Developmental Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Developmental Biology

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

Mid-gestational sevoflurane exposure inhibits fetal neural stem cell proliferation and impairs postnatal learning and memory function in a dose-dependent manner

Yuan Wang[a], Shaowei Yin[b], Hang Xue[a], Yating Yang[a], Nan Zhang[c], Ping Zhao[a,*]

[a] Department of Anesthesiology, Shengjing Hospital of China Medical University, China Medical University, Shenyang 110004, China
[b] Department of Obstetrics, Shengjing Hospital of China Medical University, China Medical University, Shenyang 110004, China
[c] Department of Neuroendocrine Pharmacology, School of Pharmacy, China Medical University, Shenyang 110122, China

ARTICLE INFO

Keywords:
Sevoflurane
Mid-gestation
Neural stem cells
Proliferation
Wnt signaling pathway
Pregnancy

ABSTRACT

Advancements in fetal intervention procedures have led to increases in the number of pregnant women undergoing general anesthesia during the second trimester—a period characterized by extensive proliferation of fetal neural stem cells (NSCs). However, few studies have investigated the effects of mid-gestational sevoflurane exposure on fetal NSC proliferation or postnatal learning and memory function. In the present study, pregnant rats were randomly assigned to a control group (C group), a low sevoflurane concentration group (2%; L group), a high sevoflurane concentration group (3.5%; H group), a high sevoflurane concentration plus lithium chloride group (H + Li group), and a lithium chloride group (Li group) at gestational day 14. Rats received different concentrations of sevoflurane anesthesia for 2 h. The offspring rats were weaned at 28 days for behavioral testing (i.e., Morris Water Maze [MWM]), and fetal brains or postnatal hippocampal tissues were harvested for immunofluorescence staining, real-time PCR, and Western blotting analyses in order to determine the effect of sevoflurane exposure on NSC proliferation and the Wnt/β-catenin signaling pathway. Our results indicated that maternal exposure to 3.5% sevoflurane (H group) during the mid-gestational period impaired the performance of offspring rats in the MWM test, reduced NSC proliferation, and increased protein levels of fetal glycogen synthase kinase-3 beta (GSK-3β). Such treatment also decreased levels of β-catenin protein, *CD44* RNA, and *Cyclin D1* RNA relative to those observed in the C group. However, these effects were transiently attenuated by treatment with lithium chloride. Conversely, maternal exposure to 2% sevoflurane (L group) did not influence NSC proliferation or the Wnt signaling pathway. Our results suggest that sevoflurane exposure during the second trimester inhibits fetal NSC proliferation via the Wnt/β-catenin pathway and impairs postnatal learning and memory function in a dose-dependent manner.

1. Introduction

Advancements in fetal intervention procedures have led to increases in the number of pregnant women undergoing general anesthesia during the second trimester. Therefore, it is important to investigate the neurodevelopmental consequences of maternal exposure to anesthetics during this period (Kuczkowski, 2004; Reitman and Flood, 2011). To date, studies regarding the neurodevelopmental consequences of maternal exposure to anesthetics have focused primarily on hippocampal changes in offspring, demonstrating that increased neuroapoptosis in the hippocampus induces neurobehavioral abnormalities (Kong et al., 2011a, 2012). Although such changes in the postnatal hippocampus are likely initiated during the embryonic period, few research groups have investigated fetal changes following maternal exposure to anesthetics.

The mid-gestational period is critical for hippocampal development, which begins during this stage under strict control: Neural precursor cells in the neural epithelia of the hippocampus and dentate gyrus undergo proliferation, migration, and differentiation—a process completed prior to birth (Sanes et al., 2005; Khalaf-Nazzal and Francis, 2013). In addition, fetal hippocampal development is extremely sensitive to changes in the external environment as well as drugs, so any interference during this period can result in abnormal hippocampal development or long-term behavioral abnormalities (Berman and Hannigan, 2000; Ishola and Adeniyi, 2013; Noorlander et al., 2014). Several studies have demonstrated that neural stem cell (NSC)

