Mechanisms of Anesthesia Induced Neurodegeneration: Roles of IP$_3$ Receptors

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Anesthesia: A medical mainstay re-examined
Some worry about brain cell death in studies of young animals. Human trials are planned.

Possible link between exposure to anesthesia and behavioral and developmental disorders before the age of 3 who had received general anesthesia.
Evidence of Anesthesia Toxicity (1)

Cell and Tissue Culture:

Cell damage in variety of cells: lymphocytes, neuroglioma, liver cells, gingival fibroblasts, PC12 cells, cortical and striatal neurons, hippocampal slices etc.

Neurons featured with Alzheimer’s disease (AD) and Huntington’s disease (HD) are more vulnerable.
Evidence of Anesthesia Toxicity (2)

**Animal Studies:**

Wide spread neurodegeneration in the rodent developing brains

Persistent memory and learning impairment in adult and aged brains

Increased plaque-load in the Alzheimer’s Disease transgenic mice
Evidence of Anesthesia Toxicity (3)

Clinical Studies:

Link between exposure to anesthesia and behavioral and developmental disorders before the age of 3

Duration of Anesthesia is a risk factor in POCD

Inverse relationship between surgical experience and age of onset of AD

Anesthesia unmask symptoms of Parkinson’s disease, Anesthesiologists were more likely to die from Parkinson’s disease than age-matched internists
Proposed Mechanisms for Anesthesia Neurotoxicity

- GABA receptor activation: Excitotoxicity in the developing brains
- NMDA receptor antagonism: Withdrawal of trophic factors in the developing brains
- Increased β-amyloid production and aggregation: Alzheimer pathology
- Disruption of intracellular calcium homeostasis: General mechanism
Intracellular Calcium Homeostasis

IP$_3$ Receptor: Physiology and Apoptosis

Coordinated rhythmic movements (Flight, balancing on a rotating rod)

Physiology

Neuronal activity

InsP$_3$R

Normal Ca$^{2+}$ release

Abnormal Ca$^{2+}$ release

Pathology

Neural death

Un-coordinated rhythmic movements

(Huntington’s Chorea, SCA-1)

Behavior

Circuit

Molecule

Circuit

Behavior

IP$_3$ Receptors and Apoptosis

Hanson et al., Current Biology, 2004, 14: R933-35
Hypothesis

Inhalational anesthetics induce apoptosis by causing excessive calcium release from ER via direct activation of IP₃ receptors
Isoflurane Induce Apoptosis in DT40 WT but not IP₃R TKO Cells Dose-Dependently

Yang et al., Anesthesiology, 2008, 108:251-60
Isoflurane Induce Apoptosis in DT40 WT but not IP₃R TKO Cells Dose-Dependently

Yang et al., Anesthesiology, 2008, 108: 251-60
Isoflurane Induce Apoptosis in DT40 WT but not IP₃R TKO Cells Time-Dependently

Yang et al., Anesthesiology, 2008, 108:251-60
Isoflurane Induced Greater Apoptosis Than Sevoflurane or Desflurane only in DT40 WT Cells

Yang et al., Anesthesiology, 2008, 109: 243-50
Isoflurane Induced Significant Greater Decrease of ER And Increase of Cytosolic and Mitochondrial Calcium Than Sevoflurane or Desflurane
Isoflurane Decreases Mitochondrial Membrane Potential Only in DT40 WT But Not IP₃R TKO Cells

![Graph showing optical density over time for different cell types](image_url)
Suppression of IP$_3$R Expression Inhibited Isoflurane-Mediated Neuronal Damage

**Figures:**

- **MTT (% Control):**
  - Con
  - Con+Iso
  - IP$_3$ siRNA+Iso

- **LDH (% Control):**
  - Con
  - Con+Iso
  - IP$_3$ siRNA+Iso

**Rat Primary Cortical Neurons**
Suppression of IP$_3$R Activity Inhibited Isoflurane-Mediated Neuronal Damage

Rat Hippocampus Neurons  Mouse Cortical Neurons
Suppression of IP₃R Activity Inhibited Isoflurane-Mediated Elevation of Cytosolic Calcium

Wild Type mice CTX treated with 0.7mM Iso

- Ca²⁺+Iso (n=5)
- No Ca²⁺+EGTA+Iso (n=4)
- No Ca²⁺+EGTA+Xc+Iso (n=5)
- Ca²⁺+Xc+Iso (n=5)

Peak [Ca²⁺] Elevation (% over Baseline)
Isoflurane Activated IP$_3$ Receptors

DT40 cells with type 3 IP$_3$R
### Channel detection rate of isoflurane-activated InsP3R

