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Research report

Isoflurane and sevoflurane affect cell survival and BCL-2/BAX ratio differently

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Abstract

Depletion of calcium from the neuronal endoplasmic reticulum (ER) induces apoptosis. Isoflurane depletes calcium from sarcoplasmic reticulum (SR) of muscle, an analogue of ER in neurons, while sevoflurane maintains or increases SR calcium. We hypothesized that isoflurane, but not sevoflurane, induces apoptosis by depleting the ER calcium. Rat PC12 pheochromocytoma cells and primary cortical neurons were treated with equipotent doses of isoflurane and sevoflurane. Isoflurane, but not sevoflurane, at equipotent doses induced cell damage determined by both LDH release and MTT reduction assays, dose and time dependently, in both types of cells. Isoflurane at 2.4% for 24 h induced cytotoxicity in both cell types, which was characterized by nuclear condensation and fragmentation and activation of caspases 3 and 9. Isoflurane cytotoxicity was suppressed by dantrolene, a ryanodine receptor antagonist that inhibits abnormal calcium release from the ER. Isoflurane decreased the Bcl-2/Bax ratio by as much as 36% ($P < 0.05$). However, sevoflurane did not cause neuronal damage by apoptosis nor did it decrease the Bcl-2/Bax ratio. These results suggest that isoflurane and sevoflurane differentially affect the Bcl-2/Bax ratio and cell survival. At equipotent concentrations, isoflurane, but not sevoflurane, induces cytotoxicity in both PC12 cells and primary cortical neurons and decreases the Bcl-2/Bax ratio.

Theme: Disorders of the nervous system
Topic: Neurotoxicity

Keywords: Volatile anesthetic; Isoflurane; Sevoflurane; Apoptosis; Calcium; Bcl-2

1. Introduction

Maintenance of a sufficient amount of calcium inside the endoplasmic reticulum (ER) plays a critical role in normal ER structure, protein synthesis, cell growth, and death [9,10,49]. Excessive calcium release, from the ER by over activation of the ryanodine receptor and/or reduced calcium uptake by inhibition of calcium ATPase on the ER membrane, leads to ER calcium depletion and subsequent cell death by apoptosis, a programmed cell death [9,31,44,52,53]. Meanwhile, inhibition of excessive calcium release from the ER by dantrolene, a selective ryanodine receptor antagonist, has been shown to inhibit neuronal toxicity evoked by glutamate and NMDA excitotoxicity, trauma peptide, HIV coat protein, beta-amyloid, and ER calcium depletion in vitro [14,16,37,45,52,53]. Dantrolene also inhibits cell damage after muscle or cerebral ischemia, seizure, and sepsis in animal studies [8,23,28,34,46,52].

The Bcl-2 family members are active mediators of apoptosis that either inhibit (e.g., Bcl-2) or facilitate (e.g., Bax) apoptotic cell death [1,42]. Bcl-2 may inhibit apoptosis by suppressing abnormal Ca^{2+} release from the ER and by

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preventing ER Ca^{2+} depletion [15,22,31,53]. The balancing effect of Bcl-2 and Bax on the ER Ca^{2+} homeostasis plays an important role in determining the fate of the cells to either undergo proliferation or apoptosis [4,5]. Any agent that decreases the Bcl-2/Bax ratio may promote apoptosis.

Volatile anesthetics seem to affect calcium homeostasis differently. Isoflurane induces Ca^{2+} release from the ER reversibly in cerebrocortical and hippocampal neurons [27]. In mouse cerebral neurons, sevoflurane (0.5 mM) increased base line and NMDA-induced cytosolic calcium elevation, but in a less degree than isoflurane (0.35 mM) [17]. Isoflurane also induces Ca^{2+} release from the sarcoplasmic reticulum (SR), analogous to the ER in neurons, via activation of the ryanodine receptor [2,47], or by inhibition of Ca^{2+} uptake into SR [47]. Isoflurane has been shown to decrease the SR Ca^{2+} content [13,26]. However, sevoflurane has either no effect [6], or inhibits Ca^{2+} release from the SR [13]. Thus, sevoflurane either maintains [13] or increases the SR Ca^{2+} content [19] in muscle cells. Isoflurane inhibits protein synthesis and secretion, and induces cytotoxicity in both human and rat hepatocytes [32], and induces apoptosis in human lymphocytes [33]. In developing rat brains, isoflurane at clinical concentrations (0.75% isoflurane for 6 h) causes widespread neurodegeneration and persistent learning deficits [25]. Although blockade of NMDA receptors and/or activation of GABA receptors have been proposed for isoflurane-induced neurodegeneration in developing brain [24,25], it is plausible that isoflurane neurotoxicity is caused by depletion of ER Ca^{2+}. In contrast, sevoflurane does not seem to deplete the ER Ca^{2+} or may in fact elevate the ER Ca^{2+} content and therefore may have less potency to induce cytotoxicity as compared to isoflurane. The current study aims at answering the following questions: (1) Will isoflurane cause cell death by apoptosis in different type of cells? (2) Will sevoflurane have different effects on cytotoxicity based on its different effects on the ER calcium content? (3) What are the effects of volatile anesthetics on changes in the ratio of apoptotic/antiapoptotic proteins (e.g., Bcl-2 and Bax)?

