The Common Inhalational Anesthetic Sevoflurane Induces Apoptosis and Increases β-Amyloid Protein Levels

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Objective: To assess the effects of sevoflurane, the most commonly used inhalation anesthetic, on apoptosis and β-amyloid protein (Aβ) levels in vitro and in vivo.

Subjects: Naive mice, H4 human neuroglioma cells, and H4 human neuroglioma cells stably transfected to express full-length amyloid precursor protein.

Interventions: Human H4 neuroglioma cells stably transfected to express full-length amyloid precursor protein were exposed to 4.1% sevoflurane for 6 hours. Mice received 2.5% sevoflurane for 2 hours. Caspase-3 activation, apoptosis, and Aβ levels were assessed.

Results: Sevoflurane induced apoptosis and elevated levels of β-site amyloid precursor protein–cleaving enzyme and Aβ in vitro and in vivo. The caspase inhibitor Z-VAD decreased the effects of sevoflurane on apoptosis and Aβ. Sevoflurane-induced caspase-3 activation was attenuated by the γ-secretase inhibitor L-685,458 and was potentiated by Aβ. These results suggest that sevoflurane induces caspase activation which, in turn, enhances β-site amyloid precursor protein–cleaving enzyme and Aβ levels. Increased Aβ levels then induce further rounds of apoptosis.

Conclusions: These results suggest that inhalational anesthetic sevoflurane may promote Alzheimer disease neuropathogenesis. If confirmed in human subjects, it may be prudent to caution against the use of sevoflurane as an anesthetic, especially in those suspected of possessing excessive levels of cerebral Aβ.


Excessive β-amyloid protein (Aβ) accumulation is a major pathological hallmark of Alzheimer disease (AD). β-amyloid protein is produced via serial proteolysis of the amyloid precursor protein (APP) by aspartyl protease β-site APP-cleaving enzyme (BACE; β-secretase) and γ-secretase. β-site APP-cleaving enzyme cleaves APP to generate a 99-residue membrane-associated C-terminus fragment (APP-C99). This fragment is further cleaved by γ-secretase to release the 4-kDa Aβ and APP intracellular domain. Increasing evidence suggests a role for caspase activation and apoptosis in AD neuropathogenesis. Recent studies suggested that caspase activation and apoptosis may enhance BACE levels to facilitate APP processing, leading to increases in Aβ levels. An estimated 200 million patients worldwide have surgery with anesthesia each year. Several studies showed an odds ratio of between 1.2 and 1.6 for the association of previous general anesthesia/surgery and AD. Moreover, the age of onset of AD has been inversely correlated with cumulative exposure to general anesthesia prior to 50 years of age. A recent study illustrated that patients having coronary artery bypass graft surgery with general anesthesia are at greater risk for the emergence of AD than those having percutaneous transluminal coronary angioplasty with local anesthesia. Though there have been no conclusive studies to strongly suggest an association between anesthesia and AD, there have been studies suggesting that anesthetics such as isoflurane may promote AD neuropathogenesis in vitro and in vivo. A recent study showed that an insult from a middle cerebral artery occlusion for 2 hours in rats caused temporary increases in APP and Aβ staining in a brain area near the ischemic region as well as long-term (up to 9 months) APP and Aβ deposits in a brain area distant from the ischemic region. These findings suggest that...
a transient insult, eg, ischemia or anesthesia with isoflu- 
rane, could lead to secondary and persistent brain inju-
dies. However, whether inhalation anesthetics other than 
isofluorane can promote AD neuropathogenesis remains 
unknown. We therefore set out to determine the effects of sevo-
flurane, currently the most commonly used inhalational an-
esthetic, on caspase activation, apoptosis, APP processing, 
and levels of BACE and Aβ in H4 human neuroglioma cells 
as well as in naive mice.

**METHODS**

**CELL LINES**

We used H4 human neuroglioma cells (naive H4 cells) and H4 
human neuroglioma cells stably transfected to express full-
length (FL)–APP (H4-APP cells). All cell lines were cultured in 
Dulbecco Modified Eagle Medium (high glucose) containing 9% 
heat-inactivated fetal calf serum, 100-U/mL penicillin, 100-
µg/mL streptomycin, and 2mM L-glutamine. Stably transfected 
H4 cells were additionally supplemented with 200-µg/mL G418.

