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Neuroprotections and mechanisms of inhalational anesthetics against brain ischemia

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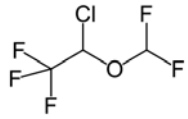
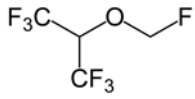
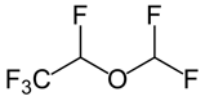
1. ABSTRACT

The new generation of inhalational anesthetics has been widely used for general anesthesia in both clinical and experimental settings because of their safety, reliability and potency. A neuroprotective role has recently been revealed for some of these anesthetics, including the volatile gases isoflurane, sevoflurane, and desflurane, as well as the inert gas xenon. *In vivo* and *in vitro* studies have demonstrated that these gases were able to protect brain against ischemic injury, indicated by the decreases in infarct volumes and neuronal apoptosis. In this review, we

Table 1. Structures and properties of inhalational anesthetics used as neuroprotective agents in cerebral ischemia

Agent	Molecular formula	Chemical structure	Blood/gas partition coefficient	Minimum alveolar concentration
Isoflurane			1.4	1.15 vol %
Sevoflurane			0.68	2.05 vol %
Desflurane	$C_3H_2F_6O$		0.42	6 vol %
Xenon	Xe	$_{54}Xe$	0.115	63%

will briefly introduce the properties of these gases, and discuss in detail their effects on brain ischemia, effective treatment regimens, and neuroprotective mechanisms. Perspectives are also discussed on future study and use of inhalational anesthetics.

2. INTRODUCTION

Stroke is the second most common cause of death and a major cause of disability worldwide. Stroke mortality, by estimation, will increase greatly in the next ten years due to the aging population (1). The two main pathological forms of cerebral ischemia are ischemic stroke and hemorrhagic stroke. Ischemic stroke accounts for approximately 80% of all stroke lesions (2). It is well known that some neurosurgical, vascular, and cardiovascular procedures present a high risk for transient focal cerebral ischemia (3, 4). Carotid endarterectomy (CEA), for example, has a reported perioperative stroke incidence from 0.25% to 7% (5, 6). According to the North American Symptomatic Carotid Endarterectomy Trial, perioperative strokes occurred intraoperatively or postoperatively, and approximately 56% of postoperative events occurred within 24 hours after CEA (7). The substantial size of the at-risk population has fostered great interest in seeking neuroprotective agents to increase the brain's tolerance to ischemia.

For decades anesthetics have been considered as natural candidates to promote brain protection under ischemic circumstances, since clinical observations have indicated that patients under general anesthesia are more tolerant of ischemia than unanesthetized patients (8). The majority of studies has validated the neuroprotective effects of inhalational anesthetics on ischemic brain, although both neutral and detrimental findings have also been observed in experimental studies and clinical settings. However, data from hemorrhagic strokes, until now, have not been available.

Currently, isoflurane (introduced into clinical practice in 1981), desflurane (introduced in 1992), and sevoflurane (introduced in 1995) constitute the primary inhaled anesthetic gases used either alone or in combination with other inhaled gas (nitrous oxide) or intravenous anesthetics/analgesics or muscle relaxants in clinical settings (9) (Table 1). Isoflurane is a clear and colorless liquid with a slightly pungent smell. Isoflurane causes minimal cardiovascular depression at concentrations below 2 minimum alveolar concentration (MAC), but decreases systemic vascular resistance more than the other agents. Desflurane differs from isoflurane by just one atom, which decreases its solubility and potency. The similar blood/gas partition coefficients of desflurane and sevoflurane provide rapid induction and recovery properties. A rapid increase in the inspired concentration of desflurane causes an increase in heart rate and blood pressure. Isoflurane and desflurane have a dose-related depressive effect on cerebral autoregulation. Sevoflurane is particularly useful for induction in children due to its low solubility, lack of airway irritability and moderate potency. Sevoflurane causes myocardial depression and a reduction in vascular resistance similar to isoflurane, but has minimal effects on heart rate. Sevoflurane has been observed to increase cerebral blood flow while dose-independently preserving cerebral autoregulation (10-13).

The noble gas xenon has been proposed as an alternative to classic anesthetics partly because of its favorable pharmacokinetic, analgesic, cardiovascular and safety properties (14). Xenon provides very rapid induction and recovery characteristics due to its lower blood/gas partition coefficient of 0.115, compared with that of isoflurane, sevoflurane and desflurane (15, 16). In recent years, the hemodynamic stability and organ protective properties of xenon, frequently observed at subanesthetic concentrations, has encouraged a wider use of this gas as a

routine anesthetic agent in Europe despite its high cost (17-25). Other gaseous agents are not considered in this review because they are being phased out at many centers due to their undesirable side effects (9).

Accumulating evidence has shown that inhalational anesthetic-induced immobility is mediated primarily by the spinal cord (26), whereas hypnosis and amnesia are mediated within the brain (27). To date, the mechanism of how inhaled anesthetics reversibly alter central nervous system function has not been completely defined. In early research, the predominant theories were the unitary hypothesis and the Meyer–Overton rule, both of which suggested that volatile anesthetics acted nonspecifically on hydrophobic lipid components of cells (27). However, subsequent studies have indicated that the effects of inhaled anesthetics depend on multiple features of their molecular structure, including hydrophobicity, electrostatics, and molecular size of anesthetics (28, 29). Many studies have been focused on ion channels which are sensitive to inhaled anesthetics at relevant clinical concentrations. Inhaled anesthetics are thought to possibly enhance inhibitory postsynaptic channel activity (*e.g.* gamma-aminobutyric acid type A receptors and glycine receptors) and inhibit excitatory synaptic activity (*e.g.* neuronal nicotinic acetylcholine receptors, serotonin type 3 receptors, and glutamate receptors). Weak inhibition of voltage- and non-voltage-active potassium, sodium and calcium channels have also been observed during inhalational anesthetic administration (26, 27). In addition, recent studies have demonstrated that activation of two-pore-domain potassium channels TREK-1 may play a critical role in inhaled anesthesia (30, 31, 32). However, none of these receptor types is central to inhalational anesthesia and the true target in the central nervous system has yet to be discovered.

The present discussion will focus on impacts of these inhalational anesthetics (isoflurane, sevoflurane, desflurane and xenon) on ischemic strokes, their underlying mechanisms, and future research perspectives.

3. EFFECTS ON ISCHEMIC BRAIN

3.1. Experimental studies

3.1.1. Impact during brain ischemia

Cerebral ischemia-reperfusion injury is involved in various surgical procedures including carotid endarterectomy, intracranial aneurysm exclusion and aortic repair under deep hypothermic circulatory arrest. The development and refinement of numerous experimental (mostly rodent) models of cerebral ischemic injury allow researchers to conduct perioperative neuroprotection studies (33, 34). This section will present the neuroprotective effects of inhaled anesthetics administered during brain ischemia *in vivo* and *in vitro*. Some neutral and detrimental findings will also be addressed. Animal gender, ischemic model, ischemic severity and inhaled anesthetic dose may play critical roles in brain histological and functional outcomes post-insult.

3.1.1.1. Neuroprotective effects during brain ischemia

3.1.1.1.1. Isoflurane

A large body of studies has shown that isoflurane either delays or reduces brain injury up to 7 days post ischemia. These studies compare histological changes and neurological deficits with "awake" ischemic controls or with other inhalational or injectable anesthetics (35). Recently, Sakai and his colleagues pointed out that isoflurane repeatedly improved long-term neurologic and histologic outcomes from focal ischemia up to 8 weeks after ischemic insult (36). In *in vitro* models, the studies observed a dose-dependent therapeutic effect of isoflurane during ischemia or other forms of neuronal injury, which include hypoxia, oxygen–glucose deprivation (OGD), a-amino-3-hydroxyl-5-methyl-4-isoxazol propionic acid (AMPA) induced- and N-methyl-D-aspartate (NMDA)-induced excitotoxicity. For example, isoflurane administered concurrently with OGD reduced neuronal cell death and degeneration as measured from 5 hours to 14 days post-insult in rat cerebellar and hippocampal slices (37-41). In rat primary mixed neuronal-glial cultures, isoflurane exposure increased the tolerance to NMDA-induced excitotoxocity by reducing the release of the intracellular enzyme lactate dehydrogenase (42).

3.1.1.1.2. Sevoflurane

Although sevoflurane has been less well studied than isoflurane, the available data suggest that sevoflurane may provide short-term (72 to 96 hours) and long-term (28 days) neuroprotective effects in the setting of focal or global cerebral ischemia (43-45). Sevoflurane has also been observed to prevent a deficit in cognitive function induced by incomplete global ischemia up to 10 days post-insult compared with fentanyl/N_2O (46). The protection has been investigated in rat corticostriatal brain slices and mixed cerebrocortical neuronal-glial cell cultures concurrently with OGD and sevoflurane exposure (47, 48).

