EXPLORING THE MODULATION OF HYPOXIA-INDUCIBLE FACTOR (HIF)-1 α BY VOLATILE ANESTHETICS AS A POSSIBLE MECHANISM UNDERLYING VOLATILE ANESTHETIC-INDUCED CNS INJURY

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Abstract

This review summarizes recent research on the potential cognitive and behavioural abnormalities induced by exposure to volatile anesthetics and suggests a role of hypoxia-inducible factor (HIF)-1 α in mediating these events. Volatile anesthetics are widely utilized in clinical and research settings, yet the long-term safety of exposure to these agents is under debate. Findings from various animal models suggest volatile anesthetics induce widespread apoptosis in the central nervous system (CNS) that correlates with lasting deficits in learning and memory. Longitudinal analysis of clinical data highlight an increased risk of developmental disorders later in life when children are exposed to volatile anesthetics, particularly when exposures occur over multiple sessions. However, the mechanisms underlying these events have yet to be established. Considering the extensive use of volatile anesthetics, it is crucial that these events are better understood. The possible role of HIF-1 α in volatile anesthetic-induced CNS abnormalities will be suggested and areas requiring urgent attention will be outlined.

Key words: apoptosis; hypoxia; isoflurane; sevoflurane

Introduction

Each year millions of individuals undergo surgeries which require the use of anesthetics. The majority of these anesthetic agents are general anesthetics, used to induce and/or maintain unconsciousness, immobility, muscle relaxation, amnesia, and analgesia [1] with the intention of providing a safe and pain-free environment. General anesthetics include both inhalational (nitrous oxide, isoflurane, sevoflurane, desflurane, and xenon) and intravenous (propofol, etomidate, ketamine, methohexital, and thiopental) agents [1]. In the clinical setting, isoflurane and sevoflurane are among the most commonly-used volatile anesthetics [2]; however, general anesthetics are usually used in combinations to produce the desired effects. Furthermore, exposure to general anesthetics can vary in duration from minutes to several hours, with many individuals being repeatedly exposed due to multiple surgical procedures. This review focuses primarily on isoflurane and sevoflurane, with reference to other volatile and intravenous anesthetics where appropriate.

Although widely used and generally considered safe, a plethora of research exists debating the long-term consequences of exposure to volatile anesthetics on the central nervous system (CNS), with many researchers suggesting that exposure may be detrimental to neurological function. While the mechanisms by which volatile anesthetics may produce long term alterations in brain functioning have not been established, Jiang and colleagues suggest that mediation of endogenously expressed hypoxia-inducible factor (HIF)-1 α may be involved [3]. Indeed, research suggests that volatile anesthetics “hijack” the regulation of HIF-1 α pathway [4], which is normally activated under physiological conditions of oxygen deprivation. Therefore, this review explores research in this area in order to demonstrate that the regulation of HIF-1 α by volatile anesthetics should be further explored as a possible mechanism underlying volatile anesthetic-induced adverse effects on the CNS.

Mechanisms underlying the clinical actions of volatile anaesthetics on the CNS

Upon inhalation, volatile anesthetics rapidly enter the circulation and are able to affect the CNS due to their ability to cross the blood-brain barrier [5]. Once in the brain, volatile anesthetics act to induce and maintain their clinically-desired effects. However, despite the widespread utilization of volatile anesthetics, the mechanisms mediating these effects are not well understood. Several volatile anesthetics share a common site of action on the alpha subunit of gamma-aminobutyric acid (GABA)_A receptors [6-7], the most abundant fast inhibitory neurotransmitter receptor in the CNS. Thus, GABA receptors are a major current research target for studying the mechanisms underlying many of the clinical effects of volatile anesthetics [1]. Research suggests that volatile anesthetics enhance GABA_A receptor activity, resulting in inhibition of neurotransmission that ultimately contributes to producing the hypnosis, reflex depression, and amnesia seen during general anesthesia [1]. However, the role of GABA_A receptor modulation in mediating other aspects of general anesthesia, including analgesia and immobility, is not well established; in this arena, it has been suggested that volatile anesthetics produce and/or maintain immobility via inhibition of voltage-gated Na⁺ channels, which disrupts axonal conductance, synaptic integration, and neuronal excitability [8]. Although emerging evidence suggests that volatile anesthetics also affect glutamatergic function [9-10], the specific targets of this interaction have yet to be established. One of the prevailing