**CELL TREATMENT**

The cells were treated with 21% oxygen, 5% carbon dioxide, 
and 4.1% sevoflurane (2 minimum alveolar concentration) for 
6 hours, during which time the cells were incubated in serum-
free cell culture media, as described by Xie et al.24 21% O2, 5% 
CO2, and 4.1% sevoflurane were delivered from an anesthesia 
machine to a sealed plastic box in a 37°C incubator containing 
6-well plates seeded with 1 million cells in 1.5-mL cell culture 
media. A Datex infrared gas analyzer (Puritan-Bennett, Tewks-
bury, Massachusetts) was used to continuously monitor the con-
centrations of delivered CO2, O2, and sevoflurane. In the in-
teraction studies, the cells were treated with Z-VAD (100µM), 
AB40 (7.5µM) plus AB42 (7.5µM), and L-685,458 (0.5µM) 1 
hour before the treatment with 4.1% sevoflurane. Control 
conditions included 3% CO2 plus 21% O2, which did not affect 
caspase-3 activation, cell viability, APP processing, or Aβ gen-
eration (data not shown).

**CELL LYSIS AND PROTEIN AMOUNT QUANTIFICATION**

Cell pellets were detergent-extracted on ice using immuno-
precipitation buffer (10mM Tris-hydrochloride [HCl]; pH, 7.4; 
150mM sodium chloride [NaCl]; 2mM EDTA; 0.5% Nonidet 
P-40) plus protease inhibitors (1-µg/mL aprotonin, 1-µg/mL leu-
peptin, and pepstatin A). The lysates were collected, centrifuged 
of 12,000 revolutions per minute (rpm) for 10 minutes, and quantified for total proteins by the BCA (bicinchoninic acid) 
protein assay kit (Pierce, Iselin, New Jersey).

**MOUSE ANESTHESIA AND TREATMENT**

The animal protocol was approved by the Standing Commit-
tee on Animals at Massachusetts General Hospital. Mice (C57/ 
BL6, aged 3–9 months; The Jackson Laboratory, Bar Harbor, 
Maine) were randomly assigned to an anesthesia or control 
group. Mice randomized to the anesthesia group received 2.5% 
sevoflurane in 100% O2 for 2 hours in an anesthetizing cham-
ber, whereas the control group received 100% O2 at an iden-
tical flow rate for 2 hours in an identical chamber. The mice 
breathed spontaneously, and concentrations of anesthetic and 
O2 were measured continuously (Datex, Tewksbury, Massa-
chussetts). The temperature of the anesthetizing chamber was 
controlled to maintain a mean (SD) rectal temperature in the 
animals of 37°C (0.5°C). Mean arterial blood pressure was mea-
sured noninvasively using a tail cuff (Kent Scientific Corpora-
tion, Torrington, Connecticut) in the anesthetized mice. An-
esthesia was terminated by discontinuing sevoflurane and placing 
animals in a chamber containing 100% O2 until 20 minutes af-
ter the return of their righting reflex. They were then returned 
to individual home cages until they were humanely killed. Mice 
were killed by decapitation 6, 12, and 24 hours after sevoflu-
rane anesthesia. The brain was removed rapidly and the pre-
ternal cortex was dissected out and frozen in liquid nitrogen 
for subsequent processing to determine caspase activation and 
levels of FL-APP, APP-C99, APP-C83, BACE, and Aβ.

**BRAIN TISSUE LYSIS AND PROTEIN AMOUNT QUANTIFICATION**

The harvested brain tissues were homogenized on ice using 
immunoprecipitation buffer (10mM Tris-HCl; pH, 7.4; 150mM 
NaCl; 2mM EDTA; 0.5% Nonidet P-40) plus protease inhibi-
tors (1-µg/mL aprotonin, 1-µg/mL leupeptin, 1-µg/mL pep-
statin A). The lysates were collected, centrifuged at 12,000 rpm 
for 10 minutes, and quantified for total proteins by BCA pro-
tein assay kit.

