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Research report

The decrease of NMDAR subunit expression and NMDAR EPSC in hippocampus by neonatal exposure to desflurane in mice

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HIGHLIGHTS

- 1.5 MAC desflurane impairs working memory and contextual fear memory of neonatal mice;.
- 1.5 MAC desflurane can decrease NMDAR subunits expression in mice hippocampus;.
- 1.5 MAC desflurane can decrease NMDAR EPSC in mice hippocampus.

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ABSTRACT

Desflurane is one of the third generation inhaled anesthetics and can be used in obstetric and pediatric medicine. However, effects of exposure to desflurane on neonatal brain are largely unknown. In this work, 6-day-old C57BL/6J mice were exposed to 1 MAC or 1.5 MAC desflurane for 2 h. When the mice were 28-day-old, the open-field, spontaneous alternation Y-maze and fear conditioning tests were performed to evaluate general activity, working memory and long term memory, respectively. Levels of NMDAR subunits NR1, NR2A, and NR2 B expression in hippocampus were evaluated by western blot. NMDAR-mediated excitatory postsynaptic current (EPSC) in mouse hippocampal slice was recorded by whole-cell patch clamp record. Mice exposed to 1.5 MAC desflurane had significantly impaired working memory and fear conditioning memory. The protein expression of NMDAR subunits (NR1, NR2B) and NMDAR-mediated EPSC in hippocampus were significantly decreased. However no significant difference was detected between mice exposed to 1.0 MAC desflurane and control mice. In conclusion, in an animal model, 6-day-old mice exposed to 1.5 MAC desflurane have significant impairments in working memory and contextual fear memory at postnatal day 28, and the decrease of NMDAR subunits expression and NMDAR EPSC in hippocampus may be involved in this process.

1. Introduction

For decades, used alone or as a part of balanced anesthesia, inhalation anesthetics have been the most widely administered drugs in most anesthesia communities. However, several preclinical reports have demonstrated that inhalation anesthetics are neurotoxic to the neonatal brains in mice [1,2], rats [3–6] and primates [7]. Those data obtained in newborn animals have raised serious safety concern regarding current inhalation anesthetics practice in young children. Desflurane is one of the latest inhaled anesthetics and can be used in obstetric and pediatric medicine. However, the effect of neonatal exposure to desflurane is still largely unknown.

The N-methyl-D-aspartate receptor (NMDAR), a major class of ionotropic glutamate receptors, is considered to be crucially involved in synaptic plasticity and neurogenesis in the neonatal brain [8,9]. Functional NMDARs are heteromultimers formed by the assembly of the NR1 and NR2 subunits. Recently, several lines of evidence have suggested that abnormal levels of NMDAR subunits NR1, NR2A and NR2B protein expression in the neonatal brain correspond to memory dysfunction performance [10–12]. As NMDAR is one of molecular targets of anesthetics [13], NMDAR may play an important role in anesthesia induced cognitive deficits in the neonatal brain. Therefore, it is beneficial to study the effect of desflurane anesthesia on neonatal brain from the perspective of NMDAR.

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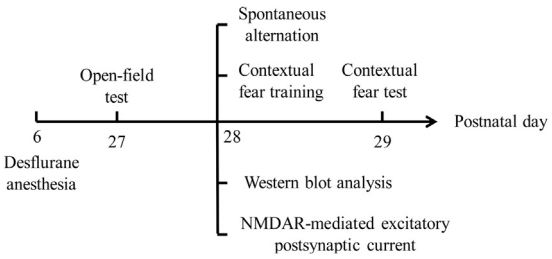

Fig. 1. Schematic time-line of the experimental design.

The present study aims to investigate the effect of desflurane anesthesia on the neonatal mice brain, and the underlying mechanism of side effect on the NMDAR. To address this issue, 6-day-old mice were exposed to a clinically relevant concentration of desflurane, and then behavioral tests were performed to evaluate learning and memory of mice when they were 28-day-old. Moreover, the level of NMDAR protein expression and NMDAR-mediated excitatory postsynaptic current (EPSC) in hippocampus were evaluated when the mice were 28-day-old.

2. Methods

2.1. Animals

All experiments were conducted according to the institutional ethical guidelines for animal experiments of Tianjin Medical University and were approved by the Committee for Animal Research at Tianjin Medical University (Tianjin, China). The C57BL/6J mice (both male and female) used in this study were maintained on a 12 h light-dark cycle with room temperature at 22° ± 1 °C. Mice had ad libitum access to water and food.