difficulties encountered when studying the mechanisms of volatile anesthetics has been the chemical and structural diversity among the various compounds that are involved in induction and maintenance of anesthesia [11]. This potentially increases the number of sites within the CNS with which volatile anesthetics can interact, complicating our ability to compare findings from independent studies [12-13].

Adverse Effects from volatile anesthetics

Despite the widespread use of volatile anesthetics in the clinical setting, adverse effects on the CNS can occur, with repeated exposures believed to be more detrimental than single, long exposures [14-15]. Analysis of clinical data suggest that repeated exposure to volatile anesthetics prior to two years of age is associated with increased risk of development of Attention Deficit Hyperactivity Disorder (ADHD) later in life [15] and learning disorders that require additional educational assistance, particularly to address speech and language deficits [16]. Both single and multiple exposures to general anesthesia prior to the age of three also correlate with an increased risk of developing language and abstract reasoning deficits [17]. Longitudinal analysis of clinical data demonstrates an association between exposure to volatile anesthetics during surgery and subsequent cognitive decline, particularly in elderly (aged ≥ 60 years old) patients [18]. Animal models corroborate these findings; rodents exposed to volatile anesthetics exhibit impairments in long-term memory [19] and deficits in spatial learning and memory [20] that persist into adulthood [21]. These cognitive deficits are associated with decreased neurogenesis [20], impaired long-term potentiation in the hippocampus [21] and dose-dependent increases in neuronal apoptosis [14, 20-22]. In primates, a clinically relevant five-hour exposure to isoflurane induces significant apoptosis of neurons and oligodendrocytes throughout the cerebral cortex, suggesting isoflurane adversely affects both grey and white matter. These effects are seen in both fetal [23] and 6 day old [24-25] rhesus macaques, which is approximately equivalent to the third trimester in pregnancy and 6 months postnatal age in humans, respectively. While the mechanisms underlying these events are not established, it is possible that the effects of volatile anesthetics on HIF-1 α are involved in these adverse consequences.

Hypoxia-Inducible Factor-1

When subjected to low oxygen levels (hypoxia), mammalian cells initiate several survival processes. One of these, capable of occurring in all nucleated cells [26-27], is the activation of HIF-1, which is a global regulator of oxygen homeostasis and cell-survival under low-oxygen conditions [28]. HIF-1 is a heterodimeric transcription factor that is composed of two subunits a beta subunit (HIF-1 β) and an alpha subunit, (HIF-1 α) [29]. Under conditions of normal oxygen supply, HIF-1 α protein is actively transcribed and translated in the cytoplasm. However, it is rapidly degraded in the cytoplasm via the ubiquitin-proteasome pathway interacting with the von Hippel-Lindau tumor suppressor protein [30]. Proline hydroxylases hydroxylate HIF-1 α in the presence of O₂, Fe²⁺, 2-oxoglutarate and ascorbate, allowing HIF-1 α to interact with the von Hippel-Lindau tumor suppressor protein. HIF-1 α is subsequently tagged with polyubiquitin and recognized for degradation. This results in little-to-no HIF-1 α protein expression during periods of oxygen homeostasis (Figure 1). Conversely, during hypoxia HIF-1 α

degradation ceases resulting in HIF-1 α stabilization and translocation to the nucleus. Here it binds with HIF-1 β to form an active HIF-1 complex (Figure 1).