* Correspondence to: Department of Anesthesiology, Shengjing Hospital of China Medical University, China Medical University, No.36, Sanhao Street, Heping District, Shenyang, Liaoning Province 110004, China.
E-mail address: zhaop@sj-hospital.org (P. Zhao).

https://doi.org/10.1016/j.ydbio.2018.01.022
Received 19 August 2017; Received in revised form 23 January 2018; Accepted 30 January 2018

proliferation in the hippocampus during the second trimester is crucial for hippocampal-dependent learning (Sun et al., 2013; Leraci and Herrera, 2007). Moreover, previous studies have reported that treatment with anesthetic agents decreases the self-renewal capacity of cultured NSCs in vitro (Culley et al., 2011; Nie et al., 2013; Zhang et al., 2013). Therefore, we hypothesized that neurobehavioral abnormalities in offspring following maternal exposure to anesthesia during the second trimester are caused by the inhibition of NSC proliferation. However, no studies to date have investigated whether maternal anesthesia influences NSC proliferation in the fetus.

The Wnt signaling pathway is a classical pathway involved in the regulation of NSC proliferation (Logan and Nusse, 2004). Glycogen synthase kinase 3 beta (GSK-3β) and β-catenin are key molecules in this pathway. Previous studies have demonstrated that GSK-3β inhibits long-term potentiation (LTP), causes tau hyperphosphorylation, and generates β-amyloid (Hooper et al., 2007; Yuskaitis and Jope, 2009; Bayod et al., 2011; Hong et al., 2012). Additional studies have revealed that exposure to sevoflurane on postnatal day 7 (P7) increases GSK-3β levels in neonatal rats, and that sevoflurane exposure impairs memory consolidation in adult rats, possibly by inhibiting the phosphorylation of GSK-3β in the hippocampus (Liu et al., 2010). Furthermore, sevoflurane has been reported to increase GSK-3β levels, decrease β-catenin levels, and inhibit NSC proliferation in mouse neural progenitor cells (Zhang et al., 2013). To investigate the mechanisms underlying these effects, we therefore assessed GSK-3β and β-catenin levels in the fetal rat brain. As *CD44* and *Cyclin D1*—downstream target genes of the Wnt signaling pathway that are directly involved in the regulation of NSC proliferation—are closely related to neurogenesis as well as learning and memory (Wu et al., 2012; Raber et al., 2014; Su et al., 2017), we also investigated the expression of *CD44* and *Cyclin D1* in the fetal rat brain.

Sevoflurane is the most commonly used anesthetic agent in clinical practice. Moreover, high concentrations of sevoflurane (e.g., 1.0–1.5 minimum alveolar concentration [MAC]) are typically used in clinical settings to facilitate uterine quiescence and minimize the risk of preterm labor (Gursoy et al., 2004; Sviggum and Kodali, 2013; Heesen and Klimek, 2016). One previous study reported that treatment of pregnant mice with 2.5% sevoflurane anesthesia induced neuroinflammation, neuroapoptosis, and learning and memory dysfunction in offspring (Zheng et al., 2013). In the present study, we utilized sevoflurane concentrations of 2% or 3.5% (1 MAC or 1.5 MAC) to investigate whether anesthetic concentration influences learning and memory abnormalities. Moreover, we examined the effect of sevoflurane on fetal NSC proliferation and explored the potential mechanisms underlying the observed effects by assessing levels of GSK-3β, β-catenin, *CD44* RNA, and *Cyclin D1* RNA. Finally, we investigated whether lithium chloride—a GSK-3β inhibitor—can ameliorate sevoflurane-induced abnormalities.

2. Materials and methods

The present study was approved by the Ethics Committee of Shengjing Hospital of China Medical University. Healthy Sprague-Dawley (SD) rats weighing 380–420 g were provided by the Animal Breeding Center of Shengjing Hospital of China Medical University. Rats were raised in an environment with a temperature of 20–25 °C under natural lighting, and were allowed ad libitum access to food and water. Female and male rats were paired, and the presence of a positive vaginal smear was considered indicative of gestation day 0.