3.1.1.1.3 Desflurane

Desflurane has been observed to be neuroprotective during deep hypothermic circulatory arrest (49) and low-flow cardiopulmonary bypass (50) in newborn pigs. Post-injury outcomes were also improved in desflurane-anesthetized rats concurrently with focal ischemic (51, 52) and incomplete global cerebral ischemic (53) models. In cortex neuronal cell cultures subjected to OGD, desflurane was observed to significantly attenuate neuronal cell death regardless of concentration (54).

3.1.1.1.4. Xenon

The noble gas xenon, with an atomic number of 54, is currently the subject of research investigating its profound impact on neurological outcomes during brain ischemic insult. As a noncompetitive antagonism of NMDA subtype of glutamate receptors, xenon affords anesthetic effects (17) as well as neuroprotective effects during ischemic injury (18). Xenon exerts a dose-dependent neuroprotective effect on rat neuronal-glial cocultures with NMDA-induced toxicity, glutamate-induced toxicity, hypoxic injury and oxygen-glucose deprivation, as reflected by reducing lactate dehydrogenase release (18-20). The protective effects of xenon have also been

identified in rodents subjected to focal ischemia and neonatal hypoxic-ischemic injury (21, 22).

3.1.1.2. No effect and detrimental effects during brain ischemia

Although isoflurane administered during brain ischemia has been reported to possess sustained protection against ischemic injury, some studies have indicated that isoflurane exposure provides either little or no protection, and may even worsen outcomes evaluated from 2 hours to 3 months post injury (55-59). For example, data from Kawaguchi and his team demonstrated that isoflurane reduced cerebral infarction 2 days after focal ischemia in comparison with the awake state. However, after a 7-day recovery period, this neuroprotective efficacy was no longer apparent as assessed by infarct size, TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling) staining, and active caspase-3 and caspase-9 staining (56, 57). These data suggest that isoflurane reduced the development of apoptosis early after ischemia but did not prevent it at later stages of post-ischemic recovery. Decreased neuroprotection by isoflurane was observed in OGD brain slices from aging rats, which might involve the decrease of the level of phosphorylated Akt and p42/44 (57). Larger concentrations of isoflurane (more than 1.5 MAC) were also observed to be either numerically or statistically associated with a greater magnitude of ischemic injury *in vivo* (58) and *in vitro* (59). Xenon has been reported to exacerbate ischemic brain damage and aggravate neurological dysfunction in a rat model combining cardiopulmonary bypass and cerebral air emboli (60). No research has been reported on detrimental effects of sevoflurane and desflurane in brain ischemic injury.

3.1.2. Preconditioning effects

The concept of ischemic preconditioning was introduced about 23 years ago (61, 62). Prior exposure of organs, such as brain and heart, to brief periods of sub-lethal ischemia can initiate endogenous ischemic tolerance via a preconditioning phenomenon. There are two distinct patterns of ischemic tolerance: 1) acute preconditioning, which is mediated by post-translational protein modifications and is short-lived; and 2) delayed preconditioning, which is protein synthesis-dependent and sustained for days to weeks (63, 64). To date, a number of preconditioning stimuli have been reported to improve brain ischemic tolerance, including sublethal hypoxia, brief periods of global ischemia, cortical spreading depression, hypothermia, hyperthermia, long-term hyperbaric oxygen and inflammation (65-71). However, these triggers of ischemic tolerance, if more prolonged or more severe, can cause cellular damage (72, 73). In addition, many preconditioning methods are difficult to implement in the clinic due to the dangers or the complex biological effects of the stimuli. Inhalational anesthetics are relatively safer and have been reported to precondition the brain against ischemic injuries by both early administration (when applied minutes before an injury) and late administration (a day or more before) in vivo (74-76) and in vitro (77, 78). This section will address the effects of four inhalational anesthetics on ischemic brain in experimental studies (Table 2).

3.1.2.1. Neuroprotective effects of anesthetic preconditioning

3.1.2.1.1. Isoflurane

Isoflurane has been one of the most extensively studied volatile anesthetic brain preconditioning agents. Isoflurane preconditioning before permanent or transient focal cerebral ischemic insult significantly reduced brain infarct size in adult male rodents (74, 76, 79-81). In a cardiac arrest-induced global ischemic model, isoflurane pretreatment improved neurological deficit scores 20 hours post ischemia in female dogs (82). Isoflurane preconditioning in a rodent neonatal hypoxia-ischemia model also reduced preweaning male and female brain cell loss and damage, and partially improved adult perirhinal cortex and striatal dependent functions (75, 83, 84).

Neuroprotective effects of isoflurane pretreatment have also been observed in *in vitro* models. Exposure of primary cortical neuronal cultures to isoflurane administered up to 24 hours before OGD with or without continued exposure during OGD resulted in a concentration-dependent reduction of neuronal cell apoptosis, death and degeneration (74, 85-88). Beneficial effects of isoflurane preconditioning have also been observed in rat cerebellar and hippocampal slice models of ischemia (76, 78, 89).

3.1.2.1.2. Sevoflurane

Studies on sevoflurane preconditioning are much fewer than those on isoflurane pretreatment. In a male rat global ischemic model, sevoflurane exposure 15 minutes or 24 hours before ischemic insult significantly diminished neuronal damage (90, 91). In a transient focal cerebral ischemic model (60 minutes), sevoflurane preconditioning was observed to induce effective but transient neuroprotective effects, which improved functional outcomes and reduced infarct volume up to 3 days after injury, and decreased ischemia-induced apoptosis up to 7 days post ischemia (92). A study on a neonatal rodent hypoxic and ischemic model showed that late pretreatment with sevoflurane partially protected perirhinal cortex and striatal-dependent functions as assessed by a battery of behavioral tests without any significant histological change against moderate to severe neonatal hypoxia-ischemia (84). The neuroprotection of sevoflurane preconditioning has also been identified in rat hippocampal slices subjected to OGD by a marked reduction in the increase in propidium iodide (PI) fluorescence and cleaved caspase-3 expression (93) and by a dose-dependent increase in recovery of neuronal function (77).

3.1.2.1.3. Desflurane

At this time, only a few studies have been performed evaluating the preconditioning effects of desflurane on ischemic brain injury. McAuliffe indicated that desflurane pretreatment 24 hours before ischemic insult partially protected perirhinal cortex and striatal-dependent functions against moderate to severe neonatal hypoxia-ischemia up to 3 months post injury (84).

3.1.2.1.4. Xenon

Recently, xenon has been investigated as a brain preconditioning agent. Ma *et al.* observed that xenon-

Table 2. Preconditioning effects of inhaled anesthetics on experimental stroke models *in vivo* and *in vitro*

