Duplicate preconditioning with sevoflurane *in vitro* improves neuroprotection in rat brain via activating the extracellular signal-regulated protein kinase

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Abstract: Objective Sevoflurane preconditioning has been demonstrated to reduce cerebral ischemia–reperfusion (IR) injury, but the underlying mechanisms have not been fully elucidated. Besides, different protocols would usually lead to different results. The objective of this study was to determine whether dual exposure to sevoflurane improves the effect of anesthetic preconditioning against oxygen and glucose deprivation (OGD) injury *in vitro*. **Methods** Rat hippocampal slices under normoxic conditions (95% O₂/5% CO₂) were pre-exposed to sevoflurane 1, 2 and 3 minimum alveolar concentration (MAC) for 30 min, once or twice, with 15-min washout after each exposure. The slices were then subjected to 13-min OGD treatment (95% N₂/5% CO₂, glucose-free), followed by 30-min reoxygenation. The population spikes (PSs) were recorded in the CA1 region of rat hippocampus. The percentage of PS amplitude at the end of 30-min reoxygenation to that before OGD treatment was calculated, since it could indicate the recovery degree of neuronal function. In addition, to assess the role of mitogen-activated protein kinases (MAPKs) in preconditioning, U0126, an inhibitor of extracellular signal-regulated protein kinase (MEK-ERK1/2, ERK1/2 MAPK), and SB203580, an inhibitor of p38 MAPK, were separately added 10 min before sevoflurane exposure. **Results** Preconditioning once with sevoflurane 1, 2, and 3 MAC increased the percentage of PS amplitude at the end of 30-min reoxygenation to that before OGD treatment, from (15.13±3.79)% (control) to (31.88±5.36)%, (44.00±5.01)%, and (49.50±6.25)%, respectively, and twice preconditioning with sevoflurane 1, 2, and 3 MAC increased the percentage to (38.53±4.36)%, (50.74±7.05)% and (55.86±6.23)%, respectively. The effect of duplicate preconditioning with sevoflurane 3 MAC was blocked by U0126 [(16.23±4.62)%]. **Conclusion** Sevoflurane preconditioning can induce neuroprotection against OGD injury *in vitro*, and preconditioning twice enhances this effect. Besides, the activation of extracellular signal-regulated protein kinase (MEK-ERK1/2, ERK1/2 MAPK) may be involved in this process.

Keywords: electrophysiology; hippocampal slice; oxygen and glucose deprivation; neuronal damage; sevoflurane preconditioning; mitogen-activated protein kinases

1 Introduction

Anesthetic preconditioning (APC) has raised more at-

tention nowadays, in both basic science and clinical research in brain. Research in this field may ultimately have an impact on anesthesia practice for patients with a risk of stroke. Accumulative evidence has suggested that volatile anesthetics exert neuroprotective effect when given before and after ischemia^[1-7]. This neuroprotection is believed to occur through

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complex signal transduction pathways involving the activations of adenosine receptors^[8,9], protein kinase C^[10-13] and mitochondrial adenosine triphosphate-regulated potassium (K_{ATP}) channels^[2,14]. Interestingly, the effect of ischemic preconditioning (IPC) of brain^[15,16] was demonstrated before that of heart IPC^[17]. Brain and heart share common signal transduction pathways, including activation of K_{ATP} channel^[18,19]. APC is as effective as IPC and they seem to share many signal transduction pathways.

Compared with IPC, one advantage of APC is that it does not need ischemia to initiate preconditioning. Furthermore, volatile anesthetics are routinely used for general anesthesia. Their dosage and duration of action in the body can be easily controlled, compared with other preconditioning intravenous drugs. It has been shown that repeated transient ischemia-reperfusion (IR) periods are more effective in triggering IPC than a single IR period, which suggests greater accumulation of critical IPC mediators within each IR period^[20]. Therefore, reperfusion after transient ischemia may be as critical as the IPC-stimulating agents for triggering IPC. Although single and repeated APC-induced neuroprotection is found to be dosage-dependent^[2,14], the role of repeated anesthetic exposure in improving neuroprotection, compared with that of single anesthetic exposure has not been reported. The aim of this study was to investigate whether dual exposure to sevoflurane at clinically relevant concentrations is more effective than single exposure in inducing neuroprotection against oxygen and glucose deprivation (OGD).

