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Absence of Abundant Binding Sites for Anesthetics in Rabbit Brain: An In Vivo *NMR Study*

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Using magnetic resonance spectroscopy, the authors tested whether cerebral concentrations of inhaled anesthetics do not increase proportionately at inspired concentrations exceeding 3% 1) because anesthetics bind to and saturate specific sites in the brain or 2) because anesthetic-induced depression of ventilation limits the increase in alveolar anesthetic partial pressure. New Zealand White rabbits were anesthetized with methohexital, 70% nitrous oxide, and local infiltration of 1% lidocaine. Cerebral concentrations of anesthetic were determined from ^{19}F spectra acquired with nuclear magnetic resonance (NMR). Inspired, end-tidal, and arterial anesthetic concentrations, and end-tidal and arterial partial pressure of carbon dioxide were measured. Blood/gas partition coefficients were determined and used to convert arterial anesthetic concentration to partial pressures. In seven spontaneously breathing animals, halothane (1%; n = 5) or isoflurane (0.8%; n = 2) was administered at a constant inspired concentration for 20 min; NMR spectra were acquired between 10 and 20 min. Thereafter, the inspired concentration was increased and the process repeated until apnea occurred. Two additional rabbits were anesthetized with isoflurane and studied similarly but with higher inspired concentrations during mechanical ventilation. In spontaneously breathing animals, ventilatory depression occurred, documented by marked increases in $PaCO_2$, and cerebral concentrations of anesthetic did not increase proportionately at inspired concentrations exceeding 3%. In contrast to an absence of a correlation of inspired and cerebral concentrations during spontaneous ventilation, arterial and cerebral concentrations correlated linearly during both spontaneous and mechanical ventilation ($R^2 > 0.969$). These results are consistent with depression of ventilation, rather than binding to specific cerebral sites as an explanation for the nonlinear relationship between cerebral and inspired anesthetic concentrations. (Key words: Anesthetics, volatile: halothane, isoflurane. Pharmacokinetics: binding, elimination, effects of ventilation. Measurement techniques, Magnetic resonance spectroscopy: ^{19}F, spin-echo.)

FROM ^{19}F NUCLEAR magnetic resonance (NMR) spectroscopy studies, Evers *et al.*[1] reported that inhaled anesthetics bind to the brain and suggested that these agents may act by binding to and altering abundant saturable molecular sites. In deeply anesthetized, spontaneously breathing rats, they found that cerebral concentrations of halothane did not increase in proportion to inspired concentrations. Cerebral concentrations were comparable at inspired concentrations of 2.5 and 4% halothane. ^{19}F NMR spectroscopy of excised brain tissue also identified two molecular cerebral environments. "Bound" anesthetic molecules were distinguished from those freely-tumbling by differences in spin-spin relaxation times (T_2).§§ More importantly, higher inspired halothane concentrations saturated the environment for bound molecules, but not for more freely tumbling molecules. Approximately 80% of the halothane molecules inhabited the bound environment. Halothane occupied this bound environment coincident with induction of anesthesia, half-saturating it at concentrations that produce clinical anesthesia. Accordingly, halothane's anesthetic effect was attributed to its occupancy of this environment. An editorial by Franks and Lieb[2] asserted that these results, "if confirmed, will be seen to represent an important step toward unraveling the mystery of general anaesthesia."

We offer a different viewpoint, suggesting that the "saturation" observed by Evers *et al.*[1] results not from binding of inhaled anesthetics to specific sites but rather from ventilatory depression.¶¶ High concentrations of halothane (and isoflurane) produce ventilatory depression.[3] At anesthetizing concentrations ≥1 MAC (the anesthetic ED_{50}, approximately 1%), ventilation is depressed more than cardiac output, thereby increasing the difference between inspired and alveolar anesthetic partial pressures.[4] The alveolar partial pressure determines the

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§§ A description of NMR terminology and concepts is provided in the appendix of Litt *et al.*[6]