Animal species and/or cell types	Inhaled anesthetics and concentrations	Precondition duration	Intervals between preconditioning and ischemia	Ischemia models	Effects on ischemic brain	Reference (number)
Neuronal-glial coculture Hippocampal slices Rat (7-day-old)	25%-75% xenon 70% xenon	2 hours 2 hours	24 hours 2, 4, 8, 24 hours	OGD[1] (75 minutes) Hypoxia/ischemia (90 minutes)	Xenon PC[2] reduced LDH[3] release and propidium iodide staining *in vitro*, reduced infarction size and improved neurologic outcomes *in vivo*. CREB[4], BDNF[5] and Bcl-2 were upregulated by anesthetic PC.	Ma D, 2006 (23)
Wistar rats (adult male) Primary cortical neuronal cultures	1.4% isoflurane 1.2% halothane	3 hours	0, 12, 24, 48 hours 24 hours	pMCAO[6] OGD (120 minutes)	Anesthetic PC within 24 hours significantly reduced infarct volumes 4 days after pMCAO and decreased the OGD-induced release of LDH 24 hours post insult. The protection was able to be eliminated by an iNOS[7] inhibitor.	Kapinya KJ, 2002 (74)
Sprague-Dawley rats (6- or 7-day-old)	1% or 1.5% isoflurane	30 minutes	24 hours	Hypoxia/ischemia (1, 2, 2.5 hours)	Isoflurane PC improved the survival of neonates with brain hypoxia/ischemia for 1 hour and the weight ratio of left/right cerebral hemispheres. Isoflurane induced a time-dependent increase in iNOS proteins.	Zhao P, 2004 (75)
Sprague-Dawley rats (adult male)	2% isoflurane	30 minutes	24 hours	pMCAO	Isoflurane PC reduced brain infarct sizes and improved neurological deficit scores assessed 6, 24, and 72 hours after pMCAO. Isoflurane induced a rapid and prolonged increase in phosphorylated p38 MAPK[8] in cerebral neocortex.	Zheng S, 2004 (76)
Rat hippocampal slices	0, 1, 2 or 3 MAC[9] sevoflurane	30 minutes	15 minutes	Hypoxia (13 minutes)	Sevoflurane PC improved the recovery of evoked CA1 population spikes at the end of 30-minute reoxygenation. The neuroprotection was blocked by a mitochondrial K_{ATP} channel inhibitor.	Kehl F, 2004 (77)
Rat cerebellar slices	1-4% isoflurane	15 minutes	15 minutes	OGD (20 minutes)	Isoflurane PC decreased Purkinje cell injury and death by hematoxylin and eosin staining. The protection was blocked by a glutamate transporter inhibitor.	Zheng S, 2003 (78)
C57BL/6 mice (male)	1% isoflurane	3 hours	0, 12, 24 hours	tMCAO[10] (1 hour)	Mean infarct volumes were significantly smaller in mice with isoflurane PC. Mild hypoxia during or desferrioxamine administered at the onset of isoflurane pretreatment completely abrogated the development of delayed tolerance (12 hours) against focal cerebral ischemia.	Kapinya KJ, 2002 (79)
Sprague-Dawley rats (adult male)	1.5% isoflurane	1 hour	1 hour	tMCAO (2 hours)	Isoflurane PC reduced brain infarction and improved neurological outcomes. The protection was attenuated by an adenosine A1 receptor antagonist.	Liu Y, 2006 (80)
Sprague-Dawley rats (adult male)	0.75%, 1.5%, 2% or 2.25% isoflurane	1 hour/day ×5 days	24 hours	tMCAO (2 hours)	Repeated isoflurane PC reduced brain infarction and improved neurological outcomes in a dose-response manner. An ATP-regulated potassium channel blocker abolished the isoflurane PC-induced tolerance.	Xiong L, 2003 (81)
Beagle-like dogs (female)	1.5% isoflurane	30 minutes	0 hour	Cardiac arrest (8 minutes)	Isoflurane PC reduced neurological deficit scores 20 hours post insult.	Blanck T, 2000 (82)
C57 mice (9-day-old)	1.8% isoflurane	2 hours	24 hours	Hypoxia/ischemia (65 minutes)	Isoflurane PC reduced preweaning mortality and improved striatal function in adult mice with equal injury in hippocampus compared with non-PC.	McAuliffe JJ, 2007 (83)
C57 mice (9-day-old)	8.4% desflurane 1.8% isoflurane 3.1% sevoflurane *vs.* room air	3 hours	24 hours	Hypoxia/ischemia (1 hour)	Anesthetic PC partially improved perirhinal cortex and striatal-dependent functions, without significant histologic changes.	McAuliffe JJ, 2009 (84)
Primary rat cortical neurons	1.4% isoflurane	3 hours	24 hours	OGD (2 hours)	Isoflurane PC decreased LDH release, maintained neuronal viability and increased expression of EAAC1 (neuronal glutamate transporter) mRNA and protein compared with control.	Kaneko T, 2005 (85)

Primary rat cortical neurons	1.13%, 2.3% or 3.3% isoflurane	30 minutes plus during OGD	0 hour	OGD (30, 60, 90 minutes)	Isoflurane PC attenuated OGD-induced neuronal apoptosis by TUNEL[11] assessment in a concentration-dependent manner.	Wise-Faberowski L, 2001 (86)
Primary mouse neuronal-glial cultures	1.5% isoflurane	3 hours	24-96 hours	OGD (3 hours)	Isoflurane PC reduced OGD-mediated toxicity by LDH release assessment at 72 hours after PC, and upregulated MT-1/2[12] messenger RNA. MT-1/2 protein transfection significantly decreased OGD-mediated toxicity. Knockdown and knockout of MT-1/2 diminished and abolished isoflurane-mediated protection, respectively.	Edmands SD, 2009 (87)
Primary hippocampal neuron culture	1.4% isoflurane	3 hours	24 hours	OGD (2 hours)	Isoflurane PC increased the number of surviving neurons and their viability by assessing LDH release, and increased HO-1[13] expression in OGD-induced injury. The neuroprotection was able to be partially abolished by tin protoporphyrin (inhibition of HO activity).	Li Q, 2008 (88)
Rat hippocampal slices	0.5% or 1.5% isoflurane	2 hours	24 hours	OGD (1 hour)	Isoflurane PC decreased neuron death in CA1 and CA3 regions, increased basal intracellular Ca^{2+} concentration and transient phosphorylation of MAPK p42/44 and Akt. The MAPK inhibitor and calmidazolium eliminated the PC protection.	Bickler PE, 2005 (89)
Rat hippocampal slices	2.4% sevoflurane	30 minutes/day×1 day 30 minutes/day×4 days	15 minutes 24 hours	Cardiac arrest (7 minutes)	Both early and late PC reduced ischemic neuronal damage and improved the recovery of evoked CA1 population spikes.	Payne RS, 2005 (90)
Rat cerebellar slices	Various concentrations of isoflurane, halothane, sevoflurane and desflurane	15 minutes	15 minutes	OGD (10 minutes)	All four anesthetics PC improved cell survival by TTC[14] staining. The EC50 for PC effects was linearly correlated with the aqueous concentration of one MAC.	Wang C, 2007 (91)
Sprague-Dawley rats (adult)	2.7% sevoflurane	45 minutes	1 hour	tMCAO (1 hour)	Sevoflurane PC significantly improved functional outcome and reduced infarct volume 3 days after tMCAO, with no differences 7 days, 14 days post insult. Apoptotic cells were significantly reduced 3 days, 7 days post insult; no difference in 14 days post ischemia.	Codaccioni JL, 2009 (92)
Rat hippocampal slices	Sevoflurane 10^{-4} M	1 hour	3 hours	OGD (10, 20, 30, 45, 50, 60 minutes)	Sevoflurane PC increased focal adhesion kinase (FAK) expression and markedly reduced the increase in propidium iodide fluorescence and cleaved caspase-3 expression 1 hour after 10, 20, and 30 minutes OGD. The neuroprotection was decreased by PP2 (an inhibitor of src tyrosine kinases). No protection was observed for periods of ischemia more than 30 minutes.	Sigaut S, 2009 (93)
C57 mice (young and middle-aged, male and female)	1% isoflurane	4 hours	24 hours	tMCAO (2 hours)	Isoflurane PC decreased ischemic damage in male mice but increased infarction in young female mice. The protection was lost in Akt1-deficient male mice.	Kitano H, 2007 (94)

[1]oxygen-glucose deprivation; [2]preconditioning; [3]lactate dehydrogenase; [4]cAMP-response element binding protein; [5]brain-derived neurotrophic factor; [6]permanent middle cerebral artery occlusion; [7]inducible nitric oxide synthase; [8]mitogen-activated protein kinase; [9]minimum alveolar concentration; [10]transient middle cerebral artery occlusion; [11]terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; [12]metallothioneins-1/2; [13]heme oxygenase-1; [14]2,3,5-triphenyltetrazolium chloride.

induced pretreatment in neonatal hypoxic-ischemic rats decreased infarction size and improved neurological function (23). However, high cost and limited availability restrict clinical use of xenon.

3.1.2.2. No effect and detrimental effects of anesthetic preconditioning

Sevoflurane preconditioning in rat acute hippocampal slices was observed to provide no protection for periods of OGD longer than 30 minutes (93). In a transient focal cerebral ischemic model (2 hours), isoflurane preconditioning markedly increased infarction in young female mice and had no effect in middle-aged female mice, and significantly decreased neuronal damage in young and middle-aged male mice (94).

3.1.3. Post-treatment effects

Interventions with volatile agents applied after the onset of brain ischemia will have broad applications, since the occurrence of brain ischemia in patients with stroke and brain trauma is not predictable. However, studies on volatile anesthetic post-treatment, to date, have been published less than those on preconditioning and peri-treatment with volatile agents. This section will review the protective, neutral or detrimental effects of inhaled agent post-treatment on ischemic brain.

3.1.3.1. Neuroprotective effects of anesthetic post-treatment

Isoflurane treatment started at the onset of reperfusion was observed to reduce 2, 3, 5-triphenyltetrazolium chloride (TTC) conversion and improved neurologic outcome in focal cerebral ischemic rats (95). Sevoflurane and desflurane were also indicated to have cerebral protective effects when given after incomplete cerebral ischemia in rats (96, 222). In a rat neonatal hypoxic-ischemic model, three hours of xenon post-administration was demonstrated to provide significant global protection, including cortex/white matter, hippocampus, basal ganglia and thalamus, up to 7 days post insult (22). Cooling combined with immediate or delayed xenon inhalation was reported to provide equivalent gender-independent long-term neuroprotection after neonatal hypoxia-ischemia (24, 25). The dose-dependent neuroprotective effects of volatile anesthetic post-treatment were also reported in corticostriatal slices subjected to OGD (95). To date, there is little evidence that post-ischemic therapy with volatile agents long after ischemia would be beneficial.