Once active, the HIF-1 complex recruits transcriptional co-activators and is able to affiliate with hypoxia-responsive elements (HREs) on enhancers and promoters of target genes (see Hong and colleagues, figure 3 [32] for an exhaustive list of HIF-1 target genes). This interaction allows HIF-1 to regulate a wide variety of genes including erythropoietin, vascular endothelial growth factor [33] and glucose transporters [34], which are involved in oxygen delivery to tissues, angiogenesis, and glucose metabolism, respectively (see Figure 1). Thus under hypoxic conditions, up-regulation of these genes is intended to be a protective mechanism aimed at promoting cell-survival, despite inadequate oxygen availability. For example, during oxygen deprivation the HIF-1-dependent up-regulation of the glucose transporter-1 (Glut-1) increases glucose uptake in order to generate adequate adenosine triphosphate (ATP), a vital energy-storage molecule for the cell [29]. Up-regulation of vascular endothelial growth factor contributes to increased vascular permeability and angiogenesis [35], the latter of which is accomplished via the stimulation of endothelial cell proliferation and migration in hypoxic regions [36]. This ultimately aids in restoring oxygen levels in hypoxic areas [30]. However, severe hypoxic insults can result in significant damage to the affected tissues, rendering these areas incapable of being sufficiently reoxygenated [37]. Furthermore, when the brain is subjected to hypoxia, the regions responsible for breathing and circulation become vulnerable to oxygen deprivation. When these structures are deprived of oxygen and subsequently damaged, blood flow remains inadequate and the oxygen levels in hypoxic regions may not be restored.

The hypoxia-induced up-regulation of HIF-1 α and the subsequent increase in expression of its target genes are transient. The literature suggests that HIF-1 α up-regulation and accumulation occurs rapidly. Nuclear HIF-1 α is present within two minutes in HeLa cells subjected to reduced oxygen environments [38] and HIF-1 α up-regulation is immediately detectable in rodent cortical tissue following middle cerebral artery occlusion [39]. In rodent models of cardiac arrest, HIF-1 α up-regulation and accumulation occurs as early as one hour following resuscitation [40]. The duration of HIF-1 α up-regulation varies significantly between models and has been reported to be as short as 32 minutes following reoxygenation from hypoxia [38], between 8 and 24 hours following reoxygenation from middle cerebral artery occlusion [39], and at least 7 days following transient global ischemia [40]. The subsequent increase in HIF-1 target gene expression following a hypoxic insult can vary substantially. Hypoxia-ischemia models show up-regulation of vascular endothelial growth factor [36] and Glut-1 [41] in rodent cortical tissue within a few hours following reperfusion, with effects lasting for at least 24 hours and 72 hours, respectively. In short, single-exposure hypobaric chamber models, vascular endothelial growth factor up-regulation has been observed within the first few hours following exposure, continuing for at least 3 days [42]. The variability reported in the initiation and persistence of the up-regulation of both HIF-1 α and its downstream targets is likely due to the various durations and severities of the hypoxic events utilized across different studies.

HIF-1 α up-regulation: beneficial or deleterious?

