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# Dual Effects of Neuroprotection and Neurotoxicity by General Anesthetics on Neural Stem Cells: Role of Autophagy

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**Abstract**

General anesthetics (GAs) are widely used for various essential surgical or medical procedures. Recent studies implicate the GAs has dual effects of neuroprotection and neurotoxicity on neurogenesis with unclear mechanisms. This minireview summarizes recent studies on GAs mediated effects on neurogenesis and proposed mechanisms, with focus on autophagy regulation and intracellular calcium homeostasis.

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## Introduction

Each year, millions of fetuses, infants and preschool children are exposed to general anesthetics (GAs) worldwide for various essential surgical or medical procedures. Unfortunately, a large number of preclinical works have demonstrated that prolonged exposure to most, if not all, general anesthetics, either volatile or intravenous, to the developing brain, can cause widespread neuronal cell death, which may be associated with long-term memory and learning disabilities.[1-3] Many mechanisms have been proposed to explain GAs mediated neurotoxicity, including activation of NMDA and GABA receptors[4,5], mitochondrial damage with excessive free radicals[6-8], activation of P75 growth factor[9,10], excessive inflammation[11,12], and disruption of intracellular $Ca^{2+}$ homeostasis[13-15]. Recent studies have also suggested neurogenesis impairment may play a role, since neurotoxic effects of anesthetics occur both *in vitro*[16-19] and *in vivo*[20-22]. As methods improve, a full mechanistic understanding of neurotoxicity becomes more realistic. For example, with the development of an *in vitro* neurogenesis system using hESCs, induced pluripotent stem cells (iPSCs), and neural stem cells (NSCs), investigators can now study the mechanisms underlying brain development and screen the toxic effects of various anesthetics under controlled conditions (dose, number of exposures, or developmental stage). New neuroprotective strategies to avoid the anesthetics mediated toxicity, then, can be generated through neurogenesis modeling. [23]

Creeley et al [24] reported exposure of third-trimester fetal macaque monkeys to isoflurane in utero caused widespread apoptosis of neurons and oligodendroglia critical for myelination. They use high concentrations of isoflurane, which was adjusted by painful stimulation. The volatile anesthetic concentration was titrated according to a predefined clinical endpoint that represents an intermediate surgical plane of anesthesia, where there was no motor response and only a mild sympathetic response with an increase of 10% or less in heart rate or blood pressure. Researchers achieved this endpoint via deep nail-bed stimulation at the hand and foot [mosquito-clamp pinch]. The turning points of anesthetic concentration and duration varied among different animal species and in human beings and depended on the combination of both anesthetic concentration and exposure duration. Our previous study by Li et al[25], showed 1.3% isoflurane for 6 hours reduced apoptosis in the rat fetal brain, while our follow up study by Wang et al[26] demonstrated that 3% isoflurane for only 1 hour significantly increased neuroapoptosis in the fetal developing brains. These studies supported our view of an association between the dual effects of GA-mediated neuroprotection and neurotoxicity and anesthetic concentration and exposure duration. Our previous studies in both cell cultures[27] and animals[28,29] demonstrated that isoflurane for short exposure did not induced neuronal cell damage by itself, but significantly inhibited neurodegeneration induced by isoflurane for prolonged use. Unfortunately, we did not examine the possible dual effects of general anesthetics on stem or neuroprogenitor cells in these studies.

Recent stem cell studies have opened up avenues for research in GA induced developmental toxicity[16,18,20]. Accumulated data indicate that ketamine can cause neuronal damage in several major brain regions in animal models during certain periods of development[30,31]. On the other hand, ketamine at concentrations ranging from 1 to 500 mM did not cause significant toxicity in NSCs.[32] In addition, ketamine may have dual effects of both increasing and inhibiting human NSCs proliferation and inducing neuronal death in a time- and dose-dependent manner.[33-36]

