The Inhalation Anesthetic Desflurane Induces Caspase Activation and Increases Amyloid β-Protein Levels under Hypoxic Conditions*

Bin Zhang, Yuanning Dong, Guohua Zhang, Robert D. Moir, Weiming Xia, Yun Yue, Ming Tian, Deborah J. Culley, Gregory Crosby, Rudolph E. Tanzi, and Zhongcong Xie

Perioperative factors including hypoxia, hypocapnia, and certain anesthetics have been suggested to contribute to Alzheimer disease (AD) neuropathogenesis. Desflurane is one of the most commonly used inhalation anesthetics. However, the effects of desflurane on AD neuropathogenesis have not been previously determined. Here, we set out to assess the effects of desflurane and hypoxia on caspase activation, amyloid precursor protein (APP) processing, and amyloid β-protein (Aβ) generation in H4 human neuroglioma cells (H4 naive cells) as well as those over-expressing APP (H4-APP cells). Neither 12% desflurane nor hypoxia (18% O₂) alone affected caspase-3 activation, APP processing, and Aβ generation. However, treatment with a combination of 12% desflurane and hypoxia (18% O₂) (desflurane/hypoxia) for 6 h induced caspase-3 activation, altered APP processing, and increased Aβ generation in H4-APP cells. Desflurane/hypoxia also increased levels of β-site APP-cleaving enzyme in H4-APP cells. In addition, desflurane/hypoxia-induced Aβ generation could be reduced by the broad caspase inhibitor benzyloxycarbonyl-VAD. Finally, the Aβ aggregation inhibitor clioquinol and γ-secretase inhibitor L-685,458 attenuated caspase-3 activation induced by desflurane/hypoxia. In summary, desflurane can induce Aβ production and caspase activation, but only in the presence of hypoxia. Pending in vivo confirmation, these data may have profound implications for anesthesia care in elderly patients, and especially those with AD.

An estimated 200 million patients worldwide undergo surgery each year. Several reports have suggested that anesthesia and surgery may facilitate development of Alzheimer disease (AD) (1–3). A recent study also reported that patients having coronary artery bypass graft surgery under general anesthesia are at increased risk for AD as compared with those having percutaneous transluminal coronary angioplasty under local anesthesia (4).

Genetic evidence, confirmed by neuropathological and biochemical findings, indicates that excessive production and/or accumulation of amyloid β-protein (Aβ) play a fundamental role in the pathophysiology of AD (reviewed in Refs. 5 and 6). Aβ is produced via serial proteolysis of amyloid precursor protein (APP) by aspartyl protease β-site APP-cleaving enzyme (BACE), or β-secretase, and γ-secretase. BACE cleaves APP to generate a 99-residue membrane-associated C terminus fragment (APP-C99). APP-C99 is further cleaved by γ-secretase to release 4-kDa Aβ and β-secretase amyloid precursor protein intracellular domain (7–9). Presenilin and γ-secretase co-fractionate as a detergent-sensitive, high molecular weight complex (10) that includes at least three other proteins, nicastrin/APH-2, APH-1, and PEN-2, all of which are necessary and sufficient for γ-secretase activity (11–13). Increasing evidence indicates that apoptosis is associated with a variety of neurodegenerative disorders, including AD (Refs. 14–17; reviewed in Ref. 18). Aβ has been shown to cause caspase activation and apoptosis, which can in turn potentiate Aβ generation (16, 19–28). Finally, fibrillar aggregates of Aβ and oligomeric species of Aβ are more neurotoxic (29–37).

* These authors contributed equally to this work.

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1 To whom correspondence may be addressed: Genetics and Aging Research Unit, Massachusetts General Hospital/Harvard Medical School, 114 16th St., C3009, Charlestown, MA 02129-4404. Tel.: 617-726-6845; Fax: 617-724-1949; E-mail: tanzi@helix.mgh.harvard.edu.

2 To whom correspondence may be addressed: Genetics and Aging Research Unit, Dept. of Neurology, Dept. of Anesthesia and Critical Care, Massachusetts General Hospital/Harvard Medical School, 114 16th St., 3750, Charlestown, MA 02129-4404. Tel.: 617-724-9308; Fax: 617-724-1823; E-mail: zxie@partners.org.