Mitogen-activated protein kinases (MAPKs) belong to a family of highly conserved proteins in multicellular organisms. They are the most ancient signaling molecules and control a vast array of physiological processes^[21]. By phosphorylating specific serine and threonine residues in cellular targets, they affect gene expression, mitosis, cytokinesis, metabolism, and cell death. Many studies demonstrate that modulations of extracellular signal-regulated protein kinases (MEK-ERK1/2, ERK1/2 MAPK) and p38 MAPK activities either by ischemia or by pharmacological agents are important in the genesis of the cytoprotective phenotype^[22-26]. To date, no data are available with respect to the role of MAPKs in APC. Therefore, the present study

also aimed to determine the significance of ERK1/2 MAPK and p38 MAPK signaling pathways in APC-induced neuroprotection and to characterize the activity profiles of these key kinases in APC.

2 Materials and methods

2.1 Animals and slice preparation Sprague-Dawley rats (weighting 100-120 g) were anesthetized with ethyl ether and decapitated. Hippocampal slices were prepared as previously described^[26]. Briefly, brain was rapidly removed, and hippocampus was dissected and sliced transversely to its longitudinal axis (400 μ m thick) in cold artificial cerebrospinal fluid (ACSF) with a Rotorslicer DTY-7700 (DSK, Osaka, Japan). Slices were placed on a nylon mesh in a recording chamber and incubated at 34 °C for 90 min, continuously supplied with humidified gas mixture (95% O₂/5% CO₂) at a flow rate of 1 L/min and ACSF at a rate of 1.5 mL/min. All the procedures were approved by the Animal Research Committee of Xuzhou Medical College.

2.2 Drug administration Sevoflurane was obtained from Dinabot (Osaka, Japan). Other chemicals except U0126 and SB203580, were from Sigma (St. Louis, Mo, USA). U0126 and SB203580 were purchased from Promega Corp (Madison, Wis, USA). Sevoflurane was applied as vapors to the ACSF via prewarmed and humidified 95% O₂/5% CO₂ gas stream, using Tec 3 vaporizer (Omeda, Steeton, West Yorkshire, United Kingdom). Sevoflurane concentration was expressed as minimum alveolar concentration (MAC), referring to dial settings on the vaporizer. The vaporizer had been previously calibrated with an anesthetics gas analyzer (Capnomac; Datex, Helsinki, Finland). Sevoflurane was applied for a minimum of 20 min to reach the equilibrium before it was perfused into the chamber, as the preliminary experiments demonstrated that the onset time for sevoflurane effects was less than 2 min and the maximum effects reached a plateau within 10 min (data not shown). Concentrations of sevoflurane in the perfusate were determined using gas chromatography (Shimadzu, Kyoto, Japan). The concentrations of sevoflurane in solution were found to be linear with the volume percentage in gas phase (0.64 mmol/L per 1.0%), up to 6.0%.

2.3 Experiment groups Slices were randomly assigned into

7 groups: OGD control slices ($n=12$) without exposure to sevoflurane; Sevo1-1 slices ($n=12$) that were exposed to 1 MAC sevoflurane once for 30 min; Sevo1-2 slices ($n=12$) that were exposed to 1 MAC sevoflurane twice, each lasting for 30 min, with a 15-min washout period interspersed; Sevo2-1 slices ($n=12$) that were exposed to 2 MAC sevoflurane once for 30 min; Sevo2-2 slices ($n=12$) that were exposed to 2 MAC sevoflurane twice, each lasting for 30 min, with a 15-min washout period interspersed; Sevo3-1 slices ($n=12$) that were exposed to 3 MAC sevoflurane once for 30 min; and Sevo3-2 slices ($n=12$) that were exposed to 3 MAC sevoflurane twice, each lasting for 30 min, with a 15-min washout period interspersed. All slices, except those in the control group, were exposed to sevoflurane, followed by 15-min washout of sevoflurane. Then they underwent a 13-min period of OGD and 30-min reperfusion.