3.1.3.2. No effect and detrimental effects of anesthetic post-treatment

In a porcine cardiac arrest and cardiopulmonary resuscitation (CPR) model, early administration of xenon and isoflurane was observed to have no significant effects on reducing neurological dysfunction and histopathological alterations induced by transient global brain ischemia (97). These results contrast with the findings of Fries' previous study (98). Pure oxygen administrated may contribute to worsening functional and histopathological outcomes after cardiac arrest and CPR.

3.2. Clinical studies

Clinically, there is much interest in determining the feasibility of pharmacologically protecting the human brain with anesthetics and the potential impact on perioperative stroke, since anesthetic cardioprotection has already been observed during human cardiac surgery. However, few clinical studies have been undertaken to assess the neuroprotective effect of inhaled anesthetics on populations undergoing CEA and cardiac bypass surgeries with potential perioperative stroke risks. In patients subjected to temporary middle cerebral artery occlusion (MCAO) longer than 15 minutes as part of an extracerebral-artery-to-intracerebral-artery bypass procedure, desflurane has been observed to increase brain tissue oxygen pressure and reduce acidosis compared with etomidate, which suggests that desflurane improves tissue metabolic status in ischemic brain regions, likely *via* enhancement of tissue perfusion (99). Several researches have determined the effects of inhalational anesthetics on critical regional cerebral blood flow (rCBF), defined as that flow below which the majority of patients developed ipsilateral EEG changeds of ischemia within 3 min of carotid ccclusion via a single extracranial collimated scintillation detector over the posterior parietal boss (MCA distribution). The critical rCBF during isoflurane anesthesia was observed to be much lower than critical rCBF during halothane or enflurane anesthesia in CEA patients, which is consistent with electroencephalogram ischemic changes (100, 101). Conversely, isoflurane was reported to induce more infarction compared with a mixed intravenous anesthetic in MCAO (102). In coronary artery bypass grafting patients, serum S100-beta protein levels, an early marker for neuronal degeneration and blood-brain-barrier disruption, were reported not to be significantly increased with isoflurane anesthesia as compared with propofol anesthesia (103).

In aggregate, the clinical evidence is less convincing, although experimental data support the neuroprotective effects of volatile anesthetics. The clinical results may be explained by the view that a single pharmacological approach can hardly affect the multiple simultaneous pathological events in patients with the parallel variability of coexisting disease (104). Furthermore, most studies have utilized indirect markers, such as critical rCBF and S100-beta protein, for clinical assessment of volatile anesthetic neuroprotection. These markers may not be as sensitive, predictive, or reproducible as histopathology and cognitive function tests. Therefore, further studies with appropriate and sensitive assessments are needed to investigate volatile anesthetic effects on perioperative stroke and neurological outcomes post insult.

4. MECHANISMS OF IMPACT ON ISCHEMIC BRAIN

4.1. Common mechanisms for pre- and peri-treatment

Much work has been performed evaluating the molecular and cellular mechanisms of inhalational anesthetics in ischemic brain injury. Several mechanisms have been implicated in both preconditioning and peri-treatment of volatile agents on brain ischemic injury, including electrophysiological and metabolic modulation, reduction of glutamate-mediated excitotoxicity, moderate increases in intracellular calcium, antioxidant mechanisms and anti-apoptosis mechanisms. This section will focus on the common mechanisms for pre- and peri-treatment with volatile anesthetics.

4.1.1. Electrophysiological and metabolic modulation

A series of electrophysiological changes induced by hypoxia and ischemia have been demonstrated in cerebral tissues. These are characterized by an initial hyperpolarization, followed by a slow depolarization and then a rapid depolarization in extreme vulnerable hippocampal CA1 pyramidal neurons. At the time of depolarization, the ionic changes, a rapid influx of Na^+ and

Ca^{2+} and an efflux of K^+, lead to cell swelling and secondary damaging biochemical events (105-107). The irreversible damage will occur when the membrane completely depolarizes, even if sufficient reperfusion is supplied (108).

Early data from both clinical settings and experimental studies has shown that inhalational anesthetics not only decrease cerebral metabolism (109-112) but also maintain the cerebral energy state during ischemia (109). Subsequent studies have demonstrated that the cerebral metabolic changes produced by inhaled agents are secondary to an effect on cortical electrical activity (109). In rat hippocampal slices, administration of isoflurane, desflurane and sevoflurane before and during hypoxia was observed to enhance the initial hyperpolarization, delay the hypoxic rapid depolarization, reduce peak hypoxic cytosolic calcium concentration and improve recovery of the resting and action potentials and postsynaptic evoked population spikes after hypoxia (113, 114). The anesthetic agents also significantly attenuated the fall in ATP and K^+ concentrations and the increase in Na^+ induced by hypoxia in CA1 pyramidal neurons (115). The protective effect and electrophysiological changes by pre- and peri-treatment with inhaled agents might be mediated by protein kinase C / protein kinase M (PKC/PKM) and by opening K_{ATP} channels, since either chelerythrine, a PKC/PKM inhibitor, or glybenclamide, a K_{ATP} channel blocker, has been observed to prevent anesthetic-mediated electrophysiological changes (116, 117).

4.1.2. Reduciton of glutamate excitotoxicity

4.1.2.1. Glutamate release and uptake

Glutamate is the major excitatory neurotransmitter in the central nervous system. Glutamate transporters, named excitatory amino acid transporters (EAATs), play a critical role in maintaining the extracellular homeostasis of glutamate (118). Brain ischemia can cause reversed transport of glutamate *via* EAATs from intracellular to extracellular space according to the glutamate concentration gradient (119). Ischemia can also activate glutamate-permeable volume-activated anion channels, which are proposed as a source of ischemia-evoked glutamate efflux (120). Increased extracellular glutamate concentrations can cause excitotoxicity (121), which plays a major role in the initiation and evolution of ischemic brain injury (122).

Quantitative *in vivo* studies have been undertaken to investigate the peri- or pretreatment effects of volatile anesthetics on ischemia-induced glutamate release and uptake compared with halothane, fentanyl-N_2O, or "awake" ischemic treatment (123-127). For example, isoflurane exposure with 0.5 MAC or an electroencephalographic burst-suppression dose (approximately 2 MAC) during rat forebrain ischemia significantly reduced hippocampal glutamate accumulation 70 minutes post ischemia by *in vitro* microdialysis and high-performance liquid chromatography (HPLC) (126). However, isoflurane peri-treatment, with which a burst-suppressed electroencephalogram pattern was achieved, did not decrease hippocampal glutamate accumulation during repeated global cerebral ischemia estimated by the same methodology (124). The reasons for these conflicting *in vivo* findings are not known. The location of the microdialysis probe (in the ischemic core vs. in the penumbra) and brain ischemic model (global ischemia *vs*. incomplete global ischemia), may contribute to the different findings. In focal cerebral ischemia, intra-ischemic isoflurane was observed to prevent ischemia-induced efflux of glutamate without reduction of infarct volume 24 hours post ischemia compared with pentobarbital, suggesting that inhibition of glutamate release may not be enough to improve brain outcomes after cerebral ischemia (127).

Several *in vitro* studies have supported the reduction of ischemia-induced glutamate release by inhalational agents pre- and peri-exposure under hypoxic, chemically anoxic, and ischemic (OGD) conditions (19, 47, 48, 128, 129). A few studies have evaluated how volatile anesthetic preconditioning may modulate glutamate efflux during ischemia. For example, peri-treatment with sevoflurane was observed to reduce OGD-induced decreases in extracellular glutamate uptake, probably depending on the glial transporter GLT1, in rat mixed cerebrocortical neuronal–glial cell cultures (48). The neuroprotection of isoflurane preconditioning was observed to reduce Purkinje neuronal injury from glutamate excitotoxicity in rat cerebellar slices, which was abolished by a specific glutamate transporter inhibitor during OGD (76, 78). However, Jung *et al.* observed that isoflurane, sevoflurane and desflurane intra-ischemic exposure at clinically relevant concentrations did not affect OGD-induced extracellular glutamate accumulation estimated by HPLC from adult rat brain slices. These results suggest that volatile agents do not affect reversed transport of glutamate *via* GLT1 and swelling-induced efflux of glutamate *via* volume-activated anion channels under ischemic condition, independent of the severity of the OGD and the concentrations of the volatile agents (130). More research is needed to validate the role of inhibition of ischemic glutamate release in inhalational anesthetic neuroprotection and preconditioning.

4.1.2.2 NMDA and AMPA receptor antagonism

Extracellular glutamate accumulation induced by brain ischemia stimulates postsynaptic glutamate receptors. The activation of these glutamate receptors results in excessive calcium accumulation in neurons, ultimately leading to their death (131). Inhalational anesthetics have been observed to attenuate ischemia-induced excitotoxicity through antagonism of glutamate receptors, such as AMPA and NMDA receptors, in many experimental animal settings (18, 40, 132-135).