¶¶ Lockhart S, Cohen Y, Kim F, Yasuda N, Litt L, Eger EI, Freire B, Johnson BH, Chang LH, James TL: A ^{19}F In vivo NMR study to test for halothane binding and saturation in the rabbit brain (abstract). Eighth Annual Meeting of the Society of Magnetic Resonance in Medicine, 1989, p 338.

partial pressure of anesthetic in arterial blood, which in turn determines the partial pressure in all perfused organs, including the brain.[5] During profound ventilatory depression, the inspired partial pressure of anesthetic can be increased without the alveolar, arterial, or cerebral partial pressures increasing.[4] Thus, ventilatory depression may account for the apparent existence of saturable cerebral binding sites.

Because steady-state arterial and cerebral partial pressures of anesthetic are equal, saturation of cerebral sites should be assessed by comparing simultaneous measurements of steady-state arterial partial pressures and cerebral anesthetic concentrations. If cerebral concentrations and arterial partial pressures are proportional over a wide range, then "saturation" of abundant cerebral binding sites is excluded.

Methods and Materials

With approval from the University of California, San Francisco Committee on Animal Research, we anesthetized 9 4–5-kg New Zealand White rabbits by intramuscular and intravenous injection of methohexital, inhalation of 70% nitrous oxide, and infiltration with 1% lidocaine. A catheter was inserted into a vein in the ear and a catheter into the femoral artery. We intubated the trachea *via* a tracheostomy with a 4.0–4.5-mm Portex® tube designed for sampling end-tidal gases from a distal (tracheal) port. A craniectomy exposed a 2–2.5-cm diameter circle of dura. Infusion of Ringer's® lactate solution (4 $ml \cdot kg^{-1} \cdot h^{-1}$) supported intravascular volume, and infusion of dopamine HCl and epinephrine was added as needed to maintain blood pressure. A servo-controlled water-jacketed cradle maintained rectal temperature between 37° and 40° C.

Cerebral concentrations of anesthetic were measured *in vivo* from ^{19}F NMR spectra acquired at 188.2 MHz on a horizontal 4.7-T Nalorac® spectrometer. A 0.9-cm by 1-cm elliptical, two-turn radio-frequency surface coil with a balanced matched circuit[6,7] was placed over the exposed dura. Magnetic field homogeneity was maximized by adjusting room-temperature shimming coils until the water proton line width was less than 35 Hz. A vial of potassium fluoride in D_2O placed above the surface coil served as a chemical shift and numerical integration reference. ^{19}F NMR spectra were obtained for 10-min epochs by averaging 360 acquisitions separated by a 1.8-s recycle time. A one-pulse sequence with quadrature detection, an acquisition time of 256 ms, and a dwell time of 62.5 μs was used. The spectral width was ±8000 Hz and the pulse width was chosen to give the maximum signal intensity.

Spin-spin NMR relaxation times (T_2) were measured *in vivo* by the Hahn spin-echo method,[8] by averaging 280 acquisitions separated by a recycle time of 3.2 s. Parameters were otherwise the same as for the one-pulse sequence, resulting in a total collection time of 15.2 min for each echo delay time. Echo delay times for halothane and isoflurane were 1, 1.5, 2, 3, 5, 10, 20, and 30 ms. The study for the 1-ms delay was then repeated and compared with the first determination to confirm that results were not affected by the duration of the experiment. T_2 values were derived from the relationship between the natural logarithm of the ^{19}F signal intensity and echo evolution time with the use of linear regression. Values for T_2 were defined as the reciprocal of the negative slope of the lines derived from curve stripping.