3 The abbreviations used are: AD, Alzheimer disease; FL, full-length; APP, amyloid β precursor protein; BACE, β-site APP-cleaving enzyme; Aβ, amyloid β-protein; CQ, clioquinol; Z, benzyloxycarbonyl; NS, not significant.
isoflurane enhances the oligomerization and cytotoxicity of Aβ (44) and induces apoptosis (48–51). Our recent studies have shown that a clinically relevant concentration of isoflurane can lead to caspase-3 activation, decrease cell viability, alter APP processing, and increase Aβ generation in human H4 neuroglioma cells overexpressing human APP (45–47). Loop et al. (49) reported that isoflurane and sevoflurane, but not desflurane, can induce caspase activation and apoptosis in human T lymphocytes. However, effects of desflurane and desflurane plus other perioperative risk factors, e.g., hypoxia, on APP processing and Aβ generation have not been assessed.

In the present study, we set out to determine effects of desflurane, hypoxia, and the combination of the two (desflurane/hypoxia) on caspase-3 activation, APP processing, and Aβ generation in H4 human neuroglioma cells (H4 naïve cells) and H4 naïve cells stably transfected to express full-length (FL) APP (H4-APP cells). We also investigated whether the caspase inhibitor, Z-VAD, the γ-secretase inhibitor L-685,458, and the Aβ aggregation inhibitor clioquinol could attenuate desflurane/hypoxia-induced caspase-3 activation and Aβ generation.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—We employed H4 human neuroglioma cells (H4 naïve cells) and H4 naïve cells stably transfected to express full-length (FL) APP (H4-APP cells) in the experiments. All of the cell lines were cultured in Dulbecco’s modified Eagle’s medium (high glucose) containing 9% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Stably transfected H4 cells were additionally supplemented with 200 μg/ml G418.

**Cell Treatment**—The cells were treated for 6 h with following three conditions: desflurane alone (21% O₂, 5% CO₂, and 12% desflurane), hypoxia alone (18% O₂ and 5% CO₂), and desflurane/hypoxia (18% O₂, 5% CO₂, and 12% desflurane). Control conditions included 5% CO₂ plus 21% O₂, which did not affect caspase-3 activation, cell viability, APP processing, and Aβ gen-

Perioperative factors, including hypoxia (38–42), hypocapnia (43), and anesthetics (44–47), have been reported to potentially contribute to AD neuropathogenesis. These perioperative factors may also cause post-operative cognitive dysfunction, a dementia associated with surgery and anesthesia, by triggering AD neuropathogenesis.

Isoflurane, sevoflurane, and desflurane are the most commonly used inhalation anesthetics. It has been reported that...
Desflurane/Hypoxia, Apoptosis, Aβ Generation

![Image](https://via.placeholder.com/150)

**FIGURE 2.** Desflurane/hypoxia induces caspase-3 activation, affects APP processing, and increases Aβ levels in H4-APP cells. A, treatment with 12% desflurane/hypoxia (18%) for 6 h (lanes 4–6) induces caspase-3 cleavage (activation) as compared with control conditions (lanes 1–3) in H4-APP cells. There is no significant difference in the amounts of β-actin in control conditions or desflurane/hypoxia-treated H4-APP cells. B, quantification of the Western blot shows that desflurane/hypoxia treatment (black bar) (**, p = 0.002) increases caspase-3 activation compared with control conditions (white bar), normalized to β-actin levels. C, desflurane/hypoxia (lanes 4 and 5) reduces levels of APP-CTFs, but not FL-APP, as compared with control conditions (lanes 1–3) in H4-APP cells. There is no significant difference in amounts of β-actin in control conditions or desflurane/hypoxia-treated H4-APP cells. D, quantification of the Western blot shows that desflurane/hypoxia (black bar) does not alter levels of FL-APP as compared with control conditions (white bar) (p = 0.789, NS), normalized to β-actin levels. E, quantification of the Western blot shows that desflurane/hypoxia (black bar) decreases levels of APP-CTFs as compared with control conditions (white bar) (*, p = 0.019), normalized to β-actin levels. F, desflurane/hypoxia (black bar) increases generation of both Aβ40 (**, p = 0.002) and Aβ42 (*, p = 0.018) as compared with control conditions (white bar).