Several *in vivo* studies support the neuroprotective mechanism for volatile anesthetics against excitotoxic injury. For example, isoflurane and xenon exposure reduced rat cortical excitotoxic injury mediated by NMDA in a dose-dependent manner (18, 136). Isoflurane anesthesia in rats decreased AMPA-induced cortical injury *in vivo* (137).

Most of the work on inhalational anesthetics and NMDA/AMPA receptors has been performed in brain slices or cultures. Isoflurane intra-ischemic treatment and preconditioning has been shown to significantly reduce neuronal cell death caused by NMDA overstimulation in rat hippocampal and cerebellar slices (40, 133, 135). This protective effect was also validated by Bickler *et al.*, who found that isoflurane exposure during stimulated ischemia temperature-independently reduced calcium fluxes and slowed the rate of calcium influx mediated by both L-glutamate and NMDA in rat cortical slices (134). No evidence indicated that neuron cells protected by isoflurane had become apoptotic or apoptotic-like, suggesting that isoflurane protection against acute excitotoxic necrosis does not allow residual apoptotic cell death to proceed (138). Xenon, an NMDA receptor antagonist (17, 139), also exerted concentration-dependent neuroprotection against NMDA-induced injury at subanesthetic doses *in vitro* (18). In rodent *in vitro* models, isoflurane was also observed to reduce AMPA-induced excitotoxic injury and AMPA receptor-linked cyclic guanosine monophosphate production (38, 135, 140, 141). Since the use of different experimental protocols, receptor subtypes, and/or tissue sources confounds quantitative comparisons of the NMDA receptor inhibitory potencies of inhaled anesthetics, Solt *et al.* defined the extent to which different clinical inhaled anesthetics inhibit the NR1/NR2B subtype of the human NMDA receptor expressed in *xenopus laevis* oocytes. The inhalational agents tested (xenon, cyclopropane, enflurane, isoflurane, desflurane, halothane, and sevoflurane) inhibited NMDA receptors at equianesthetic concentrations (1 MAC) but to different extents, with xenon having the greatest effect and sevoflurane the least (142).

However, isoflurane has been observed to have a dramatically different effect on NMDA-mediated Ca^{2+} influx in old compared with young rat hippocampal slices. A protein kinase C-mediated increase in NMDAR activity may result in increased excitotoxicity and decreased neuroprotection by isoflurane in the aging brain (57).

In summary, these data suggest that the aging-specific protective mechanism of volatile agents against brain ischemic injury appears to involve attenuation of glutamate excitotoxicity *via* inhibition of postsynaptic excitatory amino-acid pathways.

4.1.3. Intracellular calcium and calcium-dependent processes

Good evidence exists that calcium (Ca^{2+}) is an important intracellular signal for cell survival and death. In hypoxic and ischemic conditions, activation of postsynaptic glutamate receptors, like NMDA receptor, results in excessive calcium accumulation in cytoplasm, mitochondria, or the endoplasmic reticulum, eventually leading to neuronal cell death (122, 131, 143). Volatile anesthetic treatment before, during or after brain ischemic injury was validated to reduce ischemic-induced cell death *via* reduction of NMDA-receptor-mediated calcium influx *in vivo* and *in vitro* (134, 144, 145). The protective effect afforded by inhalational agents prevents calcium overloading in ischemic/hypoxic neurons, enabling intracellular calcium concentration ($[Ca^{2+}]i$) to remain in a survivable range.

Moderate increases in $[Ca^{2+}]i$ have been shown to trigger important calcium-dependent survival signals by mechanisms involving phosphorylation of the anti-apoptotic factor Akt (146), the mitogen-activated protein kinase (MAP) kinase extracellular signal-regulated kinases (ERK) (p42/44) (147), the transcription of brain-derived neurotrophic factor (148) and changes in Ca^{2+}-calmodulin-dependent gene expression (149). Isoflurane was observed to induce small to moderate increases in $[Ca^{2+}]i$ in cortical brain slices, hippocampal brain slices, and isolated cortical and hippocampal neurons in hypoxic/ischemic condition, as well as in oxygenated condition (37, 150). Pre-, peri- and post-treatment with inhalational agents was demonstrated to decrease hypoxic/ischemic-induced neuron cell death through avenues including release of Ca^{2+} from the endoplasmic reticulum, transient moderate increases in intracellular Ca^{2+} concentration and Ca^{2+}-calmodulin-mediated processes (134, 150, 151). Some studies showed that Ras and the MAP kinase-ERK pathway were involved in acute neuroprotection with isoflurane preconditioning in a calcium-dependent manner (37, 82, 89). Xenon exerted its neuroprotective effects partially by activation of the Ca^{2+} calmodulin-activated kinase 2 (CaMK2) system implicated in cellular death and over-release of neurotransmitter *in vitro* (19).

4.1.4. Antioxidant mechanisms

The neuroprotective effects of volatile anesthetics have been supposed to include decreasing reperfusion-induced oxidative injury, since volatile agents have been observed to decrease extracellular glutamate accumulation and subsequently prevent intracellular calcium overload. However, volatile anesthetics, unlike propofol and thiopental, have been shown to be devoid of direct antioxidant properties (152). They could not prevent primary mixed neuronal-glial cell culture death from H_2O_2-induced oxidative stress (153). Moreover, sevoflurane has been observed to induce reactive oxygen species (ROS) generation in normoxic brain (48) and cardiac tissue (154).

Recent studies, however, have reported that the generation of ROS was decreased by sevoflurane peri-treatment in mature mixed cerebrocortical neuronal-glial cell cultures subjected to 90 minutes of OGD (48). Yurdakoc *et al.* examined the post-treatment effects of halothane, isoflurane, and sevoflurane on the time course of lipid peroxidation, measured by malondialdehyde (MDA) formation, in a rat closed head trauma model. Only isoflurane exposure 15 minutes post insult significantly reduced concentration of MDA (155). Isoflurane preconditioning was also indicated to promote the survival of cultured ischemic hippocampal neurons by mechanisms involving significantly increasing heme oxygenase-1 (HO-1) mRNA and HO-1 protein expression (88). HO-1 represents an important endogenous antioxidative defense mechanism against post-ischemic tissure damage (156), since biliverdin and bilirubin produced by HO-1 may act as physiological antioxidants and potent scavengers of oxygen radicals (157). Metallothioneins-1/2 (MT-1/2) were

indicated to play an important role in isoflurane-mediated delayed preconditioning against OGD toxicity of neuronal and glial cells *in vitro* (87), which has an ability to decrease both reactive oxygen species levels and peroxidation of cellular proteins and lipids (158).

Nevertheless, additional investigation is needed to support the antioxidation effects of volatile anesthetics in ischemic brain.

4.1.5. Anti-apoptosis

Apoptosis, or programmed cell death, can be induced by low-level neuronal injuries including ischemia, hypoxia, radiation and others. Apoptosis is an active form of cell death morphologically characterized by shrinked cell bodies and nuclei, condensed nuclear chromatin, blebbing membrane and apoptotic bodies (159, 160). Li *et al.* observed that the number of cells undergoing apoptosis peaked at 24-48 hours post ischemia and continued to increase as late as 4 weeks after initiation of ischemia (161).

Pre- and peri-treatment with volatile anesthetics were observed to reduce the number of apoptotic cells and the concentration of apoptosis-regulating proteins after brain ischemia *in vivo* and *in vitro*. For example, TUNEL-positive or eosinophilic-positive neurons were significantly decreased by treatment with volatile agents (isoflurane, desflurane and sevoflurane) before and during ischemic injury in rodent neuronal cortical cell cultures and intact animal models (43, 54, 86). However, both TUNEL staining and hematoxylin and eosin (HE) staining have been validated not to be specific for apoptosis, since cells with either apoptotic or necrotic morphology have been stained TUNEL-positive (162, 163).

Apoptosis-regulating proteins are more specific for measuring apoptotic cell death as they are triggered further downstream in the apoptotic cascade. Sevoflurane exposure during incomplete cerebral ischemia (30 min) in rats was observed to reduce concentration of the apoptosis-inducing protein Bax in hippocampal tissue as early as 4 hours after ischemic insult. Bcl-2, p53 and Mdm-2 were not changed at this time point (164). In a rat moderate hemispheric ischemia/reperfusion (45 minutes) plus hypotension model, sevoflurane peri-treatment was detected to increase the hippocampal concentration of the anti-apoptotic proteins Bcl-2 and Mdm-2 and inhibited the ischemia-induced upregulation of the pro-apoptotic protein Bax in the first 3 days after ischemic insult compared with a fentanyl and N_2O/O_2 group. No difference in p53 and active caspase-3 was detected between two groups over time (43). The data is consistent with Bickler's findings. Bickler and his group recently have reported that expression of signal transduction genes differs after isoflurane preconditioning of rat hippocampal slices. Isoflurane preconditioning did not increase p53 mRNA, but it increased Mdm-2 mRNA, suggesting isoflurane would inhibit p53-mediated apoptosis *via* Mdm-2 (165).

