Anesthesiology
1998; 89:929-41

Mechanistic Aspects of Carbon Monoxide Formation from Volatile Anesthetics

Pamela J. Baxter, Ph.D., Kyle Garton, B.S.,† and Evan D. Kharasch, M.D., Ph.D.‡*

Background: **Desflurane, enflurane and isoflurane can be degraded to carbon monoxide (CO) by carbon dioxide absorbents, whereas sevoflurane and halothane form negligible amounts of CO. Carbon monoxide formation is greater with drier absorbent, and with barium hydroxide, than with soda lime. The mechanism, role of absorbent composition and water, and anesthetic structures determining CO formation are unknown. This investigation examined sequential steps in anesthetic degradation to CO.**

Methods: **Carbon monoxide formation from anesthetics and desiccated barium hydroxide lime or soda lime was determined at equimole and equiMAC concentrations. Carbon monoxide formation from deuterium-substituted anesthetics was also quantified. Proton abstraction from anesthetics by strong base was determined by deuterium isotope exchange. A reactive chemical intermediate was trapped and identified by gas chromatography–mass spectrometry. The source of the oxygen in CO was identified by ^{18}O incorporation.**

Results: **Desflurane, enflurane, and isoflurane (difluoromethyl-ethyl ethers), but not sevoflurane (monofluoromethyl ether), methoxyflurane (methyl–ethyl ether), or halothane (alkane) were degraded to CO. The amount of CO formed was desflurane ≥ enflurane > isoflurane at equiMAC and enflurane > desflurane > isoflurane at equimole concentrations. Proton abstraction from the difluoromethoxy carbon was greater with potassium than with sodium hydroxide, but unmeasurable with barium hydroxide. Carbon monoxide formation was correlated (r = 0.95–1.00) with difluoromethoxy (enflurane > desflurane > isoflurane ≧ methoxyflurane = sevoflurane = 0) but not ethyl carbon proton abstraction. Deuterium substitution on enflurane and desflurane diminished CO formation. Chemical trapping showed formation of a difluorocarbene intermediate from enflurane and desflurane. Incorporation of $H_2{}^{18}O$ in barium hydroxide lime resulted in $C^{18}O$ formation from unlabeled enflurane and desflurane.**

Conclusions: **A difluoromethoxy group is a structural requirement for haloether degradation to CO. Results are consistent with initial base-catalyzed difluoromethoxy proton abstraction (potassium > sodium hydroxide, thus greater CO formation with barium hydroxide lime *vs.* soda lime) forming a carbanion (reprotonated by water to regenerate the anesthetic, hence requirements for relatively dry absorbent), carbanion decomposition to a difluorocarbene, and subsequent difluorocarbene reaction to form CO. (Key words: Desflurane; difluorocarbene; enflurane; isoflurane; toxicity.)**

RECOGNITION of carbon monoxide (CO) production in anesthesia circuits resulting from volatile anesthetic degradation has necessitated changes in clinical practice and product labeling.[1-7] Intraoperative CO formation from desflurane, enflurane, and isoflurane has been reported, with CO concentrations exceeding Environmental Protection Agency safety limits.[8] There are no clinical reports of CO formation from halothane or sevoflurane. Prospective analyses have suggested that the incidence of patient CO exposure (> 30 ppm) is 0.46% for the first case of the day (2.9% in remote locations other than operating rooms), and the overall incidence is 0.26%.[4-7] Desflurane, enflurane, and isoflurane degradation to CO occurs when these anesthetics interact with relatively dry barium hydroxide lime and soda lime and is thought to be catalyzed by the strong bases in these carbon dioxide absorbents.[1,3,4,6,9] Practitioners have been cautioned by the Food and Drug Administration to replace carbon dioxide absorbent, which they suspect may be desiccated.§ Ohmeda (Liberty Corner, NJ) has changed the package insert for desflurane and has volunteered to attach an informational tag to their anesthesia machines to reflect this caution.§

The mechanism by which CO formation from volatile anesthetics occurs is unknown. Absorbent water is

* Research Fellow, Department of Anesthesiology.

† Research Technician, Department of Anesthesiology.

‡ Professor, Departments of Anesthesiology and Medicinal Chemistry (adjunct).

Received from the Departments of Anesthesiology and Medicinal Chemistry, University of Washington, and the Puget Sound Veterans Affairs Health Care System, Seattle, Washington. Submitted for publication September 19, 1997. Accepted for publication June 8, 1998. Supported by a grant from Abbott Laboratories and a Clinical Pharmacology Faculty Award from the Pharmaceutical Research and Manufacturers of American Foundation. Presented in part at the 11th World Congress of Anesthesiologists, Sydney, Australia, April 14-20, 1996. Dr. Kharasch is a consultant to Abbott Laboratories and Zeneca Pharmaceuticals.

Address reprint requests to Dr. Kharasch: Department of Anesthesiology, Box 356540, University of Washington, Seattle, Washington 98195-6540. Address electronic mail to: kharasch@u.washington.edu

§Bedford RF: Note from the FDA. ANESTHESIOLOGY 1995; 83(4):33A

known to be a major factor, because CO production is inversely proportional to absorbent water content.[5,6,9,10] Carbon monoxide forms with partially dry and desiccated barium hydroxide lime and soda lime, but not from fresh absorbent or desiccated absorbent, which has been rehydrated by adding water.[5,6,9,10] Nevertheless, the mechanism by which water affects CO formation is unknown. Generation of CO and carboxyhemoglobin concentrations are greater with barium hydroxide lime than with soda lime,[4-6,9] yet this difference also remains unexplained. At equiMAC concentrations, the rank order of CO formation is desflurane > enflurane > isoflurane, whereas sevoflurane and halothane form negligible[11] or unmeasurable[5] amounts of CO. The reason for the difference in CO production from various anesthetics is not apparent. Desflurane, enflurane, and isoflurane all contain a difluoromethyl ether group, whereas sevoflurane and halothane do not, and this feature is thought to be important.[4,9] Nevertheless, the reason for its apparent importance is unknown, and sevoflurane and halothane differ structurally from desflurane, enflurane, and isoflurane in other potentially important ways. Furthermore, differences in CO formation between the three difluoromethyl-ethyl ethers are unexplained. Thus, the factors that determine volatile anesthetic degradation to CO remain unidentified.

This investigation characterized CO formation from several volatile anesthetics, and examined several chemical steps in a potential mechanistic sequence. We tested the hypothesis that the initial event in CO production is base-catalyzed proton abstraction (removal) from the methyl carbon of methyl-ethyl ethers and that difluorocarbene is an intermediate in CO formation. Anesthetics with deuterium substituted for hydrogen, which are more stable and have been used previously to determine mechanisms of metabolism, were similarly used to determine mechanisms of degradation. Finally, barium hydroxide lime labeled with ^{18}O was used to identify the source of oxygen in CO.

Materials and Methods

Chemicals

Enflurane, isoflurane, desflurane (Ohmeda), sevoflurane, methoxyflurane (Abbott Laboratories, Abbott Park, IL), halothane (Ayerst Laboratories, New York, NY), and chlorotrifluoroethylene (Lancaster, Wyndham, NH) were used as supplied. Anesthetic structures are provided in figure 1. Sodium and potassium deuteroxide (NaOD, KOD; 40% in D_2O), Aliquat 336, sodium carbonate, difluorodibromomethane, α-methylstyrene, zinc metal, iodine, and 2-methoxyethyl ether (diglyme, anhydrous) were obtained from Aldrich (Milwaukee, WI). Reagents were the highest quality available and were used without further purification. Deuterium oxide (D_2O, 99.9% D) and water enriched with ^{18}O ($\geq$ 70%) were from Cambridge Isotope Laboratories (Andover, MA). Solutions (1.0 M) of NaOD and KOD were prepared by dilution with D_2O. $Ba(OD)_2 \cdot 8D_2O$ was prepared by adding 1.5g BaO to 4 ml D_2O. Then 0.173g $Ba(OD)_2 \cdot 8D_2O$ was added to 1 ml D_2O to make a 1 M solution. Barium hydroxide lime (Baralyme, Chemtron Medical Division, Allied Healthcare, St. Louis, MO) and soda lime (Sodasorb, W. R. Grace & Co., Cambridge, MA) were dried to constant weight.[10] A CO standard of 952 ppm in air (Matheson Gas Products, Montgomeryville, PA) was used to prepare calibration curves for quantitative analysis.