To study the role of MAPKs, slices were also assigned into 7 groups. Control group: OGD slices ($n=12$) without exposure to sevoflurane or the inhibitors of MAPKs. Sevo3-2 group: slices were exposed to 3 MAC sevoflurane twice, each for 30 min, with a 15-min washout period interspersed and then underwent 15-min washout, 13-min OGD and 30-min reoxygenation. U0126 group: slices were subjected to ACSF supplemented with 10 $\mu\text{mol/L}$ U0126 for 10 min before OGD, without sevoflurane treatment. SB203580 group: slices were subjected to ACSF supplemented with 2 $\mu\text{mol/L}$ SB203580 for 10 min before OGD, without sevoflurane treatment. Sevo+U group: slices were subjected to ACSF supplemented with 10 $\mu\text{mol/L}$ U0126 for 10 min, followed by twice exposure to 3 MAC sevoflurane. Sevo+SB group: slices were subjected to ACSF supplemented with 2 $\mu\text{mol/L}$ SB203580 for 10 min, followed by twice exposure to 3 MAC sevoflurane. Vehicle group: slices were subjected to ACSF supplemented with 1 mmol/L dimethyl sulfoxide (DMSO).

For OGD treatment, slices were maintained in glucose-free ACSF and gassed with 95% $\text{N}_2/5\% \text{CO}_2$ for 13 min. After that, a 30-min period of reoxygenation was employed. Recovery responses were recorded automatically at each 10 s after the washout of anesthetic-equilibrated ACSF from the chamber. To study the role of MAPKs in APC, stock solutions of the ERK1/2 MAPK inhibitor U0126 and the p38 MAPK

inhibitor SB203580 were prepared in DMSO and diluted in ACSF before they were perfused into the chamber. The concentration of DMSO used in the experiments alone did not affect the PS amplitudes.

2.4 Electrophysiologic techniques Bipolar nichrome stimulating electrodes (0.1 ms pulse duration, 8–9 V in amplitude) were placed in the region of CA3 to activate Schaffer collateral inputs (orthodromic stimulation) to CA1 pyramidal neurons. Pulse duration was fixed at 0.1 ms, and the stimulus intensity (8–9 V) that elicits maximum PS amplitude was used to evoke a response. Glass microelectrodes (3–5 M Ω filled with 2 mmol/L NaCl) were placed in the cell body region of CA1 neurons for extracellular recording of the CA1 population spikes (PSs). Square-wave stimuli (8–9 V, 0.1 ms, 0.1 Hz) were delivered with a SEN-3301 stimulator (Nihon Kohden, Tokyo, Japan). PSs were amplified (MEZ-8301, Nihon Kohden, Tokyo, Japan), filtered (1 Hz to 10 kHz), and digitally converted (100 kHz) using an iNet system (GWI, Somerville, MA, USA). Data were stored in computer for later analysis. Only the slices in which orthodromic stimulation evoked a PS response of more than 3.5 mV were chosen for further investigations^[14]. The PS amplitudes were measured from peak positive to peak negative. Results were shown as the percentage of the PS amplitude measured at the end of 30-min reoxygenation to that measured before OGD (after 15-min sevoflurane washout). This percentage was used to reflect the degree of neuronal function recovery post OGD period^[27,32,33].