However, several investigations have shown that volatile anesthetics delayed but did not prevent apoptosis caused by severe brain ischemia. Kawaguchi *et al.* reported that apoptosis induced by 70-minute focal cerebral ischemia increased gradually during reperfusion and peaked at 4 days in the isoflurane group, and there was no difference in the number of caspase-3 and -9 positive neurons and brain infarction between the isoflurane and control groups up to 7 days post insult (55). By transiently protecting neurons, isoflurane might increase the therapeutic window for other drugs that can reduce neuronal injury.

Bickler and Gray's studies have indicated that isoflurane neuroprotection involves calcium-dependent intracellular signaling *via* Ras and the MAP kinase p42/44 pathway and the anti-apoptotic factor Akt (37, 89). Moderate increases in $[Ca^{2+}]i$ observed in the presence of volatile anesthetics might be linked to the phosphorylation of the anti-apoptotic protein Akt, because anesthetic preconditioning neuroprotection in CA1 neurons can be eliminated by LY294002, a compound that prevents the phosphorylation of Akt by PI3 kinase. Consistent with a critical role for ERK and Akt in cell survival signaling, other studies have reported that Akt and related proteins, such as focal adhesion kinases, are associated with isoflurane neuroprotection (37, 93, 165). The level of phosphorylated Akt and p42/44 was decreased by isoflurane in normally oxygenated and OGD brain slices from aging rats, which is consistent with decreased neuroprotection by isoflurane in the aging brain (57). Isoflurane preconditioning in a rat permanent right middle cerebral arterial occlusion (MCAO) model has been shown to induce a rapid and prolonged increase in phosphorylated p38 MAPK in cerebral neocortex (76). This neuroprotection has also been observed by the same research team to induce phosphorylation/activation of ERK and increase the expression of early growth response gene 1 (Egr-1) and Bcl-2 in human neuroblastoma SH-SY5Y cells subjected to OGD (166). Xenon preconditioning was indicated to promote survival against neuronal injury by a phosphorylated cAMP (cyclic adenosine 3' ,5'-monophosphate)-response element binding protein (pCREB)-regulated synthesis of proteins (23). These data suggest a role of MAPK/P38, MAPK/ERK/RSK/CREB and MAPK/ERK/Egr-1/Bcl-2 pathways in inhaled anesthetic preconditioning-induced protection.

Although the exact mechanisms and pathways of apoptosis remain unclear, the role of mitochondria *in vitro* and *in vivo* has been extensively described (167). Disruption of mitochondrial membrane permeability and alterations in mitochondrial membrane potential (MMP) have been observed to result in release of intramitochondrial proteins, such as cytochrome *c*, which activate the apoptotic process (168, 169). Desflurane showed better preservation of mitochondrial function at 4 hours after cerebral ischemia reperfusion injury, indicated by inhibition of mitochondrial swelling, increase of membrane potential, and improvement in functions of mitochondria respiratory complexes 1-4 when compared with halothane (170). Further study is needed to clarify the exact anti-apoptotic mechanism for volatile anesthetics on ischemic brain.

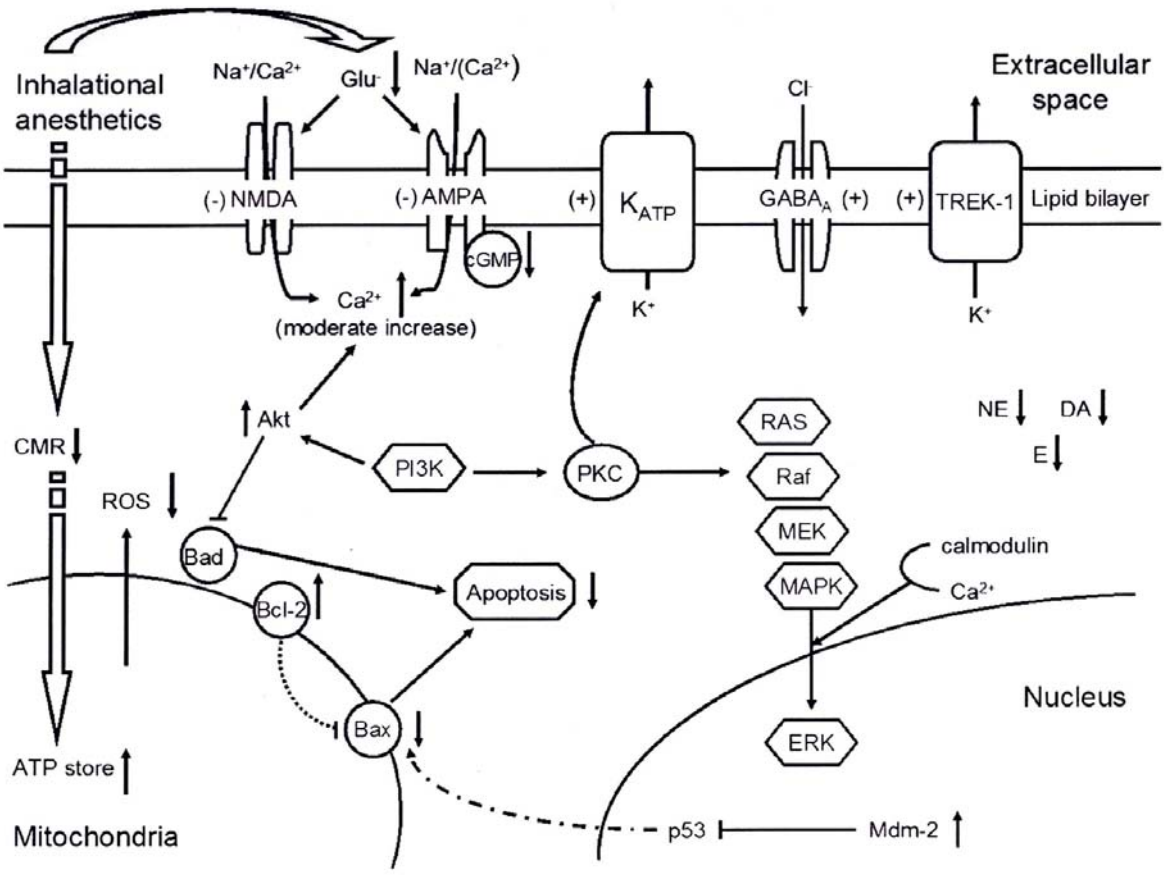


Figure 1. Schematic diagram illustrating the mechanisms of inhalational anesthetic peri-treatment in cerebral ischemia, which include: reduction of cerebral metabolic rate (CMR), increase in energy store, reduction of glutamate release, NMDA and AMPA receptor antagonism, moderate increase in intracellular calcium, reduction of ROS, increase of anti-apoptotic factor (Bcl-2, Mdm-2 and Akt), reduction of apoptotic-induced protein (Bax), activation of RAS/MAPK/ERK pathway, activation of TREK-1, $GABA_A$ receptor potentiation, reduction of cerebral catecholamine concentrations (epinephrine, norepinephrine and dopamine), and activation of plasmalemmal K_{ATP} channels mediated by PKC activation. Abbreviations: E: epinephrine; NE: norepinephrine; DA: dopamine.

4.2. Mechanisms for peri-treatment

Much work has been done to delineate the exclusive mechanisms of volatile anesthetics administered during brain ischemia. This section will briefly review the molecular mechanism of volatile anesthetic peri-treatment for ischemic brain (Figure 1).

4.2.1. Two-pore-domain potassium channels (K_2P channels)

Two-pore-domain potassium channels (K_2P channels), found in various types of neuron, have been validated to play a key role in shaping the characteristics of neuronal excitability (171-174). TREK-1, an important member of K_2P channels, is opened by polyunsaturated fatty acids (PUFAs) and lysophospholipids (LPLs) (175, 176). PUFAs and LPLs have been reported to potentially protect neuronal cells from ischemia and seizures partly *via* TREK channels (177-179), suggesting that activation of TREK-1 might be a novel mechanism involved in neuroprotection.

TREK-1 is also activated by volatile anesthetics (halothane, sevoflurane, desflurane (30), and xenon (31, 180)) and has been suggested to be an important target in the action of these volatile agents, since TREK-1 knockout mice showed a marked decrease in sensitivity to the volatile agents (30). However, the literature is devoid of direct evidence supporting neuroprotective effects of volatile anesthetics *via* activating the TREK-1 channel.

