Neuroscience Letters 562 (2014) 13–18

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Sevoflurane induced amnesia inhibits hippocampal Arc expression partially through 5-hydroxytryptamine-7 receptors in the bilateral basolateral amygdala in rats☆

Fujun Zhang[a], Xiaomei Feng[a], Qingwen Zeng[b], Bo Wang[b], Kevin Wilhelmsen[c], Qiang Li[a], Xiaohua Cao[b], Buwei Yu[a,*]

[a] Department of Anesthesiology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200025, China
[b] Key Laboratory of Brain Functional Genomics, Ministry of Education, Shanghai Key Laboratory of Brain Functional Genomics, East China Normal University, Shanghai 200062, China
[c] Department of Anesthesia and Perioperative Care, San Francisco General Hospital, University of California, San Francisco, CA 94143, USA

HIGHLIGHTS

- Sevoflurane could induce amnesia and inhibit hippocampal Arc expression after fear conditioning.
- The effects could be reversed by BLA infusion of SB269970 and exacerbated by AS-19.
- Sevoflurane might not induce neuroapoptosis in the hippocampus.

ARTICLE INFO

Article history:
Received 30 July 2013
Received in revised form 5 December 2013
Accepted 28 December 2013

Keywords:
Sevoflurane
Basolateral amygdala
Learning and memory
5-Hydroxytryptamine receptor
Activity-regulated cytoskeletal protein
Neural apoptosis

ABSTRACT

This study aimed to investigate whether the regulation of 5-hydroxytryptamine-7 (5-HT_7) receptors in the bilateral basolateral amygdala (BLA) could alter the amnesic effects of sevoflurane and change the hippocampal expression of Arc and neural apoptosis. Male Sprague-Dawley rats were randomized into ten groups. First, the animals received bilateral injection of SB269970 (20, 50, or 100 pmol/0.2 μl) or saline (0.2 μl) or AS-19 (2, 10, or 50 pmol/0.2 μl), followed by inhalation of 2% sevoflurane or air for 2 h. Then, fear conditioning training was carried out, and the percentage of freezing was detected 24 h later. Furthermore, hippocampal Arc protein level and neural apoptosis were measured. Pre-training inhalation of sevoflurane reduced the extent of freezing, and hippocampal Arc expression. The largest dose of SB269970 (100 pmol) could block sevoflurane-induced amnesia and reverse the inhibitive effect of sevoflurane on Arc expression, while the maximal dose of AS-19 could exacerbate the amnesic effect, and further inhibit Arc expression. Furthermore, pre-training inhalation of 2% sevoflurane for 6 h could not induce neural apoptosis in the hippocampus. The amnesic effect of sevoflurane might partly attribute to its impairment of memory formation in the hippocampus via activation of 5-HT_7 receptors in the BLA.

1. Introduction

Amounting evidence indicates that general anesthetics induce amnesic effects [1,22]. It has been shown that propofol and the inhalational anesthetics including sevoflurane, and isoflurance, at sub-anesthetic concentrations, could block emotional memory [2,3]. Sevoflurane is the most commonly used inhalational anesthetic, and our previous study shows that memory consolidation in rats can be impaired by inhaling 2% sevoflurane [11,12]. However, due to the large amount of targets that general anesthetics possess, and the complexity of learning and memory processes, more studies are warranted to investigate the effects of general anesthetics on memory.

Many studies have demonstrated that the basolateral amygdala (BLA) complex plays an important role in memory modulation by many drugs and hormones [2]. The BLA could regulate the consolidation of memory in other brain regions, and the BLA-hippocampus pathway was the most likely candidate. Our previous studies have shown that the BLA-hippocampus pathway is involved in the mechanisms associated with amnesia induced by propofol

* Corresponding author. Tel.: +86 21 64370045; fax: +86 21 63047221.
E-mail addresses: buweiyu@gmail.com, whx_0224@hotmail.com (B. Yu).

0304-3940/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.12.066

and sevoflurane [11,22]. Further studies provided evidence that the mechanism mediating the memory-impairing effect of sevoflurane involves an interplay between the BLA noradrenergic system and expression of activity-regulated cytoskeletal (Arc) protein in the hippocampus. There are many receptors in the BLA, such as 5-hydroxytryptamine (5-HT) receptors [4,9]. Whether these receptors are involved in the BLA-hippocampus pathway and can play an important role in the memory-impairing effect of general anesthetics remains to be determined.

Many studies have reported a role for the 5-HT_{1A} receptor in learning and memory [16]. Indeed, most of the 5-HT receptors characterized so far, i.e., 5-HT_1 through 5-HT_7, show a regional distribution in brain areas involved in learning and memory, such as the hippocampal formation (HF), amygdala and cortex. Although 5-HT_7 receptor functions still need to be clarified, it has been suggested that it might play a role in the control of learning and memory. Since, midazolam and propofol have been found to increase central serotonergic activity and provoke retrograde amnesia which could be completely abrogated by a 5-HT antagonist, it appears that facilitation of the serotonergic system may be involved in retrograde amnesia caused by these agents [24]. Thus, this study aimed to investigate whether facilitation of the serotonergic system is also involved in the amnesia caused by sevoflurane.

