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Internalization of GluA2 and the underlying mechanisms of cognitive decline in aged rats following surgery and prolonged exposure to sevoflurane

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ABSTRACT

Background: We revealed that a high concentration of sevoflurane exacerbated cognitive impairment in aged rats, and the inhibition of GluA2 subunit internalization facilitated neuroprotection after a cerebral ischemic injury. However, the trafficking of GluA2 in POCD and its underlying mechanism are not clear. We thus detected the effects of sevoflurane for different inhalation durations on postoperative cognitive function and investigated the role of GluA2 subunit trafficking in this process.
Methods: A rat model of orthopedic surgery was performed with different durations of 1.5 MAC sevoflurane inhalation. Cognitive function was evaluated by manipulating the Y maze and fear conditioning tests for 7 days after experiments. Western blot, ELISA and coimmunoprecipitation were applied to analyze GluA2 internalization, PI3K expression and its activity, as well as alterations to the MEF2-Arc pathway in the hippocampus. Neuron apoptosis and the spine morphology in the hippocampus were also observed.
Results: We found that neuron apoptosis and GluA2 internalization increased following surgery and 1.5 MAC sevoflurane inhalation for 2 h, possibly due to the decrease of the PI3K-GluA2 complex and PI3K activity in the hippocampus after prolonged 1.5 MAC sevoflurane inhalation. We also observed that the MEF2-Arc pathway contributed to long-term cognitive function, which also impaired the spinal morphology after 1.5 MAC sevoflurane inhalation for 2 h.
Conclusion: The above results suggest that 1.5 MAC sevoflurane *inhalation for 2 h* potentiated surgery-impaired cognitive function and that the *inhibition of PI3K-AMPAR GluA2 as well as activation of the MEF2-Arc signal pathway* contributes to different stages of POCD.

1. Introduction

Postoperative cognitive decline (POCD) is a common clinical complication after major surgery, especially in aged patients. It is associated with the inability to live independently, with possible permanent dementia, and even with an increased risk of mortality (Moller et al., 1998). It is therefore necessary to explore the definite mechanisms of POCD and to create strategies to avert it.

A number of studies have indicated that surgery impairs learning and memory (He et al., 2012; Terrando et al., 2011), whereas the effects of volatile anesthetics remain controversial. Because 1) low concentrations or short duration to volatile

Abbreviations: PI3K, phosphoinositide-3-kinase; MCAO, middle cerebral artery occlusion; AMPAR, a-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; GluA2, glutamate receptor subunit 2; MEF2, Myocyte enhancer factor 2; Arc, activity-regulated cytoskeleton-associated protein; POCD, postoperative cognitive decline; PIP2, phosphatidylinositol-(4,5)-bisphosphate; PIP3, phosphatidylinositol-(3,4,5)-bisphosphate.

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anesthesia were reported to be produce little effects (Zhang et al., 2014) and 2) our previous study revealed that inhalation of *1.5 MAC sevoflurane* for 2 h exacerbated brain function, including learning and memory in geriatrics (Hu et al., 2014), we aimed to determine whether a definite inhalation exposure duration of 1.5 MAC sevoflurane could worsen cognitive dysfunction after orthopedic surgery.

a-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) mediate a majority of fast excitatory neurotransmission in the central nervous system (CNS) and are critically important for nearly all aspects of brain function, including learning and memory (Cao et al., 2014; Wang et al., 2014). AMPARs comprise heterotetrameric complexes that are composed of glutamate receptor 1–4 (GluA1-4). They are highly mobile proteins that are recycled at synapses (Henley and Wilkinson, 2013). In recent years, it was claimed that AMPAR GluA2 subunit internalization may contribute to cognitive decline in healthy aging, addiction and Alzheimer's disease (Henley and Wilkinson, 2013; Liu et al., 2010). We therefore hypothesized that AMPAR GluA2 subunit trafficking was involved in POCD.

Additionally, to clarify how AMPAR GluA2 subunits internalized, we tested phosphoinositide-3-kinase (PI3K), which was previously shown to positively regulate the cell surface level of GluA2 by forming PI3K-AMPAR GluA2 subunit complexes (Wang et al., 2013). We also detected another important factor, myocyte enhancer factor 2 (MEF2), which was recently shown to negatively modify GluA2 surface expression by promoting its endocytosis. The spine density and morphology were also observed after surgery and inhalation to be regulated by MEF2 (Christina et al., 2012). The studies were completed to reveal the effect of different exposure durations on cognitive functions and the role of GluA2 as well as its underlying mechanism in modulating receptor trafficking.

