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The Role of Calcium Dysregulation in Anesthetic-Mediated Neurotoxicity

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Increasing evidence suggests that general anesthetics, either volatile or IV, can induce cell death by apoptosis in a concentration- and time-dependent manner in different types of cells, including neurons, in various animal models.^{1–8} Neurons in the developing brain are especially vulnerable to anesthetic-mediated neurodegeneration.^{4,9} For example, isoflurane, at clinically relevant concentrations, induces widespread neuronal apoptosis in the developing rat brain with subsequent learning deficits.^{4,9} This cognitive dysfunction after exposure to isoflurane has been shown to persist for several weeks in both adult and aged rats and mice.^{10–12} Commonly used IV anesthetics, such as ketamine and propofol, also induce cell death in cell culture and animal models, including primates.^{5,6,13} The mechanisms of anesthetic-mediated neuroapoptosis are still not clear. One emerging mechanism for anesthetic-mediated neurotoxicity, as illustrated by new work from Zhao et al.¹⁴ and Sinner et al.¹⁵ in this issue of the Journal, is disruption of the intracellular calcium homeostasis resulting in neuronal apoptosis.^{2,3,8,16–19}

The concentration of cytosolic free calcium ($[Ca^{2+}]_c$), one of the most widely used intracellular messengers, is tightly controlled at around 100 nM, about 10,000 fold lower than the extracellular calcium concentration (~1.2 mM) and at least 1000 times lower than the primary intracellular calcium stores in the endoplasmic reticulum (ER) (~300 to 500 μ M).^{20,21} Normal fluctuations in the cytosolic Ca^{2+} concentration are easily sensed by cells and serve to control many cellular physiological functions, including muscle contraction, metabolism, protein synthesis, fertilization, exocytosis, proliferation, differentiation, and neuronal synaptic plasticity.²² On the other hand, disruption of the intracellular calcium homeostasis, particularly due to a persistent and excessive increase in the $[Ca^{2+}]_c$, can induce cell death by apoptosis.²¹ As shown in Figure 1, an abnormal increase in $[Ca^{2+}]_c$ can result from excessive calcium influx from the extracellular space via either voltage-dependent calcium channels, agonist-dependent calcium channels, such as N-methyl-D-aspartate (NMDA) glutamate receptors, or from calcium release from the ER via either the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) or ryanodine receptor (RYR) calcium

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channels.^{20,21} In addition, increased calcium influx from the extracellular space can cause calcium release from the ER, via calcium-induced calcium release by subsequent activation of the InsP₃Rs and/or RYRs. An elevated cytosolic Ca²⁺ concentration can induce apoptosis by: 1) activating apoptotic-related enzymes, such as calpain²³; 2) causing an overload of mitochondrial Ca²⁺ resulting in the collapse of the mitochondrial membrane potential and release of cytochrome C from the mitochondria into the cytosolic space which activates caspase 9 and 3 and subsequent apoptosis^{20,23}; and 3) inhibiting normal protein synthesis as a result of the depletion of ER calcium stores by excessive calcium release via either the InsP₃R and/or the RYR.²³

Previous studies have demonstrated that volatile anesthetics, especially isoflurane, can induce apoptosis by significantly increasing both the cytosolic and mitochondrial calcium concentrations and decreasing the ER calcium concentration by over-activation of the InsP₃R^{2,3,16,17} or RYR calcium channels¹ (Fig. 1). Isoflurane appears to be more potent than sevoflurane or desflurane at causing calcium release from the ER and in promoting aggregation of pathological proteins and apoptosis or neurodegeneration.^{8,17,18} Further studies using the single channel patch clamp technique demonstrated that isoflurane alone can activate InsP₃Rs.²⁴

Consistent with this focus on calcium, Zhao et al.¹⁴ demonstrate that isoflurane causes neuroapoptosis in hippocampus neuronal cell culture via an increase in the intracellular calcium concentration. However, in this case, it is downstream of gamma-aminobutyric acid A (GABA_A) receptor induced activation of L-type calcium channels, and is amplified by calcium-induced calcium release from the ER. The possibility that anesthetic-induced GABA_A receptor activation underlies neurotoxicity and apoptosis has previously been proposed for immature neurons (Fig. 1), because this is predicted to cause the reversed chloride gradient (chloride efflux rather than influx), plasma membrane depolarization, calcium influx and subsequent excitotoxicity.²⁵ This study strongly supports this idea as the initial mechanism of isoflurane-mediated neurotoxicity and fills in the blanks of the subsequent downstream events. These findings are useful, in that they may help develop strategies to minimize anesthetic-mediated neurotoxicity through the use of L-type calcium channel blockers to inhibit excessive calcium influx, as just one example.

