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BRAIN RESEARCH

Research Report

Sevoflurane postconditioning involves an up-regulation of HIF-1α and HO-1 expression *via* PI3K/Akt pathway in a rat model of focal cerebral ischemia

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ABSTRACT

Administration of sevoflurane at the onset of reperfusion has been confirmed to provide a cerebral protection. However, little is known about the mechanism. In this study, we tested the hypothesis that sevoflurane postconditioning induces neuroprotection through the up-regulation hypoxia inducible factor-1α (HIF-1α) and heme oxygenase-1 (HO-1) involving phosphatidylinositol-3-kinase (PI3K)/Akt pathway. In the first experiment, male Sprague–Dawley rats were subjected to focal cerebral ischemia. Postconditioning was performed by exposure to 2.5% sevoflurane immediately at the onset of reperfusion. The mRNA and protein expression of HIF-1α and its target gene, HO-1, intact neurons and the activity of caspase-3 was evaluated at 6, 24 and 72 h after reperfusion. In the second experiment, we investigated the relationship between PI3K/Akt pathway and the expression of HIF-1α and HO-1 in the neuroprotection induced by sevoflurane. Cerebral infarct volume, apoptotic neuron and the expression of HIF-1α, HO-1 and p-Akt were evaluated at 24 h after reperfusion. Compared with the control group, sevoflurane postconditiong significantly ameliorated neuronal injury, up-regulated mRNA and protein levels of HIF-1α and HO-1, inhibited the activity of caspase-3, and decreased the number of TUNEL-positive cells and infarct sizes. However, the selective PI3K inhibitor, wortmannin not only partly eliminated the neuroprotection of sevoflurane as shown by reducing infarct size and apoptotic neuronal cells, but also reversed the elevation of HIF-1α, HO-1 and p-Akt expression in the ischemic penumbra induced by sevoflurane. Therefore, our data demonstrate that the cerebral protection from sevoflurane postconditioning is partly mediated by PI3K/Akt pathway *via* the up-regulation of HIF-1α and HO-1.

1. Introduction

Ischemia–reperfusion injury in the brain is involved in various surgical procedures, including intracranial aneurysm clamping, aortic arch replacement under deep hypothermic circulatory arrest and carotid endarterectomy. Research has been extensively carried out to find effective strategies and drugs to prevent at least to ameliorate brain ischemia–

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reperfusion injury in the past few decades. Increasing evidence has demonstrated that inhalational anesthetic agents, when administered early at reperfusion, could increase ischemic tolerance in the heart (Ge et al., 2010; Lange et al., 2009; Pravdic et al., 2010) and brain (Lee et al., 2008; McMurtrey and Zuo, 2010). The administration of sevoflurane immediately at the onset of reperfusion, which was called sevoflurane postconditioning, has been shown to increase survival of neuronal cells during cerebral ischemia (Peng et al., 2010; Wang et al., 2010) in a manner similar to ischemic postconditioning. However, the molecular mechanisms underlying brain protection of sevoflurane posconditioning are not yet fully understood.

Hypoxia inducible factor-1α (HIF-1α), a key physiological sensor of oxygen level in most mammalian cells, plays an important role in cellular survival, glucose metabolism and transport and metabolic adaptation (Semenza, 2000) by regulating the expression of its target genes, such as heme oxygenase-1 (HO-1) (Lee et al., 1997), vascular endothelial growth factor (VEGF) (Milosevic et al., 2007) and erythropoietin (EPO) (Mu et al., 2005). Several recent studies have demonstrated that isoflurane reduces ischemic myocardial damage *in vivo* by up-regulating HIF-1α expression, which is mediated by extracellular signal-regulated kinases 1/2 (ERK 1/2) (Wang et al., 2006) and mammalian target of rapamycin (mTOR) (Raphael et al., 2008). Moreover, isoflurane can up-regulate HIF-1α and enhance the mRNA expression of HIF-1α-responsive gene HO-1 in Hep3B cells *in vitro* (Li et al., 2006). Whether other anesthetics, such as sevoflurane, act in a similar manner is currently unclear.