The availability of selective 5-HT_7 agonists and antagonists greatly augmented our ability to investigate the role of these receptors. Based on previous studies [7,17], where 5-HT_7 receptor antagonist SB269970 and agonist AS-19 were used, this study aimed to investigate the amnesic effects of sevoflurane on rats trained with fear conditioning, and determine the effects of sevoflurane on the hippocampal expression of Arc. In addition, the effects of sevoflurane on hippocampal neural apoptosis were analyzed.

2. Materials and methods

2.1. Subjects

Adult male Sprague-Dawley rats (250–280 g) were housed on a 12/12-h light/dark cycle at 22 °C with food and water available ad libitum. All procedures were approved by Institutional Animal Care and Use Committee of Shanghai Jiaotong University School of Medicine, Shanghai, China and conformed to the guidelines for ethical treatment of animals. Rats were acclimatized to the colony room for 1 week before surgery, and underwent behavioral procedures 7 days after surgery. All behavioral testing was performed between 9:00 AM and 4:00 PM during the lights on period.

2.2. Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg). Bilateral guide cannulae (23 gauge) were implanted dorsal to the BLA as previously described [22]. After 2 days of recovery from surgery, rats were handled daily for 5 days (5 min/day).

2.3. Drug administration

Rats were bilaterally intra-BLA infused with SB269970 hydrochloride (Tocris Bioscience, Minneapolis, MN) (20, 50, or 100 pmol/0.2 μl), AS-19 (Tocris Bioscience) (2, 10, or 50 pmol/0.2 μl) or saline (0.2 μl) through a 30-gauge needle extending 2 mm beyond the cannulae. SB269970 solution was microinjected by a minipump (0.1 μl/min). The injection needle remained in place for an additional 2 min to maximize diffusion of the solution.

Thirty minutes later, animals were received 2% sevoflurane in a humidified 30% oxygen carrier gas or 30% oxygen without inhalational anesthetic for 2 h or 6 h, and the rats' body temperature was maintained at 37 ± 0.5 °C. The inhalational agents, oxygen and carbon dioxide levels in the chamber, were monitored continuously as previously described [12]. The rats were immediately removed from the chamber after termination of anesthesia, and all animals recovered rapidly from anesthesia and displayed no neurologic symptoms or signs of discomfort. There was no mortality during or after anesthesia.

2.4. Behavioral studies

Sevoflurane and control rats underwent open-field (OF), and fear conditioning training and test.

2.4.1. Habituation

Rats were habituated to the apparatus for 15 min three days before training.

2.4.2. Fear conditioning

Fifteen minutes after the anesthesia, the rats were trained with a single conditioning trial for 240 s consisting of 180 s quiet environment (without tone and shock), 30 s tone (4.5 kHz, 80 dB, CS) that co-terminated with a 3 s shock (0.75 mA, US), and again 30 s quiet environment.

2.4.3. Contextual test and cued test

Rats were returned to the conditioning chamber 24 h after the conditioning, and observed and scored for freezing for 360 s. After 6 min in the training chamber, rats were transferred to the tone test chambers for 240 s auditory tone test, that is, 120 s with no tone stimulation followed by 120 s tone. The movement of each rat was monitored and analyzed using a computer-operated video tracking system (Coulbourn Instrument, Allentown, PA). The number of observations scored as freezing was summed and converted to a percentage (number of freezing observations/total number of observations × 100).

2.4.4. OF behavior

Basal locomotor activity was assessed 24 h after sevoflurane exposure as described previously [12].

2.5. Western blotting

One hour after sevoflurane anesthesia, the rats were anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally), decapitated, and the hippocampi were harvested. The tissues were homogenated and protein concentrations were determined by the Bradford method.

Western blotting was performed as previously described [22]. The primary antibodies were as follows (Anti-Arc, 1:200; Anti-Caspase-3, 1:1000; Santa Cruz Biotechnology Inc., Santa Cruz, CA).

2.6. Histology

After the long-term memory test, the rats were deeply anesthetized, perfused intracardially with a 0.9% saline followed by 4% polyoxymethylene solution. All the procedures followed our previous study [22].

2.7. TUNEL staining assay

The perfused rat brain tissues were exposed to immersion fixation for 24 h at 4 °C in 4% paraformaldehyde, and then made into 5-μm paraffin-embedded sections. TMRred kit (Roche, Palo Alto, CA) was used for TUNEL staining. Briefly, the brain sections were incubated in a permeabilization solution, then with a TUNEL reaction mixture, and finally with 10 μg/ml Hoechst 33342 in a