**WESTERN BLOT ANALYSIS**

The cells and brain tissues were harvested at the end of the ex-
periment and were subjected to Western blot analysis, as de-
scribed by Xie et al.25 Antibodies Aβ717 (1:2000; Sigma, St Louis, 
Missouri), C66 (1:1000; generous gift of Dora Kovacs, PhD, at 
Massachusetts General Hospital and Harvard Medical School), 
and anti–β-actin (1:5000; Sigma) were used to visualize FL-
APP (110 kDa), APP-C83 (12 kDa), APP-C99 (10 kDa), and 
BACE (42 kDa), respectively. A caspase-3 antibody (1:1000; 
Cell Signaling Technology Inc, Beverly, Massachusetts) was used 
to recognize the caspase-3 fragment (17-20 kDa) resulting from 
cleavage at asparagine position 175 and caspase-3 FL (35-40 kDa).
Rabbit polyclonal anti–BACE1 antibody ab2077 (1:1000; Ab-
cam, Cambridge, Massachusetts) was used to detect the pro-
tein levels of BACE (65 kDa). The quantification of Western 
blots was performed in 2 steps, as described by Xie et al.25 
Briefly, the intensity of signals was analyzed by using an image 
program from the National Institutes of Health (NIH ImageJ; 
Bethesda, Maryland). First, we used levels of β-actin to nor-
malize (eg, determining ratio of FL-APP amount to β-actin 
amount) the levels of FL-APP, APP-C83, APP-C99, FL-
caspase-3, caspase-3 fragment, BACE, and Aβ to control for the 
loading differences in total protein amounts. Second, we pre-
sented the changes in the levels of FL-APP, APP-C83, APP-
C99, FL-caspase-3, caspase-3 fragment, Aβ, and BACE in the 
cells or animals treated with sevoflurane, Z-VAD, Aβ, and 
L-685,458 as the percentage of those in the cells or animals 
treated with controls. We refer to 100% caspase-3 activation, 
FL-APP, APP-C83, APP-C99, Aβ, and BACE in this article as 
control levels for the purpose of comparison with experi-
mental conditions.

**QUANTIFICATION OF Aβ USING SANDWICH ELISA ASSAY**

Secreted Aβ was measured with a sandwich enzyme-linked im-
munosorbent assay (ELISA) assay using an Aβ measurement 
kit (Invitrogen, Carlsbad, California) and by the Aβ ELISA Core 
Facility at the Center for Neurological Diseases, Brigham and 
Women’s Hospital, Harvard Medical School, Boston, Massa-
Specifically, 96-well plates were coated with mouse monoclonal antibodies specific to Aβ40 (2G3) or Aβ42 (21F12). Following blocking with Block Ace, wells were incubated overnight at 4°C with test samples of conditioned cell culture media; anti-Aβ (α-Aβ-HR1) conjugated to horseradish peroxidase was then added. Plates were then developed with tetramethylbenzidine reagent and well absorbance was measured at 450 nm. Levels of Aβ in test samples were determined by comparison with the signal from unconditioned media spiked with known quantities of Aβ40 and Aβ42.
IMMUNOBLOT DETECTION OF Aβ

Brain samples were homogenized (150mM NaCl with protease inhibitor cocktail in 50mM Tris; pH, 8.0) and centrifuged (300 000g for 15 minutes), and the supernatant was removed. The pellet was then resuspended by sonication and incubated for 15 minutes in homogenization buffer containing 1% sodium dodecyl sulfate (SDS). Following pelleting of insoluble material (16 000 × g for 13 minutes), the SDS extract was electrophoresed on SDS–polyacrylamide gel electrophoresis (SDS–PAGE) (4%-12% Bis-Tris polyacrylamide gel; Invitrogen, Carlsbad, California), blotted to a polyvinylidene difluoride membrane and probed with a 1:200 dilution of 6E10 (Signet, Berkeley, California).

CELL APOPTOSIS ASSAY

Cell apoptosis was assessed by a cell death detection ELISA kit (Roche, Palo Alto, California), which assays cytoplasmic histone-associated DNA fragmentation associated with cellular apoptosis.