4.2.2. $GABA_A$ receptor potentiation

Significant protection by isoflurane has been observed in rat incomplete global brain ischemia compared with fentanyl–nitrous oxide, and was completely reversed by co-administration of trimethaphan (181) with the property of gamma-aminobutyric acid type A ($GABA_A$) receptor antagonism (182). The hypothesis that $GABA_A$ receptor potentiation may play a critical role in isoflurane neuroprotection for ischemic injury was validated *in vivo* and *in vitro*. In rat hippocampal slices subjected to an OGD and severe forebrain ischemic model, isoflurane exposure

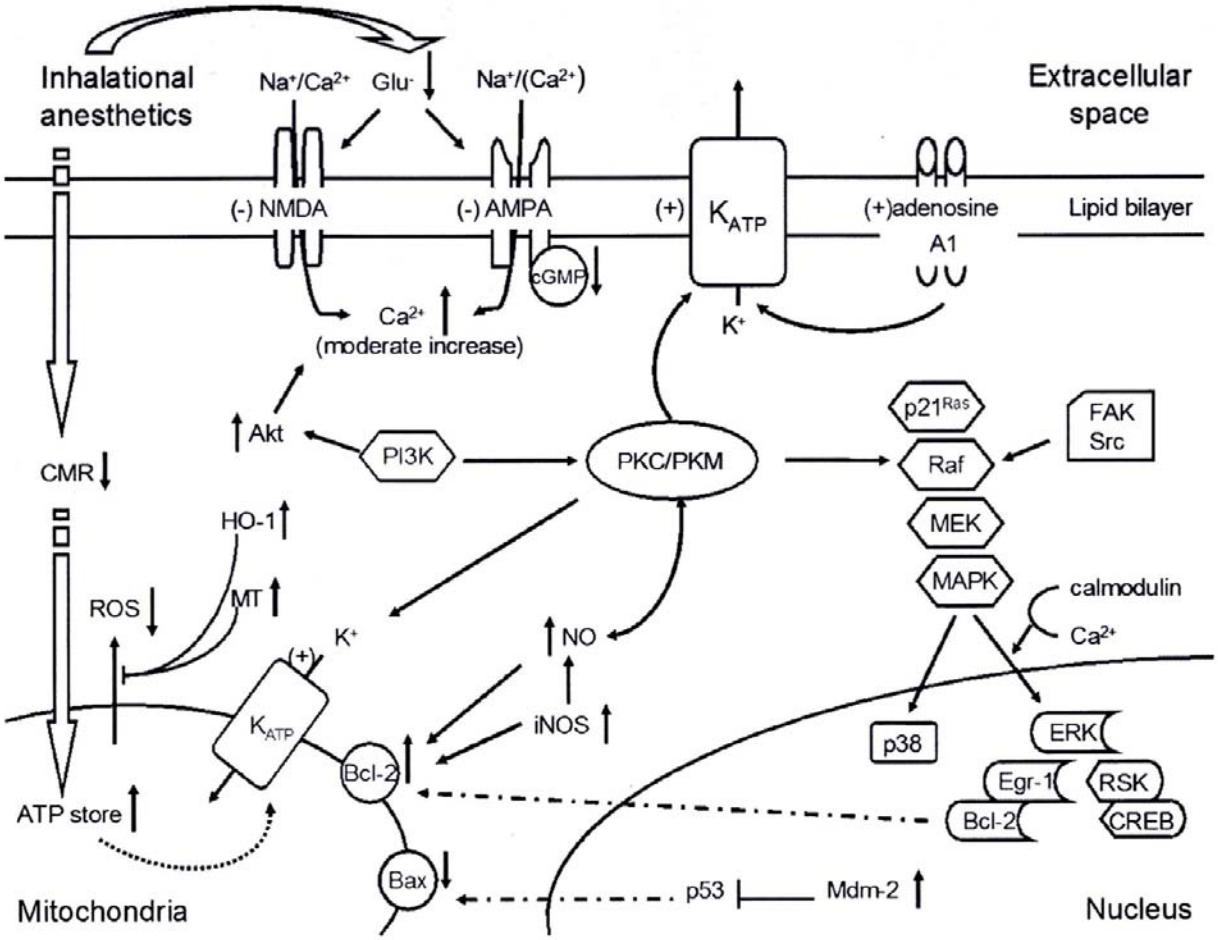


Figure 2. Schematic diagram illustrating the mechanisms of inhalational anesthetic preconditioning in cerebral ischemia, which include reduction of cerebral metabolic rate (CMR), increase in energy store, reduction of glutamate release, NMDA and AMPA receptor antagonism, moderate increase in intracellular calcium, reduction of ROS *via* increasing HO-1 and MT, increase of anti-apoptotic factor (Bcl-2, Mdm-2 and Akt), reduction of apoptotic-induced protein (Bax), activation of p21Ras and MAPK/ERK/Egr-1/Bcl-2, MAPK/ERK/RSK/CREB and MAPK/p38 pathway, activation of plasmalemmal or mitochondrial K_{ATP} channels mediated by PKC/PKM activation, nitric oxide involvement, activation of adenosine A1 receptor, and increase in the expression of focal adhesion kinase (FAK).

induced near-complete protection against ischemic injury. This neuroprotection was unaffected by co-administration of phaclofen (a $GABA_B$ receptor antagonist) but largely reversed by bicuculline (a $GABA_A$ receptor antagonist) (35, 183), which is consistent with the pharmacologic affinity of isoflurane for the $GABA_A$ receptor in a physiological condition (184-187).

Therefore, $GABA_A$ receptor potentiation may partially contribute to volatile anesthetic neuroprotection against ischemic injury.

4.2.3. Catecholamine release

Accumulating evidence has shown that there is a big increase of norepinephrine and dopamine in cerebral tissue (188, 189) during ischemia, which has adverse effects on neuronal tissue and biochemical recovery post insult (188, 190) by mechanisms that may be related to activation of L-type Ca^{2+} channel conductances and may thereby affect NMDA receptor-induced neuroexcitation (191). Reduction of catecholamine release thus represents a possible mechanism by which volatile anesthetics may exert a neuroprotective effect. Various volatile anesthetics have been observed to reduce circulating and cerebral concentration of epinephrine, norepinephrine and dopamine (47, 53, 123, 192, 193), which is related to improved neurological outcome. However, a poor correlation was observed between circulating and cerebral catecholamine concentrations (123, 193), which raises a question as to whether circulating catecholamines play a critical role in inhalational agent neuroprotection or in concentration of cerebral catecholamines.

4.2.4. Neurogenesis

Whether neurogenesis and angiogenesis serve as probable sites of action for volatile anesthetic neuroprotection still remains unproven. One *in vivo* study has investigated the effect of sevoflurane (1.4% vs. 2.8%) on endogenous neurogenesis assessed by bromodeoxyuridine, a marker of neurogenesis, in a rat forebrain ischemic model plus hemorrhagic hypotension. The data suggest that high concentrations of sevoflurane (2.8%) stimulated neurogenesis in the dentate gyrus post insult (194). However, the data was obtained by comparing BrdU-NeuN positive cells undergoing sevoflurane-treatment with or without ischemic insult, and this does not validate the notion that sevoflurane-induced neurogenesis was significant compared with stroke-induced neurogenesis, since neurogenesis and angiogenesis are known to take place after brain ischemia (195-202). More studies are needed to identify the role of neurogenesis in volatile anesthetic neuroprotection.

4.3. Mechanisms for preconditioning

Recently, more research has been focused on volatile anesthetic preconditioning mechanisms in ischemic brain. Many preconditioning mechanisms described below have not yet been tested as potential peri-treatment mechanisms of volatile agents. Further studies are needed to validate the unique mechanisms for volatile anesthetic preconditioning (Figure 2).

4.3.1. ATP-sensitive potassium channels

Adenosine triphosphate-sensitive potassium (K_{ATP}) channels have been identified to have three tissue-specific subtypes, including Kir6.2/SUR1 (beta-cell/neuroendocrine/neuronal), Kir6.2/SUR2A (cardiac and skeletal muscle type), and Kir6.2 (or 6.1)/SUR2B in smooth muscles (203, 204). Several studies have investigated the role of mitochondrial K_{ATP} (mito K_{ATP}) channels in volatile anesthetic preconditioning on ischemic brain (77, 85). In rat, these channels have a six-fold higher concentration per milligram of mitochondrial protein in brain than in heart (205). These studies indicate that mito K_{ATP} channels might be involved in mechanisms of anesthetic preconditioning neuroprotection, since the beneficial effects of isoflurane or sevoflurane can be abolished by 5-hydroxydecanoic acid, a specific mito K_{ATP} channel blocker, *in vivo* and *in vitro* (77, 81, 85, 206). However, most of these studies investigating the anesthetic preconditioning simply relied on the perceived specific inhibitors for channel subtypes without observation of the K_{ATP} current (77, 81).