We have focused on studying the role of intracellular calcium regulation in GA mediated effects on autophagy and neurogenesis. Our previous studies clearly demonstrated that GAs, especially isoflurane, at

low concentrations for short exposure, provide neuroprotection by adequate activation of inositol triphosphate receptors or ryanodine receptors ($InsP_3R$ and RYR) on the membrane of the endoplasmic reticulum (ER). However, GAs at high concentrations for prolonged use cause neurotoxicity.[37-39] Our previous study further demonstrated that isoflurane affects ReNcell CX (human neural progenitor cell (NPC) line, immortalized by retroviral transduction with the c-myc oncogene and derived from the cortical region of the human fetal brain) proliferation and differentiation via differential activation of $InsP_3R$ and RYR and elevation of cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_c$). Isoflurane at a low concentration (0.6%) increased proliferation of these neural progenitor cells, whereas no effect was seen with a higher clinically relevant isoflurane concentration (1.2%). In contrast, isoflurane at high concentration (2.4%) decreased proliferation.[16] Dual effects of cytoprotection and cytotoxicity by general anesthetics have been demonstrated in various *in vitro*[18,40-42] and *in vivo* model systems.[43-45] Our findings suggest that isoflurane may affect ReNcell CX NPC survival and neurogenesis in a dual manner through differential activation of $InsP_3$ and/or RYR.

Propofol has become one of the most widely used intravenous GAs.[46] Twaroski et al found that a high dose of propofol induced developmental toxicity.[47] On the other hand, Jeffrey et al found that propofol at clinically relevant concentrations (<7.1μmol/L) increases neuronal differentiation but is not toxic to hippocampal neural precursor cells *in vitro*.[48] Other studies showed that very low doses of propofol inhibit neuronal arborization *in vitro*[49], and increase the number of neuronal spines on differentiated cells *in vivo*.[50] It is clear that high concentrations of propofol causes cell damage, but the detailed mechanisms remain unclear. We have recently studied the role of intracellular calcium regulation on propofol mediated effects on cell death and neurogenesis and its relationship with propofol-mediated effects on autophagy in ReNcell CX NPCs. Our recent unpublished data suggested that propofol increased $[Ca^{2+}]_c$ via activation of $InsP_3R$/RYR. Like isoflurane, propofol demonstrated the dual effects of promoting and inhibiting ReNcell CX NPC neuronal proliferation and differentiation dose- and time-dependently via differential activation of $InsP_3R$ and/or RYR. This was associated with propofol's effects on autophagy regulation. Propofol induced NPC cytotoxicity in a time- and dose-dependent manner through excessive autophagy via over-activation of $InsP_3R$ and/or RYR. Particularly, high pharmacological concentrations of propofol decreased human NPC cell viability *in vitro* by excessive autophagy through a $Ca^{2+}$-mediated pathway, while clinically relevant doses of propofol enhanced proliferation of NPCs and increased neuronal fate differentiation by a $Ca^{2+}$-related non-autophagy mechanism. Additionally, autophagy biomarker microtubule-associated protein 1 light chain 3 (LC3 II) was absent in autophagy-related gene ATG 5 deficient fibroblasts ($ATG5^{-/-}$), which was not affected by the use of propofol. The effects of propofol on $ATG5^{-/-}$ cell proliferation and survival was also significantly impaired dose-dependently compared to wild type cells, suggesting physiological autophagy can inhibit propofol mediated impairment of cell survival. Both isoflurane and propofol dose-dependently impaired lysosome and autophagy flux and function in knock-in cells carrying the Familiar Alzheimer's Disease (FAD)'s presenilin-1 mutation.

In summary, from our previous studies and our recent data, we have proposed mechanisms for GA mediated dual effects of neuroprotection and neurotoxicity via their effects on cell death by apoptosis, neurogenesis and regulation of autophagy (Figure 1). GAs, such as isoflurane and propofol, at low concentrations for short exposures, may promote physiological autophagy and then inhibit apoptosis but promote neurogenesis, providing neuroprotection. On the other

hand, GAs at high concentrations for prolonged use may induce pathological autophagy, such as impairment of autophagy flux, and then promote apoptosis but inhibit neurogenesis, inducing neurotoxicity. Although it is difficult to provide clear cut on GA concentrations and durations that transform GAs from being neuroprotective to neurotoxic in clinical practice, the principle seems clear that GA exposure should be minimized to avoid its detrimental effects of apoptosis and impairment of neurogenesis.

