

Anesthetic effects on autophagy

Fan Ye, Zhi-yi Zuo*

Department of Anesthesiology, University of Virginia, Charlottesville, VA, USA

*Correspondence to: Zhi-yi Zuo, M.D., Ph.D, zz3c@virginia.edu.

orcid: 0000-0002-3542-5047 (Zhi-yi Zuo)

Abstract

Anesthetic agents provide patient comfort and optimize conditions for surgical and procedural interventions. These agents have been shown to modulate autophagy, which is a cellular mechanism that maintains tissue homeostasis by degrading and recycling excess, aged, or dysfunctional proteins. However, it is not always clear if upregulated autophagy is beneficial or harmful. This review assesses the anesthetic effects on autophagy. In the vast majority of studies, anesthetic modulation of autophagy is beneficial for cell survival.

Key words: apoptosis; autophagy; intravenous anesthetics; ischemic preconditioning; myocardium; neuroprotection; signaling molecules; volatile anesthetics

doi: 10.4103/2045-9912.215751

How to cite this article: Ye F, Zuo ZY. Anesthetic effects on autophagy. *Med Gas Res.* 2017;7(3):204-211.

Funding: This study was supported by the Robert M. Epstein Professorship endowment, University of Virginia, Charlottesville, VA, USA.

INTRODUCTION

Autophagy is a cellular mechanism that helps to maintain normal tissue homeostasis by degrading and recycling aged, excess, or dysfunctional proteins.^{1,2} However, excessive autophagy may lead to cellular death *via* induction of apoptosis (type I programmed cell death) or necrosis, or *via* a separate mechanism known as autophagic cell death (Type II programmed cell death).²⁻⁵ This process plays a key role in neurodegenerative disorders, cancers, cardiomyopathies, diabetes, liver diseases, autoimmune disorders, and infections.^{6,7} Additionally, there is evidence that autophagy decreases with aging, which may contribute to the development of age-related diseases.⁸

Anesthetic agents are widely used to provide patient comfort and optimize conditions for procedural or surgical interventions, and are an integral part of modern medicine. Agents used for general anesthesia are typically divided into volatile anesthetics, such as sevoflurane, desflurane, isoflurane, and halothane, and intravenous agents that include benzodiazepines, barbituates, local anesthetics, and opioids. Recently, there has been a growing body of literature on the effects of anesthetic agents on autophagy. This review will briefly describe autophagy and contrast it to apoptosis, provide a discussion on various commonly used anesthetics, and summarize current literature regarding anesthetic effects on autophagy.

AUTOPHAGY

Autophagy, derived from Greek for “self-eating”, is a cellular mechanism in which long-lived, dysfunctional, or excess proteins are degraded to maintain normal tissue homeostasis.^{1,2,6} Essentially, the cell “eats” itself to preserve energy and provide cellular housekeeping.⁶ Autophagy functions to 1) provide energy and 2) remove unwanted cellular material.⁸

In autophagy, a double-membraned structure known as an autophagosome nonspecifically sequesters the cytoplasmic content of a cell and then fuses with a lysosome.^{2,9} The lysosome is an organelle which allows for recycling of macromolecules by breaking down cellular structures with proteases and hydrolases into individual biochemical components, such as amino acids, fatty acids, sugars, and cholesterol.⁸ These may then be used to create new cellular structures.⁸

There are three main types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy.⁸ In macroautophagy, portions of the cytosol are sequestered inside a autophagosome, which then fuses with a lysosome.⁸ In microautophagy, the lysosomal membrane itself protrudes to isolate cytoplasmic contents as a single membrane vesicle.⁸ Macroautophagy and microautophagy may be selective or non-selective.³ Chaperone-mediated autophagy, on the other hand, is a selective pathway in which soluble cytosolic proteins are delivered to the lysosome after protein unfolding and translocation across the lysosomal membrane.^{3,8}



Autophagy is regulated by various signaling pathways. Under normal conditions, autophagy occurs constitutively at basal levels in most cells, which is thought to promote cellular upkeep.^{2,9} In times of stress or starvation (such as hypoxia), this pathway is upregulated to promote cell survival.^{4,6,10} One mechanism of this is through 5'AMP-activated protein kinase (AMPK) activation.¹¹ However, excessive and unregulated autophagy may lead to cellular death possibly due to induction of apoptosis or because of too much of the cytosolic contents and organelles of the cell being degraded.^{2-4,12} As such, autophagy must be tightly regulated, present at basal levels most of the time and be induced only when necessary.³ Target of rapamycin (TOR) kinase is a signaling control point that is active and inhibits autophagy during conditions that promote growth (such as nutrient availability).⁶ In turn, nutrient deprivation, such as during hypoxia, causes repression of TOR kinase.⁶

Autophagic flux refers to the rate of autophagic degradation, which may be regulated either up or down under various conditions.¹³ Common approaches to assess it include western blot analysis to measure the presence of specific autophagy related proteins, transmission electron microscope to evaluate presence of autophagosomes and autolysosomes, and fluorescence microscopy.¹³ Autophagy inhibition may occur in either early stage, during autophagosome formation or at later stages, during fusion between the autophagosome and lysosome.¹⁴ Inhibition during early stage autophagy may be done with 3-methyladenine (blocks the type III PI3 kinase complex) or beclin-1 or autophagy-related protein (Atg) 5 knockout with siRNAs, whereas later stage inhibition can be accomplished with chloroquine, bafilomycin A1, or Rab7 knockout.¹⁴