2. Materials and methods

2.1. Cell cultures

2.1.1. Rat pheochromocytoma cells (PC12) culture

PC12 cells were cultured as described [54,55]. Briefly, PC12 cells were maintained in RPMI 1640 medium (Invitrogen, Grand Island, NY) supplemented with 10% horse serum (Invitrogen Life Technologies, Carlsbad, CA), 5% fetal calf serum (Hyclone Laboratories, Logan, UT), and penicillin/streptomycin (Invitrogen Life Technologies, Carlsbad, CA). Monolayer cultures at a density of 0.1–0.3 × 10^6 cells/cm^2 were incubated in plastic flasks precoated with 0.01% poly-L-ornithine (Sigma-Aldrich, St. Louis, MO) in a 95% air, 5% CO_2 humidified atmosphere at 37 °C. The culture medium was changed every 48 h. The cells were passed every 4–6 days.

2.1.2. Rat primary cortical neurons culture

The use of pregnant rats for primary cortical neuronal culture has been approved by the Institutional Animal Care and Use (IACUC) at the University of Pennsylvania. Primary cultures of cortical neurons were prepared from the dissociated cortices of rat fetus at embryonic days 16–18 essentially using a protocol previously described, with some modifications [20,21]. Cortices were dissected from embryonic brain, and meninges were removed from the tissues. The cells were dissociated by trypsinization and trituration, followed by DNase treatment. The dissociated cells were resuspended in serum-free B27/neurobasal medium and were plated at a density of 150–300 cells/cm^2 on poly-D-lysine-coated 24-well plates. Cultures were maintained in serum-free B27/neurobasal medium in a humidified atmosphere (5% CO_2, 95% air) at 37 °C. More than 95% of the cells present on day 5 in vitro (DIV 5) differentiate into neurons, as characterized by the appearance of long neurites expressing neurofilament protein. Half of the medium was changed every fourth day. Neurons up to DIV 16 were used for the designed experiments.

2.2. Anesthetic exposure

PC12 cells or primary cortical neurons grown on 24-well plates at a density of 250,000 cells/well were exposed to isoflurane or sevoflurane in a tight gas chamber (Bellco Glass, Vineland, NJ), which is placed inside a cell culture incubator (Fisher Scientific, Pittsburgh, PA). Isoflurane or sevoflurane vaporized via an agent-specific vaporizer by its humidified carrying gas of 5%CO_2/21%O_2/balanced N_2 (Boc Gases, Bellmawr, NJ) passed through the tight gas chamber at a constant concentration. The gas flow to the chamber is at 5 L/min for 5 min and then 0.5 L/min for the remaining experiment period. Pilot study has confirmed that this experimental condition inside tight gas chamber itself did not affect cell viability determined by both LDH release and MTT reduction assays (data not shown). Gas phase concentrations in the gas chamber were checked with infrared absorbance of the effluent gas, and constantly monitored and maintained at the designed concentration throughout experiments, using an infrared Ohmeda 5330 agent monitor (Coast to Coast Medical, Fall River, MA). The media were aspirated and extracted into hexane for high performance liquid chromatography (System Gold, Beckmam Coulter, Fullerton, CA) to verify the anesthetic concentration. Isoflurane (2.4%) and sevoflurane (4%) in the chamber for 24 h yielded isoflurane concentration in the medium of 0.8 mM and sevoflurane concentration in the medium of 0.3 mM, respectively, by HPLC. Control 24-well plates were placed outside the tight gas chamber but inside the same cell culture incubator. After exposures, the 24-well plates or dishes were immediately used to perform