There is enough evidence that the phosphatidylinositol-3-kinase (PI3K)/Akt pathway, an anti-apoptotic prosurvival kinase signaling cascade, plays a pivotal role in anesthetic (Chiari et al., 2005; Prasad et al., 2011) and ischemic (Zhu et al., 2006) postconditioning. Recently, we have demonstrated that ischemic postconditioning protected brain from the injury induced by focal cerebral ischemia *via* the suppression of endoplasmic reticulum (ER) stress-induced apoptosis and the activation of PI3K/Akt survival pathway (Yuan et al., 2011). In addition, it has shown that phosphorylation by PI3K/Akt leads to increase HIF-1α activation (Li et al., 2008; Zhang et al., 2009). Volatile anesthetics are also known to increase PI3K/Akt activity. Wang et al. (2010) recently reported that sevoflurane (1.0 and 1.5 MAC) postconditioning not only reduced infarct volume but also improved learning and memory. This neuroprotective effect may be due to the activation of PI3K/Akt pathway and the inhibition of neuronal apoptosis. Whether PI3K/Akt pathway is involved in HIF-mediated sevoflurane postconditioning has not yet been investigated.

In the present study, we hypothesized that the underlying mechanism responsible for neuroprotection from sevoflurane postconditioning involves the up-regulation of HIF-1α and HO-1 expression *via* PI3K/Akt pathway. To test this hypothesis, we used a rat model of focal cerebral ischemia–reperfusion injury induced by 60-min occlusion of bilateral common carotid artery (CCA) combined with permanent occlusion of middle cerebral artery (MCA).

2. Results

2.1. *Physiological variables during the experiment*

Physiological parameters such as blood pressure, blood gas and glucose, and temperature were closely monitored and controlled. There were no significant differences in these physiologic parameters among the rat groups before occlusion, 60 min after occlusion and 60 min after reperfusion (data not shown).

2.2. *Effect of sevoflurane postconditioing on histopathology after ischemia–reperfusion*

In Nissl-stained sections, which was measured in 24 and 72 h after stroke, many atrophic neurons with shrunken cytoplasm and damaged nuclei were observed in the rats of control group while no apparent morphological changes in the sham group. Administration of sevoflurane preserved the integrity of neurons within the ipsilateral ischemic brain (Fig. 1A). The number of intact neurons in the control group was significantly decreased compared with that in the sham group ($P<0.01$). Treatment with sevoflurane significantly prevented the neuron loss by approximately 25.5% and 20.9%, respectively compared with that in the control group ($P<0.05$) (Fig. 1B).

2.3. *Effect of sevoflurane postconditioning on the mRNA and protein expression of HIF-1α and HO-1 in ischemic penumbra*

The results of RT-PCR showed that mRNA coding for HIF-1α and its target gene, HO-1 in penumbra cortex of rats was detected at the predicted molecular sizes in each group at 6, 24 and 72 h after cerebral focal ischemia (Fig. 2A). HIF-1α and HO-1 mRNA levels were barely detected in the rats of sham group. Compared with the sham group, the mRNA expression of HIF-1α and HO-1 in the control group was up-regulated at 6 h, peaked at 24 h and returned to basal levels at 72 h. Sevoflurane postconditioning significantly increased HIF-1α and HO-1 mRNA expression in penumbra cortex at 6 and 24 h after post-ischemia as compared with the control group ($P<0.05$). However, the mRNA expression of HIF-1α and HO-1 had no significant differences between rats in the control and rats in sevoflurane groups at 72 h after stroke (Fig. 2B) ($P>0.05$).

Western blot analysis showed that the protein level of HIF-1α and HO-1 in the penumbra cortex was very low in sham group rats. However, the protein levels of HIF-1α and HO-1 in the control and sevoflurane postconditioning group were markedly increased at all time points after cerebral ischemia, especially at 24 h of reperfusion compared with those of the sham group (Fig. 2C) ($P<0.05$). Compared with the control group, sevoflurane postconditioning group had much higher protein expression of HIF-1α and HO-1 at 72 h after stroke (Fig. 2D) ($P<0.05$).