Cell Lysis and Protein Amount Quantification—Cell pellets were detergent-extracted on ice using immunoprecipitation buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 0.5% Nonidet P-40) plus protease inhibitors (1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin A). The lysates were collected, centrifuged at 12,000 rpm for 10 min, and quantified for total proteins by BCA protein assay kit (Pierce).

Western Blots Analysis—The cells were harvested at the end of the experiments and were subjected to Western blots analyses as described by Xie et al. (52). Antibodies A8717 (1:1,000; Sigma) and C66 (1:1,000, generous gift of Dr. Dora Kovacs at Massachusetts General Hospital and Harvard Medical School) were used to visualize FL-APP (110 kDa) and APP-CTFs (10–12 kDa). Antibody anti-β-actin (1:2,000, Sigma) was used to detect β-actin (42 kDa). A caspase-3 antibody (1:1,000 dilution; Cell Signaling Technology, Inc., Beverly, MA) was used to recognize FL-caspase-3 (35–40 kDa) and caspase-3 fragment (17–20 kDa) resulting from cleavage at asparate position 175. Rabbit polyclonal anti-BACE-1 antibody (1:1,000; Abcam, Cambridge, MA) was used to detect protein levels of BACE (65 kDa). The quantification of Western blots was performed as described by Xie et al. (52). Briefly, intensity of signals was analyzed by using the National Institutes of Health image program (NIH Image 1.62). We quantified Western blots using two steps. First, we used levels of β-actin to normalize (e.g. determining ratio of FL-APP amount to β-actin amount) levels of FL-APP, APP-CTFs, FL-caspase-3, caspase-3 fragment, and BACE to control for any loading differences in total protein amounts. Second, we presented changes in levels of FL-APP, APP-CTFs, FL-caspase-3, caspase-3 fragment, and BACE in treated cells as a percentage of those in cells treated with controls.

Quantification of Aβ Using Sandwich Enzyme-linked Immunosorbent Assay—Secreted Aβ was measured with a Sandwich enzyme-linked immunosorbent assay by using an Aβ measurement kit (Invitrogen), and by the Aβ enzyme-linked immunosorbent assay core facility at the Center for Neurological Dis-

ery (45–47). We used 18% O₂ to establish mildly hypoxic conditions. In the interaction studies, the cells were treated with Z-VAD (50 μM), L-685,458 (0.5 μM), clioquinol (CQ) (1 μM), and Aβ40 (7.5 μM) plus Aβ42 (7.5 μM) 1 h before desflurane/hypoxia treatment. The controls for Z-VAD, L685,458, and CQ were Me₂SO, whereas the control condition for Aβ was phosphate-buffered saline. Control conditions for desflurane, hypoxia, and desflurane/hypoxia were 5% CO₂ plus 21% O₂, which did not affect caspase-3 activation, cell viability, APP processing, and Aβ generation (data not shown).
Desflurane/Hypoxia, Apoptosis, Aβ Generation

Desflurane Does Not Cause Caspase-3 Activation, APP Processing, or Secreted Aβ Levels in H4-APP Cells—We previously reported that isoflurane can induce apoptosis and increase secreted Aβ levels in H4-APP cells (45–47). The effects of other commonly used inhalation anesthetics, including sevoflurane and desflurane, on cellular apoptosis, APP processing, and Aβ generation have not been reported. We therefore set out to determine these effects in H4-APP cells. Because caspase-3 activation is one of the final steps of cellular apoptosis (54), we assessed effects of desflurane on caspase-3 activation by quantitative Western blot analyses. In the present experiment, treatment with 12% desflurane for 6 h only induced modest caspase-3 cleavage (activation) (Fig. 1A), as assessed by determining the ratio 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 desflurane treatment did not significantly induce caspase-3 activation as compared with control conditions (100% versus 127% (p = 0.14) (Fig. 1B). APP immunoblotting revealed no significant differences in levels of FL-APP and APP-CTFs between desflurane-treated and control H4-APP cells (Fig. 1, C–E). Finally, the desflurane treatment did not increase secreted levels of Aβ40 or Aβ42 as compared with control conditions (Fig. 1F). These results indicate that desflurane alone neither induces apoptosis nor increases Aβ generation.