Inspired and end-tidal concentrations of anesthetic were measured with a Beckman® LB-2 infrared analyzer, calibrated with premixed tanks the concentration of which was determined by reference to primary volumetric standards. Blood/gas partition coefficients were determined for each animal at the beginning and end of each experiment.[9] To determine anesthetic partial pressure in arterial blood, a 1-ml sample of arterial blood anticoagulated with EDTA was injected into an evacuated (approximately ⅓ atm) flask of precisely known volume (approximately 600 ml). The flask was placed in a water bath at 39° C for 1 h and shaken vigorously at 15-min intervals before equilibrating its contents with ambient pressure. At the end of the hour, 20 ml air was added to the flask and barbotaged 20 times. The mixture then was analyzed by gas chromatography. The results permitted calculation of the concentration of anesthetic in blood.[9] The blood/gas partition coefficient (corrected for body temperature) was used to convert anesthetic concentration to partial pressure. End-tidal carbon dioxide measurements were obtained with a Puritan-Bennett® analyzer, and arterial blood gases were determined with a Corning 178® analyzer calibrated before each use. In every case, a background ^{19}F spectrum was obtained before administration of a volatile agent.

In spontaneously breathing rabbits, anesthesia was maintained with 1% (inspired) halothane (n = 5) or 0.8% isoflurane (n = 2) for 20 min, followed by measurement of inspired, end-tidal, and arterial anesthetic partial pressures, end-tidal CO_2, arterial blood gases, and rectal temperature. Cerebral anesthetic concentration, defined by ^{19}F NMR signal intensity, was computed from spectra acquired between 10 and 20 min. Signal intensity was defined as the area of the single peak for halothane or of the trifluoromethyl peak for isoflurane, normalized by the area of the potassium fluoride reference peak. The inspired concentration was then increased in increments of 0.5–1%, and the entire protocol repeated at each inspired concentration. This process was repeated until apnea occurred. The existence of a steady state was confirmed by consistent signal intensities obtained between 10–15 min and 15–20 min, differing by less than 4% across the entire range of concentrations studied.

Two additional rabbits were anesthetized with isoflurane and studied during mechanical ventilation, with arterial P_{CO_2} maintained between 35 and 45 mmHg. Inspired anesthetic concentrations imposed during controlled ventilation exceeded those during spontaneous ventilation. The highest inspired concentrations applied during controlled ventilation were those producing circulatory collapse. The T_2 also was measured at each inspired concentration. Consequently, each concentration of isoflurane was maintained for 130 min to allow for collection of data at eight different echo delay times. The inspired concentration then was increased by 1.5% and the process repeated.

All regressions and correlation coefficients were estimated by the method of linear least squares.

Results

In spontaneously breathing rabbits, a lack of proportionality was observed both between inspired concentration and cerebral concentration (fig. 1) and between inspired concentration and arterial partial pressure (fig. 2) at inspired concentrations exceeding 3% for halothane and 4% for isoflurane. Ventilatory depression was documented by marked increases in end-tidal carbon dioxide (CO_2) and arterial carbon dioxide tension (P_{CO_2}) at these concentrations. By contrast, cerebral anesthetic concentration and arterial partial pressure correlated linearly for both halothane and isoflurane (fig. 3). For any animal, the smallest squared correlation coefficient relating arterial partial pressure and cerebral concentration was 0.969.

In the two rabbits anesthetized with isoflurane and studied during mechanical ventilation, the absence of ventilatory depression resulted in a linear correlation between anesthetic inspired concentration and arterial partial pressure over a very large range of arterial anesthetic partial pressures. The highest partial pressure (0.067 atm) was nearly two-fold greater than the highest partial pressure (0.038 atm) found with spontaneous ventilation. The correlation between inspired and cerebral isoflurane concentrations also was linear (R^2 = 0.987; 0.981) (unlike that observed during spontaneous ventilation), as was the correlation between arterial partial pressure and cerebral concentration (R^2 = 0.998; 0.989) (fig. 4). Spectral changes observed during spontaneous ventilation showed a linear correlation with inspired concentrations below 4.0% but an absence of correlation at higher concentrations (fig. 5, top). In contrast, a linear correlation is apparent for all concentrations when ventilation is controlled (fig. 5, bottom).