STATISTICS

Data were expressed as mean (SD). The number of samples varied from 3 to 10, and the samples were normally distributed. We used a 2-tailed t test to compare the difference between the experimental groups. P < .05 and P < .01 were considered statistically significant.

RESULTS

SEVOFLURANE INDUCES CASPASE-3 ACTIVATION AND APOPTOSIS, ALTERS APP PROCESSING, AND INCREASES LEVELS OF BACE AND Aβ IN H4-APP CELLS

Sevoflurane has previously been reported to induce cytotoxicity in various cell lines.27-32 We have previously reported that isoflurane can induce caspase activation and apoptosis and increase Aβ levels in H4-APP cells.19,24,33 We therefore asked whether the currently most commonly used inhalational anesthetic, sevoflurane, also affects apoptosis and APP processing in H4-APP cells. The H4-APP cells were treated with a clinically relevant concentration (4.1%) of sevoflurane for 6 hours. Because caspase-3 activation is one of the final steps of cellular apoptosis,34 we assessed the effects of sevoflurane on caspase-3 activation by quantitative Western blot analysis. Sevoflurane treatment led to caspase-3 activation (Figure 1A), as evidenced by increased ratios of cleaved (activated) caspase-3 fragment (17-19 kDa) to FL-caspase-3 (35-40 kDa). Quantification of the Western blots, based on the ratio of caspase-3 fragment to FL-caspase-3, revealed that the 4.1% sevoflurane treatment (Figure 1B) led to a 275% increase in caspase-3 activation compared with control cells (Figure 1B) (P = .001). Given that caspase-3 activation alone cannot represent apoptotic cell damage,35 we also assessed the effects of sevoflurane on cellular apoptosis by detecting cytoplasmic histone-associated DNA fragmentation using a cell death detection ELISA kit. We showed that treatment with 4.1% sevoflurane (Figure 1C) led to cellular apoptosis compared with control conditions (Figure 1C) (100% vs 134%; P < .001). We also found that sevoflurane treat-

Table. Blood Pressure and Blood Gas in Mice Under Control Condition and Sevoflurane Anesthesia

<table>
<thead>
<tr>
<th>Mean (SD) Blood or Gas Pressure, mm Hg</th>
<th>Controlb</th>
<th>2.5% Sevofluraneb</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP</td>
<td>107 (2.5)</td>
<td>95.4 (3.3)</td>
</tr>
<tr>
<td>pH</td>
<td>7.31 (0.04)</td>
<td>7.33 (0.04)</td>
</tr>
<tr>
<td>PVO2</td>
<td>226 (55)</td>
<td>234 (75)</td>
</tr>
<tr>
<td>PCO2</td>
<td>42.2 (4.1)</td>
<td>43.8 (3.1)</td>
</tr>
</tbody>
</table>

Abbreviations: MAP, mean arterial pressure; PVO2, mixed venous oxygen tension; PCO2, mixed venous carbon dioxide tension.

SEVOFLURANE INDUCES CASPASE-3 ACTIVATION AND INCREASES LEVELS OF BACE AND Aβ IN NAIVE MICE

We then assessed the in vivo relevance of these effects of sevoflurane. Naive mice were given anesthesia with 2.5% sevoflurane for 2 hours. The mice exhibited no significant effects on blood pressure or blood gas (Table). Anesthesia with 2.5% sevoflurane for 2 hours led to caspase-3 activation for 6 (Figure 2A) and 12 hours (Figure 2B) after anesthesia. Quantification of these results revealed that sevoflurane anesthesia led to a 150% (Figure 2B) (P = .04) and 159% (Figure 3B) (P = .002) increase in the ratio of caspase-3 fragment to FL-caspase-3 levels 6 and 12 hours, respectively, but not 24 hours (data now shown) after anesthesia compared with control conditions. Anesthesia with 2.5% sevoflurane for...
2 hours also led to poly (adenosine diphosphate-ribose) polymerase (PARP) cleavage, as evidenced by a 149% (Figure 2D) \((P = .01)\) and 140% (Figure 3D) \((P = .02)\) increase in levels of PARP fragment at 6 (Figure 2C) and 12 hours (Figure 3C), respectively, following anesthesia. Finally, sevoflurane anesthesia increased levels of caspase-cleaved APP–N (N-terminal)–fragment (Figure 2, E and F, a 135% increase) \((P < .001)\) 6 hours after anes-