Apoptosis, first described by Kerr et al.¹⁵ in 1972, is widely known as programmed cell death.^{4,15} Morphologically, this occurs in two distinct stages: formation of apoptotic bodies followed by phagocytosis and cellular degradation.¹⁵ Hallmarks of apoptosis include chromatin condensation, DNA fragmentation, cellular swelling, and activation of specific proteases known as caspases.^{2,4,15} There are two apoptotic signaling cascade pathways, the intrinsic and extrinsic pathways.¹⁶ The intrinsic pathway is triggered from cellular stress, such as DNA damage, starvation, and hypoxia, whereas the extrinsic pathway is initiated through death receptor activation.^{16,17}

Although autophagy and apoptosis are morphologically different processes, the two share several similarities. Both occur under conditions of cellular stress, although autophagy typically happens first to attempt to maintain cellular homeostasis.^{17,18} Several proteins play a role in both autophagy and apoptosis.¹⁶ These include Atgs, Bcl-2/beclin-1, caspases, p53, and FADD-like interleukin-1 β -converting enzyme-inhibitory protein (FLIP).¹⁶ The Bcl-2/beclin-1 complex inhibits both autophagy and apoptosis.¹⁷ While Atgs are necessary for autophagy, Atg5 and Atg12 may induce apoptosis during stress, and in turn, apoptosis

inhibits Atg5.¹⁷ Caspases and FLIP are involved in the death receptor pathway of apoptosis, and their activation blocks autophagy.¹⁷ p53 may promote proteins that trigger either the intrinsic or extrinsic pathway of apoptosis, but also downregulates autophagy via inactivating AMPK and activating mechanistic TOR (mTOR).¹⁶

ANESTHETIC AGENTS

Agents used for general anesthesia are divided into two categories: inhalational anesthetics and intravenous anesthetics. There are four components necessary to provide general anesthesia in humans: hypnosis, amnesia, immobility, and analgesia. While inhalational anesthetics may provide all of these components (albeit to different degrees), there is no single intravenous agent that may do the same. Currently, the four most commonly used inhalational anesthetics in the United States are nitrous oxide, isoflurane, sevoflurane, and desflurane. Isoflurane, sevoflurane, and desflurane are all halogenated hydrocarbons, and are derivatives of ether.¹⁹ The exact mechanism of action of volatile anesthetics is unknown, although they are thought to modify neuronal function by decreasing excitatory neurotransmission and/or increasing inhibitory neurotransmission through interactions with protein targets, such as γ -aminobutyric acid (GABA) receptor and voltage-gated sodium channels.^{20,21}

Drugs that may be used for intravenous anesthesia include propofol, etomidate, ketamine, sodium thiopental, midazolam, and opioids. These are commonly used for induction of anesthesia but they may be used for maintenance of anesthesia as well. Etomidate, propofol, midazolam, and sodium thiopental produce anesthesia by enhancing the inhibitory neurotransmitter GABA in the central nervous system (CNS). Ketamine primarily antagonizes N-methyl-D-aspartate (NMDA) receptors, the site of action of the excitatory neurotransmitter NMDA, but also acts on nicotinic receptors, muscarinic receptors, monoaminergic receptors, opioid receptors, voltage-gated sodium channels, and L-type calcium channels.²² Opioids work on opioid receptors to produce analgesia. These drugs are used in a variety of combinations to produce the desired effects.

A PubMed search was performed using keywords “anesthesia” and “autophagy” as well as “anesthesiology” and “autophagy.” Non-English texts were excluded. Additionally, relevant citations from texts were reviewed and included.

Since 2010, there are 15 studies linking anesthesia with autophagy. Among them, 13 studies were animal studies; whereas the other two used cultured human cells. Six studies utilized intravenous anesthetic agents, seven studies used volatile anesthetic agents, one investigation studied both volatile and intravenous anesthetic agents, and one study determined the effects of local anesthetics (**Table 1**).

ANESTHETIC EFFECTS ON CARDIAC AUTOPHAGY

There is a well-established literature that volatile anes-



thetics result in cardiac preconditioning,³⁷⁻⁴¹ which is a phenomenon in which a short period of ischemia protects against a subsequent more drastic ischemic event.^{41,42} Similar to ischemic insult, volatile anesthetics induce cardiac preconditioning by activation of protein kinase C (PKC) and modulation of nitric oxide synthase, ultimately stimulating the mitochondrial adenosine triphosphate-sensitive potassium (mitoKATP) channel and triggering reactive oxygen species (ROS) release.⁴¹ Sevoflurane preconditioning also enhanced AMPK activation in an ROS-dependent manner, which provides cardioprotection.⁴³ Anesthetic preconditioning, like ischemic preconditioning, provides both early and delayed protection as well.⁴¹ However, cardioprotection from anesthetic preconditioning is lost during prolonged ischemic periods.¹

Several studies suggest that autophagy plays a role in anesthetic preconditioning of myocardium (**Table 1**). Qiao et al.²⁶ in 2013 found that sevoflurane preconditioning resulted in not only nuclear factor-kappa B (NF- κ B) activation, but also the formation of autophagosomes in rat myocardium, suggesting upregulated autophagy. There was also a concomitant decrease in inflammatory markers tumor necrosis factor-B (TNF- κ B) and interleukin-1 α (IL-1 α), as well as apoptosis markers (active caspase-3). Administration of a NF- κ B inhibitor parthenolide prior to anesthetic preconditioning decreased autophagy during delayed anesthetic preconditioning. However, Qiao's group did not use an autophagy inhibitor as part of their study. Shiomi et al.¹ published two studies relating to autophagy and anesthetic preconditioning. In one study, they examined the involvement of autophagy in the loss of sevoflurane preconditioning effects during prolonged ischemia. They found that sevoflurane preconditioning in guinea pig hearts was lost during longer ischemic insult (45 minutes of ischemia compared to 30 minutes), but was restored with administration of chloramphenicol, an autophagy inducer. Administration of an autophagy inhibitor, on the other hand, abolished protective effects. In another study, Shiomi et al.²⁸ found that upregulation of autophagy was mediated by ROS during sevoflurane preconditioning.