2. Materials and methods

2.1. Animals

We purchased male Wistar rats (weighing 500–700 g, aged 18–20 months) from the Academy of Military Medical Science of the Chinese People's Liberation Army and fed them under controlled conditions on a 12 h light/dark cycle with ad libitum access to food and water. All experimental procedures were approved by the Chinese Small Animal Protection Association. All rats were held to acclimate to in their housing area for 1 week before experiments were initiated.

2.1.1. Experimental protocol

To test the effects of different durations of sevoflurane exposure on cognition, old rats were first divided into 6 groups – C: control ($n = 16$), P: propofol ($n = 16$), PS: surgery + propofol ($n = 16$), S1: surgery + 30 min of 1.5 MAC sevoflurane ($n = 16$), S2: surgery + 1 h of 1.5 MAC sevoflurane ($n = 16$), and S3: surgery + 2 h of 1.5 MAC sevoflurane ($n = 16$). Half of the animals in each group underwent the Y-maze test, and the rest of rats underwent fear conditioning tests.

2.1.2. Experimental protocol 2

We then divided old rats into 4 groups – C: naive control ($n = 12$), P: propofol (without surgery) ($n = 18$), PS: surgery + propofol ($n = 18$), and SS: surgery + 2 h of 1.5 MAC sevoflurane ($n = 18$). After the experiments, the animals were sacrificed for TUNEL staining, western blotting, Golgi staining and co-immunoprecipitation at day 1, 3, and 7.

2.1.3. Experimental protocol 3

After surgery and anesthesia, at 1 month and 2 months, Golgi staining and western blotting were applied to investigate the differences between animals in group PS ($n = 12$) and group SS ($n = 12$).

2.2. Anesthesia and surgery

Animals were anesthetized with either propofol infusion or received sevoflurane inhalation. Sevolfurane-treated rats were placed in a translucent chamber (W 25 cm × D 15 cm × H 10 cm) that was connected to a vaporizer. Rats in the control group were induced with 5% sevoflurane plus 40% oxygen. After the rat's righting reflex disappeared, the chamber was replaced by a mask. The sevoflurane concentration was reduced to 3.60% (1.5 MAC), and the inhalation duration was 30 min, 1 h and 2 h. The concentrations of sevoflurane were detected continuously by a gas monitor (Ohmeda Excel 210 SE anesthetic machine, Datex Instrumentarium Corp., Helsinki, Finland). Propofol-treated animals received a propofol infusion through the tail vein at a rate of 0.6 ± 0.1 (mg kg^{-1} min^{-1}) while 40% oxygen was given. We stopped administering propofol when the surgery ended. During anesthesia, all of the vital signs were monitored and the mean arterial pressure (MAP) was detected through a femoral artery catheter, and blood gas analysis was performed by apical puncture. Rats in the control group were placed in the same chamber and flushed with 40% oxygen without anesthesia for 2 h.

Surgical model (Vizcaychipi et al., 2011): Under different types of anesthesia, rats received an open tibial fracture on their left hind paw with intramedullary fixation. Analgesia was provided by buprenorphine (0.3 mg/kg in saline) intraperitoneally in no more than 1 mL. Throughout all of the procedures, the surgical area was kept sterile. The left hindpaw of the surgical animals was shaved and sterilized with povidone iodine. After a median incision on the surgical area, a 0.38-mm pin was inserted in the intramedullary canal. Then, we stripped the periosteum and performed an osteotomy. After cleaning the wound, the skin was sutured with 8/0 Prolene sutures. Post-intervention rats were moved to heated pads to recover and then moved to their own cage in which food and water were adequate.

2.3. Fear conditioning

Two animals were counterbalanced for group assignment and trained at the same time (Li et al., 2012). The chambers were manufactured of acrylic. Rats were delivered foot shocks through a grid floor formed by 19 stainless steel bars. The shock delivery system was connected to the floors (Anymaze associates). Before/after each session, the chambers were wiped with pine-scented cleaner before and after each session. The room in which training occurred was lit with fluorescent bulbs, and a ventilation fan provided a background noise (65 dB). The odor, appearance, and material of the chambers as well as the room formed the training context.

Training was executed 24 h before the experiments. Animals were first allowed to explore for a 3-min baseline period, and then, they received 3 tone (2000 Hz, 90 dB)-shock (1 mA, 2 s) pairings, which were separated by 1 min to establish the fear response. After all of the experiments, rats were observed for fear to context based fear that is believed to reflect the hippocampal-dependent memory. During the context test, each animal was placed into the chamber in which it was previously trained for 8 min (without tone or shock). Episodes of freezing was recorded by two observers who were blinded to the group assignment.

2.4. Y-maze

The Y-maze was performed to investigate spatial working memory in aged rats (Lee et al., 2008). The Y-maze consisted of