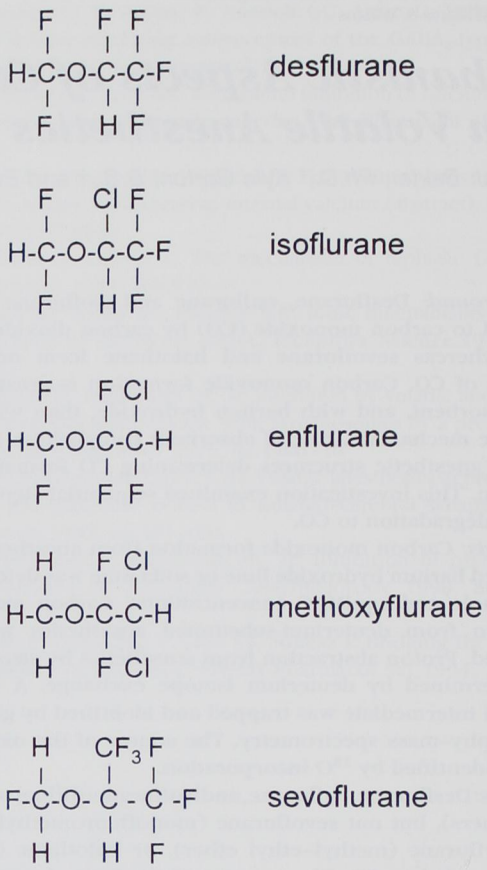

Fig. 1. Anesthetic structures.

Synthesis of Deuterated Anesthetics

Perdeuterated and selectively deuterated enflurane were synthesized by base-catalyzed exchange, as described previously.[12] Analysis by gas chromatography–mass spectrometry (GC-MS) showed deuterium incorporation on the difluoromethyl and chlorofluoroethyl (β-ethyl) carbons was: DF_2C-O-CF_2-CDClF (perdeuterated, 90 and 97%), DF_2C-O-CF_2-CHClF (methyl deuterated, 92 and 13%), HF_2C-O-CF_2-CDClF (ethyl deuterated, 13 and 94%). Perdeuterated desflurane was prepared similarly, however, it was necessary to substitute 0.1 M NaOD and 0.1 M NaOH to synthesize the monodeuterated forms. Analysis by GC-MS showed that deuterium incorporation on the difluoromethyl and α-ethyl carbons was DF_2C-O-CDF-CF_3 (perdeuterated, 86 and 89%), DF_2C-O-CHF-CF_3(methyl deuterated, 62 and 14%), and HF_2C-O-CDF-CF_3(ethyl deuterated, 48 and 92%). The smaller difference in relative acidities of the two carbons of desflurane, compared with enflurane, precluded more selective monodeuteration.

Synthesis of 1,1-Difluoro-2-methyl-2-phenylcyclopropane

A modification of previous methods was used.[13] Zinc metal was activated by sequentially washing powdered zinc in a filter funnel with dilute HCl, water, acetone, and diethylether, dried under high vacuum to remove residual solvent, stored in a sealed container, and used within two days of activation. A stir-bar and activated zinc (1.5 g; 22.8 mmol) were placed into a dry 250-ml flask and 2 to 3 crystals of iodine were added. The flask was then sealed with a rubber septum and purged with nitrogen. Diglyme (40 ml) and α-methylstyrene (0.9 g, 7.6 mmol) were added *via* syringe. The last 10 ml of diglyme was mixed with difluorodibromomethane (4.78 g, 22.8 mmol) and transferred to the reaction flask slowly over 10 min. The reaction was stirred on ice for 24 hr. Two volumes of ether were added, the mixture was filtered through Whatman #40 paper, and the filtrate evaporated to a white film that was redissolved in ether. The final product (54% yield) was characterized by GC-MS and ^{19}F NMR. GC-MS: (t_R= 20.1 min; see below for chromatographic conditions) *m/z* 168 ($[M]^+$, 28%), 153 ($[M\text{-}CH_3]^+$, 100%), 133 ($[M\text{-}CH_3\text{-}HF]^+$, 35%), 103 ($[M\text{-}CH_3\text{-}CF_2]^+$, 18%), 77 ($Ph^+$, 15%), 51 ($CF_2H$, 9%); Lit: *m/z* 168 (78%), 153 (100%)[14] ^{19}F NMR: ($CDCl_3$, δ relative to hexafluorobenzene) 24.4 ppm (dd, J_{FF} = 150 Hz, J_{FH} = 12 Hz), 27.5 ppm (dd, J_{FF} = 151 Hz, J_{FH} = 13 Hz) Lit: dd, J_{FF} = 150 Hz, J_{FH} = 13 Hz; dd, J_{FF} = 150 Hz, J_{FH} = 12 Hz.[13]

Carbon Monoxide Formation

Experiments were performed in triplicate at room temperature (23°C) in sealed 20.7-ml vials containing 4 g desiccated barium hydroxide lime or soda lime and a small strip of Whatman #1 filter paper, onto which anesthetics were injected to promote volatilization and prevent direct contact between anesthetic and absorbent. For each replicate a pair of vials was prepared and samples were collected alternately to avoid oversampling of the headspace volume from a single vial. A blank gas sample (0.5 ml) was drawn from one vial of each pair and injected into a 12-ml sealed headspace vial for analysis. Anesthetic (either at equimole or equiMAC concentration) was then injected into each 20-ml vial, the vials were placed on a wheel revolving at 60 rpm, and a 0.5 ml headspace sample was drawn at timed intervals (1, 4, 7, 10, 20, 30, 45, 60, 120, 180, 240 and 300 min after anesthetic introduction alternating between the vial pairs for each replicate) and injected into a fresh, sealed headspace vial. Carbon monoxide was quantified by GC-MS, as described previously.[10] Results were not corrected for minor dilution caused by repetitive sampling. The detection limit was approximately 2 ppm and because all samples were diluted 24-fold, the actual detection limit was approximately 50 ppm. For experiments at equivolume (approximately equimole concentrations), 5 μl of each anesthetic was injected, yielding desflurane (38 μmol), enflurane (41 μmol), isoflurane (39 μmol), sevoflurane (38 μmol), methoxyflurane (43 μmol), and halothane (47 μmol). For experiments at equiMAC concentrations (1.5 MAC), the following amounts were added: desflurane (10.2 μl), enflurane (2.6 μl), isoflurane (2.0 μl), sevoflurane (3.4 μl), methoxyflurane (0.3 μl), and halothane (1.0 μl). Experiments with deuterated anesthetics were conducted with 5 μl anesthetic, and CO formation was determined after 300 min. Anesthetic concentrations in the vials were confirmed by GC-MS and were within 5% of predicted.

Experiments were performed to evaluate the source of the oxygen atom in the CO resulting from anesthetic degradation. Absorbent labeled with ^{18}O was prepared by rehydrating desiccated barium hydroxide lime (2 g) with 300 μl $H_2{}^{18}O$, then again drying to constant weight. This was repeated three more times, followed by final desiccation to constant weight. Formation of CO was determined as described before after adding 5 μl enflurane or desflurane and sampling 1, 3, and 5 hr after anesthetic addition. Samples were analyzed for $C^{16}O$ and $C^{18}O$ by GC-MS, monitoring *m/z* 12 and 30, respectively. In addition, *m/z* 16, 18, and 28 were also monitored.

Deuterium Exchange

Base-catalyzed proton abstraction from the methyl, ethyl, or isopropyl carbons of the volatile anesthetics was determined by deuterium exchange. Sodium, potassium or barium deuteroxide (1.0 M, 1.0 ml) and 13 μl of the phase transfer catalyst Aliquat 336 were added to a 20-ml vial. The vial was sealed and 100 μl unlabeled anesthetic was added through the septum *via* syringe. A 0.5-ml headspace sample was immediately drawn and injected into another sealed vial. Experimental vials were continuously shaken at 23°C, and headspace samples were withdrawn 5, 10, 20, 30, 45, 60, and 90 min after anesthetic addition. The extent of deuterium incorporation into the anesthetics was determined by GC-MS, using a Hewlett-Packard (Wilmington, DE) 5890 II+ GC/5971 mass selective detector with 7694 headspace sampler and a DB-VRX column (30 m × 0.32 mm × 1.8 μm; J&W Scientific, Folsom, CA). The GC injector and detector temperatures were 150 and 250°C, respectively, and the column head pressure was 2.5 psi. For anesthetic analysis, the headspace sampler parameters were high agitation, 0.5-min sample equilibration, 0.05-min vial pressurization, 0.35-min loop fill, 0.15-min loop equilibration, 0.35-min sample injection time = 0.35 min, and oven, loop, and transfer line temperatures were 70, 75, and 85°C, respectively. The GC oven temperature was held at 30°C for 3 min, increased by 40°C/min to 185°C, and held at 185°C for 5 min. The following ions were monitored: desflurane *m/z* 52/51 $[CF_2D]^+/[CF_2H]^+$ and 102/101 $[CFDCF_3]^+/[CFHCF_3]^+$; enflurane *m/z* 52/51 $[CF_2D]^+/[CF_2H]^+$ and 118/117 $[CF_2CDClF]^+/[CF_2CHClF]^+$; isoflurane *m/z* 52/51 $[CF_2D]^+/[CF_2H]^+$ and 118/117 $[CDClCF_3]^+/[CHClCF_3]^+$; sevoflurane *m/z* 34/33 $[CFDH]^+/[CFH_2]^+$ and 152/151 $[C(CF_3)_2D]^+/[C(CF_3)_2H]^+$; halothane *m/z* 118/117 $[CF_3CClD]^+/[CF_3CClH]^+$; methoxyflurane *m/z* 82/81 $[CF_2OCDH_2]^+/[CF_2OCH_3]^+$ and 84/83 $[CDCl_2]^+/[CHCl_2]^+$. Proton abstraction was determined by deuterium incorporation into the anesthetic, calculated by dividing the peak height of the $(M + 1)^+$ ion by the sum of the peak heights of the M^+ and $(M + 1)^+$ ion and multiplying by 100 to obtain percent exchange. Baseline levels of the M^+ and natural isotope abundances $(M + 1)^+$ ions were determined for all the anesthetics and subtracted from the experimental values.

