether the cognitive function impairment was associated with the mAChR M_1 expression levels. The altered expression of mAChR M_1 in the hippocampus was carried out to compare its transcripts by RT-PCR analysis. As is shown in Fig. 1, the results showed that 3% sevoflurane induced the decline of cognitive function and significantly deceased the mAChR M_1 expression at mRNA levels at 1 day in the 3.0% sevoflurane I group (be tested after received 3.0% sevoflurane for 1 day) when compared with the normal control group ($P < 0.05$). However, there was no significant difference in 1.5% sevoflurane I group (be tested after received 1.5% sevoflurane for 1 day), 1.5% sevoflurane II group (be tested after received 1.5% sevoflurane for 7 days) and 3.0% sevoflurane II group (be tested after received 3.0% sevoflurane for 7 days) when compared with mAChR M_1 expression the of rats in normal saline group ($P > 0.05$). Therefore, administration of sevoflurane may temporally affect the ability of cognitive function of rats through suppressing the mAChR M_1 expression at mRNA levels in hippocampus.

Discussion

In the present study, the pharmacological characteristics of sevoflurane as the theoretical basis to further explore the effect of sevoflurane on the cognitive function in 12-month-old SD rats and its possible mechanisms. The results demonstrated that administration of sevoflurane might temporally affect the ability of cognitive function of rats through suppressing the mAChR M_1 expression at mRNA levels in hippocampus.

Using the Y-maze test, we found that the effect of different doses of sevoflurane on cognitive function of rats showed that the cognitive function of rats in 3% sevoflurane I group was lower than in normal saline group. However, there was no significant difference in 1.5%

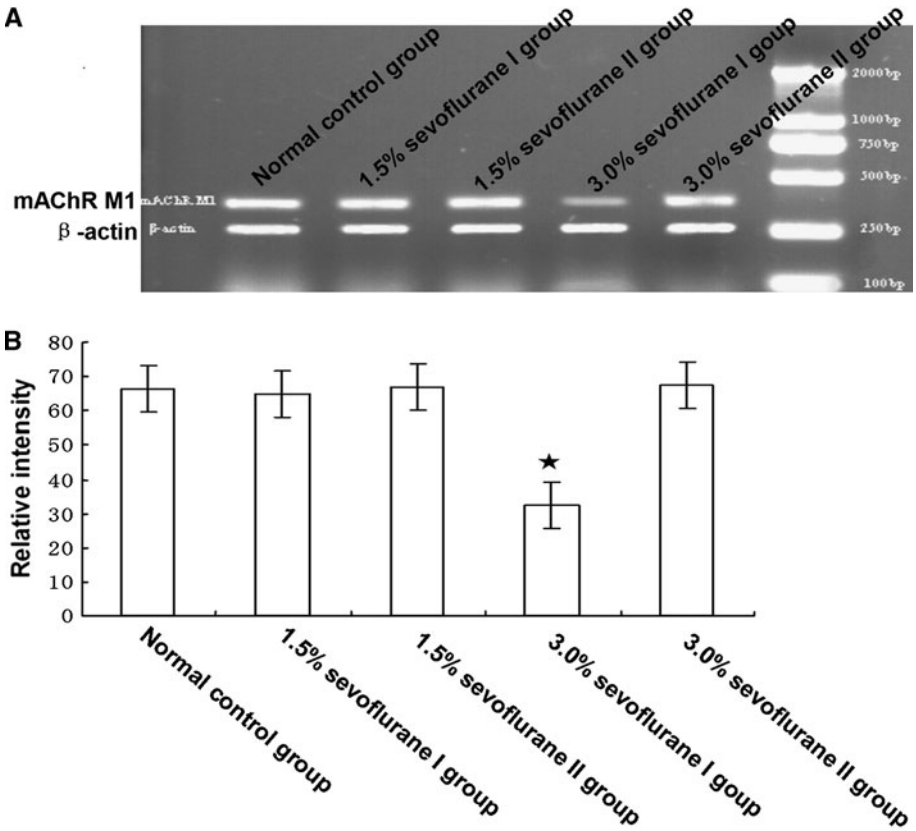

Fig. 1 Analysis of mAChR M_1 expression by semi-quantitative RT-PCR. 3% sevoflurane induced the decline of cognitive function and significantly deceased the mAChR M_1 expression at mRNA levels at 1 day in the 3.0% sevoflurane I group (be tested after received 3.0% sevoflurane for 1 day) when compared with the normal control group ($P < 0.05$). There was no significant difference in 1.5% sevoflurane I group (be tested after received 1.5% sevoflurane for 1 day), 1.5% sevoflurane II group (be tested after received 1.5% sevoflurane for 7 days) and 3.0% sevoflurane II group (be tested after received 3.0% sevoflurane for 7 days) when compared with mAChR M_1 expression the of rats in normal saline group ($P > 0.05$)