On the 14th day of gestation, pregnant rats were randomly divided into five groups: a control group (C group, n = 56), a low sevoflurane concentration group (L group, n = 56), a high sevoflurane concentration group (H group, n = 56), a high sevoflurane concentration plus lithium chloride group (H + Li group, n = 56), and a lithium chloride group (Li group, n = 56). We choose two different concentrations of anesthetic commonly used in clinical practice to examine whether clinically relevant levels of sevoflurane anesthesia in pregnant rats inhibit fetal NSC proliferation and produce neurobehavioral deficits. The Li and H + Li groups received an intraperitoneal injection of 2 mmol/kg lithium chloride 30 min before anesthesia.

Pregnant rats were then placed in the anesthesia box, and the concentration of anesthetic gas in the box was measured using an anesthetic gas monitoring system. An anesthetic exposure time of 2 h was chosen because this represents the minimum time required to detect apoptosis or injury in the fetus (Wang et al., 2009) and because the duration of fetal surgery in clinical practice is commonly 2 h. Rats in the L group were treated with 2% sevoflurane for 2 h, while rats in the H group were treated with 3.5% sevoflurane for 2 h (inhaled oxygen concentration in both groups: 30%). Rats in the C group were treated with 30% oxygen only for 2 h. All rats maintained spontaneous breathing.

During the anesthesia process, the MAC value of exhaled gas was automatically calculated by a gas monitor. Heart rate and blood pressure were measured, and arterial blood was intermittently extracted via the internal carotid artery for blood gas analysis. Anal temperature was maintained at 37 ± 0.5 °C. To determine the effects of sevoflurane on fetal NSC proliferation and Wnt signaling, three time points after anesthesia were chosen: 6 h, 24 h, and 48 h. At each time point, samples of fetal brain tissue were harvested for immunofluorescence, real time polymerase chain reaction (PCR), and Western blot analysis. The remaining pregnant rats in each group were allowed to carry their pregnancies to full-term. To evaluate the effects of sevoflurane on postnatal NSC proliferation, the hippocampal tissues of offspring were harvested at P0, P14, and P28.

2.1. Neurobehavioral test: Morris Water Maze

Rat offspring were reared for 28 days prior to behavioral analysis via the Morris Water Maze (MWM) task (Shenyang Furui Infrared Technology, Ltd.; Shanghai Science and Technology Co., Ltd., China). Eight litters from each group were randomly selected for the MWM task, with three offspring from each litter serving as replicates. The MWM was conducted in a circular, stainless steel bucket (diameter: 120 cm; height: 60 cm) containing a platform (diameter: 6 cm) that had been submerged 1.5 cm below the surface of the water. Water temperature was maintained at 24 ± 0.5 °C. The MWM task included a 5-day navigation test and a spatial exploration test.

2.1.1. Navigation test

The navigation task was conducted over 5 days, and each rat underwent four rounds (90 s each) of testing per day. Rounds were separated by an interval of at least 30 min to ensure adequate rest for each rat. Rats were randomly placed into the bucket from each quadrant of the middle wall, and the image acquisition system and analysis software automatically recorded the time required for rats to find and remain on the platform for 5 s (i.e., escape latency). Swimming speed and distance were automatically recorded by the computer system. If the rat was unable to find the platform within 90 s, the computer automatically stopped recording, and the tester gently guided the rat to the platform and ensured that it remained on the platform for 30 s.

2.1.2. Spatial exploration test

After the navigation experiment, we removed the underwater platform to perform the spatial exploration test, during which the midpoint of the original quadrant area was designated as the entry point. Rats were placed into the water facing the bucket wall, and the image acquisition and analysis system automatically recorded the number of times rats crossed the original area during the 90-second task. Rat body temperature was maintained by active heating as described by Bianchi et al. (2008): After each trial, rats were placed in a holding cage under a heat lamp for 1–2 min until dry before being returned to their regular cages.