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Research Report

Melatonin pretreatment prevents isoflurane-induced cognitive dysfunction by modulating sleep–wake rhythm in mice

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ABSTRACT

Background: Sleep plays an important role in memory processing. However, its role in anesthesia-induced cognitive dysfunction was not revealed. Our study sought to investigate the connection between the cognition decline and sleep–wake rhythm disorders after long-term isoflurane anesthesia in mice. Also, we examined the effect of exogenous melatonin pretreatment on both cognitive function and circadian rhythm. Furthermore, we discussed whether NR2B (N-methyl–D-aspartate receptor 2B subunit)–CREB (cAMP-response element binding protein) signaling pathway was involved in this course.

Methods: 2-month-old male C57/BL-6J mice were submitted to long-term anesthesia using 1% isoflurane from CT (Circadian Time) 14 to CT20. Melatonin pretreatment were conducted before anesthesia for 7 Days. Intellicage for mice and Mini-Mitter were applied to monitor spatial memory and gross motor activity which can reflect cognition and sleep–wake rhythm. Messenger RNA and protein expression of right hippocampus NR2B and CREB were examined by RT-PCR and Western blot.

Results: 6 h isoflurane anesthesia led to impaired spatial memory from Day 3 to Day 10 in mice accompanied by the disruption of sleep–wake rhythm. Meanwhile, the hippocampus CREB and NR2B expression declined in step. Melatonin pretreatment ameliorated disturbed sleep–wake cycle, improved isoflurane-induced cognitive dysfunction, and reversed the down-regulation of CREB and NR2B expression.

Conclusions: Our data demonstrate that sleep–wake rhythm is involved in the isoflurane-induced cognition impairment and pretreatment of melatonin has a positive effect on circadian normalization and cognition reversal. Also, NR2B–CREB signaling pathway has a critical role in this process. This study provides us a new strategy for anesthesia-induced cognitive dysfunction therapy.

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1. Introduction

Postoperative cognitive dysfunction (POCD) is a common and well-known complication after surgery (Steinmetz et al., 2009). It features disturbance of memory, attention, consciousness, information processing and sleep–wake cycle, leading to postoperative morbidity and mortality (Bekker and Weeks, 2003). The etiology of POCD is likely multifactorial. Different preoperative and operative factors are associated with the development of cognitive dysfunction. Factors such as increasing age, presence of diabetes, hypotension during surgery and anesthesia were believed to be involved (Shaw et al., 1989; Grocott et al., 2005).

Sleep, which shares some common neuronal mechanisms with general anesthesia (Allada, 2008; Kelz et al., 2008) is important to memory processing (Turner et al., 2007). It is known to be poor in the hospital (Vico-Romero et al., 2014), and the prevalence of sleep disorders showed a marked increase in patients admitted to the Intensive Care Unit (Gomez, 2013). The disturbance of sleep–wake rhythm is considered to be one of the most important causes of sleep disorders. This rhythm is influenced by various factors, including light (Friedman et al., 2012), melatonin (Koch et al., 2009) and orexin (Diniz et al., 2010). It has also been reported that general anesthesia could impact the sleep–wake rhythm. Propofol anesthesia, for instance, induced phase advances when administered at the rest/activity transition point(Challet et al., 2007).Moreover, inhalational anesthetics sevoflurane and isoflurane caused phase delays when administered during the subjective Day (Ohe et al., 2011; Cheeseman et al., 2012). However, whether the disruption of sleep–wake rhythm resulted from anesthesia plays an important role in cognitive dysfunction remains unknown.

Melatonin, a hormone secreted by the pineal gland during the dark period of the Day, mediates a diverse array of biological and physiological actions. In addition to its effects on antioxidant, immunomodulatory, and oncostatic activities (Pandi-Perumal et al., 2006), melatonin has an important role in sleep and circadian regulations. Small dosages of melatonin were found to regulate sleep–wake rhythm (Hughes et al., 1998) and ameliorate the sleep quality by normalizing sleep–wake cycle (Finati et al., 2013). Despite these advances, little is known about the effect of melatonin on cognitive function after anesthesia.