<table>
<thead>
<tr>
<th>Isoflurane (0.4 mM)</th>
<th>IP$_3$ (μM)</th>
<th>Heparin (100μg/ml)</th>
<th>Xestospongin C (1μM)</th>
<th>Channel Detection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0/14 (0)</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>30/60 (0.5)</td>
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<tr>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>13/15 (0.87)</td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>18/20 (0.9)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>1/10 (0.1)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>1/10 (0.1)</td>
</tr>
</tbody>
</table>

**InsP$_3$R, Inositol 1,4,5-Trisphosphate Receptors**
Anesthetics and Neurodegeneration

Effects of Isoflurane on Blood Glucose Level in New Born Rodents

Blood Glucose

7 day old Rat blood glucose levels

- Control
- 1% Isoflurane

*** p<0.001
Suppression of IP3R Activity Inhibited Isoflurane-Induced Apoptosis in the Hippocampus
Xestospongin C Inhibited Isoflurane-Induced Apoptosis in Cerebral Cortex

17KD

Cleaved Caspase-3

42KD

Beta Actin

**++

Cleaved Caspase-3 (% Control)

Control, Sham, Xc only, 1%ISO, V+1%ISO, Xc+1%ISO

**

++
Suppression of IP3R Activity Inhibited Isoflurane-Induced Activation of BACE

- Control
- Sham
- Xc only
- V+1.5%ISO
- Xc+1.5%ISO

BACE (70KD) vs Beta Actin (42KD)

Graph showing BACE (% over Control) for different groups:

- Control
- Sham
- Xc only
- V+1.5%ISO
- Xc+1.5%ISO

Significant increase in BACE expression in V+1.5%ISO and Xc+1.5%ISO compared to Control and Sham.

*** indicates statistical significance.
The Morris Water Maze: A sensitive test of rodent memory, learning and behavior.

- Cued test (Swimming ability)
- Place test (Learning and memory)
- Probe test (Memory)
Suppression of IP₃ Receptors Inhibited Isoflurane-Mediated Learning Impairment
Summary

- Isoflurane induced cell apoptosis by causing abnormal calcium release from the ER via direct activation of IP$_3$ receptor in tissue cultures and newborn rats hippocampus and cerebral cortex.

- Isoflurane dose-dependently impaired learning in rats, which may be associated with over activation of IP$_3$ receptors.
Anesthesia and Alzheimer’s Disease

- Anesthetics increase β-amyloid production and aggregation.
- Isoflurane induced β-amyloid aggregation and apoptosis form a vicious cycle.
- Intracellular calcium dysregulation may play a critical role in amyloidosis and neurodegeneration in AD.
- Isoflurane worsens neurodegeneration in AD by disrupting intracellular calcium homeostasis.
Mechanism of Ca\textsuperscript{2+} Disruption in Alzheimer’s Disease by Presenilin Regulation of InsP\textsubscript{3} Receptor Channel Gating

King-Ho Cheung,\textsuperscript{1} Diana Shineman,\textsuperscript{2,4} Marioly Müller,\textsuperscript{1} César Cárdenas,\textsuperscript{3} Lijuan Mei,\textsuperscript{1} Jun Yang,\textsuperscript{1} Taisuke Tomita,\textsuperscript{5} Takeshi Iwatsubo,\textsuperscript{6} Virginia M.-Y. Lee,\textsuperscript{2,4} and J. Kevin Foskett\textsuperscript{1,3,*}

The Common Inhalational Anesthetic Isoflurane Induces Apoptosis via Activation of Inositol 1,4,5-Trisphosphate Receptors

Huafeng Wei, M.D., Ph.D.,* Ge Liang, M.D.,† Hui Yang, M.D.,‡ QiuJun Wang, M.D.,§ Brian Hawkins, Ph.D.,‖ Muniswamy Madesh, Ph.D.,# Shouping Wang, M.D.,** Roderic G. Eckenhoff, M.D.††

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A Presenilin-1 Mutation Renders Neurons Vulnerable to Isoflurane Toxicity

**Diagrams:**
- Lactate Dehydrogenase (LDH) levels in WT, Vector, and L286V under isoflurane and sevoflurane conditions.
- Data show significantly increased LDH levels in L286V with isoflurane.

**References:**
A Presenilin-1 Mutation Renders Neurons Vulnerable to Isoflurane Toxicity

Alzheimer’s Presenilin-1 mutation render cell vulnerable to anesthetics mediated toxicity by increased calcium release from the ER via over activation of IP$_3$ receptors.

Isoflurane is more potent than sevoflurane or desflurane to induce cell damage by its greater potency to disrupt Intracellular calcium homeostasis
Isoflurane preconditioning inhibited isoflurane-induced neurotoxicity

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![Graph showing LDH (% Control) for different treatments and DIV stages.]

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