Reactive Intermediate Trapping

The hypothesis that difluorocarbene is a reactive intermediate in CO formation was tested. Specifically, experiments sought to trap and to identify this reactive intermediate. A 12-ml vial containing 0.5 g desiccated barium hydroxide lime, 10 μl Aliquat 336, and a stir-bar was sealed with a rubber septum, then tetrahydrofuran (2 ml) and the carbene trap α-methylstyrene (65 μl) were added *via* syringe. A blank sample (25 μl) was withdrawn and 270 μl desflurane was added *via* syringe. For enflurane, a 25-ml flask containing 1.5 g desiccated barium hydroxide lime, 30 μl Aliquat 336, and a stir-bar was sealed, then diglyme (6 ml) and α-methylstyrene (160 μl) were added, a blank sample was withdrawn, and 900 μl enflurane was added *via* syringe. Reaction mixtures were stirred before withdrawing a sample, and heated to facilitate α-methylstyrene reaction with any formed intermediate. Duplicate liquid samples (25 μl) were withdrawn 10, 20, 30, 45, 60, 75, 90, 120, 150, 180, and 240 min after anesthetic addition and injected into a sealed headspace vial. All samples were analyzed by electron ionization GC-MS for the presence of the difluorocarbene adduct 1,1-difluoro-2-methyl-2-phenylcyclopropane. Analyses were performed on the described headspace GC-MS using a DB-VRX column. The GC injector and detector temperatures were 150 and 250°C, respectively and the column head pressure was 2.5 psi. The headspace sampler parameters were high agitation, 3.0-min sample equilibration time, 0.05-min vial pressurization, 0.4-min loop fill, 0.15-min loop equilibration, 0.4-min sample injection, and oven, loop, and transfer line temperatures were 110, 115, and 120°C, respectively. The GC oven was 30°C for 3 min, increased by 10°C/min to 200°C, and held for 5 min. The difluorocarbene adduct eluted at 20.1 min. For time course experiments, selected ion monitoring at *m/z* 133, 153, and 168 was used.

Analysis

Results are expressed as the mean ± standard deviation. Correlations were examined by linear least squares regression analysis.

Results

Carbon Monoxide Formation

Carbon monoxide formation catalyzed by desiccated barium hydroxide lime and soda lime was measured at equiMAC and at equivolume (approximately equimole concentrations). The former is more clinically relevant, whereas equimole concentrations are appropriate for mechanistic inferences. The rank order of CO formation at equiMAC concentrations was desflurane > enflu-

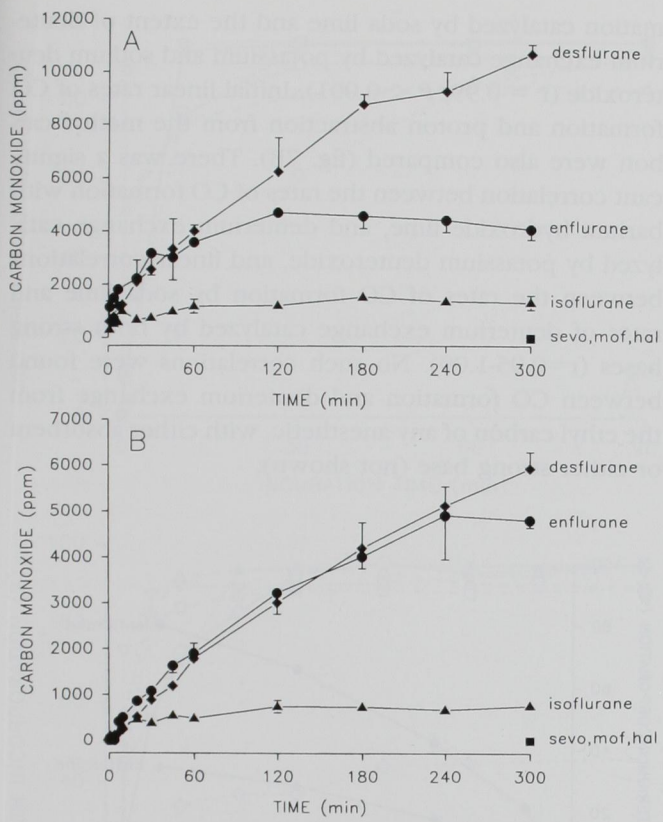

Fig. 2. Anesthetic degradation to carbon monoxide at equiminimum alveolar concentrations (1.5 MAC) concentrations by (*A*) barium hydroxide lime and (*B*) soda lime. Each data point is the mean ± SD (n = 3). The single data point at 300 min reflects undetectable carbon monoxide from sevoflurane, methoxyflurane, or halothane.

rane > isoflurane with barium hydroxide lime (fig. 2A), and desflurane = enflurane > isoflurane with soda lime (fig. 2B). The rank order of CO formation at approximately equimole concentrations was enflurane > desflurane > isoflurane with barium hydroxide lime (fig. 3A) and soda lime (fig. 3B). No CO was detectable from sevoflurane, halothane or methoxyflurane using either carbon dioxide absorbent.

Carbon monoxide concentrations from barium hydroxide lime degradation of perdeuterated or selectively deuterated enflurane and desflurane were determined to assess the presence and magnitude of an isotope effect (fig. 4). Carbon monoxide formation from enflurane after 5 hr was reduced by approximately 50% by perdeuteration or by selective deuteration of either the difluoromethyl or the β-ethyl carbon. Carbon monoxide formation from desflurane was also reduced by perdeuteration or selective deuteration of the difluoromethyl or α-ethyl carbon. The magnitude of the isotope effect was less than with enflurane, consistent with the lower deuterium incorporation in desflurane. Effects of perdeuteration and selective deuteration were similar, both for enflurane and desflurane.

Deuterium Exchange

Proton (methoxy or ethyl) abstraction is a likely initial step in anesthetic degradation to CO. The ability of the strong bases in carbon dioxide absorbents to abstract a proton from the volatile anesthetics, reflecting relative hydrogen acidities, was measured by deuterium exchange. Anesthetics, strong base, and a phase transfer catalyst were reacted in deuterium oxide. Recombination of an anesthetic anion with a deuterium atom from the medium reflects proton abstraction by the strong base.

Deuterium exchange on the methoxy carbon of enflurane, desflurane, isoflurane, sevoflurane, and methoxyflurane catalyzed by potassium (fig. 5A) and sodium (fig.

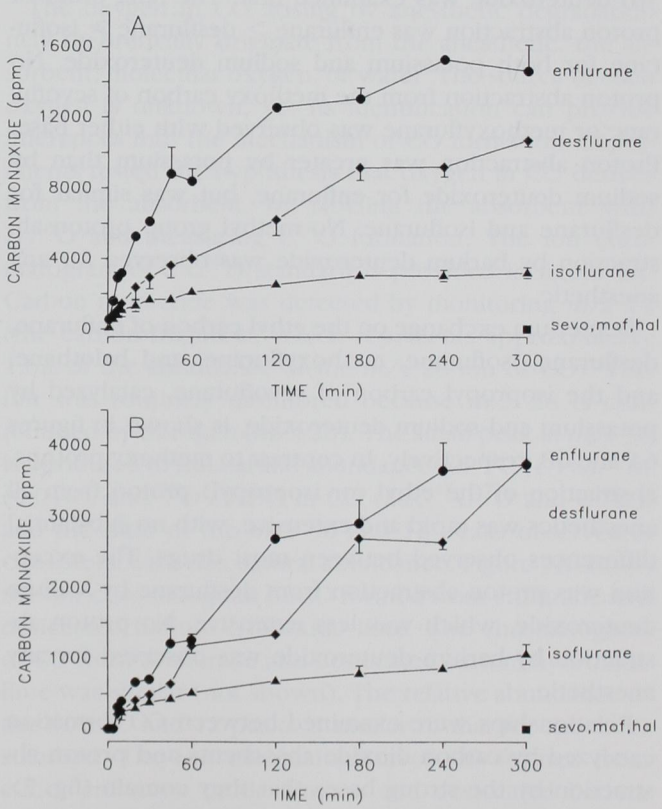

Fig. 3. Anesthetic degradation to carbon monoxide at equivolume (approximately equimolar concentrations) by (*A*) barium hydroxide lime and (*B*) soda lime. Each data point is the mean ± SD (n = 3). The single data point at 300 min reflects undetectable carbon monoxide from sevoflurane, methoxyflurane, or halothane.