Desflurane/Hypoxia Induces Caspase-3 Activation, Affects APP Processing, and Increases Secreted Aβ Levels in H4-APP Cells—Because hypoxia has been reported to potentiate AD neuropathogenesis (43, 55), we assessed the effects of desflurane/hypoxia on caspase-3 activation, APP processing, and secreted Aβ levels in H4-APP cells. We chose 18% O2 to assess the effects of desflurane on apoptosis and Aβ generation under mildly hypoxic conditions. Caspase-3 immunoblotting showed visible increases in protein levels of caspase-3 fragment following treatment with 12% desflurane/hypoxia (18%) for 6 h as compared with control conditions (Fig. 2A). Quantification of the results by determining ratio of cleaved (activated) caspase-3 fragment (17–19 kDa) to FL-caspase-3 (35–40 kDa) revealed that desflurane/hypoxia treatment led to a 306% increase in caspase-3 cleavage (activation) as compared with control conditions (##, p = 0.0017). DMSO (net bar) reduces the desflurane/hypoxia-induced increases in secreted Aβ levels in H4-APP cells (##, p = 0.00038). DMSO, dimethyl sulfoxide.

Statistics—Given the presence of background caspase-3 activation and cell death in cells cultured in serum-free medium, we did not use absolute values to describe changes in caspase-3 activation. Instead, 100% caspase-3 activation, FL-APP, APP-CTFs, and BACE in the manuscript refer to control levels for the purpose of comparison to experimental conditions. The data were expressed as the means ± S.D. The number of samples varied from 3 to 10, and the samples were normally distributed. We used a two-tailed t test to compare differences between experimental groups and control groups. p values less than 0.05 (* or #) and 0.01 (** or ##) were considered statistically significant.

RESULTS

FIGURE 3. Caspase inhibitor Z-VAD attenuates caspase-3 activation induced by desflurane/hypoxia in H4-APP cells. A, treatment with desflurane (12%) plus hypoxia (18%) for 6 h (lanes 3 and 6) induces caspase-3 cleavage (activation) as compared with control conditions (lanes 1 and 2) or Z-VAD treatment (lane 7 and 8). Z-VAD treatment (lane 7 and 8) attenuates caspase-3 cleavage induced by desflurane/hypoxia. There is no significant difference in amounts of β-actin in H4-APP cells with above treatments. B, quantification of the Western blot shows that desflurane/hypoxia (black bar) increases caspase-3 activation as compared with control conditions (white bar) (*, p = 0.016), normalized to β-actin levels. The desflurane/hypoxia-induced caspase-3 activation is reduced by Z-VAD (50 μm) treatment (lane 3 and 4). Z-VAD treatment (lane 7 and 8) attenuates caspase-3 cleavage induced by desflurane/hypoxia. There is no significant difference in amounts of β-actin in H4-APP cells with above treatments. C, quantification of the Western blot shows that desflurane/hypoxia (black bar) increases caspase-3 activation as compared with control conditions (white bar) (**, p = 0.0032). Z-VAD (net bar) reduces the desflurane/hypoxia-induced increases in secreted Aβ levels in H4-APP cells (##, p = 0.00038). DMSO, dimethyl sulfoxide.
Desflurane/Hypoxia, Apoptosis, Aβ Generation

Caspase Inhibitor Z-VAD Attenuates the Desflurane/Hypoxia-induced Caspase-3 Activation—Given the findings that desflurane/hypoxia can induce apoptosis and increase Aβ generation, we next asked whether the desflurane/hypoxia-induced alteration in APP processing and Aβ generation is dependent on caspase-3 activation. For this purpose, we set out to assess the effects of the caspase inhibitor Z-VAD on desflurane/hypoxia-induced caspase-3 activation and Aβ generation. Z-VAD treatment attenuated caspase-3 activation induced by desflurane/hypoxia treatment, whereas Z-VAD alone did not induce caspase-3 activation as compared with control conditions (Fig. 3A). Quantification of the Western blots revealed that Z-VAD attenuated desflurane/hypoxia-induced caspase-3 activation: 304% versus 94%, \( p = 0.009 \) (Fig. 3B). Z-VAD also reduced desflurane/hypoxia-induced increases in secreted Aβ levels in H4-APP cells, \( p = 0.00038 \) (Fig. 3C).

