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Isoflurane inhibits occludin expression via up-regulation of hypoxia-inducible factor 1α

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ABSTRACT

The blood–brain barrier (BBB) is a functional structure which regulates and restricts the transfer of circulating molecules and immune cells into the central nervous system. The barrier is formed by the presence of tight junctions (TJ) between the specialized brain endothelial cells. The volatile anesthetic isoflurane may affect the permeability of the BBB. Previous studies have proven that isoflurane alters hypoxia-inducible factor-1α (HIF-1α) expression, which may affect the TJ proteins; however, the mechanism of how TJ proteins are affected by isoflurane is still unclear. Primary human brain vascular endothelial cells (HBVEC) were exposed to isoflurane at various concentrations (0–2.5%) and different time periods (0–6 h). The cell viability, occludin expression, paracellular permeability, VEGF expression, TGF-β3 expression and occludin protein endocytosis were quantified. Isoflurane treatment induced a time- and concentration-dependent decrease in occludin mRNA and protein levels in HBVEC. This effect was partially abrogated by silencing the HIF-1α expression. Isoflurane could activate HIF-1α, and the overexpression HIF-1α up-regulated the level of VEGF and TGF-β3, VEGF decreased the expression of occludin and TGF-β3 accelerated the endocytosis of occludin. RNA interference targeting HIF-1α reduced both VEGF and TGF-β3 expression after isoflurane treatment.

Conclusion: This study provides direct evidence *in vitro* that exposing isoflurane to HBVECs can trigger HIF-1α activation, leading to lower protein levels of occludin, and increased permeability of the BBB.

1. Introduction

The blood–brain barrier (BBB) is a diffusion barrier which is composed of endothelial cells, astrocyte end-feet and pericytes (PCs) (Ballabh et al., 2004). The BBB is a functional structure that hinders the influx of most compounds from blood to the brain, and the BBB plays an important role in regulating and restricting the transfer of circulating molecules into the brain that can

Abbreviations: BBB, blood–brain barrier; TJ, tight junctions; HIF-1α, hypoxia-inducible factor-1α; VEGF, vascular endothelial growth factor

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interfere with the internal milieu of the brain (Chi et al., 2001; Murakami et al., 1997). Moreover, the BBB forms a neurovascular unit which could protect the brain from circulating neurotoxic agents and inflammatory factors (Yan et al., 2012). A variety of factors were able to alter the characteristics of the BBB, including the use of osmotic agents (Neuwelt et al., 1980), anoxia, trauma, inflammation and so on (Beggs and Waggener, 1976; Dux et al., 1984). Evidence suggested that anesthetic agents can affect the permeability (Chi et al., 1992) and transfer coefficient of the BBB for small molecules (Saija et al., 1989).

Isoflurane, a commonly used and potent inhalational anesthetic agent in balanced anesthesia, was discovered by Vitcha (1971). Isoflurane has been considered relatively safe as an anesthetic agent frequently used in humans. However, a series of studies have demonstrated that isoflurane may result in some adverse effects, such as decreased cerebral metabolism (Maekawa et al., 1986; Newberg et al., 1983), heart rate and arterial pressure (Eger, 1984), and little depression of cardiac contractility. Furthermore, extensive numbers of studies have suggested that isoflurane has an effect on the basal permeability of the BBB (Chi et al., 1992; Saija et al., 1989; Tetrault et al., 2008). However, the specific mechanism by which isoflurane leads to alteration of the permeability of the BBB is unclear.

There have been several prior reviews of the impact of isoflurane on the expression of several genes concerned with cell survival including hypoxia-inducible factor (HIF)-1 (Li et al., 2008; Semenza and Wang, 1992). This heterodimeric transcriptional factor is a transcription factor of the basic helix-loop-helix-Per-Arnt-Sim super-family, and consists of two subunits: HIF-1α and HIF-1β. HIF-1α is the specific and oxygen-regulated subunit of the HIF-1 complex and determines the level of HIF-1 activity, whereas HIF-1β is constitutively expressed (Huang et al., 1998). Cell response to the volatile anesthetic isoflurane was regarded as inducing HIF-1 gene expression (Tavare et al., 2012). An *in vitro* study using Hep3B cells demonstrated that isoflurane could up-regulate HIF-1α activity and increase endothelial permeability (Yan et al., 2012).