Only one study in our literature search used an intravenous agent to study ischemia/reperfusion injury in myocardium and autophagic cell death.²³ In Noh et al's²³ 2010 study, they induced ischemia/reperfusion injury in rat myocardium and then randomized the rats to one of four groups, sham operation (no ischemia), and the following three groups had left coronary artery occlusion associated myocardial ischemia for 25 minutes followed by reperfusion and either infusion of saline, propofol, or intralipid. They found that myocardial infarction size was reduced in the ischemia/reperfusion group treated with propofol. The autophagy related proteins LC3-II and beclin-1 were upregulated following ischemia/reperfusion injury but

decreased in the myocardium of rats treated with propofol. The inhibitory pathway of autophagy mediated by mTOR phosphorylation and beclin-1/Bcl-2 interaction was also promoted by propofol, suggesting that propofol attenuated autophagic cell death following ischemia/reperfusion.

These studies suggest that autophagy plays a role following ischemia/reperfusion injury in the myocardium. Interestingly, while all studies suggest protection with volatile anesthetics, the role of autophagy differs. With volatile anesthetic preconditioning, autophagy induction was found to be protective whereas Noh's study²³ suggests that intravenous anesthetic agents attenuates autophagic cell death. This may be explained by autophagy playing two roles during ischemia/reperfusion; during ischemia, autophagy is protective as a result of AMPK activation and mTOR inhibition; whereas during reperfusion following ischemia autophagy can lead to autophagic cell death.²³

Anesthetic Effects on Neuronal Autophagy

Propofol has widely been thought of as a neuroprotective agent.²⁴ Proposed mechanisms for this effect include anti-inflammatory/antioxidant effects against free radicals and reactive oxygen species.^{23,44} reduction in cerebral metabolic rate of oxygen,^{24,45} activation of GABA_A receptors,⁴⁶ calcium channel inhibition,²⁵ and inhibition of NMDA subtype of glutamate receptors.⁴⁷

In the adult hippocampus, studies have suggested that hypoxia-ischemia injury or electroconvulsive shock therapy (ECT) may result in autophagy.^{25,33} In Cui et al's²⁴ 2012 study, treatment of neuronal PC12 cells or rats with an autophagy inhibitor 3-methyladenine (3-MA) resulted in increased cell survival, decreased autophagy related proteins (LC3-II, beclin-1, and class III PI3K), and decreased amounts of autophagosomes and autolysosomes, suggesting that excessive autophagy is deleterious to hippocampal neurons. Additionally, Cui et al.^{24,25} demonstrated that administration of propofol also resulted in autophagy inhibition and increased cell survival in the hippocampus following hypoxia-ischemia injury. One potential explanation for this effect is inhibition of the p53 pathway by propofol, as a study showed that following ischemia/reperfusion in hippocampal cells, propofol, p53 inhibitor (pifithrin- γ , PFT- α) and autophagy inhibitor (3-methyladenine, 3-MA) all resulted in p53 and autophagy related protein inhibition following ischemia/reperfusion injury.²⁵ ECT is a treatment modality used for depression. Its side effects include memory and learning impairment.⁴⁸ Propofol has been shown to reduce these side effects.⁴⁹ Li et al.³³ in 2016 showed that propofol resulted in reduction of autophagy and autophagy related proteins (beclin-1 and LC-II/I) in depressed rats that underwent ECT, suggesting that autophagy may be responsible for attenuation of memory and learning deficits after ECT.