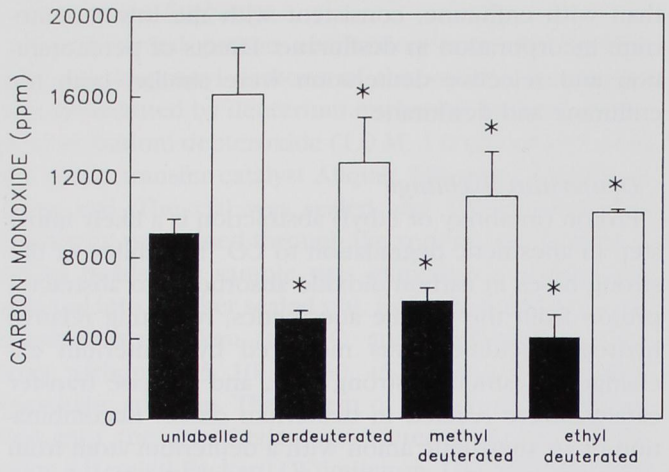

Fig. 4. Effect of deuterium substitution on enflurane (solid bars) or desflurane (open bars) degradation to carbon monoxide barium hydroxide lime. Carbon monoxide formation after 300 min is shown (mean ± SD, n = 3). *Significantly different from control by analysis of variance ($P < 0.05$).

5B) deuteroxide was examined first. The rank order of proton abstraction was enflurane > desflurane > isoflurane for both potassium and sodium deuteroxide. No proton abstraction from the methoxy carbon of sevoflurane or methoxyflurane was observed with either base. Proton abstraction was greater by potassium than by sodium deuteroxide for enflurane, but was similar for desflurane and isoflurane. No methyl group proton abstraction by barium deuteroxide was observed for any anesthetic.

Deuterium exchange on the ethyl carbon of enflurane, desflurane, isoflurane, methoxyflurane, and halothane, and the isopropyl carbon of sevoflurane, catalyzed by potassium and sodium deuteroxide, is shown in figures 6A and 6B, respectively. In contrast to methoxy protons, abstraction of the ethyl (or isopropyl) proton from all anesthetics was rapid and extensive, with no substantial differences observed between most drugs. The exception was proton abstraction from desflurane by sodium deuteroxide, which was less extensive. No proton abstraction by barium deuteroxide was observed for any anesthetic.

Relationships were examined between CO formation catalyzed by carbon dioxide absorbents and proton abstraction by the strong bases that they contain (fig. 7). There was a significant linear correlation between the extent of CO formation with barium hydroxide lime and methyl carbon deuterium exchange catalyzed by potassium deuteroxide ($r = 1.0$, $P < 0.001$) (fig. 7A). Similar correlations were found between the extent of CO formation catalyzed by soda lime and the extent of deuterium exchange catalyzed by potassium and sodium deuteroxide ($r = 0.99$, $P < 0.001$). Initial linear rates of CO formation and proton abstraction from the methyl carbon were also compared (fig. 7B). There was a significant correlation between the rates of CO formation with barium hydroxide lime, and deuterium exchange catalyzed by potassium deuteroxide, and linear correlations between the rates of CO formation by soda lime and rates of deuterium exchange catalyzed by both strong bases (r=0.95-1.00). No such correlations were found between CO formation and deuterium exchange from the ethyl carbon of any anesthetic, with either absorbent or either strong base (not shown).

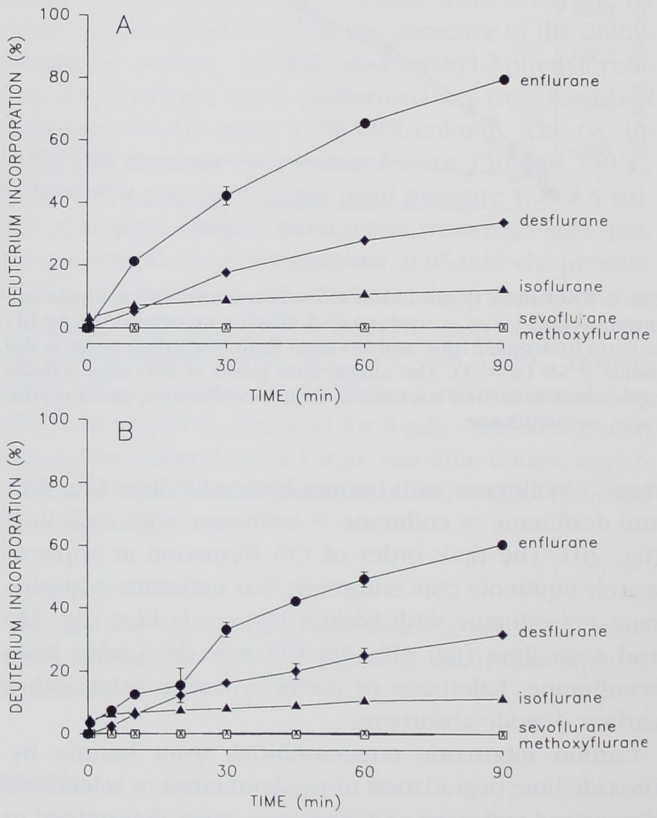

Fig. 5. Deuterium exchange on the methoxy carbon of volatile anesthetics catalyzed by strong base. Proton abstraction catalyzed by either (*A*) potassium or (*B*) sodium deuteroxide in deuterium oxide was measured by incorporation of deuterium into the anesthetic molecule. Equimolar anesthetic concentrations were used. Results are mean ± SD (n = 3 for potassium deuteroxide, n = 5 for sodium deuteroxide). No deuterium incorporation was observed with barium deuteroxide and any anesthetic (not shown). Results indicate the relative ease of methoxy proton abstraction is enflurane > desflurane > isoflurane ≧ sevoflurane = methoxyflurane = 0.

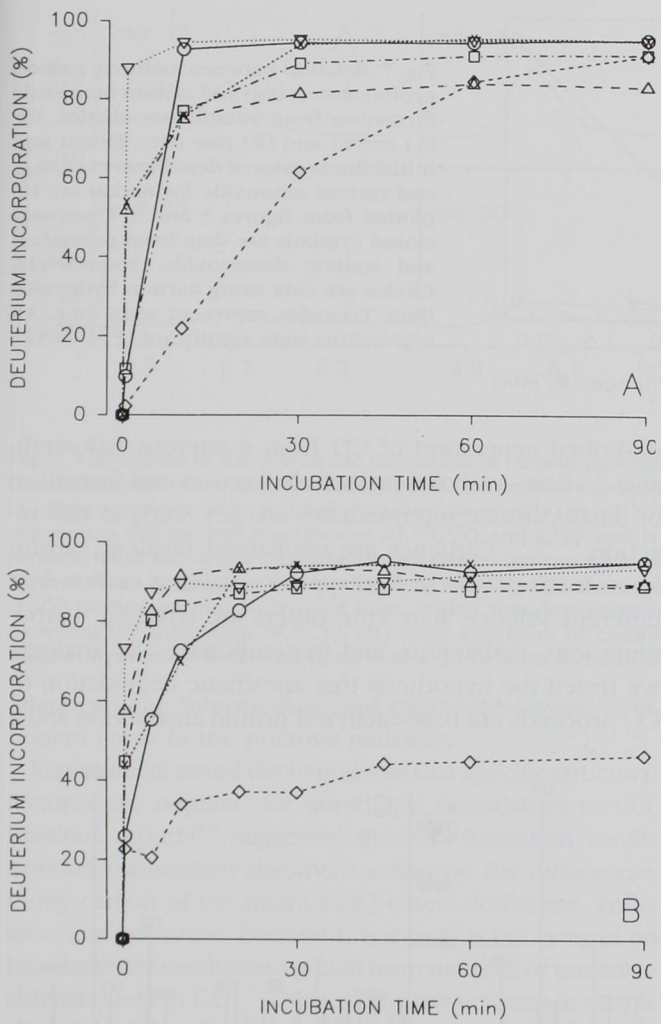

Fig. 6. Deuterium exchange on the ethyl carbon (isopropyl carbon for sevoflurane) of volatile anesthetics catalyzed by strong base. Proton abstraction catalyzed by either (*A*) potassium or (*B*) sodium deuteroxide was measured by deuterium incorporation into the anesthetic molecule. Equimolar anesthetic concentrations were used. Mean values are shown (n = 3 for potassium deuteroxide, n = 5 for sodium deuteroxide); SD is omitted for clarity. No deuterium incorporation was observed with barium deuteroxide and any anesthetic (not shown). Symbols denote enflurane (○), desflurane (◇), isoflurane (△), methoxyflurane (□), sevoflurane (X), and halothane (▽). Results indicate that ethyl proton abstraction is facile from all anesthetics (less so with desflurane).