2.5 Triphenyltetrazolium chloride (TTC) staining To assess the slice viability, some slices were reperfused in 30 mL ACSF gassed with 95% $\text{O}_2/5\% \text{CO}_2$ for 30 min after the recording. At the end of reperfusion, slices were stained with 2% (w/v) TTC solution for 30 min at 35 °C, followed by washout with normal saline. The residual solution on slice surface was removed by absorbent paper, and slices were weighted. The red product formazan in the slices was extracted by a 1:1 mixture of ethanol: DMSO at 4 °C overnight. The red solvent extract was placed into a 96-well plate (200 μL /well), and the absorbance was read at 490 nm with a Universal Microplate Recorder, to reflect the amount of formazan in the sample. The absorbance was normalized by the weight of the slice,

and results were presented as A_{490}/g . Slices without OGD injury and not exposed to sevoflurane served as normal controls. Other groups were the same as those stated in experiment groups.

2.6 Data analysis Data were from at least three replications, and presented as mean $\pm$ SD. Data of slice function among groups were analyzed with one-way analysis of variance (ANOVA) followed by *post hoc* Duncan test (SPSS 11.0). $P < 0.05$ was considered as statistically significant.

3 Results

PS amplitudes were analyzed before, during and after the 13-min period of OGD^[27,32]. In the control group, PS amplitude at the start of the experiment was (6.2 ± 0.8) mV. Following the OGD period, PS amplitude vanished and showed a restricted recovery during the reoxygenation period to (0.96 ± 0.03) mV, (15.13 ± 3.79)% of that before OGD. In the other groups, the PS amplitudes at the start of experiment were not significantly different from that in the control group ($P > 0.05$). In all sevoflurane groups, there was no significant difference in PS amplitudes between during the initial data acquisition period and after washout of sevoflurane, indicating that sevoflurane itself had no effect on PS amplitude. Changes in PS during and following ischemia (13-min OGD and 30-min reoxygenation, respectively) in slices from control, Sevo3-2, Sevo+U, and Sevo+SB groups were shown in Fig. 1.

Our investigation suggested that the effect of preconditioning induced by once exposure to sevoflurane *in vitro* was dosage-dependent. Compared with the control slices, exposure to sevoflurane for once dosage-dependently increased the degree of neuronal function recovery, from (15.13 ± 3.79)% in the control group to (31.88 ± 5.36)%, (44.00 ± 5.01)%, and (49.50 ± 6.25)% with exposure to sevoflurane 1, 2, and 3 MAC, respectively ($P < 0.01$ vs control) (Fig. 2). Moreover, twice exposure to sevoflurane 1, 2 and 3 MAC increased the degree of neuronal function recovery to (38.53 ± 4.36)%, (50.74 ± 7.05)% and (55.86 ± 6.23)%, respectively ($P < 0.01$ vs control) (Fig. 2). In this study, duplicate exposure to sevoflurane at the same concentration achieved better effects than did single exposure ($P < 0.01$, Sevo1-2 vs Sevo1-1; $P < 0.05$, Sevo2-2 vs Sevo2-1, Sevo3-2 vs Sevo3-1).

Fig. 1 Representative extracellular population spike (PS) traces before (after washout of sevoflurane), during (at 10 min of OGD) and after (at the end of 30-min reoxygenation) OGD were recorded in control, Sevo3-2, Sevo+U and Sevo+SB groups. In control group, after OGD, PS recovered only a little, while in Sevo3-2 group (preconditioned with 3 MAC sevoflurane twice), the recovery of PS was better than that in the control group. In Sevo+U group, the recovery of PS amplitude was much attenuated, showing that U0126 (10 μ mol/L) abolished the effect of preconditioning induced by twice exposure to 3 MAC sevoflurane. In Sevo+SB group, the PS changes of amplitude was not significantly influenced by SB203580 (2 μ mol/L), as compared with the Sevo3-2 group. The bottom panel showed how to measure PS amplitude.

Meanwhile, there was no significant difference between Sevo2-2 and Sevo3-1 groups, suggesting that duplicate exposure to a lower concentration of sevoflurane might achieve an outcome similar to that by single exposure to a higher concentration.