In vivo spin-echo determinations for halothane T_2 yielded a biexponential decay, whereas determinations for isoflurane T_2 yielded a single exponential decay for echo delays between 1 and 30 ms. Our finding of one T_2 environment for isoflurane but two for halothane *in vivo* is consistent with *in vitro* results reported by Evers *et al.*[1,10] The single T_2 for isoflurane results from either one environment or an average of multiple environments among which anesthetic molecules can rapidly exchange. Our T_2 values *in vivo* were 4.2 and 49 ms for halothane and 5 ms for isoflurane, consistent with *in vitro* values reported by Evers *et al.* of 3.6 and 43 ms for halothane and 2.4 ms for isoflurane.[1,10] Our results indicate that, *in vivo*, 95% of the halothane inhabited the short T_2 environment. *In vivo* spin-echo measurements using a surface coil (Hahn spin-echo method[8]) are not as accurate as *in vitro* high-resolution measurements (Carr-Purcell-Meiboom-Gill sequence[1,10]). However, these measurements do confirm that the population of anesthetic molecules detected by our surface coil is the same as that observed by Evers *et al.*[1]

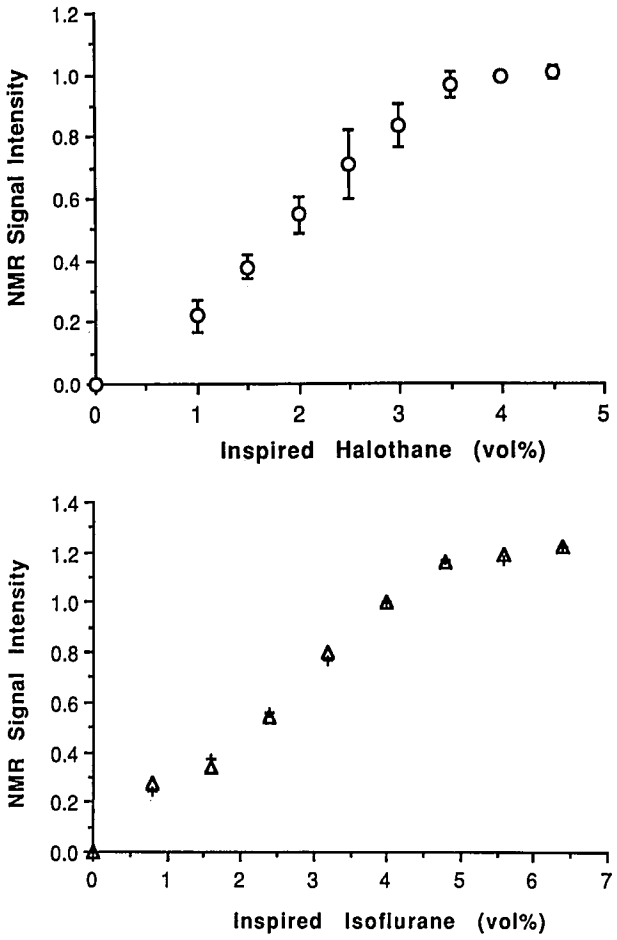

FIG. 1. In spontaneously breathing rabbits, cerebral anesthetic concentrations (mean ± SD) do not correlate with inspired concentrations exceeding 3% halothane (*top*, n = 5) or 4% isoflurane (*bottom*, n = 2). Cerebral concentrations (defined as NMR signal intensity) were normalized by dividing by the value obtained at an inspired concentration of 4%.