sevoflurane I group, 1.5% sevoflurane II group and 3.0% sevoflurane II group when compared with the cognitive function of rats in normal saline group. As expected, the changes in cognitive function of SD rats are in parallel with the variations of the mAChR M_1 expression; i.e., reduced cognitive function of SD rats upon administration of 3.0% sevoflurane I group is accompanied by decreased expression of the mAChR M_1. The mAChR M_1 expression of pseudotraining group was higher than those in the normal control group; however, there was no significant difference in 1.5% sevoflurane I group, 1.5% sevoflurane II group and 3.0% sevoflurane II group when compared with mAChR M_1 expression the of rats in normal saline group. This finding suggests that mAChR M_1 expression may be involved in the mechanical stress-induced and sevoflurane-induced cognitive impairment.

The decrease in mAChR M_1 mRNA we observed might be due to specific downregulation of transcription of the mAChR M_1 gene. Several researches have demonstrated that the disrupting effects of intracerebral application of pirenzepine, a relatively selective M1 antagonist, on acquisition of inhibitory avoidance response in mice [22] as well as on spatial learning [23, 24], and representational memory in rats [25, 26]. Bymaster et al. [27] have confirmed a clear relationship between the neurochemical anticholinergic activity of subcutaneous administration of pirenzepine and trihexyphenidyl with the behavioral blockade of working memory performance in rats. Meanwhile, Roldán et al. [28] have revealed that selective blockade of the M1 muscarinic receptor subtype produced a dose-related impairment in memory consolidation of inhibitory avoidance, which indicated that the selective blockade of the central M1 muscarinic receptors interfered with memory consolidation of inhibitory avoidance and suggested that this receptor subtype was important involved in mnemonic functions. The effect of sevoflurane on the cognitive function was associated with the expression of mAChR M_1, which observed in the present study was expected. Recently, it has been reported that voltage-gated sodium channels have important roles in anesthetic mechanisms. Much attention has been paid to the effects of sevoflurane on voltage-dependent sodium channels. To elucidate this, Yokoyama et al. [29] have examined the effects of sevoflurane on Na(v) 1.8, Na(v) 1.4, and Na(v) 1.7 expressed in Xenopus oocytes. The effects of sevoflurane on Na(v) 1.8, Na(v) 1.4, and Na(v) 1.7 sodium channels were studied by an electrophysiology method using whole-cell, two-electrode voltage-clamp techniques in Xenopus oocytes. The results revealed that sevoflurane appears to have inhibitory effects on Na(v)1.8, Na(v)1.4, and Na(v) 1.7 by PKC pathways. However, it is still unknown to what the extent of mAChR M_1 expression participates in this process. Therefore, further research needs to be done to unravel the underlying mechanisms.

In our current study, the results demonstrate that administration of over-anesthetic sevoflurane may impair cognitive function of old rats. mAChR M_1 expression may be involved in the cognitive function. The decreased levels of mAChR M_1 expression may be one of the mechanisms of the impairment of cognitive function by sevoflurane. However, whether or not other factors were also involved the process needed to be investigated in the future researches.

Acknowledgments This work was supported by Medical Science Research Foundation of Jiangsu Province, China (Grant No. H200645) and Science Foundation of the Health Bureau of Wuxi City, China (Grant No. XM0805).