Next, we asked whether desflurane/hypoxia can induce caspase-3 activation in the absence of APP overexpression in naïve H4 cells. Caspase-3 immunoblotting showed that desflurane/hypoxia induced caspase-3 activation as compared with control conditions in both H4 naïve and H4-APP cells (Fig. 4A). Quantification of the Western blot revealed that desflurane/hypoxia treatment led to a 306% and a 195% increase in caspase-3 activation as compared with control conditions in H4 naïve cells (Fig. 4B, \( p = 0.025 \)) and H4-APP cells (Fig. 4C, \( p = 0.007 \)), respectively. APP immunoblotting did not show detectable decreases in protein levels of APP-CTFs (10–12 kDa), but not FL-APP (110 kDa), following the desflurane/hypoxia treatment as compared with control conditions (Fig. 2C). Quantification of the results showed that the desflurane/hypoxia treatment led to a 49% decrease in levels of APP-CTFs as compared with control conditions (Fig. 2E, \( p = 0.019 \)). Finally, the desflurane/hypoxia treatment increased secreted Aβ40: 5.3 pg/ml versus 15.9 pg/ml, \( p = 0.002 \) and Aβ42: 1.9 pg/ml versus 6.7 pg/ml, \( p = 0.018 \) (Fig. 2F). These findings suggest that the combination of desflurane and hypoxia induces apoptosis, alters APP processing, and increases Aβ generation.

Changes in caspase-3 activation in H4 naïve cells (100%).

Changes in caspase-3 activation in H4-APP cells (100%).

Quantification of the Western blot shows that desflurane/hypoxia (black bar) increases caspase-3 activation as compared with control conditions (white bar) (*, \( p = 0.025 \), normalized to β-actin levels, in H4 naïve cells). C, quantification of the Western blot shows that desflurane/hypoxia (black bar) (**, \( p = 0.007 \)) increases caspase-3 activation as compared with control conditions (white bar), normalized to β-actin levels, in H4-APP cells. D, desflurane/hypoxia (lane 2) does not alter levels of APP-CTFs as compared with control conditions (lane 1) in H4 naïve cells. Desflurane/hypoxia (lane 4) reduces levels of APP-CTFs as compared with control conditions (lane 3) in H4-APP cells. E, quantification of the Western blot shows that desflurane/hypoxia (black bar) does not alter ratio of APP-CTFs to FL-APP as compared with control conditions (white bar) in H4 naïve cells (\( p = 0.35 \), NS). F, quantification of the Western blot shows that desflurane/hypoxia (black bar) decreases ratio of APP-CTFs to FL-APP in H4-APP cells (*, \( p = 0.03 \)).

FIGURE 4. Desflurane/hypoxia treatment induces caspase-3 activation independent of APP processing in H4 naïve cells. A, treatment with desflurane (12%) plus hypoxia (18%) for 6 h (lanes 3 and 4) induces caspase-3 cleavage (activation) as compared with control conditions (lanes 1 and 2) in H4 naïve cells. The same treatment (lanes 7 and 8) also induces caspase-3 activation in H4-APP cells as compared with control conditions (lanes 5 and 6). B, quantification of the Western blot shows that desflurane/hypoxia (black bar) increases caspase-3 activation as compared with control conditions (white bar) (*, \( p = 0.007 \)) in naïve H4 cells.