Figure 2. Anesthesia with 2.5% sevoflurane for 2 hours induces caspase-3 activation and increases β-site amyloid precursor protein (APP)–cleaving enzyme (BACE) levels 6 hours after anesthesia in naive mice. A, Sevoflurane anesthesia induces caspase-3 cleavage (activation) by decreasing full-length (FL)–caspase-3 levels and increasing caspase-3 fragment compared with control conditions in Western blot analysis. B, Quantification of the Western blots shows that sevoflurane anesthesia increases the ratio of caspase-3 fragment to FL–caspase-3 levels compared with control conditions. C, Sevoflurane anesthesia induces poly–(adenosine diphosphate–ribose) polymerase (PARP) cleavage by increasing PARP fragment compared with control conditions in Western blot analysis. D, Quantification of the Western blots shows that sevoflurane anesthesia increases levels of PARP fragment compared with control conditions. E, Sevoflurane anesthesia increases levels of caspase-cleaved APP–N-fragment (APP-N-caspase fragment) compared with control conditions in Western blot analysis. F, Quantification of the Western blots shows that sevoflurane anesthesia increases levels of APP–N-caspase fragment compared with control conditions. G, Sevoflurane anesthesia increases BACE levels compared with control conditions in Western blot analysis. H, Quantification of the Western blots shows that sevoflurane anesthesia increases BACE levels compared with control conditions. There is no significant difference in amounts of β-actin in control conditions or sevoflurane-treated mouse brain tissue. Data are means (SD); \(n = 3\) to 6 for each experimental group; the \(t\) test was used to compare the difference between control conditions and sevoflurane anesthesia; values are significant at * \(P < .05\) and ** \(P < .01\).
thesia compared with control conditions. Collectively, these results suggest that sevoflurane can induce caspase activation in the brain tissues of naive mice for up to 12 hours after anesthesia.

We next assessed whether sevoflurane can elevate levels of BACE and Aβ in the mouse brain. Western blot analyses revealed that exposure to 2.5% sevoflurane anesthesia increased levels of BACE for 6 (Figure 2G), 12

Figure 3. Anesthesia with 2.5% sevoflurane for 2 hours induces caspase activation and increases levels of β-site amyloid precursor protein (APP)–cleaving enzyme (BACE) and β-amyloid protein (Aβ) 12 hours after anesthesia. A, Sevoflurane anesthesia induces caspase-3 cleavage (activation) by decreasing full-length (FL)–caspase-3 levels and increasing caspase-3 fragment compared with control conditions in Western blot analysis. B, Quantification of the Western blots shows that sevoflurane anesthesia increases the ratio of caspase-3 fragment to FL-caspase-3 levels compared with control conditions. C, Sevoflurane anesthesia induces poly–(adenosine diphosphate–ribose) polymerase (PARP) cleavage by increasing PARP fragment compared with control conditions in Western blot analysis. D, Quantification of the Western blots shows that sevoflurane anesthesia increases PARP fragment levels compared with control conditions in Western blot analysis. E, Sevoflurane anesthesia increases BACE levels compared with control conditions in Western blot analysis. F, Quantification of the Western blot shows that sevoflurane anesthesia increases BACE levels compared with control conditions. G, Sevoflurane anesthesia increases Aβ levels compared with control conditions in Western blot analysis. Synthetic Aβ was used as a marker to identify position of Aβ in Western blot analysis. H, Quantification of the Western blot shows that sevoflurane anesthesia increases Aβ levels compared with control conditions. There is no significant difference in amounts of β-actin in control conditions or sevoflurane-treated mouse brain tissue. Data are mean (SD); n=3 to 6 for each experimental group; the t test was used to compare the difference between control conditions and sevoflurane anesthesia; values are significant at *P < .05 and **P < .01.
duced caspase-3 activation, which was attenuated by treatment with 4.1% sevoflurane for 6 hours. Sevoflurane increased β-actin (100µM), a caspase inhibitor, for 1 hour, followed by treatment with Z-VAD sulfoxide treatment (Figure 5D). Treatment with Z-VAD attenuated the effects of sevoflurane on levels of APP-C83 (44% reduction vs no reduction; P = .01) and APP-C99 (54% reduction vs 24% reduction; P = .04) compared with dimethyl sulfoxide treatment (Figure 5D).