As shown in Fig. 3, administration of either U0126 or SB203580 alone had no effect on the recovery of slices from 13-min OGD [(18.50 ± 5.23) % and (16.25 ± 6.20) %, respectively], as compared with the control group. However, U0126 blocked the preconditioning effect of twice exposure to sevoflurane 3 MAC when administered 10 min before sevoflurane expo-

Fig. 2 Sevoflurane-induced preconditioning was dosage-dependent and protocol-dependent *in vitro*. Neuronal function recovery was reflected by the percentage of PS amplitude at the end of 30-min reoxygenation to that before OGD. All data were presented as mean $\pm$ SD. * $P < 0.01$ vs control, # $P < 0.05$, ## $P < 0.01$ vs Sevo2-1 group (exposed to 2 MAC sevoflurane once); & $P < 0.05$ vs Sevo2-2 group (exposed to 2 MAC sevoflurane twice); \$ $P < 0.05$, \$\$ $P < 0.01$ vs once exposure to sevoflurane at the corresponding concentration.

Fig. 3 U0126 blocked the effect of duplicate conditioning with sevoflurane 3 MAC on the PS amplitude recovery *in vitro*, while SB203580 did not. PS amplitude recovery was reflected by the percentage of PS amplitude at the end of 30-min reoxygenation to that before OGD injury. Note that data from control and twice exposure to 3 MAC sevoflurane were the same as those in Fig. 2. Data were presented as mean $\pm$ SD. * $P < 0.01$ vs control, DMSO, U0126, SB203580, and Sevo+U groups. There was no difference between sevoflurane and Sevo+SB groups.

sure [(16.23 $\pm$ 4.62)%]. This suggested that sevoflurane could activate the ERK1/2 MAPK signaling pathway, and the activation of this pathway may play an important role in sevoflurane-induced preconditioning. In addition, SB203580 had no effect on the preconditioning of duplicate exposure to sevoflurane 3 MAC [(51.25 $\pm$ 7.03)%]. Similarly, 1 mmol/L DMSO alone had no effect on neuronal function recovery of

control slices (Fig. 3).

As shown in Table 1, TTC conversion into formazan in hippocampal slices was markedly decreased by OGD treatment, as compared with that in normal group (without OGD treatment) [(12.5 $\pm$ 3.9) A_{490}/g vs (45.2 $\pm$ 10.8) A_{490}/g , $P < 0.01$]. Duplicate preconditioning with sevoflurane 3 MAC attenuated the effects of OGD insult, from (12.5 $\pm$ 3.9) A_{490}/g to (38.5 $\pm$ 8.7) A_{490}/g ($P < 0.01$). Although U0126 or SB203580 alone had no effect on OGD injury [(13.6 $\pm$ 2.9) A_{490}/g and (11.9 $\pm$ 3.2) A_{490}/g , respectively, $P > 0.05$], TTC conversion into formazan in Sevo+U group was significantly decreased, as compared to that in Sevo3-2 group [(14.1 $\pm$ 2.6) A_{490}/g vs (38.5 $\pm$ 8.7) A_{490}/g , $P < 0.01$, Table 1]. Besides, there was no significant difference in OGD injury between Sevo+SB and Sevo3-2 groups ($P > 0.05$, Table 1). These results suggested that U0126 could abolish the neuroprotection by sevoflurane preconditioning, probably through inhibiting the activation of MEK-ERK1/2 which is known to be involved in the neuroprotection induced by sevoflurane preconditioning.