It has become increasingly clear that the BBB is formed by the presence of tight junctions (TJs) between adjacent capillary endothelial cells. Recent evidence suggests that many factors increase BBB permeability, possibly by specific alterations in TJ proteins such as occludin (Hawkins et al., 2007). Occludin (also known as OCLN) is an important cellular component of TJs, and can restrict permeability to small molecules. Occludin plays a critical role in maintaining permeability of the BBB; not only loss of occludin expression but also translocation of occludin could increase the permeability. High levels of HIF were associated with lower levels of occludin and resulted in increased TJ permeability (Harten et al., 2009). Previous studies showed that VEGF and TGF-β3 are two HIF-1 target genes (Li et al., 2006; Scheid et al., 2002). HIF-1 regulates the expression of vascular endothelial growth factor (VEGF) and VEGF increases BBB permeability possibly via alterations to occludin (Yeh et al., 2007). Recent studies using Sertoli cells cultured *in vitro* to permit TJ assembly have shown that TJ dynamics are regulated, at least in part, by TGF-β3. This, is turn, regulates the production of occludin, by Sertoli cells (Lui et al., 2003). HIF activation mediates both paracellular permeability and localization of TJ proteins in cerebral microvessel endothelial cells (Mark and Davis, 2002). However, it is unclear whether HIF-1α is induced in or involved in permeability dysfunction of brain endothelial cells exposed to isoflurane. This study provides direct evidence *in vitro* that primary human brain vascular endothelial cells (HBVEC) exposed to isoflurane can trigger HIF-1α activation leading to lower levels of occludin, and increased permeability of the BBB.

2. Results

2.1. *The cell viabilities were invariable with various concentrations of isoflurane preconditioning*

We investigated the effects of isoflurane preconditioning in different concentrations on the viabilities of HBVEC. The HBVECs were incubated with 0, 0.5, 1, 1.5, 2 or 2.5% of isoflurane for 0, 3 and 6 h, rinsed with PBS 3 times to remove isoflurane after isoflurane pretreatment. Cell viability was detected by MTT assay. The results showed that 0.5, 1, 1.5, 2% isoflurane did not affect the viability of HBVECs, while 2.5% isoflurane reduced cell viability (albeit tiny amount); however, the difference was not statistically significant (Fig. 1).

2.2. *Isoflurane preconditioning induced a significant decrease in expression of occludin protein*

Occludin is an important cellular component of the TJ, and plays a critical role in maintaining the BBB permeability. To evaluate changes in expression of occludin after isoflurane exposed in brain endothelial cells *in vitro*, western blot and RT-PCR assays were used. The results showed that isoflurane reduced the level of occludin gene transcription and protein expression in a dose- and time-dependent manner. Occludin mRNA levels were significantly decreased after 2 and 2.5% isoflurane exposure (Fig. 2A). 0–1% isoflurane did not significantly change the total occludin protein levels, whereas 1.5–2.5% isoflurane dramatically reduced the protein expression

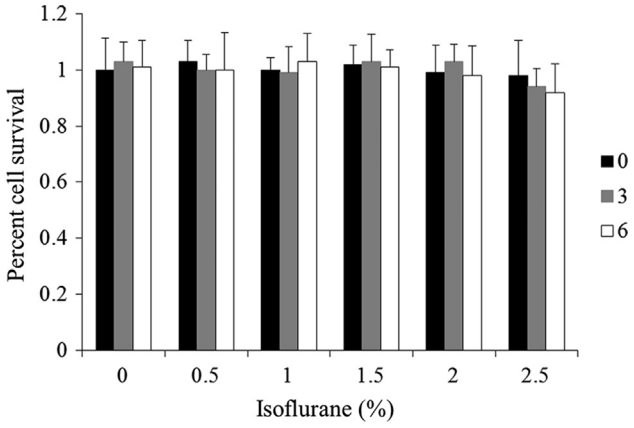

Fig. 1 – Isoflurane does not affect the viability of primary human brain vascular endothelial cells (HBVECs). HBVECs were exposed to various concentrations of isoflurane (0–2.5%) for the indicated time. All experiments were repeated at least three times. Data are presented as means ± SEM. ($n=3$) (Dunnett's test, $^*P<0.05$ compared to the controls).