**Table 1: Studies involving anesthesia and autophagy**

Study	Year	Animals/cells	Model	Results
Noh et al. ²³	2010	Male Sprague-Dawley rats (myocardium)	Rats were randomized to sham procedure, or left coronary artery occlusion with 25 minutes of ischemia and reperfusion with saline, propofol, or intralipid. Western blot assay, immunohistochemistry, and transmission electron microscope (TEM) were performed.	Myocardial infarction size reduced in the ischemia/reperfusion (I/R) group treated with propofol. Autophagy related proteins microtubule-associated protein1 lightchain3-II (LC3-II) and Beclin-1 was upregulated following I/R injury but decreased in rat myocardium treated with propofol. Inhibitory pathway of autophagy mediated by mechanistic target of rapamycin (mTOR) phosphorylation and Beclin-1/Bcl-2 interaction was promoted by propofol.
Cui et al. ²⁴	2012	Male Sprague-Dawley rats (hippocampus) and neuronal PC12 cells	In <i>in vivo</i> model, rat carotid arteries were transiently clamped to induce I/R and hippocampal cells harvested, exposed to control, propofol, or autophagy inhibitor. In <i>in vitro</i> model, PC12 cells were deprived of oxygen and glucose. Western blot assay and TEM were performed in each.	Exposure of the PC12 cells or rats to either propofol or 3-methyladenine (3-MA; an autophagy inhibitor) resulted in decreased creation of autophagosomes and autolysosomes, as well as autophagy-related proteins. Bcl-2 (cytoprotective protein) was increased in the propofol and 3-MA groups.
Cui et al. ²⁵	2013	Male Sprague-Dawley rats (hippocampus)	Rat carotid arteries transiently were clamped to induce I/R, hippocampus cells harvested, injected with intralipid, propofol, p53 inhibitor (pifithrin- α , PFT- α), or autophagy inhibitor 3-MA. Real-time polymerase chain reaction (RT-PCR), immunofluorescent, histochemical, western blot analysis were performed.	Propofol reduced I/R-induced autophagy activation, pyramidal neuronal death, and upregulation of p53.
Qiao et al. ²⁶	2013	Adult male Sprague-Dawley rats (myocardium)	Rats were exposed to 2.5% sevoflurane or control for anesthetic preconditioning 24 hours prior to I/R of myocardium with coronary artery occlusion for 30 minutes; then 2 hours of reperfusion, nuclear factor-kappa B (NF- κ B) DNA-binding activity tested, western blot assay and TEM analysis were performed.	Anesthetic preconditioning with sevoflurane was cardioprotective, leading to reduced infarct size, reduction of inflammation and apoptosis, upregulation of autophagy prior to I/R, and activation of NF- κ B transcription factor.
Shiomi et al. ¹	2013	Male guinea pigs (myocardium)	Guinea pig hearts were isolated and subjected to 30 or 45 minutes of ischemia followed by 120 minutes of reperfusion. Four groups: control, preconditioning with 2% sevoflurane, 2% sevoflurane + autophagy upregulator (chloramphenicol), or 2% sevoflurane + autophagy inhibitor (3-MA). Western blot assay and TEM analysis were performed.	Myocardial infarction size was reduced in group with anesthetic preconditioning with sevoflurane with 30 minutes of ischemia but with more prolonged ischemia (45 minutes) this protective effect was gone. Addition of chloramphenicol prior to sevoflurane resulted in restoring the loss of sevoflurane induced cardioprotective effects with prolonged ischemia. 3-MA abolished this.
Sheng et al. ²⁷	2014	Embryonic day 15-16 embryos of CD1 mice (brain)	Cells were cultured for 7 days, exposed to either 2% isoflurane preconditioning or 4% oxygen hypoxic preconditioning for 30 minutes and then harvested 12, 24, 48, and 72 hours later. Cell viability, western blot assay, immunofluorescence, immunohistochemistry, and TEM analysis were performed.	Preconditioning with both isoflurane and hypoxia led to induction of autophagy and resistance to cell death due to oral glucose deprivation or glutamate; whereas treatment with autophagy inhibitors abolished this response. Pretreatment with sphinokinas-2 inhibitor or in sphinokinas-2 knock-out mice induction of autophagy was abolished.
Shiomi et al. ²⁸	2014	Male guinea pigs (myocardium)	Guinea pig hearts were isolated and subjected to 30 minutes of ischemia followed by 120 minutes of reperfusion. Four groups: control, preconditioning with 2% sevoflurane, 2% sevoflurane + reactive oxygen species (ROS) scavenger (N-methylpurine DNA glycosylase, MPG), or MPG alone, analyzed by western blot assay and TEM.	2% sevoflurane preconditioning led to less ischemia and increased autophagy compared to control. Concomitant administration of MPG abolished these protective effects, suggesting that cardiac preconditioning with sevoflurane is influenced by ROS mediation of autophagy.
Chang et al. ²⁹	2015	Cultured human umbilical vascular endothelial cells (HUVECs)	HUVECs were exposed to lipopolysaccharide (autophagy inducer) or propofol, analyzed by western blot analysis.	Propofol and lipopolysaccharides augmented expression of autophagic markers including mTOR, Beclin-1, Atg5, LC3, and Rab7. Functionally, propofol improved the angiogenic capabilities of HUVECs, but effect was significantly diminished with autophagy inhibitors including bafilomycin A, 3-MA, and chloroquine.