Table 1. Effect of U0126 and SB203580 on formazan production (A_{490}/g) after OGD-induced injury

Groups	<i>n</i>	Formazan production (A_{490}/g)
Normal (without OGD)	14	45.2 $\pm$ 10.8
Control (OGD alone)	8	12.5 $\pm$ 3.9**
Sevo3-2	10	38.5 $\pm$ 8.7#
U0126	9	13.6 $\pm$ 2.9**&
SB203580	6	11.9 $\pm$ 3.2**&
Sevo+U	7	14.1 $\pm$ 2.6**&
Sevo+SB	8	35.2 $\pm$ 9.9#

* $P < 0.05$, ** $P < 0.01$ vs normal group; # $P < 0.01$ vs control group; & $P < 0.01$ vs Sevo3-2 group.

4 Discussion

The rat hippocampal slice preparation serves as a useful model system to study the mechanisms of general anesthetic action. It may be one of the major targets for anesthetic action^[27]. In the studies of Weber *et al.*^[28] and Tian *et al.*^[33], the amplitudes of extracellular field potentials^[28] and PS^[33]

were analyzed before, during, and after the OGD period, and the percentage was used to reflect the recovery of neuronal function. In another report of Libien *et al.*^[34], the recovery of synaptic function was calculated by comparing the PS amplitude at 1 h post-anoxic to the pre-anoxic recording. In the present study, the PSs are also the extracellular field potentials, and our experiment method is the same as theirs. The neuronal function could be well indicated by PS amplitude, and the change of amplitude stands for the change of neuronal function. In the present study, we found that sevoflurane-induced preconditioning *in vitro* is dependent not only on the concentration but also on the protocol of preconditioning. Furthermore, our study furnishes the first direct evidence that APC can be improved by dual exposure, compared with single exposure, at the same anesthetic concentration. Kehl *et al.*^[14] have found that APC with sevoflurane 15 min before hypoxia can significantly increase the degree of neuronal function recovery in hippocampal slices, as reflected by PS recovery. The volatile anesthetics isoflurane and halothane have been reported to be capable of reducing regional brain infarcts in rats by inducing an ischemia-tolerant preconditioned state^[1,2]. Recently, Xiong *et al.* have demonstrated that the rats receiving 1.5% and 2.25% isoflurane in oxygen 1 h per day for 5 d exhibit dosage-dependent effects of APC in middle cerebral artery occlusion^[2]. Their results also demonstrate that anesthetic could induce preconditioning in the brain. In the present study, by investigating the effects of duplicate exposure to anesthetic *in vitro* and the relationships between single and repeated exposure to anesthetic, we confirmed and extended those results. Our study reveals that at least 2 cycles of exposure to a clinically relevant concentration of a volatile anesthetic followed by washout can enhance neuroprotection afforded by APC. This may decrease the required anesthetic concentrations and thus minimize the adverse cardiovascular and cerebrovascular effects of anesthetic exposure in patients with a higher risk of stroke. The observed enhancement of neuroprotection by duplicate exposure to sevoflurane may be attributed to the accumulation of mediators, but the underlying mechanisms still need to be further elucidated.