Reactive Intermediate Trapping

Trapping experiments tested the hypothesis that difluorocarbene is an intermediate in the base-catalyzed decomposition of difluoromethoxy-containing anesthetics, analogous to the reaction mechanism affording carbon dihalides as intermediates in the base-catalyzed hydrolysis of halomethanes,[15] which are identified by trapping of the reactive intermediate.[16] α-Methylstyrene was used to trap the putative reactive difluorocarbene before it could combine into a more stable species.[13] Figure 8A shows the spectrum of synthetic 1,1-difluoro-2-methyl-2-phenylcyclopropane (t_R20.1 min), the stable product of difluorocarbene reaction with α-methylstyrene (reaction shown in fig. 8D, *inset*). Analysis by GC-MS of the reaction between desiccated barium hydroxide lime and enflurane or desflurane showed a peak at 20.1 min, which was not present in the absence of anesthetic. The mass spectrum of this peak is shown in figures 8B (enflurane) and 8C (desflurane). Based on identical retention times and mass spectra of the trapped product and synthetic standard, the trapped intermediate was identified as difluorocarbene. Using selected-ion mode to improve sensitivity (fig. 8D), time-dependent formation of the difluorocarbene adduct trapped during enflurane degradation by barium hydroxide lime was demonstrated (fig. 8E).

^{18}O Incorporation

The oxygen in CO arising by anesthetic degradation may theoretically originate from the anesthetic, the absorbent, molecular oxygen, or water. The source of such oxygen is unknown, yet its identification can provide inferences into the mechanism of CO formation. Experiments tested the hypothesis that oxygen in CO derives from the absorbent, by labeling the absorbent with $H_2{}^{18}O$ and measuring $C^{18}O$ formation. The ion chromatogram of a $C^{16}O$ standard is provided in figure 9A. Carbon monoxide was detected by monitoring *m/z* 12 (the carbon fragment, which represents approximately 1.6% of the abundance of the *m/z* 28 ion of CO). This ion was routinely monitored because *m/z* 28 is confounded by nitrogen (*m/z* 28). The small peak at *m/z* 30 is attributed to the natural abundances of ^{13}C (1.1%), ^{17}O (0.04%), and ^{18}O (0.2%) in CO (*i.e.*, $^{13}C^{17}O$ and $C^{18}O$), and the ratio of the *m/z* 30 and 12 peaks observed is consistent with this natural abundance. Figure 9B shows the ion chromatogram of CO formed from enflurane and desiccated barium hydroxide lime. The chromatogram of CO from desflurane and desiccated barium hydroxide lime was similar (not shown). The relative abundance of the *m/z* 30 and 12 peaks is similar to that for the $C^{16}O$ standard. The ion chromatogram of CO formed from either enflurane or desflurane, and desiccated barium hydroxide lime labeled with $H_2{}^{18}O$, is shown in figures 9C and 9D, respectively. The ratio of *m/z* 30 to 12 is considerably greater than in figures 9A and 9B, evidence that considerably more $C^{18}O$ is present than in either the $C^{16}O$ standard or the CO formed from enflurane or

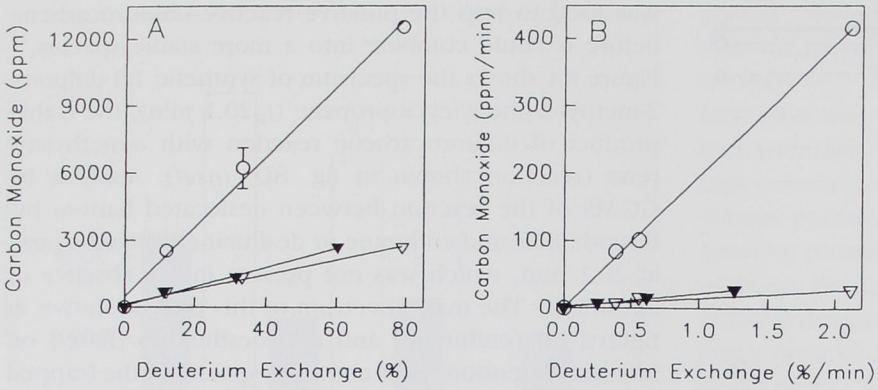

Fig. 7. Relation between methoxy carbon proton abstraction and carbon monoxide formation from volatile anesthetics, for (*A*) extent and (*B*) rate data. Extent and initial linear rates of deuterium exchange and carbon monoxide formation are replotted from figures 3 and 5. Open and closed symbols are data from potassium and sodium deuteroxide, respectively. Circles are data using barium hydroxide lime. Triangles represent soda lime. All regressions were significant ($P < 0.05$).

desflurane and native (*i.e.*, $H_2{}^{16}O$-containing) absorbent. Thus, the oxygen in CO originates, at least in part, from oxygen in the CO_2 absorbent.

Discussion

Mechanistic Implications

Chloroform is degraded to CO by strong bases in carbon dioxide absorbents, a specific example of the well-described generation of CO from numerous halomethanes.[17] Base-catalyzed proton abstraction and formation of dihalocarbene intermediates are key steps in this reaction.[15,16,18] Carbenes are uncharged divalent carbon intermediates; one of four types containing carbon in a different valence state (the others are trivalent: carbonium ions, carbanions, and free radicals).[17] By analogy, we tested the hypothesis that anesthetic degradation to CO proceeds *via* base-catalyzed proton abstraction and a

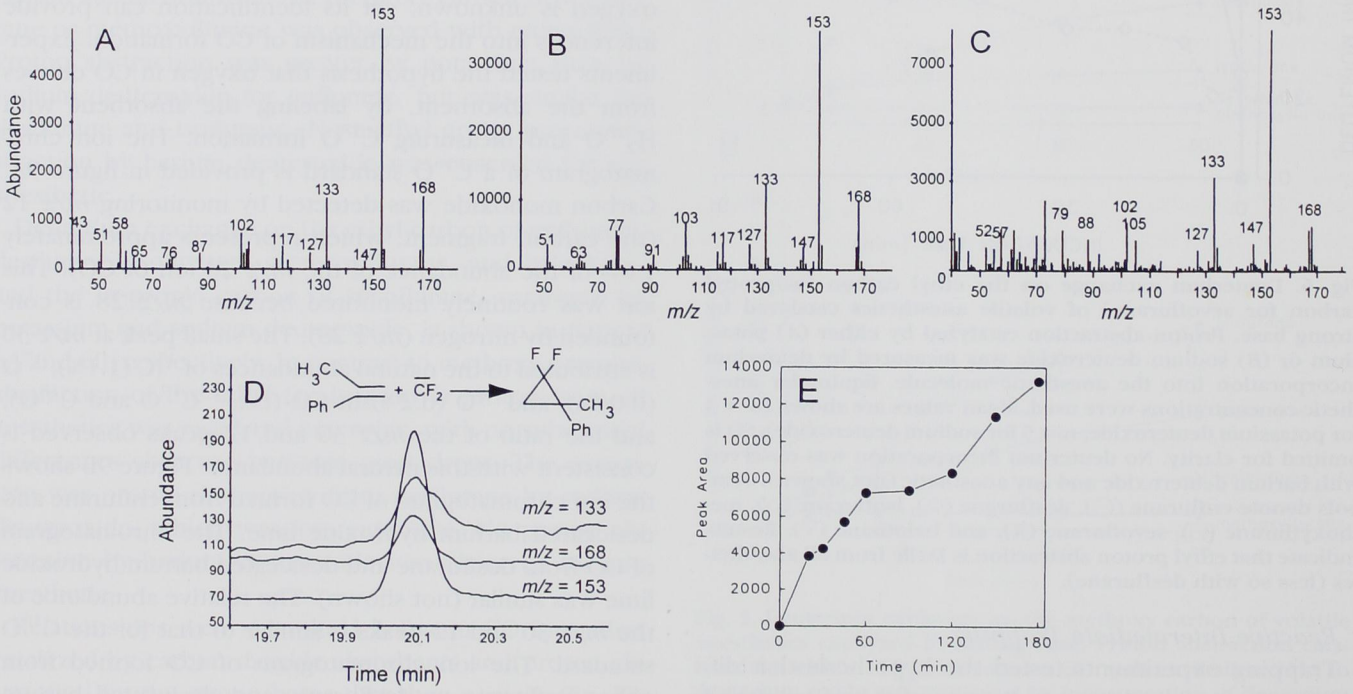

Fig. 8. Identification of a difluorocarbene intermediate formed during anesthetic degradation to carbon monoxide. (*A*) Mass spectrum of synthetic 1,1-difluoro-2-methyl-2-phenylcyclopropane (t_R=20.1 min). (*B*,*C*) Mass spectrum of a peak eluting at 20.1 min after incubation of (*B*) enflurane or (*C*) desflurane with barium hydroxide lime, with styrene added to trap reactive intermediates. (*D*) Selected-ion mode chromatogram of 1,1-difluoro-2-methyl-2-phenylcyclopropane. The insert depicts the trapping reaction between difluorocarbene and α-methyl styrene to produce 1,1-difluoro-2-methyl-2-phenylcyclopropane. Difluorocarbene may exist as a ground-state singlet or triplet, but available data suggest that it is a singlet and is drawn as such.[34] (*E*) Time-dependent formation of 1,1-difluoro-2-methyl-2phenylcyclopropane during the reaction between enflurane and barium hydroxide lime.