Table 1: Continued

Study	Year	Animals/cells	Model	Results
Kashiwagi et al. ³⁰	2015	Transgenic mice expressing an autophagosome maker, green fluorescent protein (GFP)-LC3 (skeletal muscle)	Mice were anesthetized with isoflurane, pentobarbital, ketamine, xylazine, or propofol, compared autophagy with anesthesia <i>versus</i> muscle disuse without anesthesia using tibialis anterior muscle and western blot and histological analysis.	Anesthesia with pentobarbital demonstrated time-dependent upregulation of autophagy, but muscle disuse without anesthesia did not lead to autophagy upregulation. Muscle contraction during anesthesia reduced autophagy upregulation during anesthesia. All other anesthetic agents studied upregulated autophagy.
Kwon et al. ³¹	2015	Human keratinocytes	Human keratinocytes underwent hypoxia-reoxygenation while treated with 0.01, 0.1, 0.5 and 1 ng/mL remifentanyl, analyzed by quantitative real-time PCR and western blot analysis.	Remifentanyl increased proliferation of cells damaged by hypoxia-reoxygenation, enhanced autophagy activity, and decreased apoptotic cell death. Autophagy inhibitor negated protective effects of remifentanyl.
Li et al. ³²	2015	Aged male Sprague-Dawley rats (hippocampus)	Rats were randomly exposed to 1.5% isoflurane or control for 1 or 4 hours, Morris water maze task was assigned after 24 hours and western blot analysis was performed.	Escape latencies of rats exposed to isoflurane for 4 hours was prolonged 4–5 days post anesthesia, decreased autophagy markers beclin 1 and LC3B, but autophagic flux marker p62 was unchanged. There was no difference in escape latencies or expression of markers in 1 hour isoflurane and control group. Expression of autophagy markers was initially increased in the first several hours following isoflurane exposure for 4 hours then decreased in the next 12 to 24 hours; whereas this did not occur with controls.
Li et al. ³³	2016	Male Sprague Dawley rats (hippocampus)	Rats were stressed for 40 days to create depression model, split into four groups (control, electroconvulsive shock therapy, propofol, and electroconvulsive shock therapy and propofol). Western blot analysis was performed.	Rat hippocampus of electroconvulsive shock therapy group had increased expression of autophagy related proteins Beclin-1 and LC3-II/I; whereas those in the group treated with both electroconvulsive shock therapy and propofol had decreased expression of Beclin-1 and LC3-II/I.
Zhang et al. ³⁴	2016	Male Sprague-Dawley rats (hippocampus)	Rats were randomly selected into one of six groups: control, rapamycin, chloroquine, sevoflurane, sevoflurane + rapamycin, and sevoflurane + chloroquine, then subjected to the Morris water maze test after 24 hours. TEM, terminal deoxynucleotidyl transferase (tdT)-mediated dUTP nick end labeling (TUNEL) assay, reverse transcription polymerase chain reaction (RT-PCR), and western blot analysis were performed.	Treatment of rats with sevoflurane led to impaired memory performance and resulted in apoptosis in hippocampal neuronal cells. However, treatment with autophagy inducer (rapamycin) resulted in more autophagic markers and decreased cognitive deficit; whereas treatment with autophagy inhibitor chloroquine resulted in the opposite effect.
Zhou et al. ³⁵	2016	H4 human neuroglioma cells	H4 human neuroglioma cells were exposed to 4.1% sevoflurane for 6 hours, flow cytometry was used to assess cell viability and apoptosis, TEM was used to detect autophagosomes, and western blot analysis was performed.	Sevoflurane induces autophagy in H4 cells mediated by endoplasmic reticulum stress. Additionally, exposure with 4.1% sevoflurane for 6 hours leads to apoptosis and decreased cell viability, which is attenuated by autophagy inducers and increased by autophagy inhibitors.
Xiong et al. ³⁶	2017	Human neuroblastoma SH-SY5Y cells	Human neuroblastoma cells were exposed to local anesthetics (procaine, tetracaine, ropivacaine, bupivacaine, lidocaine). Autophagy inhibition was provided by beclin-1 knockout. Neurotoxicity and autophagic flux were assessed.	Local anesthetics induced neurotoxicity and increased autophagic flux (decreased p62, increased autolysosomes, LC3-II). In cells with beclin-1 knockout, local anesthetic induced neurotoxicity was worse, suggesting autophagy upregulation attenuated neurotoxicity.

Volatile anesthetics during adulthood are typically considered safe and may even confer neuroprotection in ischemia/reperfusion injury⁵⁰; however, recently there has been a growing concern that volatile anesthetics used in animals and humans at the extremes of age may be detrimental. Some animal studies have suggested that inhalational anesthetics may increase the risk of postoperative cognitive dysfunction, a temporary or mid-term decline in cognitive function after surgery and anesthesia that primarily affects older individuals.^{32,34,51,52}

The dual effects of volatiles may however depend upon the concentration of volatile anesthetics used⁵³ or the duration of exposure,⁵⁴ in which deleterious effects occur with high concentration and long duration of use, whereas protective effects are present at low concentration and short term use. Autophagy may play a role in these effects as discussed in the following paragraphs. In this connect, impaired autophagy has been implicated in the pathogenesis of several neurodegenerative diseases due to accumulation of autophagosomes in



neurons, such as Alzheimer's disease, Huntington's disease, and Parkinson's disease.⁵⁵

There are four recent studies^{27,32,34,35} that more closely examine the relationship between volatile anesthetics, autophagy, and their ties to neurocognitive disorders. Li et al.³² in 2015 conducted a study which investigated hippocampal autophagy of aged rats exposed to 1.5% isoflurane of various durations compared to oxygen. They found that exposure of aged rats to isoflurane for 4 hours resulted in impairment of spatial learning and memory, whereas this effect did not occur with only 1 hour of isoflurane exposure. Additionally, after 4 hours of exposure to isoflurane, there was a transient activation of autophagy evidenced by increased amounts of beclin-1 and LC3B-II, followed by a decrease in levels afterwards indicating autophagy suppression.

Zhang et al.³⁴ in 2016 similarly studied the effects of sevoflurane on aged rats. Their study showed that sevoflurane in aged rats led to impaired memory and induced hippocampal neuronal apoptosis. These processes might involve autophagy. When rats were given an autophagy inhibitor (chloroquine), sevoflurane-induced neuronal apoptosis and memory impairment worsened, while conversely, treatment with an autophagy inducer, rapamycin, reduced the cognitive deficit.

Another study examining sevoflurane and possible neuronal cell damage was performed by Zhou et al.³⁵ in 2016, in which they exposed H4 human neuroglioma cells to various concentrations of sevoflurane (0, 4.1%, or 8%) for 6 hours. They noted that sevoflurane resulted in increased apoptosis, increased expression of C/EBP homologous protein and glucose-regulated protein 78 (markers of endoplasmic reticulum stress), decreased cell viability, and autophagy activation. Autophagy activation with rapamycin led to decreased apoptosis, decreased markers of endoplasmic reticulum stress, and increased cell viability; whereas the opposite effects occurred with autophagy inhibition with 3-MA. Using 4-phenylbutyrate, an inhibitor of endoplasmic reticulum stress, also increased cell viability and decreased sevoflurane-induced autophagy and apoptosis.