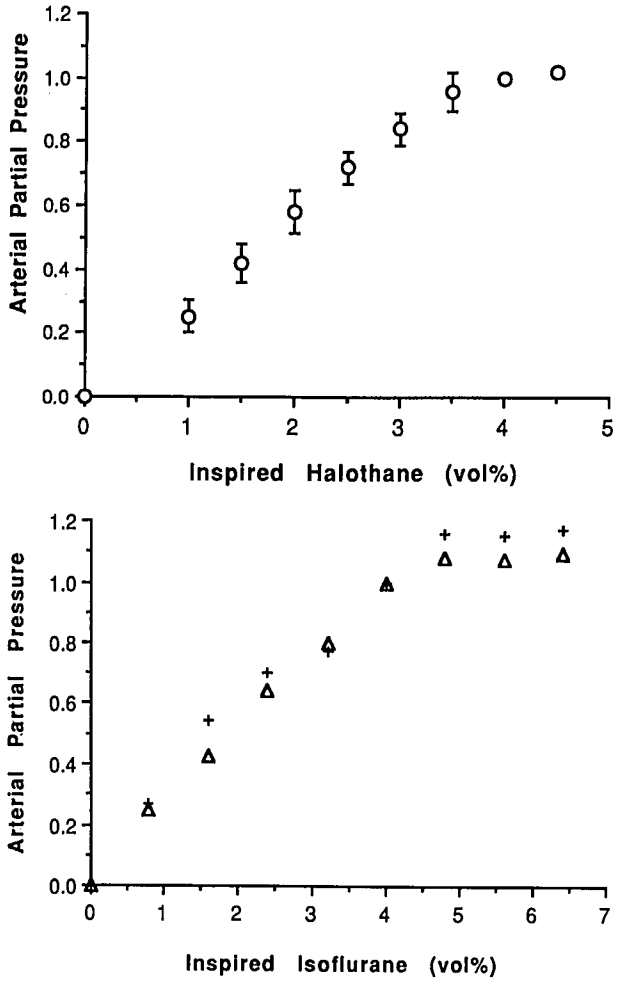

FIG. 2. Arterial partial pressure also failed to correlate with inspired concentration of halothane (*top*) and isoflurane (*bottom*) in the seven spontaneously breathing rabbits. Values for arterial partial pressure were normalized by dividing by the value obtained at an inspired concentration of 4%.

Discussion

We and Evers *et al.*[1,11] found an absence of correlation between inspired and cerebral anesthetic concentrations at high inspired concentrations of anesthetic in spontaneously breathing animals. However, our data demonstrate that this absence results from depression of ventilation rather than "saturation" of cerebral binding sites. Our finding of a linear correlation between anesthetic arterial partial pressure and cerebral concentration for halothane and isoflurane during spontaneous ventilation excludes the possibility of abundant saturable binding sites in brain. This conclusion is confirmed by data from the two animals in whom ventilation was controlled. In these animals, correlation was found among cerebral concentration, arterial partial pressure, and inspired concentration was linear for arterial partial pressures of isoflurane as high as 0.067 atm (a value well above those achieved clinically).

Our new data are consistent with a recent correction reported by Evers *et al.* for studies in rats.[11] The corrected data, however, have limitations that do not apply to our data: 1) Evers *et al.* assumed without contemporaneous documentation that their results were consequent to ventilatory depression. We obtained measurements (PA_{CO_2} and Pa_{CO_2}) documenting depression of ventilation. 2) Dose–response measurements were not obtained in the same animal; each animal in the corrected report supplied only a single point, whereas complete dose–response re-