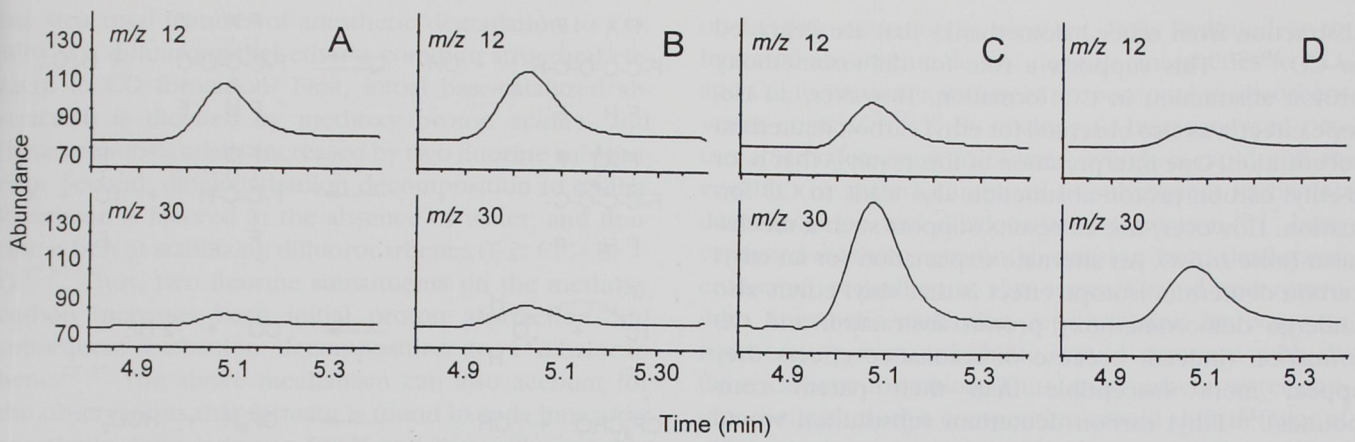

Fig. 9. The source of oxygen in the formation of carbon monoxide. $H_2{}^{18}O$ was incorporated into barium hydroxide lime, which was then desiccated. Carbon monoxide (mw 28) formation was monitored by gas chromatography–mass spectrometry at *m/z* 12 and 30. (*A*) Mass spectrum of an unlabeled carbon monoxide (*i.e.*, $C^{16}O$) standard. The *m/z* 12 peak is the carbon fragment. The *m/z* 30 peak represents the natural abundances of $^{13}C^{17}O$ and $C^{18}O$ and is small relative to the carbon fragment. (*B*) Mass spectrum of $C^{16}O$ formed from enflurane degradation by native barium hydroxide lime, which was desiccated before use. The spectrum of $C^{16}O$ formed from desflurane was similar (not shown). (*C*) Mass spectrum of carbon monoxide formed from enflurane degradation by $H_2{}^{18}O$-labeled barium hydroxide lime, which was desiccated before use. The peak at *m/z* 30 is large relative to the carbon peak, denoting the formation of $C^{18}O$. (*D*) Mass spectrum of carbon monoxide formed from desflurane degradation by $H_2{}^{18}O$-labeled barium hydroxide lime. The peak at *m/z* 30 reflects formation of $C^{18}O$.

dihalocarbene intermediate and examined several component steps in the putative pathway.

Experiments tested the hypothesis that specific structural features are required for anesthetic degradation to CO. Previous reports[4,9] suggested that CO formation results from an unspecified absorbent action on the difluoromethoxy carbon of the methylethyl ethers desflurane, enflurane, and isoflurane, because this moiety is not present on halothane or sevoflurane (which form minimal or undetectable amounts of CO).[5,9] Nevertheless, halothane (a simple alkane) and sevoflurane (a methyl–isopropyl ether) also differ in other ways from these methyl-ethyl ethers. A methyl-ethyl ether without difluoromethyl substituents was required to test the hypothesis. Methoxyflurane, with an ethyl group similar to that of enflurane but a nonfluorinated methyl carbon, was ideally suited for this purpose. Methoxyflurane degradation to CO, either by soda lime or barium hydroxide lime, was undetectable. Carbon monoxide formation from sevoflurane and halothane was also undetectable, confirming previous reports.[5] Thus, a difluoromethyl ether function (or possibly any dihalomethyl ether group) does appear to be required for halogenated anesthetic degradation to CO by carbon dioxide absorbents.

Experiments also tested the hypothesis that specific structural features are required for proton abstraction. Neither potassium nor sodium hydroxide catalyzed any measurable proton abstraction from methoxy (methoxyflurane) or monofluoromethoxy (sevoflurane) groups. Thus, difluoro substitution (or possibly any dihalo substitution) appears to be required for methoxy proton abstraction and CO formation.

The more specific hypothesis that base-catalyzed difluoromethoxy proton abstraction is an initial step in CO formation and that differences in abstraction between anesthetics may influence CO formation was also tested. Results are consistent with this hypothesis. There were excellent correlations between absorbent-catalyzed CO formation and methoxy proton abstraction by the strong bases that they contain. This relationship, however, applied only to methoxy protons. The presence of any abstractable proton *per se* was not sufficient for CO formation. Halothane, sevoflurane, and methoxyflurane contain a highly abstractable (ethyl or isopropyl) proton yet formed no detectable CO. Also, there no correlation between CO formation and ethyl proton abstraction from desflurane, enflurane, and isoflurane, although such a correlation might not occur if carbanion decay to difluorocarbene is rapid relative to the rate of initial proton abstraction (*vide infra*).

Additional evidence for initial proton abstraction derives from experiments with deuterium-substituted anesthetics. The stronger carbon-deuterium bond is more resistant to cleavage than the carbon-hydrogen bond. Deuterium substitution on the difluoromethoxy carbon of enflurane and desflurane significantly diminished CO formation, and isotope effect magnitude (1.6–1.8 for enflurane) was similar to that for base-catalyzed proton

abstraction from other halomethanes that are degraded to CO.[19–21] This supports a role for difluoromethoxy proton abstraction in CO formation. However, an isotope effect was also observed for ethyl carbon deuterium substitution. One interpretation of this result is that α- or β-ethyl carbon proton abstraction also leads to CO formation. However, not all results support such a mechanism (*vide infra*). An alternate explanation for an ethyl carbon deuterium isotope effect is that vinyl ethers also undergo difluoromethoxy proton abstraction and CO formation (indeed, because of resonance effects they appear more susceptible than their parent compounds).[22] Ethyl carbon deuterium substitution would diminish vinyl ether formation, and, hence, vinyl ether-dependent CO production. This speculation awaits haloalkene synthesis and experimental confirmation.

The strongest evidence for dihalocarbenes in chemical reactions derives from experiments in which the intermediate is trapped, usually by olefins.[17,23] Such convincing evidence for difluorocarbene formation from difluoromethoxy ether anesthetics was obtained by trapping, with α-methylstyrene, the putative difluorocarbene intermediate formed from enflurane and desflurane by barium hydroxide lime. Difluorocarbene formation from other halogenated compounds has been demonstrated previously,[21,24,25] to which difluorocarbene generation from fluoromethyl anesthetics may be similar.

Stoichiometry between anesthetic degradation and CO formation was not pursued in these experiments. Strong bases degrade anesthetics both to CO and to haloalkenes,[26,27] thus anesthetic degradation will not be reflected stoichiometrically by CO formation. Moreover, the ratio of products (CHF_3, CO, formate) varies greatly under different conditions,[4] in part because CO may react further.[18] This additionally complicates interpretation of stoichiometry.

Potential Mechanism

By analogy to CO formation from halomethanes,[15,16,18] the present results are consistent with a mechanism of CO formation from desflurane, enflurane, and isoflurane (fig. 10). These difluoromethyl ethers undergo rapidly reversible base-catalyzed difluoromethyl proton abstraction to yield a difluoromethyl ethyl ether carbanion.‖

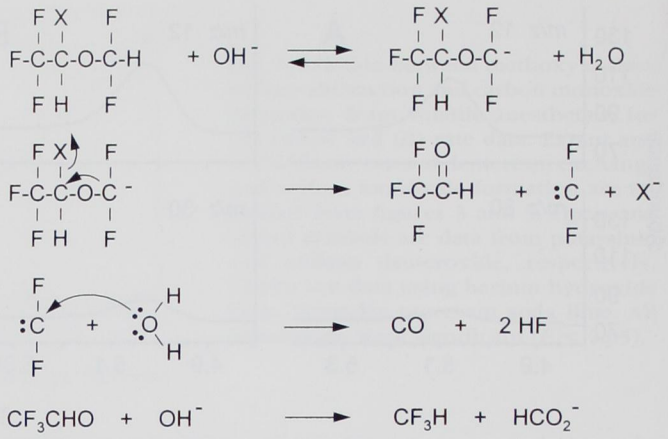

Fig. 10. Proposed mechanism of carbon monoxide formation from difluoromethyl–ethyl ether anesthetics. Shown is the backbone structure for isoflurane (X = Cl) and desflurane (X = F). Also shown is a putative mechanism for the concomitant formation of trifuoromethane. Water in line 3 may also react as OH^-.