Sheng et al.²⁷ discovered in 2014 that autophagy from anesthetic or hypoxic preconditioning in mouse cortical neurons may be mediated by sphingosine kinase 2 (SPK2). Sheng's group demonstrated similar results from the aforementioned studies, and found that preconditioning with either isoflurane or hypoxia led to increased autophagy (evidenced by increased LC3-II/LC3-I ratio) and increased cell viability (resistance to oral glucose deprivation and glutamate). Autophagy plays a key role in the protective effects of preconditioning as autophagy inhibition with 3-MA abrogated the benefits of preconditioning. Additionally, preconditioning upregulated SPK2, and SPK2 inhibitors or SPK2 knockout prevented autophagy from preconditioning, suggesting that SPK2 is essential to autophagy and preconditioning.

These studies suggested that autophagy occurs in con-

junction with administration of anesthesia, which indeed is important in modulating cellular survival. However, there is some conflicting data between these studies. In certain studies, autophagy induction resulted in increased cell survival,^{27,34} whereas in others, cell survival decreased.^{24,25,33,35} This is consistent with previous research that shows that autophagy is both pro-survival and pro-death, depending on cell type and cellular conditions.⁵⁶

ANESTHETIC EFFECTS ON AUTOPHAGY IN OTHER CELL TYPES

Chang et al.²⁹ in 2015 investigated the effects of propofol on vascular endothelium. Cultured human umbilical vascular endothelial cells (HUVECs) were exposed to either lipopolysaccharide, which is known to be an autophagy inducer in HUVECs, or propofol. Using western blot analysis, Chang et al.²⁹ found that both propofol and lipopolysaccharides augmented the expression of autophagic markers including mTOR, Beclin-1, Atg5, LC3, and Rab7. The group also showed that propofol improved the angiogenic capabilities of HUVECs. However, this effect was significantly diminished with autophagy inhibitors including bafilomycin A, 3-MA, and chloroquine.

Either prolonged general anesthesia or sedation leads to skeletal muscle atrophy.³⁰ Kashiwagi et al.³⁰ in 2015 studied whether anesthesia or muscle disuse was responsible for the atrophy. They anesthetized mice with a variety of anesthetic agents (pentobarbital, ketamine, ketamine and xylazine combined, isoflurane, and propofol) and detected upregulation of autophagy. Electrical stimulation of muscle during anesthesia attenuated autophagy. However, autophagy was not present when they immobilized muscle by denervation without anesthesia. Their study suggests that both anesthesia and muscle inactivity may be responsible for skeletal muscle autophagy upregulation.

Kwon et al.³¹ studied effects of remifentanyl on human keratinocytes and hypoxia-reoxygenation injury. Human keratinocytes underwent hypoxic injury by being cultured under 1% oxygen tension for 24 hours, exposed to several different concentrations of remifentanyl, then reoxygenated over 12 hours. Their group found that remifentanyl treatment resulted in autophagy activation, increased keratinocyte proliferation, and decreased apoptosis. 3-MA inhibited autophagy and prevented the protective actions of remifentanyl. Their results suggest that remifentanyl is protective against hypoxia-reoxygenation and that these effects were mediated through increased autophagy.

Xiong et al.³⁶ in 2017 examined local anesthetic agents-induced neurotoxicity. They used both ester (procaine and tetracaine) and amide-type (bupivacaine, lidocaine and ropivacaine) on human neuroblastoma cells and assessed neurotoxicity and presence of autophagy. Additionally, they inhibited autophagy by transfecting human neuroblastoma



cells with siRNA beclin-1, causing knockout of beclin-1. They found that local anesthetics induced neurotoxicity and increased autophagic flux (evidenced by decreased p62, increased autolysosomes, LC3-II). When autophagy was inhibited, local anesthetic-induced neurotoxicity was greater, suggesting that autophagy upregulation attenuated local anesthetic induced neurotoxicity.

CONCLUSION

Autophagy is a cellular mechanism that maintains cellular homeostasis. This is a process that must be closely regulated as too much or too little autophagy may result in cellular death. Dysregulated autophagy may play a role in many different disease processes.^{6,7} Anesthetic agents, both volatiles and intravenous, are shown to modulate autophagic flux. However, unlike apoptosis, it is not always clear whether upregulated autophagy is good or bad. In the vast majority of studies, which included *in vitro* and *in vivo* studies, anesthetic modulation of autophagy is beneficial. The only study where this was not the case was Kashiwagi's,⁵⁴ in which anesthesia resulted in upregulated autophagy and skeletal muscle atrophy. In some cases, upregulated autophagy was beneficial, whereas in others the opposite is true. Possible explanations include the degree to which autophagy is upregulated, or during when in the autophagy cycle regulation occurs. The mechanism by which anesthetic agents are able to affect autophagic flux is not entirely clear. This may be due to direct cause-effect or indirect effects mediated *via* other signaling molecules.

There are ample areas for future research. There is a growing area of research connecting autophagy with many disease processes, including neurodegenerative disorders, cancers, cardiomyopathies, diabetes, liver conditions, autoimmune disorders, and infections.^{6,7} Given that anesthetics may modulate autophagy, anesthetics may alter these disease processes *via* autophagy as well. Anesthetic modulation may depend on the amount or duration of anesthesia delivered or type of anesthetic agent. In instances where autophagy modulation is beneficial, in the future, autophagy inhibitors or inducers may be given clinically. Additionally, it is not known what the long-term effects of anesthetics are on autophagic flux, as all the studies in this review were over periods of time lasting days at the most. However, we must be cautious in applying data from animal studies and *in vitro* studies to clinical practice. Nonetheless, autophagy and anesthetic modulation of autophagy are an exciting area for future research.

