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SWIMMING EXERCISE AMELIORATES NEUROCOGNITIVE IMPAIRMENT INDUCED BY NEONATAL EXPOSURE TO ISOFLURANE AND ENHANCES HIPPOCAMPAL HISTONE ACETYLATION IN MICE

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Abstract—Isoflurane-induced neurocognitive impairment in the developing rodent brain is well documented, and regular physical exercise has been demonstrated to be a viable intervention for some types of neurocognitive impairment. This study was designed to investigate the potential protective effect of swimming exercise on both neurocognitive impairment caused by repeated neonatal exposure to isoflurane and the underlying molecular mechanism. Mice received 0.75% isoflurane exposures for 4 h on postnatal days 7, 8, and 9. From the third month after anesthesia, the mice were subjected to regular swimming exercise for 4 weeks, followed by a contextual fear condition (CFC) trial. We found that repeated neonatal exposure to isoflurane reduced freezing behavior during CFC testing and deregulated hippocampal histone H4K12 acetylation. Conversely, mice subjected to regular swimming exercise showed enhanced hippocampal H3K9, H4K5, and H4K12 acetylation levels, increased numbers of c-Fos-positive cells 1 h after CFC training, and less isoflurane-induced memory impairment. We also observed increases in histone acetylation and of cAMP-response element-binding protein (CREB)-binding protein (CBP) during the swimming exercise program. The results suggest that neonatal isoflurane exposure-induced memory impairment was associated with dysregulation of H4K12 acetylation, which may lead to less hippocampal activation following learning tasks. Swimming exercise was associated with enhanced hippocampal histone acetylation and CBP expression. Exercise most likely ameliorated isoflurane-induced memory impairment by enhancing hippocampal histone acetylation and activating more neuron cells during memory formation.

Key words: physical exercise, isoflurane, neurocognitive impairment, histone acetylation, hippocampus, cAMP-response element-binding protein-binding protein.

INTRODUCTION

Inhalational anesthetics are widely used in pediatric anesthesia, and their adverse effects on brain development are an increasing cause of concern. Several recent studies demonstrated that multiple exposures to anesthesia and surgery before 4 years of age were associated with increased risk of developing learning disabilities (Wilder et al., 2009; Flick et al., 2011). Rodent studies have shown that inhalational anesthetics have differential effects on neurocognitive function at different stages of brain development, and that the neonatal brain appears to be particularly susceptible to the neurotoxic effects of anesthetics (Stratmann et al., 2009). The mechanism underlying neurocognitive impairment induced by inhalational anesthetics was commonly thought to be associated with neurodegeneration (Jevtovic-Todorovic et al., 2003; Wang et al., 2009). Although the number of studies on prevention or attenuation of neurodegeneration (apoptosis) induced by inhalational anesthetics is growing, it is equally important to develop therapeutic strategies to improve the function of neurons that remain after neurodegeneration has occurred.

Regular physical exercise is a behavioral intervention that can ameliorate neurocognitive impairment in both the aging brain and in neurodegenerative disease (Adlard et al., 2005; van Praag et al., 2005; Nichol et al., 2007). The protective effects of exercise on neurocognitive function include increased neurogenesis, enhanced synaptic plasticity, improved neurotransmission, and increased expression of neurotrophic factors (Cotman et al., 2007; Gomes da Silva et al., 2012), all of which involve changes in gene expression. The underlying mechanisms that contribute to regulation of exercise-induced gene expression are not entirely clear. Chromatin remodeling via histone acetylation is known to play a particularly important role in the regulation of gene expression, and may thus be involved in the changes in gene expression caused by exercise. In fact, a previous study reported that increased brain-derived neurotrophic factor (BDNF) expression induced by exercise was most likely mediated by epigenetic factors such as DNA methylation and histone acetylation (Gomez-Pinilla et al., 2011).

The present study was designed to investigate the potential protective effect of regular swimming exercise on cognition impairment in mice subjected to repeated neonatal exposure to isoflurane. Mice received 0.75% isoflurane for 4 h on postnatal days 7, 8, and 9 and were

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Abbreviations: ANOVA, analysis of variance; CBP, CREB-binding protein; CFC, contextual fear condition; HAT, histone acetyltransferase; HDAC, histone deacetylase; MAC, minimum alveolar concentration; OD, optical density.

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subjected to a 4-week swimming exercise program beginning the third month after anesthesia. On completion of the swimming program, an open-field test was used to assess anxiety levels or locomotor activity, or a contextual fear condition (CFC) trial was used to assess contextual fear memory formation ability. Changes in hippocampal histone acetylation were also assessed during swimming exercise program and after CFC training. The expression of cAMP-response element-binding protein (CREB)-binding protein (CBP) and p300, which are transcriptional coactivators that both have histone acetyltransferase (HAT) activity, and of histone deacetylase (HDAC) 1–4 was assayed.