In another experiment, our results reveal that U0126, a

specific inhibitor of MEK-ERK1/2, could abolish the neuroprotection induced by duplicate exposure to 3 MAC sevoflurane, when added 10 min before sevoflurane treatment. Previous evidence indicates that U0126 could block the neuroprotection induced by isoflurane in hippocampal slice cultures^[24], suggesting that the neuroprotection of isoflurane is induced through activating the MEK-ERK1/2 signal pathway. However, this neuroprotection effect was not induced by APC. We presume that sevoflurane induces preconditioning probably through activating this signal pathway, and our results confirm this hypothesis. MEK-ERK1/2 links growth factor receptors with nerve growth factor and brain-derived neurotrophic factor, to exert neuroprotective effects such as NMDA receptor modulation and reduction of glutamate excitotoxicity^[29]. MEK-ERK1/2 also phosphorylates hypoxia-inducible factor 1 α , a transcription factor required for the cell adaptation to acute and chronic hypoxia^[30]. In addition, here p38 MAPK is found to have no effect on the preconditioning of sevoflurane, thus the role of p38 MAPK in preconditioning is disputed. Silva *et al.*^[32] have found that p38 MAPK plays different roles in IPC and APC in rat heart. In IPC-treated heart, the activity of p38 MAPK was enhanced, while in APC-treated heart, the activity of p38 MAPK was not affected, suggesting that p38 MAPK does not participate in triggering APC. Whether these two findings share the same mechanism needs to be further investigated. Moreover, Zheng *et al.* have found that isoflurane can induce a rapid and prolonged increase of phosphorylated p38 MAPK level in cerebral neocortex, suggesting that isoflurane preconditioning may improve long-term neurological outcome after focal brain ischemia, and that the effects may be mediated by activating p38 MAPK^[22]. Jiang *et al.*^[25] have reported that brain seizure tolerance involves p38 signaling pathways. In contrast, investigation by Sugino *et al.* demonstrates that pretreatment with SB203580 could reduce ischemic cell death in the CA1 region after transient global ischemia, by inhibiting the activity of p38 MAPK^[31]. Unfortunately, currently there is no easy explanation for these seemingly opposing conclusions of the 2 studies that used almost identical models. Further studies are needed to fully reveal the underlying mechanisms.

In summary, the present study demonstrates that once and dual exposure to sevoflurane can induce neuroprotection against OGD injury in the rat hippocampal slice preparation *in vitro*, in a dose-dependent way. In addition, dual exposure to sevoflurane improves the effect of preconditioning. Activation of MEK-ERK1/2 may be a key mechanism of sevoflurane-induced preconditioning. These findings suggest that perioperative administration of volatile anesthetics may induce cerebroprotection in humans. However, this proposition requires further investigations for confirmation.

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离体条件下七氟醚重复预处理增强大鼠脑的神经保护

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摘要: 目的 大量研究表明, 吸入麻醉药预处理可以降低脑缺血再灌注损伤, 但吸入预处理的机制还不甚清楚, 不同的给药方案可能会产生不同的效果。本研究旨在探讨七氟醚重复预处理是否能增强单次预处理对缺氧无糖(oxygen and glucose deprivation, OGD)损伤大脑的保护作用及其可能的机制。**方法** 大鼠海马脑片经最低肺泡有效浓度(minimum alveolar concentration, MAC)为1、2和3的七氟醚单次或双次预处理(每次持续30 min), 每次预处理后冲洗15 min, 然后进行13 min OGD损伤, 再复氧供糖30 min。在这些过程中记录CA1区群峰电位(population spikes, PSs), 损伤后PS的恢复程度代表神经功能的恢复程度。为研究丝裂原活化蛋白激酶(mitogen-activated protein kinases, MAPKs)在七氟醚预处理中的作用, 在七氟醚预处理前10 min加入细胞外信号调节蛋白激酶(extracellular signal-regulated protein kinase, MEK-ERK1/2)抑制剂U0126和p38 MAPK抑制剂SB203580。**结果** 1 MAC、2 MAC和3 MAC七氟醚单次预处理后, 神经功能的恢复程度分别为(31.88±5.36)%、(44.00±5.01)%和(49.50±6.25)%, 对照组为(15.13±3.79)%; 1 MAC、2 MAC和3 MAC七氟醚双次预处理后, 神经功能的恢复程度分别为(38.53±4.36)%、(50.74±7.05)%和(55.86±6.23)%。此外, U0126能抑制3 MAC七氟醚双次预处理的保护作用[(18.5±5.23)% vs (55.86±6.23)%]。**结论** 以上结果提示, 七氟醚预处理具有神经保护作用, 且七氟醚重复预处理诱导的神经保护作用强于单次预处理的神经保护作用。此外, MEK-ERK1/2的活化参与了七氟醚预处理对OGD损伤大脑的保护作用。

关键词: 电生理; 海马脑片; 缺氧无糖; 神经损伤; 七氟醚预处理; 丝裂原活化蛋白激酶