Author contributions

FY and ZYZ conceived the concept of the manuscript. FY drafted the paper. ZYZ revised the manuscript.

Conflicts of interest

None declared.

Plagiarism check

Checked twice by iThenticate.

Peer review

Externally peer reviewed.

Open access statement

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

Open peer reviewer

Lei Huang, Loma Linda University, USA.

REFERENCES

- Shiomi M, Miyamae M, Takemura G, et al. Induction of autophagy restores the loss of sevoflurane cardiac preconditioning seen with prolonged ischemic insult. *Eur J Pharmacol*. 2014;724:58-66.
- Martinet W, De Meyer GR. Autophagy in atherosclerosis. *Curr Atheroscler Rep*. 2008;10:216-223.
- Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature*. 2008;451:1069-1075.
- Yousefi S, Simon HU. Apoptosis regulation by autophagy gene 5. *Crit Rev Oncol Hematol*. 2007;63:241-244.
- Ginet V, Spiehlmann A, Rummel C, et al. Involvement of autophagy in hypoxic-excitotoxic neuronal death. *Autophagy*. 2014;10:846-860.
- Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. *J Pathol*. 2010;221:3-12.
- Jung KT, Lim KJ. Autophagy: can it be a new experimental research method of neuropathic pain? *Korean J Pain*. 2015;28:229-230.
- Cuervo AM. Autophagy and aging: keeping that old broom working. *Trends Genet*. 2008;24:604-612.
- Mizushima N, Klionsky DJ. Protein turnover via autophagy: implications for metabolism. *Annu Rev Nutr*. 2007;27:19-40.
- Kuma A, Hatano M, Matsui M, et al. The role of autophagy during the early neonatal starvation period. *Nature*. 2004;432:1032-1036.
- Meley D, Bauvy C, Houben-Weerts JH, et al. AMP-activated protein kinase and the regulation of autophagic proteolysis. *J Biol Chem*. 2006;281:34870-34879.
- Scott RC, Juhász G, Neufeld TP. Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. *Curr Biol*. 2007;17:1-11.
- Loos B, du Toit A, Hofmeyr JH. Defining and measuring autophagosome flux-concept and reality. *Autophagy*. 2014;10:2087-2096.
- Thorburn A. Apoptosis and autophagy: regulatory connections between two supposedly different processes. *Apoptosis*. 2008;13:1-9.
- Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*. 1972;26:239-257.
- Li M, Gao P, Zhang J. Crosstalk between autophagy and apoptosis: potential and emerging therapeutic targets for cardiac diseases. *Int J Mol Sci*. 2016;17:332.
- Liu G, Pei F, Yang F, et al. Role of autophagy and apoptosis in non-small-cell lung cancer. *Int J Mol Sci*. 2017;18:367.