EXPERIMENTAL PROCEDURES

Animals

All animal procedures were approved by the Animal Experimentation Ethics Committee of Xiangya Hospital, Central South University, China (Approval number: 2013101125). A total of 294 male C57BL/6 mice were used. The neonatal mice were raised by their mothers, and weaned mice were housed in group cages with ad libitum food and water. The animal room was maintained at 25 ± 2 °C and a 12/12-h light/dark cycle.

Inhalation anesthesia and swimming exercise program

The mice received three exposures to 0.75% isoflurane performed on postnatal days 7, 8, and 9. This administration schedule was chosen because the maximum brain growth rate in the mouse occurs at that time (Watson et al., 2006). Isoflurane was administered for 4 h in a gas-proof chamber maintained at 36 ± 1 °C by heating of the gas. The chamber was filled with 30% O_2 + 0.75% isoflurane with a fresh gas flow of 2 L/min. The isoflurane concentration was monitored with a Capnomac Ultima anesthesia monitor (Daetex-Ohmeda; GE Healthcare, Wauwatosa, WI, USA) attached to the chamber outlet. Control animals were exposed to 30% O_2-enriched air without isoflurane. Arterial blood specimens for blood gas and blood glucose analysis were obtained from the left cardiac ventricle after each exposure.

Mice in the regular exercise groups were subjected to a 4-week swimming exercise program beginning on the third month after isoflurane or air exposure. During the first 5 days (adaptive phase), the mice were acclimatized to the swimming activity in a 80-cm diameter round basin filled with the warm water (32 ± 3 °C), which helped to reduce the stress of swimming exercise. In the adaptive phase, the water depth was kept at 5 cm so that the hind limbs could easily touch the bottom of the basin and allow the mice to stand and decrease the activity intensity. The water depth was increased to 15 cm during the swimming exercise phase, and beginning in the third week, exercise intensity was increased by attaching a load to the tail. The added load was 2% of the animal's body weight, an amount that is considered to maintain the intensity below the anaerobic threshold for swimming exercise (Almeida et al., 2011), and was used in a previous study (Goes et al., 2014). The swimming exercise program was performed with groups of 12–20 mice; it began daily before 12:00 am, and each session lasted 30 min. The mice were allowed to take rest for at least 3 min on a platform in the basin between each swimming exercise, which consisted of 5 min of swimming four times/session. After each swimming exercise session, the mice were dried with towels and an electric hair dryer.

Behavioral testing

The time course of the experimental procedures is shown in Fig. 1. Three months after gas exposure and 24 h after the last swimming exercise, both treated and control mice were subjected to an open-field test or CFC trial.

Open-field test. The open-field test was carried out in white, opaque behavioral chambers 75 × 75 × 45 cm in size. Mice were initially placed in the center of the chamber and allowed to freely explore for 5 min in dim light (about 5 lux) and with continuous white noise (60 dB). Locomotor activity was recorded by a video camera and analyzed using a PC-based video-tracking system (Smart JUNIOR, Panlab Harvard Apparatus, Barcelona, Spain). Locomotor activity was recorded as the total distance traveled during the 5-min test; anxiety level was expressed as the ratio of the time spent exploring in the center of the testing zone to the total duration of the test period.

CFC trial. CFC trials were conducted in a transparent Plexiglass training box (40 × 30 × 26 cm) that was placed inside a soundproof cabinet (75 × 60 × 45 cm). The floor of the training box was comprised of 28 iron bars that transmitted electric shocks to the feet. During the training session, mice were placed in training box and allowed to explore for 4 min. After an additional 4 min of exploration, mice were given a 0.75-mA electric stimulus for 2 s. The CFC training sessions were captured by a video camera fixed to the top of the sound-proof cabinet, and the 3 min of video immediately before the foot shock was given was used to analyze freezing behavior, using ANY-maze software (Stoelting Co, Wood Dale, IL, USA). The CFC memory test was given 24 h after training. Mice were placed in the same situation as in training and freezing behavior was measured as described above to assess contextual fear memory. Freezing behavior was expressed as the ratio of freezing time to the total 3-min observation time.

Tissue extraction and immunoblot analysis

Mice were sacrificed by cervical dislocation at 1 h, 24 h, 5 days, 4 weeks (when performing the swimming exercise program), and 1 h after CFC training. The brain was quickly removed and the hippocampus was rapidly separated on an ice platform. The extraction and separation of nuclear and cytosolic protein were done with a nuclear/cytoplasmic protein extraction kit (Beyotime Biotechnology, Shanghai, China) following the manufacturer's instructions. Histone extraction, protein