Desflurane/hypoxia treatment did not show detectable changes in levels of APP-CTFs and FL-APP between desflurane/hypoxia treatment and control conditions in H4 naïve cells (Fig. 4D). As a positive control, APP immunoblotting showed that desflurane/hypoxia (lane 4) reduced levels of APP-CTFs without significant changes in FL-APP levels as compared with control conditions (lane 3) in H4-APP cells (Fig. 4D). Quantification of the Western blots revealed that desflurane/hypoxia treatment did not change the ratio of APP-CTFs to FL-APP in H4 naïve cells (Fig. 4E) but led to a 24% decrease in the ratio of APP-CTFs to FL-APP in H4-APP cells (Fig. 4F, \( p = 0.03 \)). Aβ levels were too low to be detected in H4 naïve cells in

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whether pharmacologically based reductions in Aβ generation remain prevalent concerns in perioperative care for patients, because they can result from hypotension, shunting (e.g. one lung ventilation), and other pathological conditions. Therefore, these data, once confirmed in vivo, suggest that it

**Desflurane/Hypoxia, Apoptosis, Aβ Generation**

The commonly used inhalation anesthetic isoflurane has previously been shown to promote Aβ aggregation and to enhance toxicity of Aβ (44). We have shown that isoflurane can induce cellular apoptosis and increase Aβ generation in H4-APP cells (45–47). Because desflurane is another commonly used inhalation anesthetic, we set out to assess the effects of desflurane on apoptosis, APP processing, and Aβ generation in H4-APP cells. We were able to show that a 6-h treatment with a clinically relevant concentration of desflurane (12%) did not induce caspase-3 activation and affected neither APP processing nor secreted Aβ levels in H4-APP cells. These results are consistent with findings of other studies, which illustrated that isoflurane and sevoflurane, but not desflurane, can induce apoptosis (49). However, it is still possible that different treatments of desflurane in other cell lines may lead to apoptosis and enhancement of Aβ generation.

Next, we found that treatment with 12% desflurane/hypoxia (18% O₂) for 6 h can induce caspase-3 activation, alter APP processing, and increase Aβ generation in H4-APP cells, whereas treatment with either 12% desflurane or hypoxia alone did not lead to similar effects in H4-APP cells. Collectively, these findings suggest that desflurane may promote AD neuropathogenesis only under hypoxic conditions. It is possible that treatment with 12% desflurane alone for 6 h had undetectable effects on apoptosis and APP/Aβ, which were potentiated by hypoxic conditions. Hypoxia and cerebral blood supply insufficiency remain prevalent concerns in perioperative care for patients, because they can result from hypotension, shunting (e.g. one lung ventilation), and other pathological conditions. Therefore, these data, once confirmed in vivo, suggest that it
Desflurane/Hypoxia, Apoptosis, Aβ Generation

**FIGURE 6.** γ-Secretase inhibitor L-685,458 and Aβ aggregation inhibitor CQ reduce the desflurane/hypoxia-induced caspase-3 activation, but Aβ potentiates the desflurane/hypoxia-induced caspase-3 activation in H4-APP cells. A, treatment with desflurane (12%) plus hypoxia (18%) for 6 h (lanes 5 and 6) induces caspase-3 cleavage (activation) as compared with control conditions (lanes 1 and 2) or L-685,458 (0.5 μM) treatment (lanes 3 and 4). L-685,458 (0.5 μM) treatment (lanes 7 and 8) attenuates caspase-3 cleavage induced by desflurane/hypoxia. There is no significant difference in amounts of β-actin in H4-APP cells with above treatments. B, quantification of the Western blot shows that desflurane/hypoxia (black bar) increases caspase-3 activation as compared with control conditions (white bar) (**, p = 0.0001), normalized to β-actin levels. The desflurane/hypoxia-induced caspase-3 activation is reduced by L-685,458 (0.5 μM) (net bar) (**, p = 0.0001), CQ (1 μM) (net bar) (**, p = 0.0009) levels in H4-APP cells. D, desflurane/hypoxia (lanes 5 and 6) induces caspase-3 cleavage (activation) as compared with control conditions (lanes 1 and 2) or CQ (1 μM) treatment (lanes 3 and 4). CQ (1 μM) (lanes 7 and 8) treatment attenuates caspase-3 cleavage induced by desflurane/hypoxia. There is no significant difference in amounts of β-actin in H4-APP cells with above treatments. E, quantification of the Western blot shows that desflurane/hypoxia (black bar) increases caspase-3 activation as compared with control conditions (white bar) (*, p = 0.035), normalized to β-actin levels. The desflurane/hypoxia-induced caspase-3 activation is attenuated by treatment of CQ (1 μM) (net bar) (**, p = 0.0018), L-685,458 (0.5 μM) (net bar) (**, p = 0.0009) levels in H4-APP cells. F, desflurane/hypoxia (lane 3) induces caspase-3 cleavage (activation) as compared with control conditions (lane 1). Aβ40 (7.5 μM) plus Aβ42 (7.5 μM) treatment potentiates caspase-3 activation induced by desflurane/hypoxia (lane 4). There is no significant difference in amounts of β-actin in H4-APP cells with the above treatments. G, quantification of the Western blot shows that desflurane/hypoxia (black bar) increases caspase-3 activation as compared with control conditions (white bar), normalized to β-actin levels. The desflurane/hypoxia-induced caspase-3 activation is potentiated by treatment of Aβ (net bar) (**, p = 0.0012). DMSO, dimethyl sulfoxide.
**Desflurane/Hypoxia, Apoptosis, Aβ Generation**