Classically, HIF-1 α is believed to restore tissue homeostasis and thus promote cell survival during conditions of oxygen deprivation [43]. However, up-regulation of HIF-1 α in response to chronic or severe hypoxia correlates with cell death [44-45]. This is attenuated with inhibition of HIF-1 α [46-48], suggesting that under certain circumstances HIF-1 α is actually deleterious to cell survival. Interestingly, the HIF-1 complex itself is capable of promoting both cell death or cell survival in the same cell, which appears to be dependent on the type of insult that initially induced HIF-1 expression [49] and points to a role of the insult itself in the contrasting effects of HIF-1 α up-regulation on cell survival. The ability of HIF-1 to mediate contrasting effects on cell survival is likely due to the capability of HIF-1 to promote the up-regulation of both “pro-survival” and “pro-death” genes [50]. However, a current explanation for how HIF-1 α up-regulation itself can differentially mediate cell viability relates to the effects of the hypoxic insult on HIF-1 α post-translational modification (Figure 2). Following translation in the cytoplasm, HIF-1 α can be phosphorylated or dephosphorylated. Dephosphorylated HIF-1 α appears to preferentially bind to p53 [51], a tumor suppressor that initiates the expression of various pro-apoptotic genes [52]. In contrast, phosphorylated HIF-1 α binds to HIF-1 β and forms the HIF-1 complex [51]. Suppression of dephosphorylated HIF-1 α by Geldamycin A, a benzoquinone ansamycin antibiotic that inhibits the induction of dephosphorylated HIF-1 α , suppresses p53 induction and inhibits apoptosis [51]. Importantly, severe, chronic hypoxia promotes dephosphorylation of HIF-1 α , which corresponds to the hypoxic conditions under which HIF-1 α correlates with cell death [53]. Taken together, these findings suggest that chronic, severe hypoxia promotes prolonged up-regulation of dephosphorylated HIF-1 α , which preferentially associates with p53, resulting in p53-dependent apoptosis. Interestingly, hypoxia-initiated p53-dependent cell death is not carried out by traditional p53 targets but instead appears to depend on the apoptotic-inducing protein BCL2/adenovirus E1B 19 kd-interacting protein (Bnip) 3L [54]. Ultimately, research to date suggests that the conflicting findings regarding HIF-1 α -mediated cell fate could be explained, at least partially, by the severity and/or duration of the hypoxic event.

Volatile anesthetics regulate HIF-1 α

Classically, hypoxia is the key regulator of HIF-1 α ; however volatile anesthetics are also able to regulate HIF-1 α , even under normal oxygen conditions. Isoflurane dose and time-dependently up-regulates HIF-1 α protein levels in cancer cell lines [55] and animal preconditioning models [56]. Sevoflurane regulates HIF-1 α transcriptional and translational activity. Rats exposed to sevoflurane in an ischemia model demonstrate up-regulated HIF-1 α mRNA and protein levels in the CNS [57]. Few studies have addressed the mechanisms by which volatile anesthetics regulate HIF-1 α . However, isoflurane administration in hep3b cell lines does not directly inhibit HIF-1 α degradation or affect mRNA levels, suggesting that the mechanism may not involve direct inhibition of HIF-1 α or an increase in gene expression [55]. When cycloheximide, a translation inhibitor, was applied to this model, isoflurane-induced accumulation of HIF-1 α was inhibited [55]. Together, these findings point to a role of protein translation in isoflurane-dependent regulation of HIF-1 α , however they do not exclude the possibility that isoflurane regulates HIF-1 α via translation of a protein involved in degradation inhibition [55]. While this has yet to be further explored, the regulation of protein kinase C activity is a potential candidate. Isoflurane and sevoflurane activate

multiple isoforms of protein kinase C [58], which itself has been identified as an up-regulator of HIF-1 α via inhibition of HIF-1 α degradation [59]. Therefore, isoflurane could be regulating HIF-1 α via indirectly inhibiting HIF-1 α degradation through the translation of protein kinase C. Interestingly, exposure to the intravenous anesthetic propofol results in a reduction of HIF-1 α protein levels, possibly via the suppression of HIF-1 α accumulation [60] or by inhibiting HREs [61]. Together, these findings demonstrate the ability of both volatile and intravenous anesthetics to regulate HIF-1 α .

The regulation of HIF-1 α by volatile anesthetics affects cell survival

Deleterious Effects

Animal studies suggest that isoflurane-induced HIF-1 α up-regulation coincides with neuroapoptosis in a dose-dependent manner [3]. Isoflurane-induced apoptosis is attenuated following HIF-1 α knock-down in cortical and hippocampal cultured neurons [3], strongly suggesting a key role of HIF-1 α in isoflurane-induced neurotoxicity. Moreover, down-regulation of HIF-1 α by propofol appears to be a mechanism by which propofol prevents HIF-1 α -induced apoptosis [61]. This protective effect of propofol-dependent HIF-1 α down-regulation appears to be due to the down-regulation of the mRNA and protein levels of HIF-1 α -regulated pro-apoptotic genes, including Bnip3L. These findings not only demonstrate that both volatile and intravenous anesthetics regulate HIF-1 α , but also suggest that their regulation of HIF-1 α may also be one of the mechanisms by which they affect cell viability.

