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Early exposure to sevoflurane inhibits Ca^{2+} channels activity in hippocampal CA1 pyramidal neurons of developing rats

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ABSTRACT

Sevoflurane is one of inhalation anesthetics and has been commonly used in obstetric and pediatric anesthesia. The widespread use of sevoflurane in newborns and infants has made its safety a health issue of concern. Voltage-gated Ca^{2+} channels (VGCCs) play an important role in neuronal excitability and are essential for normal brain development. However, the role of sevoflurane on regulating Ca^{2+} channels during the period of rapid brain development is still not well understood.

The aim of this study is to explore the effects of sevoflurane on voltage-gated Ca^{2+} channels for hippocampal CA1 pyramidal neurons during the period of rapid brain development. 1-week-old Sprague-Dawley rats were randomly divided into 3 groups: control group, 2.1% sevoflurane group (exposed to 2.1% sevoflurane for 6 h) and 3% sevoflurane group (exposed to 3% sevoflurane for 6 h). Whole-cell patch clamp technique was used. *I–V* curve, steady-state activation and inactivation curves of Ca^{2+} channels were studied in rats of the both 3 treated groups at 5 different ages (1 week, 2 weeks, 3 weeks, 4 and 5 weeks old).

After anesthesia with sevoflurane at 1-week-old rats, Ca^{2+} channels current density was significantly decreased at week 1 and week 2 ($p<0.01$). And 3% sevoflurane exposure resulted in a rightward shift in steady-state activation curve at week 1 and week 2, as well as the inactivation curve from week 1 to week 3. However, the 2.1% sevoflurane-induced

Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol tetraacetic acid; 4-AP, 4-aminopyridine; TEA-Cl, tetrathylammonium chlorine; TTX, tetrodotoxin; ATP, adenosine 5′-triphosphate; SNK, Student–Newman–Keuls

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rightward shift was only found in steady-state inactivation curve of Ca^{2+} channels at week 1 and week 2. Both the slope factor (k) of Ca^{2+} channels activation and inactivation curves increased by 3% sevoflurane at week 1 ($p<0.05$).

Therefore, early exposure to sevoflurane persistently inhibits Ca^{2+} channels activity in hippocampal CA1 pyramidal neurons of developing rats but the development of Ca^{2+} channels recovers to normal level at juvenile age. Moreover, the inhibition of 3% sevoflurane on VGCCs is greater than that of 2.1% sevoflurane.

1. Introduction

As one of commonly used inhalation anesthetics, sevoflurane has been widely used in obstetric and pediatric anesthesia for its hemodynamic stability, short-lasting action and excellent respiratory tolerance (Edwards et al., 2010; Gibert et al., 2012). However, there may be some side effects caused by sevoflurane. The epileptiform EEG and seizure activity have been observed during sevoflurane anesthesia in children (Conreux et al., 2001; Constant et al., 2005; Vakkuri et al., 2001). And a previous study suggested that sevoflurane would block human motional memory (Alkire et al., 2008). Several studies have reported that sevoflurane induces social behavior abnormality and learning deficits in mice (Jevtovic-Todorovic et al., 2003; Satomoto et al., 2009). Meanwhile, increasing evidence indicates that sevoflurane exposure leads to widespread neuroapoptosis and cognitive impairment on immature brain (Kodama et al., 2011; Istaphanous et al., 2011; Shen et al., 2013). This has raised serious concerns among anaesthesiologists, neuroscientists and parents regarding the safety of sevoflurane on brain development.

Developing brain is sensitive and vulnerable to general anesthetics (Liang et al., 2010). Sevoflurane might have harmful effects on the developing brain. Following numerous children investigations, Kalkman et al. (2009) has found out that early exposure to anesthetics before 2 years old can induce an increased risk of cognitive dysfunction in children. Moreover, extensive studies indicate that prolonged exposure to sevoflurane on neonatal rat results in widespread neuroapoptosis and later spatial memory damage (Fang et al., 2012; Istaphanous et al., 2011; Wang et al., 2012). However, the mechanisms of sevoflurane-mediated neuronal death and cognitive impairment on immature brain are largely unknown. Hence, the effects of sevoflurane on developing brains need to be further explored.

Voltage-gated calcium channels (VGCCs) are involved in regulating Ca^{2+} signaling in brain (Norris et al., 2010) and play an important role in neurotransmitter release, synaptic plasticity, neuronal excitability and gene expression (Li et al., 2007; Zhang et al., 2009). The alteration in Ca^{2+} signaling influences neurogenesis, neurodegeneration and neurocognitive function (Berridge, 2011, 2010). Meanwhile, the modulation of Ca^{2+} channel activity has a key function on neurons survival and the viability of hippocampal neurons might be reduced by the dysfunction of Ca^{2+} channels (Li et al., 2007). Therefore, VGCCs are essential for normal brain development. Several studies have shown that VGCCs can be inhibited by sevoflurane (Eckle et al., 2012; Gong et al., 2012; Rithalia et al., 2004). However, the role of sevoflurane on regulating VGCCs during the period of rapid brain development is still not well understood.

To explore the critical period of development, we first recorded voltage-gated calcium currents in hippocampal CA1 pyramidal neurons by using whole-cell patch clamp technique. To determine whether the sevoflurane-induced persistent inhibition on VGCCs would occur during development, we further assessed the effects of 6-h 3% sevoflurane exposure on *I–V* curve, steady-state activation and inactivation curves of VGCCs. Finally, we investigated whether the inhibition of sevoflurane on VGCCs was concentration-dependent by evaluating the properties of VGCCs after a lower concentration (2.1%) of sevoflurane administration with the same treatment time (6 h).

2. Results

2.1. *Isolated hippocampal neurons*

The hippocampal pyramidal neurons in Fig. 1A with a smooth and bright appearance and no visible organelles were selected for recording. It was hard to perform whole-cell patch clamp recording on the cell with reduced viability, whose appearance was granular (Fig. 1B). The neurons adopted in control and sevoflurane groups were showed in Fig. 1C and D.

2.2. *Changes of VGCCs properties in hippocampal CA1 pyramidal neurons during the period of rapid brain development*

Control group were used to evaluate the changes of VGCCs properties in hippocampal CA1 pyramidal neurons during the period of rapid brain development. The inward slowly-inactivating Ca^{2+} current was evoked by 250 ms depolarizing voltage steps from −60 mV to+40 mV in 10 mV increment. The Ca^{2+} currents can be blocked completely by 0.5 mmol/L $CdCl_2$ (Fig. 2E).

The current–voltage (*I–V*) relationship curve of Ca^{2+} current showed that potential-dependent Ca^{2+} current was activated from pulse potential of −40 mV and reached its maximal amplitude at approximately+10 mV in control 1 w but 0 mV in others (Fig. 2A). The current density in control group was calculated to assess the changes in Ca^{2+} currents (Table 1). With the increases of rat age from 1 week to 5 weeks, the current density increased gradually and there was a significant