We have previously shown that an amyloid fibril-binding dye, Congo Red (32), a β-sheet breaker peptide iAβ5 (57), and a metal protein attenuation compound GGA-3 (58) can attenuate, apoptosis induced by isoflurane and desflurane/hypoxia treatments might likewise reduce levels of GGA-3 to enhance BACE levels and β-secretase activity subsequent to caspase activation. We therefore assessed whether inhibition of Aβ aggregation and generation can attenuate desflurane/hypoxia-induced caspase-3 activation. These results suggest that the desflurane/hypoxia-induced alterations in APP processing and Aβ generation are largely dependent on the ability of desflurane/hypoxia to induce apoptosis. To further explore the mechanism by which desflurane/hypoxia induces apoptosis, affects APP processing, and increases Aβ generation, we assessed the effects of desflurane/hypoxia on BACE. Desflurane/hypoxia treatment for 6 h increased protein levels of BACE in H4-APP cells. Previously, we showed that inhalation anesthetic isoflurane can induce apoptosis which in turn enhances BACE levels to facilitate APP processing and to increase Aβ generation (47). Recent studies have shown that subsequent to caspase activation during ischemia, reductions in protein levels of Golgi-localized γ-ear-containing ARF-binding protein (GGA)-3, a protein that can alter trafficking and metabolism of BACE, is associated with increased levels of BACE and activity of β-secretase (56). It is interesting to speculate whether isoflurane and desflurane/hypoxia treatments might likewise reduce levels of GGA-3 to enhance BACE levels and β-secretase activity subsequent to caspase activation.

We employed 18% O₂ to assess the effects of desflurane on apoptosis and Aβ generation under mildly hypoxic conditions. Future studies will be necessary to assess whether severe hypoxic conditions, e.g., 1% O₂, alone would have similar effects on apoptosis and Aβ generation.

Z-VAD, a broad caspase activation inhibitor, was able to attenuate both caspase-3 activation and Aβ generation induced by desflurane/hypoxia. These results suggest that the desflurane/hypoxia-induced alterations in APP processing and Aβ generation are largely dependent on the ability of desflurane/hypoxia to induce apoptosis. To further explore the mechanism by which desflurane/hypoxia induces apoptosis, affects APP processing, and increases Aβ generation, we assessed the effects of desflurane/hypoxia on BACE. Desflurane/hypoxia treatment for 6 h increased protein levels of BACE in H4-APP cells. Previously, we showed that inhalation anesthetic isoflurane can induce apoptosis which in turn enhances BACE levels to facilitate APP processing and to increase Aβ generation (47). Recent studies have shown that subsequent to caspase activation during ischemia, reductions in protein levels of Golgi-localized γ-ear-containing ARF-binding protein (GGA)-3, a protein that can alter trafficking and metabolism of BACE, is associated with increased levels of BACE and activity of β-secretase (56). It is interesting to speculate whether isoflurane and desflurane/hypoxia treatments might likewise reduce levels of GGA-3 to enhance BACE levels and β-secretase activity subsequent to caspase activation.