In cancer models, isoflurane exposure has been linked to worsened cancer prognosis that is mediated by HIF-1 α [62]. Specifically, isoflurane-induced up-regulation of HIF-1 α appears to enhance migration and proliferation of cancer cells, which ultimately increases cancer growth and malignancy. Considering that patients with cancer routinely undergo surgeries involving volatile anesthetics, these findings raise concerns. Moreover, volatile anesthetics are often used on animals in cancer therapeutics research, which raises the possibility that volatile anesthetics in these paradigms could be masking beneficial effects of potential cancer therapeutics (see Xie [63] for a review of this work and its implications). However, the experiment presented does not directly test the effects of isoflurane on human cancer progression and prognosis in the clinical setting or its effects on therapeutics in the research setting. As these questions have yet to be addressed, more research is required in order to determine how isoflurane-mediated up-regulation of HIF-1 α affects cancer patients and research. Ultimately, these findings highlight the urgent need for more research on the role of HIF-1 α in isoflurane-regulated cell survival and death.

Beneficial Effects

While research indicates that volatile anesthetics can have adverse effects on the CNS, isoflurane and sevoflurane can be protective when utilized as preconditioning agents in human cell lines [56] and rat ischemia models [64-65]. Preconditioning usually involves brief episodes of restricted blood and oxygen supply to protect the brain from subsequent hypoxic-ischemic insults [66]. Preconditioning with isoflurane ameliorates neuronal loss from ischemia in rats [65] and, interestingly, dose-dependently prevents neurotoxicity from subsequent prolonged exposure to volatile anesthetics in rat primary cortical neurons and pheochromocytoma neurosecretory cells with

overexpression of Alzheimer's mutated presenilin-1 [67]. Importantly, animal research has suggested a role of HIF-1 α up-regulation in the mechanisms underlying isoflurane and sevoflurane preconditioning protection in rabbits [57] and rats [58], respectively. Taken together with the aforementioned research, it appears that volatile anesthetics, via the up-regulation of HIF-1 α , can be either neuroprotective or neurotoxic, depending on the context in which they are given.

Points to consider when interpreting the literature

Despite the evidence presented, there are several limitations to consider when evaluating the research on long-term adverse effects from exposure to volatile anesthetics. Firstly, in the clinical setting, volatile anesthetics are usually administered in combination. Thus, studies investigating the effects of a particular anesthetic agent may not be directly translatable to the clinical setting. Secondly, clinical data collected from patients contains many uncontrollable variables, including duration of exposure to the anesthetic agent, whether or not the patient has undergone repeated exposures, and the anesthetic(s) used. This suggests that whether or not volatile anesthetics result in adverse events in these patients could be affected by any number or combination of these differences. Thirdly, neonatal rodents are commonly used in studies evaluating the effects of volatile anesthetics on cognitive abnormalities. This is a concern, as neonatal rodents, compared to adults, are more susceptible to neuroapoptosis and cognitive deficits following exposure to volatile anesthetics [22-23], likely because the developing CNS is extremely vulnerable to cellular and molecular interference [68]. While neonatal rodents are intentionally utilised to address the concerns of anesthetic exposure in young humans, this age-related vulnerability to volatile anesthetic-induced CNS injury is a confounding factor that needs to be considered when extrapolating these findings to the general population.