Sevoflurane treatment, but not Z-VAD treatment, alone, significantly increased Aβ levels in the conditioned media compared with control conditions (Figure 5E). Treatment with Z-VAD attenuated the sevoflurane-induced increase in Aβ levels (152% vs 98%; P = .001) (Figure 5E). We have also found that treatment with 4.1% sevoflurane for 6 hours can induce caspase-3 activation without detectable changes in APP processing and Aβ levels in H4 naive cells (data not shown). Collectively, these results suggest that sevoflurane-induced al-

ATTENUATION OF SEVOFLURANE-INDUCED ALTERATIONS IN APP PROCESSING AND Aβ GENERATION BY THE CASPASE INHIBITOR Z-VAD IN H4-APP CELLS

Given that sevoflurane can alter APP processing and increase Aβ levels in H4-APP cells, we next asked whether these effects are dependent on caspase activation. For this purpose, we incubated H4-APP cells with Z-VAD (100µM), a caspase inhibitor, for 1 hour, followed by treatment with sevoflurane for 6 hours. Sevoflurane induced caspase-3 activation, which was attenuated by treatment with Z-VAD (Figure 5A). Quantification of the Western blots revealed that treatment with sevoflurane and Z-VAD reduced caspase-3 activation from 210% to 107% (Figure 5B) (P = .03). Treatment with Z-VAD also attenuated sevoflurane-induced alterations in APP processing and Aβ generation. As can be seen in Figure 5, APP immunoblotting revealed that sevoflurane treatment decreased protein levels of FL-APP, APP-C83, and APP-C99 compared with control conditions. While Z-VAD treatment alone had no effect, the Z-VAD treatment attenuated the sevoflurane-induced changes in FL-APP, APP-C83, and APP-C99 (Figure 5C). Quantification of the Western blots showed that treatment with sevoflurane led to 34% (P = .004), 44% (P = .007), and 54% (P = .004) reductions in levels of FL-APP, APP-C83, and APP-C99, respectively, compared with control conditions (Figure 5D).

Figure 4. Anesthesia with 2.5% sevoflurane for 2 hours increases levels of β-site amyloid precursor protein–cleaving enzyme (BACE) and β-amyloid protein (Aβ) 24 hours following anesthesia. A, Sevoflurane anesthesia increases BACE levels compared with control conditions in Western blot analysis. B, Quantification of the Western blot shows that sevoflurane anesthesia increases BACE levels compared with control conditions. C, Sevoflurane anesthesia increases Aβ levels compared with control conditions. There is no significant difference in amounts of β-actin in control conditions or sevoflurane-treated mouse brain tissue. Data are mean (SD); n = 3 to 6 for each experimental group; the t test was used to compare the difference between control conditions and sevoflurane anesthesia; values are significant at *P < .05 and **P < .01.
Figure 5. The caspase inhibitor Z-VAD inhibits caspase-3 activation and attenuates sevoflurane-induced increases in β-amyloid protein (Aβ) in H4 amyloid precursor protein (H4-APP) cells. A, Western blot analysis shows that 4.1% sevoflurane induces caspase-3 cleavage (activation) compared with control conditions or Z-VAD (100µM) treatment. B, Quantification of the Western blot shows that sevoflurane treatment increases caspase-3 activation compared with control conditions or the Z-VAD (100µM) treatment. The Z-VAD treatment inhibits the sevoflurane-induced increases in Aβ levels. C, Z-VAD inhibits the sevoflurane-induced increases in APP-C83 and APP-C99 compared with control conditions or Z-VAD treatment, normalized to β-actin levels. D, Quantification of the Western blot shows that 4.1% sevoflurane decreases the protein levels of full-length (FL–APP) and APP–C-terminal fragments (APP-C83 and APP-C99) compared with control conditions or Z-VAD (100µM) treatment in Western blot analysis. Treatment with Z-VAD inhibits the sevoflurane-induced decreases in levels of APP-C83 and APP-C99. There is no significant difference in the amounts of APP-C83 and APP-C99 between control conditions and 4.1% sevoflurane treatment. Values are significant at *P<.05, **P<.01, and the difference between dimethyl sulfoxide (DMSO) treatment and Z-VAD treatment at †P<.05 and ‡P<.01.