In another study in this issue, Sinner et al.¹⁵ show that ketamine, a commonly used IV general anesthetic, also induces neuroapoptosis and disrupts synaptic integrity in a hippocampal cell culture model by significant suppression of intracellular calcium oscillation and a decrease in the expression of the calcium regulatory protein CaMKII. Calcium oscillation in immature neurons plays an important role in regulation of gene expression, neuronal differentiation and synaptogenesis, and therefore neuronal network development and plasticity.²⁶ Because NMDA receptor activation by glutamate in immature neurons contributes to these calcium oscillations,²⁷ ketamine, an NMDA antagonist,⁶ was hypothesized to suppress oscillations, decrease CaMKII and inhibit synapse formation,¹⁵ as observed in Sinner et al's study. On the other hand, ketamine causes an upregulation in NMDA receptor NR1 subunits that may significantly increase cytosolic calcium and subsequent excitotoxicity via dysfunctional glutaminergic neurotransmission.⁶

Together, these two new studies, along with other recent reports,^{1-3,8,17-19} improve our understanding of the molecular pathways involved in anesthetic-mediated neurotoxicity. Ultimately, with agents as diverse as isoflurane and ketamine and molecular targets as different as the GABA_A and NMDA receptor, it is becoming clear that disruption of intracellular calcium homeostasis is a convergent path toward general anesthetic-induced neuroapoptosis. It should be noted that the same mechanisms, inhibition of NMDA glutamate receptors and activation of GABA_A receptors, have also been proposed to

underlie anesthetic-mediated neuroprotection.^{28,29} This startling disparity is probably explained by experimental details, especially the different anesthetic exposure paradigms. For example, short exposures and low concentrations of isoflurane may produce only a modest elevation of cytosolic calcium, resulting in the activation of endogenous neuroprotective mechanisms, such as the Akt pathway.^{30,31} Higher concentrations and longer isoflurane exposures overwhelm these pathways, as suggested by the above discussion and the papers in this issue. However, the result of these anesthetic-triggered pathways will also depend on patient vulnerabilities, whether genetic, pathologic, or developmental. Our continuing challenge is to sort out these issues so that perioperative care can be conducted without adverse long-term consequences.

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Figure 1. The proposed mechanisms of anesthetic-mediated apoptosis via disruption of intracellular calcium homeostasis in immature neurons

The cytosolic calcium concentration $[Ca^{2+}]_c$ can be increased (fat upward arrow) by either calcium influx from the extracellular space through the voltage-dependent calcium channel (VDCC) or N-methyl-D-aspartate (NMDA) glutamate receptor on the plasma membrane (PM) or by calcium release from the endoplasmic reticulum (ER) through either the inositol 1,4,5-trisphosphate receptor (InsP₃R) or the ryanodine receptor (RYR). Calcium releases from the ER through either InsP₃R and/or RYR decreases the ER calcium concentration and increase cytosolic calcium concentrations. Increased $[Ca^{2+}]_c$ also leads to an increase in the mitochondrial calcium concentration. Excessive and abnormal increase in the $[Ca^{2+}]_c$ activates calcium-dependent calpain and activates caspase, resulting in apoptosis. Abnormal elevation of mitochondrial calcium concentration can collapse mitochondrial membrane potential (MtPTP), release cytochrome C from mitochondria to cytosol, activate caspase 9 and 3 and trigger intrinsic pathway apoptosis. Depletion of ER calcium itself may induce apoptosis directly by inhibiting normal protein synthesis. Isoflurane can increase the $[Ca^{2+}]_c$ by activating gamma-aminobutyric acid A (GABA_A) receptors, which depolarize the PM and activate the L-type VDCC and result in calcium influx. Isoflurane also activates the InsP₃Rs and RYRs, causing calcium release from the ER. Excessive and abnormal activation of GABA_A, InsP₃R and RYR by isoflurane may cause the above calcium

dysregulation and cell death by apoptosis. In accordance, the antagonists for the GABA_A receptor (bicuculline), the L-type VDCC (nicardipine), InsP₃R (xestospongin C) and RYR (dantrolene) have all been shown to inhibit isoflurane-induced neuronal apoptosis. NMDA receptor activation plays an important role in formation and regulation of cytosolic calcium oscillation, which is important for gene regulation, neuronal differentiation and synaptogenesis in immature neurons. Ketamine inhibits the NMDA receptor and therefore suppresses cytosolic calcium oscillation, which results in the damage of synaptogenesis and neuronal apoptosis in immature neurons.