Future Directions

Despite the potential limitations of the current literature, it is clear that volatile anesthetics can have adverse effects on cell viability throughout the CNS that are correlated with behavioural abnormalities in animal models. The risk of anesthetic-induced behavioural abnormalities is also seen in clinical data, suggesting that these effects are not limited to models and do occur in humans. However, it is also clear that volatile anesthetics can initiate protective effects on the CNS when utilized as pre-conditioning agents. This suggests that exposure to volatile anesthetics can be both detrimental and beneficial to cell survival, with the circumstances under which these contrasting effects occur not being well characterized. From the literature presented, it is possible that HIF-1 α regulation is involved in both the adverse and beneficial effects of exposure to volatile anesthetics on cell viability and behaviour seen in animals and patients. However, further research is required to determine if indeed the ability of volatile anesthetics to regulate HIF-1 α underlies volatile anesthetic-induced effects on cell survival and/or behaviour. Determining these processes could also contribute to the understanding of mechanisms of action of other volatile solvents, such as the solvents that are abused by inhalant users or those that industrial workers are exposed to, which are also known to result in adverse effects. Future research efforts are needed to address the proposed mechanisms by which volatile anesthetics mediate HIF-1 α activity. This includes exploring the possibilities that

volatile anesthetics increase HIF-1 α protein levels beyond the speed of degradation, inhibit HIF-1 α degradation directly, or promote HIF-1 α stabilization indirectly. This will not only assist in a better understanding of volatile anesthetic-induced CNS injury, but it will also be an important contribution to the understanding of oxygen-independent regulation of HIF-1 α . A crucial future direction should involve investigating the effects of inhibiting and stimulating HIF-1 α activity while assessing volatile anesthetic-mediated cell viability in order to establish a better understanding of how these events relate to and affect each other. Research efforts utilizing primates will need to investigate whether or not the adverse effects on cell viability seen in primates following exposure to volatile anesthetics correlate with short and/or long-term behavioural abnormalities. Considering the widespread use of volatile anesthetics in both the clinical and research settings, more information on the mediation of the potential beneficial and/or detrimental effects of these agents is required.

Figure Legends

Fig. 1 A basic model (adapted from Carroll & Ashcroft [31]) illustrating the regulation of hypoxia-inducible factor-1 α (HIF-1 α) under normal and hypoxic conditions. During conditions of normal oxygenation, HIF-1 α is actively transcribed and translated but rapidly degraded via the ubiquitin-proteasome pathway interacting with the von Hippel-Lindau (VHL) tumor suppressor protein (pVHL). Proline hydroxylases (PHD) hydroxylate HIF-1 α in the presence of O₂, Fe²⁺, 2-oxoglutarate (2-OG) and ascorbate, allowing HIF-1 α to interact with pVHL. HIF-1 α is subsequently tagged with polyubiquitin (Uq) and recognized for degradation. In contrast, during hypoxia HIF-1 α is no longer degraded and accumulates in the cytoplasm. After translocating to the nucleus from the cytoplasm, HIF-1 α binds with HIF-1 β to form the HIF-1 complex, which subsequently binds to the Hypoxia Response Elements (HREs) on promoters of various genes (including glucose transporter-1 (Glut-1), vascular endothelial growth factor (VEGF) and erythropoietin (EPO)), recruits transcriptional co-activators (p300/CBP) and affects transcriptional activity

Fig. 2 A simplified model (adapted from Suzuki and colleagues [51]) depicting the effects of hypoxic insult and hypoxia-inducible factor-1 α (HIF-1 α) post-translational phosphorylation (p) on HIF-1 α –mediated cell viability. Following chronic and/or severe hypoxia, dephosphorylation of HIF-1 α is promoted. Dephosphorylated HIF-1 α preferentially binds to p53, resulting in p53-dependent apoptosis. Conversely, following acute and/or mild hypoxia, phosphorylation of HIF-1 α is promoted. Phosphorylated HIF-1 α preferentially binds to hypoxia-inducible factor-1 β (HIF-1 β) to form the hypoxia-inducible factor-1 (HIF-1) complex. HIF-1 then interacts with its target genes (including glucose transporter-1 (Glut-1) and vascular endothelial growth factor (VEGF)) to promote cell survival

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Fig. 1

Fig. 2