In this study, we asked whether the sleep–wake disorders induced by anesthesia contributed to the development of cognitive dysfunction, and whether this effect of anesthesia could be blocked by exogenous melatonin treatment. By using an international advanced cognitive behavioral instrument-automatic mouse intellicage, we found that 6 h isoflurane anesthesia induced cognitive dysfunction, accompanied by the disruption of sleep–wake rhythm. Pretreatment of melatonin had a positive effect on sleep–wake cycle normalization and cognition reversal.NR2B–CREB signaling pathway was thought to be among the molecular mechanisms.

2. Results

2.1 Cognitive performance of animals after anesthesia and melatonin treatment

Following anesthesia, animals were housed in the intellicage. Their continuous locomotive activity was tracked in terms of the number of visits and their correct rate of visits were also recorded

In Place learning phase, the results showed a reducement in locomotive activity from the first day after isoflurane anesthesia based on an decreased numbers of visits in anesthesia group ($P<0.05$). Significantly increased on correct rate of visit two days after isoflurane anesthesia were observed and later significantly decreased from Day 3 to Day 7 when compared with control group ($P<0.05$) were observed, suggesting that isoflurane anesthesia could lead to cognitive dysfunction in mice. The restoration of normal locomotive activity and correct corner visits were occurred in anesthesia group 10 days and 11 days respectively after anesthesia.

Compared with anesthesia group, pretreated with melatonin mice when underwent a 6 h isoflurane anesthesia showed a significant increased on the numbers of visits on Day 1, Day 4, day 5, Day 6 and Day 7. Meanwhile, higher correct visits were also detected from Day 3 to Day 7 ($P<0.05$). During place reversal learning phase, melatonin pretreated mice showed that the locomotive activity and correct rate of visit recovered to control level on Day 13 and Day 14. ($P>0.05$) (Figs. 1A and E).

2. 2 Gross motor activity of animals after anesthesia and melatonin treatment

A 6 h anesthetic during the subjective night caused a persistent and marked shift of motor activity rhythm. On Day 1 after anesthesia, compared with control group, anesthesia mice demonstrated greater spontaneous activity levels on CT0, 2 and 4(1771.20±270.97 vs. 1242.50±329.38, $P=0.022$; 1737.50±399.40 vs. 1203.30±123.43, $P=0.008$; 1578.70±297.58 vs. 691.6±107.49, $P<0.001$). This phenomenon lasted for at least 4 Days after anesthesia: CT4 (1498.80± 310.66 vs. 1062.20±96.64, $P=0.008$); CT6 (1173.50± 221.53 vs. 672.33±141.96, $P<0.001$).

On Day 1 after anesthesia, when compared with anesthesia group, mice which pretreated with melatonin showed lower activity levels during sleeping period on CT0 (1305.00±432.50, $P=0.048$), CT6(685.17±180.27, $P<0.001$), and this effect lasted for at least 4 Days after anesthesia on CT0, 2, 4 and 6 (828.50±180.09, $P<0.001$; 837.83±71.63, $P<0.001$; 1152.70±199.54, $P=0.048$; 668.17±191.17, $P<0.001$). These marked difference indicate that pretreatment with melatonin could normalize the disrupted sleep–wake rhythm caused by isoflurane anesthesia (Fig. 2A and C).

2.3 The expression of CREB and NR2B at the hippocampus level after anesthesia and melatonin treatment

Compared with control group, the expression of NR2B mRNA had decreased on Day 1 (0.51±0.09 vs. 1.12±0.15, $P<0.001$), Day 3 (0.62±0.15 vs. 1.17±0.16, $P<0.001$), and Day 7 (0.69±0.12 vs. 1.13±0.14, $P=0.002$) after anesthesia in the hippocampus. Compared with anesthesia group, melatonin+anesthesia group