Plasmalemmal Kir6.2/SUR1 channels are present in the brain, in areas such as hypothalamus, forebrain, and striatum, and are activated by ADP and inhibited by high or physiological intracellular ATP concentrations (207, 208). Bantel *et al.* examined the involvement of plasmalemmal K_{ATP} channels in volatile anesthetic-induced preconditioning by investigating the effects of sevoflurane and xenon directly on K_{ATP} currents. The data suggest that activation of plasmalemmal K_{ATP} channels, but not mito K_{ATP} channels, is essential for neuronal preconditioning by xenon, and verified that the opening of either plasmalemmal K_{ATP} channels or mito K_{ATP} channels was not involved in sevoflurane preconditioning *in vitro* (209). The results are consistent with those of Zheng and his group, who found that blocking K_{ATP} channels had no effect on isoflurane preconditioning in cerebellar brain slices (78).

Further studies are needed to identify the exact effects of K_{ATP} channel and probable downstream proteins involved in volatile anesthetic pretreatment on ischemic brain.

4.3.2. Nitric oxide

Accumulating evidence has shown that nitric oxide is involved in physiologically regulating cerebral blood flow and neurotransmitter as well as pathologically promoting or attenuating neuronal cell death in ischemic brain (122). NO can induce the expression of the anti-apoptotic protein Bcl-2 (210). NO was found to play a critical role in the $p21^{ras}$ /extracellular signal-regulated kinase cascade, which might be necessary for ischemic tolerance induction in neuronal cell culture (211). Nitroxyl anion (NO^-), physiologically formed from NO by interfering with redox metal-containing proteins, has been indicated to reduce Ca^{2+} influx by interacting with the NR2A subunit of NMDA receptor (212).

Nitric oxide can be synthesized by different types of nitric oxide synthases (NOS) including iNOS, endothelial NOS and neuronal NOS (213), among which iNOS may be important to mediate volatile anesthetic preconditioning-induced neuroprotection in adult rats (74, 75). Pretreatment with volatile anesthetics was demonstrated to induce iNOS-dependent prolonged neuroprotection in rat adult *in vitro* and *in vivo* models (74). An increase in iNOS protein was revealed 6 hours after anesthetic pretreatment by Western blotting from cortical tissues. Consistent with Kapinya's results, in a rat neonatal hypoxia/ischemia model, isoflurane preconditioning also provided effective protection accompanied by a time-dependent increase in iNOS expression in neonatal brains (75). This neuroprotection was abolished by aminoguanidine, an iNOS inhibitor. The expression of the anti-apoptotic protein Bcl-2 in the hippocampus increased after isoflurane pretreatment, and was reduced by inhibitors of iNOS (214). Nitric oxide, produced by iNOS, can induce Bcl-2 expression (210) *via* activating signal transducer and activator of transcription-3 (215), a transcription factor that increases Bcl-2 expression (216). These data indicate that iNOS, which in large increases may be harmful and associated with inflammation (217), was a signaling molecule upstream of Bcl-2 and might be involved in the delayed phase of ischemic tolerance induced by isoflurane preconditioning in neonates.

Further studies are needed to elucidate iNOS-dependent downstream involvement in volatile preconditioning-induced neuroprotection and to determine whether endothelial NOS or neuronal NOS plays a favorable or unfavorable part in this neuroprotective mechanism.

4.3.3. Adenosine A1 receptor activation

Adenosine A1 receptor activation may also play a critical role in the neuroprotection by volatile anesthetics against ischemic insult. In a rat focal cerebral ischemic model, preconditioning with isoflurane was shown to reduce ischemic injury, and the adenosine A1 receptor antagonist attenuated this neuroprotection (80). Activation of adenosine A1 receptor has been observed to physiologically promote the opening of neuronal K_{ATP} channels (218). The obtained data suggest that adenosine A1 receptor activity induced by isoflurane pretreatment could possibly be a trigger for K_{ATP} channel activation resulting in cerebral ischemic tolerance. Further studies are needed to demonstrate the role of the adenosine A1 receptor activation-mediated pathway in volatile anesthetic neuroprotection.

4.4. Mechanisms for post-treatment

Although volatile anesthetic preconditioning is clinically feasible for preventing at-risk populations from perioperative ischemia, it is of limited value for patients with stroke and brain trauma occurring outside the hospital. Interventions that can be applied after the onset of brain ischemia will have broad applications. Ischemic post-treatment has been indicated to promote neuronal cell survival in rodent models since 2006 (219, 220). However, limited studies have been focused on the neuroprotective effects of and mechanisms involved in volatile agent post-treatment on ischemic brain.

4.4.1. ATP-sensitive potassium channels

A recent study has indicated that activation of mitochondrial adenosine 5'-triphosphate-sensitive potassium channels might be involved in the mechanism of volatile agent post-treatment. Isoflurane administered after OGD or focal brain ischemia provided neuroprotection which was able to be abolished by glibenclamide, a general adenosine 5'-triphosphate-sensitive potassium channel blocker, or 5-hydroxydecanoic acid, a mitochondrial adenosine 5'-triphosphate-sensitive potassium channel blocker (95). More work is needed to investigate the role of plasmalemmal K_{ATP} channels and changes in different K_{ATP} currents in inhalational anesthetic post-treatment in ischemic brain.

5. SUMMARY AND PERSPECTIVES

Although inhalational anesthetic neuroprotection has been successfully tested in reproducible and well-controlled animal models of cerebral ischemia, the clinical evidence is less convincing. Clarifying the limitations of the current research may help us understand the translational loss.

Data on ischemic brain impact afforded by inhaled anesthetics has been derived mostly from rodent stroke models, since rodents closely resemble higher species in physiological parameters and cerebrovascular anatomy (33). In addition, numerous studies examining the relative neuroprotective efficacy of inhaled agents assess neuronal survival by histological and functional outcomes at short-term end points (usually within 7 days). However, the ischemic cascade associated with neuronal damage can continue for a considerably longer time after the initial ischemic insult (221). Before extrapolation to future clinical trials, further research with higher-order and gyrencephalic animals is needed to test long-term histological and functional improvement afforded by volatile anesthetics.

Most studies in animal stroke models have compared outcomes of different anesthetics with other anesthetics without normalized doses (*i.e.,* MAC). Only a few studies have compared the effects of volatile anesthetics with the unanesthetized state, a more ideal control. In future research, designers need to utilize comparable doses (MAC) of inhaled anesthetics for neuroprotective assessment.

Furthermore, studies need to be designed with strict controlled physiological variables *via* ventilation of the animals, administration of appropriate fluids, or use of warming blankets, since poor control of physiological parameters, including blood gas, body temperature, blood pressure, glucose levels and end-tidal CO_2 ($EtCO_2$), may fluctuate and lead to variability in outcomes (34), which ultimately can result in translational failure in the search for clinical neuroprotection.

Another potential reason of the failure of translatiing the success from benchtop to bedside applications is that the majority of experimental stroke studies have used young adult and male animals. The epidemiological studies have shown that stroke in humans mainly afflicts the elderly and women have a greater perioperative stroke risk than men(223, 224, 225). The experimental studies have confirmed that age and gender may modify the response to inhalational anesthetic administration (57, 94, 226). Further studies are needed to identify the gender- or age-specific effects on neuroprotective capacity of inhalational anesthetics.

In addition, many indirect parameters, including electroencephalograms and genetic, biochemical, and immunohistochemical markers, have been utilized by researchers in animal and clinical studies, but these may not be as sensitive or reproducible as histopathological and cognitive functional tests. More prospective randomized controlled clinical studies, with sensitive and appropriate end points, need to be undertaken to evaluate inhalational anesthetics and their effects on cerebral ischemia and functional outcomes.

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Abbreviations: CEA: carotid endarterectomy; OGD: oxygen–glucose deprivation; AMPA: a-amino-3-hydroxyl-5-methyl-4-isoxazol propionic acid; NMDA: N-methyl-D-aspartate; TUNEL: terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; MAC: minimum alveolar concentration; PI: propidium iodide; TTC: 2,3,5-triphenyltetrazolium chloride; CPR: cardiopulmonary resuscitation; MCAO: middle cerebral artery occlusion; rCBF: regional cerebral blood flow; EAATs: excitatory amino acid transporters; HPLC: high-performance liquid chromatography; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinases; ROS: reactive oxygen species; MDA: malondialdehyde; HO-1: heme oxygenase-1; MT-1/2: metallothioneins-1/2; HE: hematoxylin and eosin; Egr-1: early growth response gene 1; MMP: mitochondrial membrane potential; PUFAs: polyunsaturated fatty acids; LPLs: lysophospholipids; $GABA_A$: gamma-aminobutyric acid type A; K_{ATP} channels: adenosine triphosphate-sensitive potassium channels; mito K_{ATP} channels: mitochondrial K_{ATP} channels; NO—: Nitroxyl anion; NOS: nitric oxide synthases; PKC: protein kinase C; PKM: protein kinase M; cAMP: cyclic adenosine 3' ,5'-monophosphate; pCREB: phosphorylated cAMP-response element binding protein.

Key Words Anesthetics, Inhalation, Isoflurane, Sevoflurane, Desflurane, Xenon, Brain ischemia, Review

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