Figure 6. A and B) Caspase-3 activation in H4 naive cells. Addition of 7.5µM Aβ40 plus 7.5µM Aβ42 further potentiated sevoflurane-induced caspase-3 activation in H4 naive cells (460% vs 1249%; P=.01) (Figure 6, A and B). C, Caspase-3 activation induced by sevoflurane in H4-APP cells was reduced by L-685,458 (13% vs 160%; P=.02) (Figure 6, C and D). However, L-685,458 alone did not significantly increase caspase-3 activation compared with control conditions (Figure 6, C and D). These results suggest that Aβ can further potentiate the effects of sevoflurane on caspase activation.

To assess the possibility that sevoflurane-induced increases in Aβ levels can lead to further caspase activation beyond that induced by sevoflurane, we next asked whether the γ-secretase inhibitor L-685,458 could attenuate, but Aβ could potentiate, sevoflurane-induced caspase-3 activation in H4-APP and naive H4 cells. Sevoflurane treatment led to a 460% increase in caspase-3 activation over control conditions (Figure 6, A and B) (P=.02) in H4 naive cells. Addition of 7.5µM Aβ40 plus 7.5µM Aβ42 further potentiated sevoflurane-induced caspase-3 activation in H4 naive cells (460% vs 1249%; P=.01) (Figure 6, A and B). Caspase-3 activation induced by sevoflurane in H4-APP cells was reduced by L-685,458 (13% vs 160%; P=.02) (Figure 6, C and D). However, L-685,458 alone did not significantly increase caspase-3 activation compared with control conditions (Figure 6, C and D). These results suggest that Aβ can further potentiate the effects of sevoflurane on caspase activation.
We have previously shown that the commonly used inhalational anesthetic isoflurane can induce caspase activation and apoptosis and increase Aβ generation in H4-APP cells.19,24,33,37 However, it is unknown whether other inhalational anesthetics can also promote AD neuropathogenesis. Here we assessed the effects of sevoflurane, desflurane plus hypoxia, and ischemia on caspase activation, apoptosis, APP processing, and Aβ levels in H4 cells and naive mice.

First, we found that sevoflurane can induce caspase-3 activation and apoptosis in H4-APP cells. Given that isoflurane, desflurane plus hypoxia, and ischemia have all been shown to enhance BACE and Aβ levels subsequent to caspase activation,15,28,34 we next asked whether sevoflurane has similar effects. We were able to show that a clinically relevant regimen of sevoflurane anesthesia enhanced BACE levels, altered APP processing, and increased Aβ levels in H4-APP cells. These results, along with previous findings, indicate that AD neuropathogenesis can be promoted by multiple inhalational anesthetics, suggesting that it may be prudent to carry out systematic and comprehensive assessment of the effects of all currently used inhalational anesthetics on AD-related neuropathogenic events.

Because the above findings were in vitro–based, we next sought in vivo confirmation of sevoflurane effects in naive mice. We found that a clinically relevant concentration of sevoflurane induces caspase activation and PARP cleavage, and elevates levels of caspase-cleaved APP-N-fragment, BACE, and Aβ for up to 24 hours after the anesthesia. However, sevoflurane anesthesia did not significantly alter blood pressure and blood gas in naive mice (Table). These findings suggest that a clinically relevant regimen of sevoflurane anesthesia induces a time-dependent cascade of caspase activation and elevated BACE and Aβ levels in vivo.

We next showed that the broad caspase activation inhibitor Z-VAD could attenuate sevoflurane-induced caspase-3 activation, indicating that sevoflurane-induced alterations in APP processing and Aβ levels are at least partially dependent on caspase activation. We also showed that the γ-secretase inhibitor L-685,458 reduced Aβ levels (data not shown) and attenuated sevo-
flurane-induced caspase activation and apoptosis in H4-APP cells. In contrast, exogenously added Aβ potentiates sevoflurane-induced caspase-3 activation in naive H4 cells. These data suggest that enhanced Aβ generation, subsequent to sevoflurane-induced caspase-3 activation, can lead to further caspase-3 activation, resulting in additional rounds of apoptosis and Aβ generation.