18. Mariño G, Niso-Santano M, Baehrecke EH, Kroemer G. Self-consumption: the interplay of autophagy and apoptosis. *Nat Rev Mol Cell Biol.* 2014;15:81-94.
19. Chiao S, Zuo Z. A double-edged sword: volatile anesthetic effects on the neonatal brain. *Brain Sci.* 2014;4:273-294.
20. Herold KF, Sanford RL, Lee W, et al. Volatile anesthetics inhibit sodium channels without altering bulk lipid bilayer properties. *J Gen Physiol.* 2014;144:545-560.
21. Campagna JA, Miller KW, Forman SA. Mechanisms of actions of inhaled anesthetics. *N Engl J Med.* 2003;348:2110-2124.
22. Kohrs R, Durieux ME. Ketamine: teaching an old drug new tricks. *Anesth Analg.* 1998;87:1186-1193.
23. Noh HS, Shin IW, Ha JH, Hah YS, Baek SM, Kim DR. Propofol protects the autophagic cell death induced by the ischemia/reperfusion injury in rats. *Mol Cells.* 2010;30:455-460.
24. Cui D, Wang L, Qi A, Zhou Q, Zhang X, Jiang W. Propofol prevents autophagic cell death following oxygen and glucose deprivation in PC12 cells and cerebral ischemia-reperfusion injury in rats. *PLoS One.* 2012;7:e35324.
25. Cui DR, Wang L, Jiang W, Qi AH, Zhou QH, Zhang XL. Propofol prevents cerebral ischemia-triggered autophagy activation and cell death in the rat hippocampus through the NF- κ B/p53 signaling pathway. *Neuroscience.* 2013;246:117-132.
26. Qiao S, Xie H, Wang C, Wu X, Liu H, Liu C. Delayed anesthetic preconditioning protects against myocardial infarction via activation of nuclear factor-kappaB and upregulation of autophagy. *J Anesth.* 2013;27:251-260.
27. Sheng R, Zhang TT, Felice VD, et al. Preconditioning stimuli induce autophagy via sphingosine kinase 2 in mouse cortical neurons. *J Biol Chem.* 2014;289:20845-20857.
28. Shiomi M, Miyamae M, Takemura G, et al. Sevoflurane induces cardioprotection through reactive oxygen species-mediated upregulation of autophagy in isolated guinea pig hearts. *J Anesth.* 2014;28:593-600.
29. Chang CY, Chen PH, Lu SC, et al. Propofol-enhanced autophagy increases motility and angiogenic capacity of cultured human umbilical vascular endothelial cells. *Life Sci.* 2015;142:49-59.
30. Kashiwagi A, Hosokawa S, Maeyama Y, et al. Anesthesia with Disuse Leads to Autophagy Up-regulation in the Skeletal Muscle. *Anesthesiology.* 2015;122:1075-1083.
31. Kwon JY, Park BS, Kim YH, et al. Remifentanyl protects human keratinocytes against hypoxia-reoxygenation injury through activation of autophagy. *PLoS One.* 2015; 10:e0116982.
32. Li ZQ, Li LX, Mo N, et al. Duration-dependent regulation of autophagy by isoflurane exposure in aged rats. *Neurosci Bull.* 2015;31:505-513.
33. Li P, Hao XC, Luo J, Lv F, Wei K, Min S. Propofol mitigates learning and memory impairment after electroconvulsive shock in depressed rats by inhibiting autophagy in the hippocampus. *Med Sci Monit.* 2016;22:1702-1708.
34. Zhang X, Zhou Y, Xu M, Chen G. Autophagy is involved in the sevoflurane anesthesia-induced cognitive dysfunction of aged rats. *PLoS One.* 2016;11:e0153505.
35. Zhou YF, Wang QX, Zhou HY, Chen G. Autophagy activation prevents sevoflurane-induced neurotoxicity in H4 human neuroglioma cells. *Acta Pharmacol Sin.* 2016;37:580-588.
36. Xiong J, Kong Q, Dai L, et al. Autophagy activated by tuberin/mTOR/p70S6K suppression is a protective mechanism against local anaesthetics neurotoxicity. *J Cell Mol Med.* 2017;21:579-587.
37. Cason BA, Gampert AK, Slocum RE, Hickey RF. Anesthetic-induced preconditioning: previous administration of isoflurane decreases myocardial infarct size in rabbits. *Anesthesiology.* 1997;87:1182-1190.
38. Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Warltier DC. Isoflurane mimics ischemic preconditioning via activation of K(ATP) channels: reduction of myocardial infarct size with an acute memory phase. *Anesthesiology.* 1997;87:361-370.
39. Yvon A, Hanouz JL, Haelewyn B, et al. Mechanisms of sevoflurane-induced myocardial preconditioning in isolated human right atria in vitro. *Anesthesiology.* 2003;99:27-33.
40. Zaugg M, Lucchinetti E, Uecker M, Pasch T, Schaub MC. Anaesthetics and cardiac preconditioning. Part I. Signalling and cytoprotective mechanisms. *Br J Anaesth.* 2003;91:551-565.
41. Swyers T, Redford D, Larson DF. Volatile anesthetic-induced preconditioning. *Perfusion.* 2014;29:10-15.
42. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation.* 1986;74:1124-1136.
43. Lamberts RR, Onderwater G, Hamdani N, et al. Reactive oxygen species-induced stimulation of 5'AMP-activated protein kinase mediates sevoflurane-induced cardioprotection. *Circulation.* 2009;120:S10-15.
44. Braz MG, Braz LG, Freire CM, et al. Isoflurane and propofol contribute to increasing the antioxidant status of patients during minor elective surgery: a randomized clinical study. *Medicine (Baltimore).* 2015;94:e1266.
45. Vasileiou I, Xanthos T, Koudouna E, et al. Propofol: a review of its non-anaesthetic effects. *Eur J Pharmacol.* 2009;605:1-8.
46. Hollrigel GS, Toth K, Soltesz I. Neuroprotection by propofol in acute mechanical injury: role of GABAergic inhibition. *J Neurophysiol.* 1996;76:2412-2422.
47. Orser BA, Bertlik M, Wang LY, MacDonald JF. Inhibition by propofol (2,6 di-isopropylphenol) of the N-methyl-D-aspartate subtype of glutamate receptor in cultured hippocampal neurons. *Br J Pharmacol.* 1995;116:1761-1768.
48. Pigot M, Andrade C, Loo C. Pharmacological attenuation of electroconvulsive therapy--induced cognitive deficits: theoretical background and clinical findings. *J ECT.* 2008;24:57-67.
49. Li X, Li W, Luo J, et al. Effects of propofol on the activation of hippocampal CaMKIIalpha in depressed rats receiving electroconvulsive therapy. *J ECT.* 2012;28:242-247.
50. Lai Z, Zhang L, Su J, Cai D, Xu Q. Sevoflurane postconditioning improves long-term learning and memory of neonatal hypoxia-ischemia brain damage rats via the PI3K/Akt-mPTP pathway. *Brain Res.* 2016;1630:25-37.
51. Culley DJ, Baxter MG, Crosby CA, Yukhananov R, Crosby G. Impaired acquisition of spatial memory 2 weeks after isoflurane and isoflurane-nitrous oxide anesthesia in aged rats. *Anesth Analg.* 2004;99:1393-1397; table of contents.
52. Culley DJ, Baxter MG, Yukhananov R, Crosby G. Long-term impairment of acquisition of a spatial memory task following isoflurane-nitrous oxide anesthesia in rats. *Anesthesiology.* 2004;100:309-314.
53. Zhou X, Li W, Chen X, et al. Dose-dependent effects of sevoflurane exposure during early lifetime on apoptosis in hippocampus and neurocognitive outcomes in Sprague-Dawley rats. *Int J Physiol Pathophysiol Pharmacol.* 2016;8:111-119.
54. Xiao H, Liu B, Chen Y, Zhang J. Learning, memory and synaptic plasticity in hippocampus in rats exposed to sevoflurane. *Int J Dev Neurosci.* 2016;48:38-49.
55. Kiriya Y, Nochi H. The function of autophagy in neurodegenerative diseases. *Int J Mol Sci.* 2015;16:26797-26812.
56. Xu M, Zhang HL. Death and survival of neuronal and astrocytic cells in ischemic brain injury: a role of autophagy. *Acta Pharmacol Sin.* 2011;32:1089-1099.