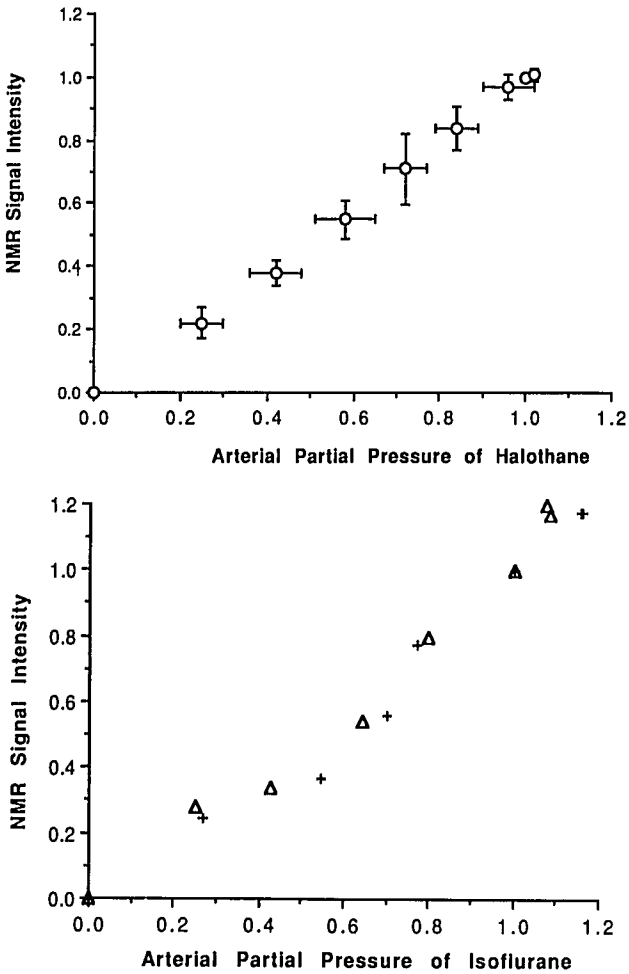

FIG. 3. In contrast to the results displayed in figures 1 and 2, cerebral concentration correlated linearly with all arterial partial pressures of halothane (*top*) and isoflurane (*bottom*). The linear correlation coefficients (estimated by least squares) are 0.998 for the combined halothane data and 0.969 (+) and 0.990 (Δ) for each rabbit anesthetized with isoflurane. Cerebral concentrations (defined as NMR signal intensity) were normalized by dividing by the value obtained at an inspired concentration of 4%. Values for arterial partial pressure were normalized by dividing by the value obtained at an inspired concentration of 4%.

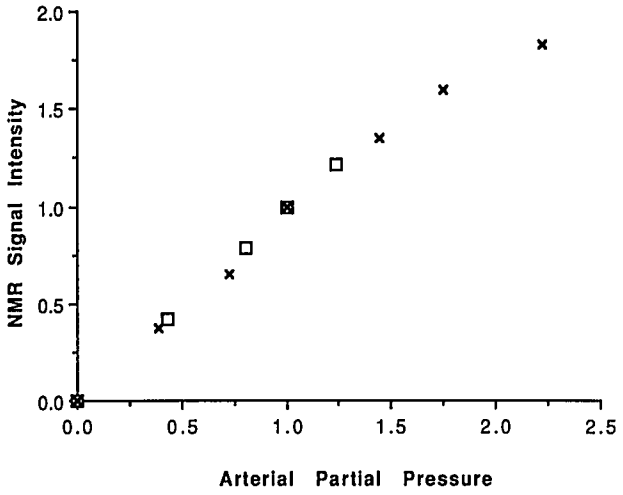

FIG. 4. Similar to the results displayed in figure 3, cerebral concentration correlated linearly with arterial partial pressure of isoflurane in the two cases in which the lungs were mechanically ventilated to maintain arterial P_{CO_2} 35–45 mmHg. The linear correlation coefficients were 0.989 (□) and 0.998 (x) for the two respective animals. Cerebral concentrations (defined as NMR signal intensity) were normalized by dividing by the value obtained at an inspired concentration of 4.5%. Values for arterial partial pressure were normalized by dividing by the value obtained at an inspired concentration of 4.5%.

lationships were obtained in the current study. 3) Only halothane was studied, and over a more limited range of concentrations than in the current study. That is, our results lend themselves to a more general interpretation than those supplied by Evers *et al.* (Also see point 4). 4) The effect of controlled ventilation was not studied. We demonstrated a linear correlation between inspired anesthetic concentration and arterial partial pressure and between arterial partial pressure and cerebral concentration when ventilation was controlled. 5) The measurements made by Evers *et al.* were made with decapitated animals rather than made *in vivo* as in the current study. In this respect, our separate reports are complementary, suggesting that the *in vitro* approach produces results similar to those obtained *in vivo*.