Wei et al.38 showed that treatment with 4.1% sevoflurane for 24 hours did not induce cell death in rat PC12 pheochromocytoma cells and primary cortical neurons. In addition, many studies have suggested that sevoflurane can protect cells from cytotoxicity.39-47 However, many other studies have suggested that sevoflurane may induce cytotoxic effects.27-32 This discrepancy could be owing to the use of different cell lines, eg, rat kidney cells vs human neural-derived cells, and the duration and concentration of sevoflurane exposure in these studies. Future studies need to assess the effects of sevoflurane on apoptosis, APP processing, and Aβ levels with different concentrations and durations. A recent study by Wei et al.38 showed that isoflurane inhibited the cytotoxicity induced by isoflurane itself. These findings suggest that isoflurane could have neuroprotective effects through induction of endogenous neuroprotective mechanisms, eg, preconditioning, while different concentrations of isoflurane with different exposure times could cause inherent neurotoxic effects. We have postulated that sevoflurane may also have dual effects on cytotoxicity. Future studies are necessary to further test this hypothesis.

The exact molecular mechanisms by which sevoflurane induces caspase activation and apoptosis, alters APP processing, and increases Aβ levels are unknown. A recent study showed that caspase activation can reduce levels of the golgi-associated, γ-adaptin ear containing adenosine diphosphate–riboseylation factor binding protein 3 (GGA-3), a protein involved in BACE degradation.15 We therefore hypothesized that sevoflurane induces caspase activation, which then reduces GGA-3 levels. The reduced GGA-3 levels will result in accumulation of BACE; the increased BACE will finally increase Aβ levels by facilitating amyloidogenic processing of APP. Our in vivo findings that sevoflurane induces caspase-3 activation at 6 or 12 hours after anesthesia but enhances Aβ levels at a later times (eg, 12 and 24 hours after anesthesia) further support this hypothesis.

Sevoflurane might also affect APP processing and Aβ generation through energy inhibition. Velliquette et al.49 reported that insulin, 2-deoxyglucose, 3-nitropropionic acid, and kainic acid can induce acute energy inhibition to enhance levels of BACE and Aβ in wild-type and AD transgenic (Tg2576) mice. A recent neuroimaging study showed that sevoflurane blocked emotional memory in humans and suppressed cerebral metabolism, as evidenced by the fact that sevoflurane induced a 17% reduction of the cerebral metabolic rate of glucose use in human brains.50 Future studies will be necessary to determine whether a sevoflurane-induced increase in levels of BACE and Aβ is dependent on sevoflurane-induced changes in glucose use or GGA-3 levels.

A recent study by Groen et al.23 showed that an insult from a 2-hour occlusion of the middle cerebral artery increased levels of APP and Aβ in axons at the corpus callosum and in neurons at the border of the ischemic re-

Figure 7. Hypothetical pathway by which sevoflurane induces apoptosis and γ-amyloid protein (Aβ) generation. Sevoflurane induces caspase-3 activation and apoptosis. Caspase activation and apoptosis, in turn, increase β-site APP-cleaving enzyme (BACE) levels, which serves to increase Aβ generation. Elevated Aβ generation can then further induce caspase-3 activation and apoptosis.
duce caspase activation and apoptosis, which then increase the levels and activities of BACE, leading to elevated Aβ levels. Finally, increased Aβ levels can further potentiate caspase activation and apoptosis, resulting in subsequent rounds of apoptosis and Aβ generation following sevoflurane treatment. We would like to emphasize that though our current findings suggest that sevoflurane may induce key aspects of AD neuropathogenesis in vitro and in vivo, the in vivo relevance of these effects in humans remains unclear. Nonetheless, our current findings should ultimately help to facilitate the design of safer anesthetics and improved anesthesia care for patients, especially elderly individuals and patients with AD.

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