This carbanion, in the presence of sufficient water, would simply reprotonate to regenerate the original anesthetic. In the absence of sufficient water, the carbanion could eliminate a halogen anion from the ethyl moiety α to the oxygen and decompose to difluorocarbene and the corresponding aldehyde. The difluorocarbene can subsequently react with hydroxide and/or residual water# in the absorbent to form CO, *via* difluoromethanol and formyl fluoride intermediates.[28–30] Difluorocarbene could also react with CO_2 or silica in the absorbent to form CO.[30,31] Formate may arise from formyl fluoride reaction with water or by subsequent reactions of CO.[18] For desflurane and isoflurane, fluoride and chloride elimination, respectively, from the α-ethyl carbon would yield trifluoroacetaldehyde. Trifluoroacetaldehyde, in the presence of strong base, may further decompose to formate and trifluoromethane.[32] Trifluoroacetaldehyde may also decompose to CO and trifluoromethane, but this seems unlikely.[33] Additional reactions and alternative mechanisms are also possible, however, these are more speculative. Difluorocarbene, in the absence of water, may combine with itself to form tetrafluoroethylene.[23,34] A concerted reaction may occur, with deprotonation and direct difluorocarbene formation occurring simultaneously, rather than initial carbanion formation and subsequent decomposition.[20,24] This has been seen with difluoromethanes[20,24,35] and dichloromethyl methyl ether[36] but not all difluorocarbene-forming compounds.[25] A concerted mechanism, however, is not supported by the ability (fig. 5) to incorporate deuterium.[20]

The difluorocarbene mechanism permits rationalizing

‖Formation of a similar carbanion, difluoromethyleneisopropylether (isopropoxyfluoromethylene), has been shown.[21]

#Defined as any water remaining after the absorbent is dried to constant weight. Desiccated absorbents may contain some residual water, as judged by their ability to scavenge CO_2.[10]

the structural features of anesthetic degradation to CO. Why is a difluoromethyl ether a common structural element in CO formation? First, initial base-catalyzed abstraction is dictated by methoxy proton acidity, and these are substantially increased by two fluorine substituents. Second, dihalocarbanion decomposition to a dihalocarbene is favored in the absence of water, and fluorine is best at stabilizing difluorocarbenes (F $\geqq$ Cl > Br > I).[17,37] Thus, two fluorine substituents on the methoxy carbon increases both initial proton abstraction and subsequent carbanion decomposition to a dihalocarbene.[17,37] The above mechanism can also account for the observations that formate is found in soda lime after anesthetic degradation to CO,** and that trifluoromethane is formed concomitantly with CO from desflurane and isoflurane but not enflurane.[4,38]

In addition to methoxy halogen substituents, ethyl halogen substituents may also influence anesthetic degradation to CO, because desflurane, enflurane, and isoflurane possess identical difluoromethoxy groups yet differ in extent of CO formation. This difference is attributable in part to α- and β-ethyl halogen substituent influence on difluoromethoxy proton susceptibility to base-catalyzed abstraction.[39] Thus, substitution of the α-ethyl carbon with two fluorines renders the difluoromethoxy hydrogen of enflurane most acidic, compared with one α-ethyl fluorine on desflurane and one less electronegative α-ethyl chlorine on isoflurane. Differences in methoxy proton acidity (enflurane > desflurane > isoflurane) were reflected in proton abstraction and CO formation (figs. 3 and 5). Ethyl halogen substituents, specifically fluorine, can also influence CO formation by double bond–no bond resonance stabilization.[22,39] Thus, the relative reactivities for proton abstraction and fluorocarbanion stability are 3° > 2° > 1° hydrofluorocarbons, and fluorine substitution on the carbon β to the carbanion is most important.[39] Consistent with this consideration, difluoromethyl isopropyl ether (lacking any fluorine substitution except that on the methoxy group) does not undergo base-catalyzed deprotonation and difluorocarbene formation.[21]

The influence of water in carbon dioxide absorbents on CO formation merits mention. Fully hydrated or rehydrated absorbents do not degrade anesthetics to CO, and CO formation is inversely proportional to absorbent water content.[5,6,9,10] By analogy to base-catalyzed proton abstraction from haloforms, which is rapid and reversible (by water) compared to subsequent carbanion decomposition and dihalocarbene formation,[16,17] base-catalyzed methoxy proton abstraction from desflurane, enflurane, or isoflurane also appears rapid and reversible. Anesthetic carbanion reprotonation by water to regenerate parent anesthetic would compete with difluorocarbene formation. Thus, as absorbent water content diminishes, this competition would shift in favor of difluorocarbene formation. The exact role of absorbent water in difluorocarbene decomposition to CO is less certain. Experiments with $H_2{}^{18}O$ clearly showed absorbent ^{18}O as a source of the oxygen in CO (fig. 9). Because, however, desiccated absorbents could contain both OH^- and some residual water, the added ^{18}O water could react as $H_2{}^{18}O$ or $^{18}OH^-$, or possibly as ^{18}O silicates, to form $C^{18}O$; the current results do not differentiate between these sources of oxygen. Dichlorocarbene reacts exclusively, or almost exclusively, with water and not hydroxide ions,[17,18] and difluorocarbene formation under anhydrous conditions does not give CO.[24] Although this supports a role for water in CO formation, oxide,†† hydroxide, or silicates might also form CO. Any residual water in desiccated absorbents, although sufficient to hydrolyze difluorocarbene and form CO, appears insufficient to shift equilibrium toward carbanion reprotonation and thereby prevent difluorocarbene formation.

An alternative mechanism for CO formation involves proton abstraction from the ethyl, rather than the methyl, carbon of desflurane, enflurane, and isoflurane. For desflurane, the resulting carbanion could (1) α-eliminate difluoromethanol (yielding CO) to give fluorotrifluoromethylmethylene (hydrolyzing to trifluoroacetaldehyde), or (2) α-eliminate fluoride to give difluoromethoxytrifluoromethylmethylene (hydrolyzing to trifluoroacetaldehyde and difluoromethanol, yielding CO). An analogous scheme for isoflurane could be proposed. For enflurane, β-elimination of difluoromethanol (yielding CO) would produce chlorotrifluoroethylene. This alternative, or coexistent mechanism, is consistent with the deuterium isotope data (fig. 4). In contrast, this mechanism is not supported by (1) inconsistencies between ethyl proton abstraction (enflurane = isoflurane $\geqq$ desflurane) and CO formation (enflurane $\geqslant$ desflurane > isoflurane),

** Moon RE, Ingram C, Brunner EA, Meyer AF: Spontaneous generation of carbon monoxide within anesthetic circuits. Anesthesiology 1991; 75:A873.

‡‡Potassium superoxide did catayze formation of CO from desflurane, enflurane and isoflurane, although the relative rates for the three compounds were much closer than those observed with soda lime or barium hydroxide lime (results not shown).

(2) the formation of a difluorocarbene intermediate from enflurane and desflurane, and (3) the inability to observe chlorotrifluoroethylene formation specifically from enflurane (results not shown). A variation of the above mechanism, for desflurane, proposed nucleophilic attack by OH^- at the α-ethyl carbon, leading to a difluoromethyl anion and trifluoroacetyl fluoride, further sequential nucleophilic attacks at α-chloride (sic), and α-fluoride sites to form trifluoroacetic acid then an α-dihydroxyl compound, and further nucleophilic attack at the terminal hydroxyl to form CO.[40] This mechanism however is also not supported by difluorocarbene formation and difluoromethoxy requirements for CO formation and does not account for enflurane degradation to CO, absence of CO formation from methoxyflurane, or trifluoromethane formation from desflurane and isoflurane. It is also energetically unfavorable: α-ethyl deprotonation would more likely lead to dehydrofluorination than to a difluoromethyl anion, hydroxylation (initial and even more so subsequent) of a trifluoromethyl group requires vigorous conditions not occurring in absorbents (whereas base-catalyzed decarboxylation of trifluoroacetic acid is more likely[41]); there is no α-chloride in desflurane. At present, both methoxy- and ethyl-proton abstraction mechanisms are plausible, but more data support the former mechanism.

Clinical Implications

Previous investigations have shown that the magnitude of CO production by carbon dioxide absorbents from equiMAC anesthetic concentrations is desflurane > enflurane > isoflurane, whereas halothane and isoflurane produced minimal or no CO, both *in vitro*[4,9,11] and in anesthesia machines.[5] The current results corroborate these findings. Furthermore, they indicate that greater CO formation from desflurane than enflurane at equiMAC concentrations results from a difference in anesthetic potency. Although at equimole concentrations enflurane produces more CO, the lower potency of desflurane (6% MAC) *versus* enflurane (1.7%) necessitates much higher clinical desflurane concentrations, and hence, greater CO formation ensues.

Carbon monoxide formation from desflurane, enflurane, and isoflurane was greater with barium hydroxide lime than with soda lime, both *in vitro*[4,9] and in anesthesia machines.[4-6] The current results confirm these findings and provide an explanation: base-catalyzed difluoromethoxy proton abstraction was greater with potassium than sodium hydroxide. Barium hydroxide lime contains 4.6% potassium hydroxide, whereas soda lime contains only 2.5% potassium hydroxide and 1.5% sodium hydroxide, hence greater initial proton abstraction with barium hydroxide lime. Although barium hydroxide is contained in barium hydroxide lime but not soda lime, this appears to be less relevant because barium hydroxide does not catalyze difluoromethoxy proton abstraction.