References

1. Fibiger HC (1991) Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. Trends Neurosci 14:220–223
2. Smith G (1988) Animal models of Alzheimer's disease: experimental cholinergic denervation. Brain Res 472:103–118
3. Izquierdo I, McGaugh JL (2000) Behavioural pharmacology and its contribution to the molecular basis of memory consolidation. Behav Pharmacol 11:517–534
4. Sitaram N, Weingartner H, Gillin JC (1978) Human serial learning: enhancement with arecholine and choline impairment with scopolamine. Science 201:274–276
5. Sweeney JE, Bachman ES, Coyle JT (1990) Effects of different doses of galanthamine, a long-acting acetylcholinesterase inhibitor, on memory in mice. Psychopharmacology 102:191–200
6. Mangelus M, Kroyter A, Galron R, Sokolovsky M (2001) Reactive oxygen species regulate signaling pathways induced by M1 muscarinic receptors in PC12M1 cells. J Neurochem 76:1701–1711
7. Robinson L, Platt B, Riedel G (2011) Involvement of the cholinergic system in conditioning and perceptual memory. Behav Brain Res 221(2):443–465
8. Hasselmo ME (2006) The role of acetylcholine in learning and memory. Curr Opin Neurobiol 16(6):710–715
9. Levey AI, Kitt CA, Simonds WF, Price DL, Brann MR (1991) Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J Neurosci 11:3218–3226
10. Eger EI II, Saidman LJ, Brandstater B (1965) Minimum alveolar anesthetic concentration: a standard of anesthetic potency. Anesthesiology 26:756–763
11. Cook TL, Smith M, Winter PM, Starkweather JA, Eger EI III (1978) Effect of subanesthetic concentration of enflurane and halothane on human behavior. Anesth Analg 57:434–440
12. Dwyer R, Bennett HL, Eger EI II, Heilbron D (1992) Effects of isoflurane and nitrous oxide in subanesthetic concentrations on memory and responsiveness in volunteers. Anesthesiology 77: 888–898
13. Ghoneim MM, Block RI (1997) Learning and memory during general anesthesia: an update. Anesthesiology 87:387–410
14. El-Zahaby HM, Ghoneim MM, Johnson GM, Gormezano I (1994) Effects of subanesthetic concentrations of isoflurane and their interactions with epinephrine on acquisition and retention of the rabbit nictitating membrane response. Anesthesiology 81: 229–237
15. Kandel L, Chortkoff BS, Sonner J, Laster MJ, Eger EI II (1996) Nonanesthetics can suppress learning. Anesth Analg 82:321–326

16. Dutton RC, Maurer AJ, Sonner JM, Fanselow MS, Laster MJ, Eger EI II (2001) The concentration of isoflurane required to suppress learning depends on the type of learning. Anesthesiology 94:514–519
17. Alkire MT, Gorski LA (2004) Relative amnesic potency of five inhalational anesthetics follows the Meyer–Overton rule. Anesthesiology 101:417–429
18. McGaugh JL (2000) Memory. A century of consolidation. Science 287:248–251
19. Veselis RA, Reinsel RA, Feshchenko VA, Wronski M (1997) The comparative amnestic effects of midazolam, propofol, thiopental, and fentanyl at equisedative concentrations. Anesthesiology 87:749–764
20. Peng S, Zhang Y, Zhang J, Wang H, Ren B (2009) Effect of ketamine on ERK expression in hippocampal neural cell and the ability of learning behavior in minor rats. Mol Biol Rep. [Epub ahead of print]
21. Ma YL, Peng JY, Liu WJ, Zhang P, Huang L, Gao BB, Shen TY, Zhou YK, Chen HQ, Chu ZX et al (2009) Proteomics identification of desmin as a potential oncofetal diagnostic and prognostic biomarker in colorectal cancer. Mol Cell Proteomics 8(8):1878–1890
22. Orsini C, Castellano C, Cabib S (2001) Pharmacological evidence of muscarinic-cholinergic sensitization following chronic stress. Psychopharmacology 155:144–147
23. Herrera-Morales W, Mar I, Serrano B, Bermúdez-Rattoni F (2007) Activation of hippocampal postsynaptic muscarinic receptors is involved in long-term spatial memory formation. Eur J Neurosci 25:1581–1588
24. von Linstow Roloff E, Harbaran D, Micheau J, Platt B, Riedel G (2007) Dissociation of cholinergic function in spatial and procedural learning in rats. Neuroscience 146:875–889
25. Ohno M, Yamamoto T, Watanabe S (1994) Blockade of hippocampal M1 muscarinic receptors impairs working memory performance of rats. Brain Res 650:260–266
26. Messer WS Jr, Thomas GJ, Hoss W (1987) Selectivity of pirenzepine in the central nervous system. II. Differential effects of pirenzepine and scopolamine on performance of a representational memory task. Brain Res 407:37–45
27. Bymaster FP, Heath I, Hendrix JC, Shannon HE (1993) Comparative behavioral and neurochemical activities of cholinergic antagonists in rats. J Pharmacol Exp Ther 267:16–24
28. Roldán G, Bolaños-Badillo E, González-Sánchez H, Quirarte GL, Prado-Alcalá RA (1997) Selective M1 muscarinic receptor antagonists disrupt memory consolidation of inhibitory avoidance in rats. Neurosci Lett 230:93–96
29. Yokoyama T, Minami K, Sudo Y, Horishita T, Ogata J, Yanagita T, Uezono Y (2011) Effects of sevoflurane on voltage-gated sodium channel Na(v)1.8, Na (v)1.7, and Na (v)1.4 expressed in Xenopus oocytes. J Anesth 25:609–613