In conclusion, our data for halothane in spontaneously breathing animals confirm those of Evers *et al.*[1,11] However, our additional data demonstrating linear correlation of arterial anesthetic partial pressures and cerebral concentrations excludes an interpretation of abundant saturable binding sites for anesthetics in brain. The similarity of our results for isoflurane and halothane suggests that these results apply to other inhaled anesthetics. The differences we observed with spontaneous versus controlled ventilation confirms our hypothesis that ventilatory depression causes the apparent "saturation." The sites and mechanisms of action of inhaled anesthetics remain unknown.

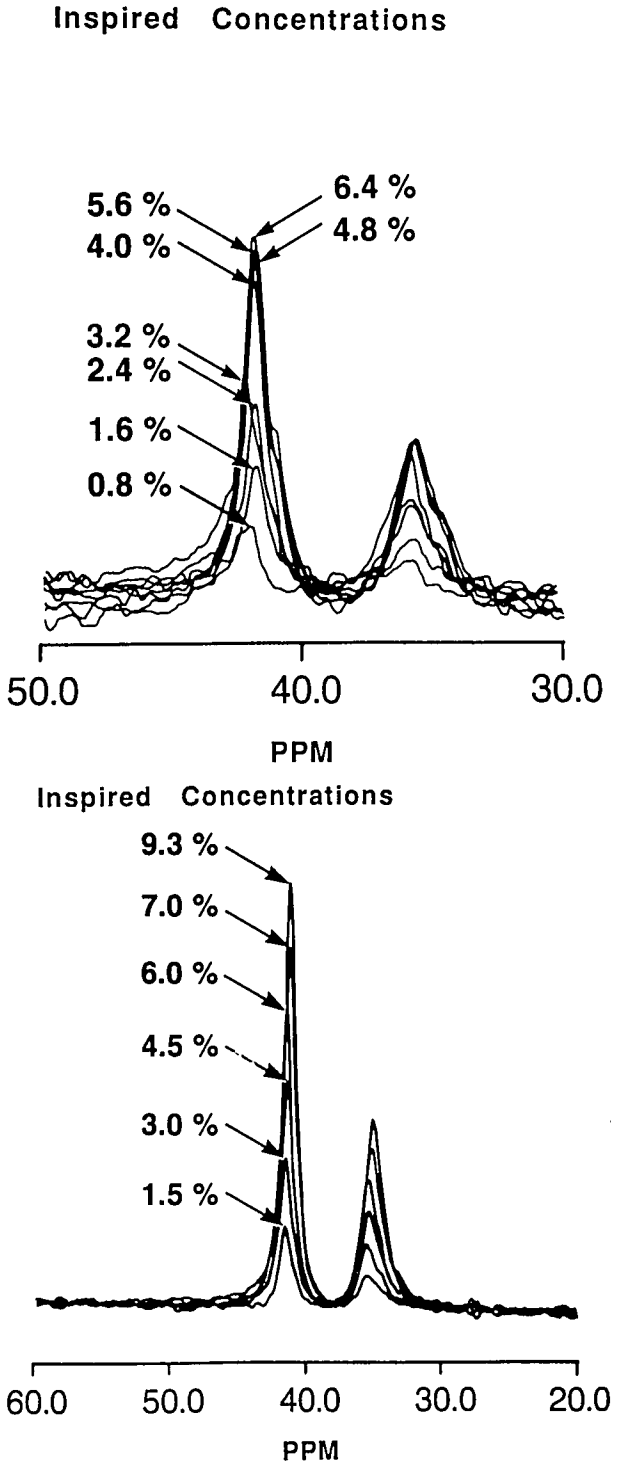

FIG. 5. Representative spectra of isoflurane at inspired concentrations ranging from 0.8 to 6.7% during spontaneous ventilation demonstrate a lack of correlation at concentrations exceeding 4.0% (*top*). In contrast, representative spectra of isoflurane at inspired concentrations ranging from 1.5 to 9.3% during mechanical ventilation demonstrate a good correlation at all concentrations (*bottom*). The x-axis denotes chemical shift in parts per million (ppm) downfield from the potassium fluoride reference.

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