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Figure 1

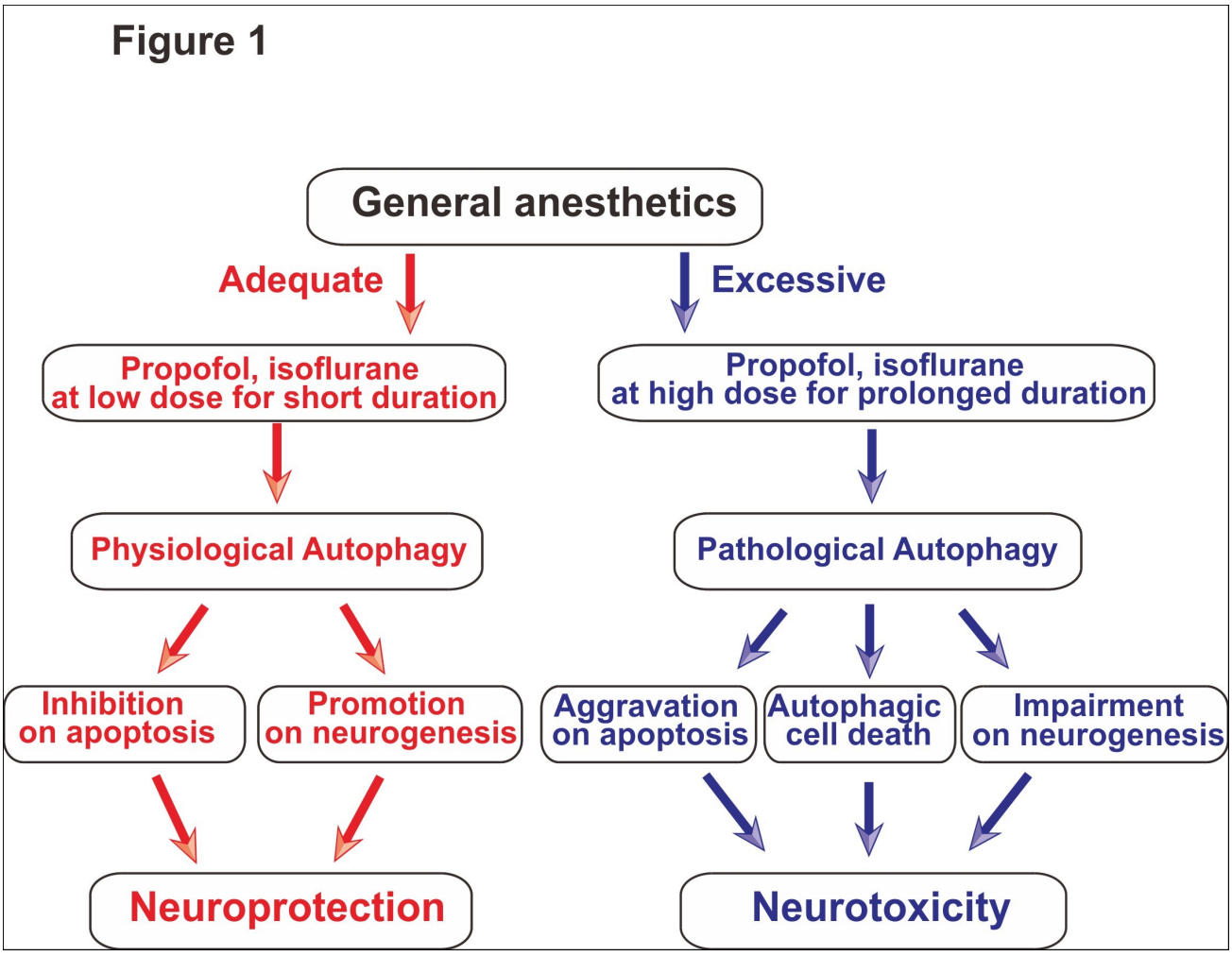


**Figure 1.** Role of autophagy in anesthetic mediated dual effects of neuroprotection and neurotoxicity. General anesthetics at low concentrations for short exposure induce physiological autophagy, which in turn inhibits apoptosis and promotes neurogenesis and eventually provides neuroprotection (left side). On the other hand, general anesthetics at high concentrations for prolonged use result in impairment of autophagy, which in turn promotes apoptosis and inhibits neurogenesis and eventually causes neurotoxicity (right side).

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