2.2. Experiment protocol

To investigate the effects of 1 minimum alveolar concentration (MAC) or 1.5 MAC desflurane anesthesia on cognition behavior, 6-day-old mice were divided into 3 groups: control (n = 24), 1 MAC desflurane (n = 24), and 1.5MAC desflurane (n = 24). When the mice were 28-day-old, all the mice received open-field test. Half of each group received spontaneous alternation Y-maze test and the rest of the mice received contextual fear conditioning test.

To investigate the effects of 1 MAC or 1.5 MAC desflurane anesthesia on NMDAR in hippocampus, we divided 6-day-old mice into 3 groups: control (n = 24), 1 MAC desflurane (n = 24), and 1.5 MAC desflurane (n = 24). When the mice were 28-day-old, they were killed by decapitation and harvested their hippocampal tissues for Western blot analysis (n = 12, per group) and electrophysiological recording (n = 12, per group).

A schedule showing the time (days) of different experiments is shown in Fig. 1.

2.3. Anesthesia treatment

Considering that minimum alveolar concentration (MAC) values in neonate rodents might decrease as a function of anesthesia duration [14], we determined 1 MAC value sing a bracketing study design with tail-clamp stimulation, as described by Loepke et al. [15].

6-day-old mice were randomly assigned to receive the following treatments: 25% oxygen for 2 h, 10% desflurane(1 MAC) for 2 h, 15% desflurane(1.5 MAC) for 2 h.The total gas flow was 2 l/min, using 25% O_2 as a carrier. The temperature of the anesthetizing chamber was controlled to maintain a 37 °C ± 0.5 °C rectal temperature in the mice.

2.4. Arterial blood gas analysis

Arterial blood analysis was performed essentially as described previously [1]. Briefly, the mice underwent a quick arterial blood sampling from the left cardiac ventricle immediately after removal from the maternal cage (0 h) or at the end of anesthesia (2 h). And the samples were analyzed immediately after blood collection by blood gas analyzer (GEM Premier 3000, Instrumentation laboratory, Bedford, MA). The mice used to determine physiologic parameters during anesthesia were not used for any further tests.

2.5. Open-field test

Emotional responses to a novel environment were measured by an open-field test as previously described method [16]. Activities of mice from the three groups (28-day-old) were measured as the total travel distance (m) in 10 min.

2.5.1. Spontaneous alternation in the Y-maze test

Spontaneous alternation was tested as described previously [16]. This task is based on the animals' innate disposition to alternates and adaptive to assess spatial working memory. The symmetrical Y maze made of acrylic consists of three arms separated by 120°. Each arm is 30 cm long, 15 cm high, 8 cm wide. Each mouse was placed in the center of the Y-maze and was allowed to explore freely through the maze during an 8 min session. The sequence and the total number of arms entered were recorded. The percentage of alternation is the number of triads containing entries into all three arms divided by the maximum possible number of alternations (total number of arm entries minus 2) × 100.

2.5.2. Fear conditioning test

The training procedures for contextual fear conditioning were performed as described in Frankland et al. [17]. During training, 28-day-old mice were placed in the transparent plastic apparatus for 7 min. After 2 min they were presented with 5 unsignaled foot-shocks (2 s duration, 0.75 mA, 1 min apart). After training, the mice were returned to their home-cage. Contextual fear conditioning test was performed in the conditioning chamber 1 day after training. During testing, mice were placed back in the conditioning chamber for 2 min. Freezing and activity were recorded using the CCD video camera and the percent of freezing was calculated to evaluate contextual fear conditioning memory. Mice used to the contextual fear conditioning test were not used for any further testing.

2.6. Western blot analysis

The membranes of hippocampus neurons were isolated, and protein analyses of NR1, NR2A, and NR2 B subunits of NMDAR were performed as previously described [18]. Briefly, the hippocampus were dissected and homogenized in cold buffer A which contained 250 mM sucrose, 20 mM HEPES (pH 7.5), 50 mM KCl, 2 mM EGTA, and protease inhibitor cocktail (Calbiochem). In order to remove the nuclei and large cell debris, the lysates were centrifuged at 800 *g* for 10 min. Then the supernatants were collected and centrifuged at 100,000 *g* for 1 h. The membrane pellet were resuspended in buffer B which contained 20 mM HEPES (pH 7.0), 150 mM KCl, 2 mM EGTA, 1% (w/v) CHAPSO, and protease inhibitor cocktail, and then were incubated at 4 °C for 1 h. The solubilized membranes were centrifuged at 100,000 *g* for 1 h, and ultimately the supernatants were collected. The proteins were separated by SDS-PAGE and transferred to a PVDF membrane. The membrane was blocked with 5% non-fat skim milk and incubated with primary antibodies against NR1, NR2A, NR2B (1:1000, Cell Signaling) and β-actin (1:5000, Sigma) and followed by incubation with HRP-conjugated