Anesthetic development and the search for an "ideal" anesthetic agent continue. A previous investigation identified structural features that predispose halogenated compounds to metabolism and metabolism-based toxicity and proposed methods for designing safer chemicals by avoiding these elements.[42] Similarly, the current investigation identifies structural features that predispose halogenated anesthetics to CO_2 absorbent-catalyzed degradation to CO, and a possible mechanism of CO formation. Safer anesthetics can be designed by avoiding structural features that facilitate CO formation.

The authors thank Dr. Jeff Jones, University of Rochester, for the helpful discussions regarding difluorocarbene chemistry and Dr. William Trager, University of Washington, for the discussions regarding deuterium isotope effects and their mechanistic implications. We also thank Drs. Trager and Rob Palmer for critical review of the manuscript.

References

1. Lentz RE: CO poisoning during anesthesia poses puzzle. J Clin Monit 1995; 11:67–71

2. Moon RE: Cause of CO poisoning, relation to halogenated agents still not clear. J Clin Monit 1995; 11:66–7

3. Woehlck HJ, Dunning M III, Gandhi S, Chang D, Milosavljevic D: Indirect detection of intraoperative carbon monoxide exposure by mass spectrometry during isoflurane anesthesia. ANESTHESIOLOGY 1995; 83:213–7

4. Woehlck HJ, Dunning M III, Nithipatikom K, Kulier AH, Henry DW: Mass spectrometry provides warning of carbon monoxide exposure via trifluoromethane. ANESTHESIOLOGY 1996; 84:1489–93

5. Frink EJ Jr, Nogami WM, Morgan SE: Production of carbon monoxide using dry Baralyme with desflurane, enflurane, isoflurane, halothane, or sevoflurane anesthesia in pigs (abstract). ANESTHESIOLOGY 1996; 85:A1018

6. Frink EJ Jr, Nogami WM, Morgan SE, Salmon RC: High carboxyhemoglobin concentrations occur in swine during desflurane anesthesia in the presence of partially dried carbon dioxide absorbents. ANESTHESIOLOGY 1997; 87:308–16

7. Woehlck HJ, Dunning M III, Connolly LA: Reduction in the incidence of carbon monoxide exposures in humans undergoing general anesthesia. ANESTHESIOLOGY 1997; 87:228–34

8. Buckley RG, Aks SE, Eshom JL, Rydman RR, Schaider J, Shayne P: The pulse oximetry gap in carbon monoxide intoxication. Ann Emerg Med 1994; 24:252–5

9. Fang Z, Eger EI II, Laster MJ, Chortkoff BS, Kandel L, Ionescu P: Carbon monoxide production from degradation of desflurane, enflu-

rane, isoflurane, halothane and sevoflurane by soda lime and Baralyme. Anesth Analg 1995; 80:1187–93

10. Baxter PJ, Kharasch ED: Rehydration of desiccated Baralyme prevents carbon monoxide formation from desflurane in an anesthesia machine. ANESTHESIOLOGY 1997; 86:1061–5

11. Fang Z, Eger II EI: Production of carbon monoxide from carbon dioxide absorbents acting on volatile anesthetics. Anesth Analg 1995; 80:S120

12. Burke TR, Pohl LR: Synthesis of deuterated and tritiated derivatives of enflurane. J Labelled Compounds 1981; 18:663–70

13. Dolbier WR Jr, Wojtowicz H, Burkholder CR: New zinc difluorocarbenoid reagent. J Org Chem 1990; 55:5420–2

14. Kamel M: *gem*-Difluorocyclopropane aus chlordifluormethan und alkanolat-ionen in geringer konzentration. Chem Ber 1976; 109: 2351–69

15. Hine J, Dowell AM Jr, Singley JE Jr: Carbon dihalides as intermediates in the basic hydrolysis of haloforms. IV. Relative reactivities of haloforms. J Am Chem Soc 1956; 78:479–82

16. Hine J: Carbon dichloride as an intermediate in the basic hydrolysis of chloroform. A mechanism for substitution reactions at a saturated carbon atom. J Am Chem Soc 1950; 72:2438–45

17. Hine J: Divalent Carbon. New York, The Ronald Press, 1964

18. Robinson EA: The reaction of dichloromethane with water and with phenoxide ions (Reimer-Tiemann reaction). J Chem Soc 1961; 1663–71

19. Hine J, Burske NW: The kinetics of the base-catalyzed deuterium exchange of dichlorofluoromethane in aqueous solution. J Am Chem Soc 1956; 78:3337–9

20. Hine J, Langford PB: Methylene derivatives as intermediates in polar reactions. IX. The concerted mechanism for α-eliminations of haloforms. J Am Chem Soc 1957; 79:5497–5500

21. Hine J, Tanabe K: Methylene derivatives as intermediates in polar reactions. XII. Isopropoxyfluoromethylene. J Am Chem Soc 1958; 80:3002–7

22. Hine J: Polar effects on rates and equilibria. VIII. Double bond-no bond resonance. J Am Chem Soc 1963; 85:3239–44

23. von E Doering W, Hoffman AK: The addition of dichlorocarbene to olefins. J Am Chem Soc 1954; 76:6162–5

24. Hine J, Porter JJ: Methylene derivatives as intermediates in polar reactions. VIII. Difluoromethylene in the reaction of chlorodifluoromethane with sodium methoxide. J Am Chem Soc 1957; 79:5493–6

25. Hine J, Porter JJ: The formation of difluoromethylene from difluoromethyl phenyl sulfone and sodium methoxide. J Am Chem Soc 1960; 82:6178–81

26. Takeyoshi S: Stability of isoflurane in soda lime. Hiroshima J Anesth 1992; 25:321–7

27. Horibe M, Fujii K, Morio M, Yuge O, Kikuchi H: Decomposition of enflurane in soda lime. J Anesth 1994; 8:84–6

28. Krauskopf KB, Rollefson GK: The photochemical reaction between chlorine and formaldehyde. The preparation of formyl chloride. J Am Chem Soc 1934; 56:2542–8

29. Barbanov VG, No LV, Fedursta MU, Kushina ID: High-energy reactions of polyfluoroalkanes. I. Mechanism and kinetics of the formation of carbon monoxide in the reaction of chlorodifluoromethane with steam. Zh Obshch Khim 1984; 54:824–7

30. Jollie DM, Harrison PG: An *in situ* IR study of the thermal decomposition of trifluoroacetic acid. J Chem Soc Perkins Trans 1997; 2:1571–5

31. Birchall JM, Haszeldine RN, Roberts DW: Cyclopropane chemistry. II. Cyclopropanes as sources of difluorocarbene. J Chem Soc Perkins Trans I 1973; 1071–8

32. Pfeil E, Stache H, Lömker F: Zur kritik reaktionskinetischer methoden: Die spaltung der trihalogenacetaldehyde durch laugen. Just Lieb Am Chem 1959; 623:74–91

33. Dodd RE, Smith JW: The photolysis of trifluoroacetaldehyde. J Chem Soc 1957; 1465–73

34. Chambers RD: Fluorine in Organic Chemistry. New York, Wiley Interscience, 1973

35. Hine J, Ketley AD: Methylene derivatives as intermediates in polar reactions. XX. The reactions of aqueous and alcoholic base with chlorodifluoromethane and difluoroiodomethane. J Org Chem 1960; 25:606–7

36. Hine J, Rosscup RJ, Duffey DC: Formation of the intermediate methoxychloromethylene in the reaction of dichloromethyl methyl ether with base. J Am Chem Soc 1960; 82:6120–3

37. Hine J, Ehrenson SJ: The effect of structure on the relative stability of dihalomethylenes. J Am Chem Soc 1958; 80:824–30

38. Eger EI II, Strum DP: The absorption and degradation of isoflurane and I-653 by dry soda lime at various temperatures. Anesth Analg 1987; 66:1312–5

39. Andreades S: Fluorocarbanions. Rates of base-catalyzed hydrogen-deuterium exchange, isotope effects, and acidity of monohydrofluorocarbons. J Am Chem Soc 1964; 86:2003–10

40. Huh BK, Passannante AN, Crimmins MT: A mechanism of carbon monoxide production from volatile anesthetics and anhydrous soda lime in anesthetic circuits (abstract). ANESTHESIOLOGY 1997; 87: A1138

41. Auerbach I, Verhoek FH, Henne AL: Kinetic studies on the decarboxylation of sodium trifluoroacetate in ethylene glycol. J Am Chem Soc 1950; 72:299–300

42. Yin H, Anders MW, Korzekwa K, Higgins L, Thummel KE, Kharasch ED, Jones JP: Designing safer chemicals: Predicting the rates of halogenated alkane metabolism. Proc Natl Acad Sci USA 1995; 92:11076–80