We have previously shown that an amyloid fibril-binding dye, Congo Red (32), a β-sheet breaker peptide iAβ5 (57), and a metal protein attenuation compound GGA-3 (58) can attenuate, apoptosis induced by isoflurane (46, 47). We therefore assessed whether inhibition of Aβ aggregation and generation can attenuate desflurane/hypoxia-induced caspase-3 activation in H4-APP cells by determining the effects of CQ (an Aβ aggregation inhibitor) and L-685,458 (a γ-secretase inhibitor) on caspase-3 activation induced by desflurane/hypoxia in H4-APP cells. L-685,458 inhibited both caspase-3 activation and Aβ generation induced by desflurane/hypoxia. CQ also inhibited caspase-activation induced by desflurane/hypoxia. These results suggest that the desflurane/hypoxia-induced alterations in APP processing and Aβ generation are largely dependent on the ability of desflurane/hypoxia to induce apoptosis. To further explore the mechanism by which desflurane/hypoxia induces apoptosis, affects APP processing, and increases Aβ generation, we assessed the effects of desflurane/hypoxia on BACE. Desflurane/hypoxia treatment for 6 h increased protein levels of BACE in H4-APP cells. Previously, we showed that inhalation anesthetic isoflurane can induce apoptosis which in turn enhances BACE levels to facilitate APP processing and to increase Aβ generation (47). Recent studies have shown that subsequent to caspase activation during ischemia, reductions in protein levels of Golgi-localized γ-ear-containing ARF-binding protein (GGA)-3, a protein that can alter trafficking and metabolism of BACE, is associated with increased levels of BACE and activity of β-secretase (56). It is interesting to speculate whether isoflurane and desflurane/hypoxia treatments might likewise reduce levels of GGA-3 to enhance BACE levels and β-secretase activity subsequent to caspase activation.

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**FIGURE 7. Hypoxia (18%) does not affect caspase-3 activation, APP processing, nor Aβ generation in H4-APP cells.** A, hypoxia treatment alone (lanes 4–6) does not induce caspase-3 cleavage (activation) as compared with control conditions (lanes 1–3) in H4-APP cells. There is no significant difference in amounts of β-actin in control conditions or hypoxia-treated H4-APP cells. B, quantification of the Western blot shows that hypoxia treatment (black bar) does not increase caspase-3 activation compared with control conditions (white bar) (p = 0.16, NS), normalized to β-actin levels. C, hypoxia (lanes 4–6) does not affect APP processing as compared with control conditions (lanes 1–3) in H4-APP cells. There is no significant difference in amounts of β-actin in control conditions or hypoxia-treated H4-APP cells. D, quantification of the Western blot shows that hypoxia treatment (black bar) does not alter levels of FL-APP as compared with control conditions (white bar) (p = 0.45, NS), normalized to β-actin levels. E, quantification of the Western blot shows that hypoxia treatment (black bar) does not alter levels of APP-CTFs as compared with control conditions (white bar) (p = 0.24, NS), normalized to β-actin levels. F, hypoxia (black bar) does not increase generation of Aβ40 as compared with control conditions (white bar) (p = 0.45, NS).
Desflurane/Hypoxia, Apoptosis, Aβ Generation

FIGURE 8. Hypothetical pathway by which desflurane/hypoxia induces a vicious cycle of apoptosis and Aβ generation/aggregation. Desflurane/hypoxia induces caspase-3 activation/apoptosis. Caspase activation, in turn, increases BACE levels, which serves to increase Aβ generation. Desflurane/hypoxia also enhances Aβ aggregation, which further induces caspase-3 activation and apoptosis. Elevated Aβ generation and Aβ aggregation then further induces apoptosis leading to a vicious cycle of desflurane/hypoxia-induced apoptosis and Aβ generation/aggregation.

activation, suggesting a vicious cycle of apoptosis and Aβ generation that can be triggered by desflurane/hypoxia.

In conclusion, we have found that the combination of desflurane and hypoxia, but neither alone, induces apoptosis, alters APP processing, and increases Aβ generation. Further investigation, especially via in vivo studies, will be necessary to assess the potential role of desflurane and/or hypoxia in triggering or driving AD neuropathogenesis. These efforts should promote further attempts to determine the effects of anesthetics on AD neuropathogenesis, ultimately leading to provision of safer anesthesia care to patients, especially elderly patients, who are particularly susceptible to the incidence of post-operative cognitive dysfunction and risk for AD.

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