Anesthesiology
67:169–173, 1987

An In Vivo ^{19}F *Nuclear Magnetic Resonance Study of Isoflurane Elimination from the Rabbit Brain*

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^{19}F nuclear magnetic resonance spectroscopy was performed at 2.0 Tesla to evaluate the washout of isoflurane from the adult rabbit brain after 90 min of anesthesia. This investigation reconciles previous *in vivo* NMR studies of others, which observed a slow anesthetic washout, with invasive non-NMR studies that found a rapid washout, as predicted by perfusion-limited models of anesthetic uptake and elimination. Two NMR surface coil experiments were performed: in the first, a 1-cm surface coil was placed directly over the exposed dura to be certain that the washout was observed only from the brain; in the second, a 3-cm coil was placed noninvasively over the intact scalp to emulate previous NMR experiments. As in previous NMR experiments, a slow washout of isoflurane was observed with the large coil. The NMR signal that is observed with the large coil cannot be attributed solely to brain tissue. Fat surrounding the brain contributes significantly to the fluorine NMR spectra that are observed with the 3-cm coil, and its contributions lengthen the apparent washout time. A rapid washout of isoflurane from the rabbit brain was observed with the small coil, whose signal unambiguously arises only from brain tissue. The observed rapid washout is consistent with previous invasive biochemical measurements of anesthetic washout from the brain. (Key words: Brain. Ions: ^{19}F. Measurement techniques: nuclear magnetic resonance spectroscopy. Pharmacokinetics.)

UNTIL RECENTLY, PHARMACOKINETIC studies of inhalation anesthetics were performed by invasive techniques, such as autoradiography,[1] or by indirect techniques, such as end-tidal gas chromatography.[2] The *in vivo* use of ^{19}F nuclear magnetic resonance (NMR) spectroscopy was pioneered by Wyrwicz *et al.*, who detected anesthetic agents in brain of living rabbits.[3,4] However, in their pharmacokinetic studies, they found 40% of the ^{19}F NMR signal present 7 h after terminating a 30-min halothane anesthetic, and 45% of the ^{19}F NMR signal present 5 h after a 90-min isoflurane anesthetic.[3,4] This slow anesthetic elimination conflicts with invasive results and with end-tidal gas chromatographic studies.[1,5] It is also inconsistent with perfusion-limited models of anesthetic uptake and distribution, which are based on the tissue solubility of anesthetics and tissue blood flow.[2] The *in vivo* ^{19}F NMR results of Wyrwicz *et al.* motivated an investigation that asked if the tissue partition coefficients of halothane and isoflurane varied with the partial pressure of the anesthetic. The investigation found that Henry's law was obeyed by isoflurane and halothane over a range of partial pressures from 0.01 to 1.0 MAC,[6] proving that there is no significant tissue binding of these agents in this partial pressure range. In another study prompted by previous *in vivo* NMR results, gas chromatography was used to measure the concentration of isoflurane in rabbit brains during and following a 90-min anesthetic.[7] A rapid elimination of isoflurane from the brain was found in that invasive study. The concordance among all invasive studies of anesthetic washout from the brain and their discordance with the washout observed with NMR spectroscopy questions the possibility of using ^{19}F surface coil spectroscopy for accurate *in vivo* measurements of anesthetic agents in the brain.

Experiments demonstrating the *ex vivo* quantitation of fluorinated anesthetic agents with ^{19}F NMR spectroscopy have been performed previously by Wyrwicz and others with tissue preparations.[8–14] We recently used ^{19}F NMR spectroscopy *in vivo* at 5.6 Tesla to verify that halothane washes out rapidly from the rat brain after 45 min of anesthetic administration.[15] In this paper, we use a small NMR coil in an animal model similar to the one used by Wyrwicz *et al.* in an attempt to reconcile previous noninvasive NMR results with those obtained with invasive biochemical techniques.

Materials and Methods

Our protocol was aproved by the University of California, San Francisco, Committee on Animal Research.

This article is accompanied by an editorial. Please see: Smith DS, Chance B: New techniques, new opportunities, old problems. ANESTHESIOLOGY 67:157–160, 1987.

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Received from the Departments of Anesthesia, Pharmaceutical Chemistry, Neurosurgery, and Radiology, the University of California School of Medicine, San Francisco, California. Accepted for publication February 27, 1987. Suported by grants from the Anaquest Clinical Research Program, the Anesthesia Research Foundation, the NIH (Grant R23-GM34767), the Parker B. Francis Foundation, the Pharmaceutical Manufacturers Association, and the UCSF Academic Senate Committee on Research. Presented in part at the annual meeting of the Association of University Anesthetists, Gainesville, April, 1986.

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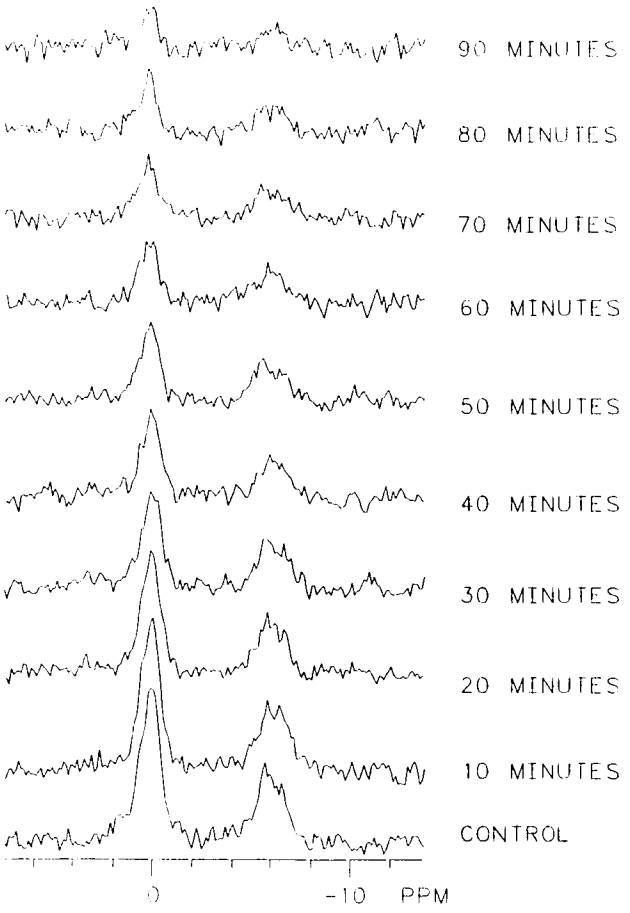

FIG. 1. Representative ^{19}F NMR washout spectra from one rabbit, obtained *in vivo* with the 1.0-cm diameter surface coil placed directly on the dura. NMR parameters are given in the text. The peak at 0 ppm is from the three identical ^{19}F trifluoromethyl nuclei. The broad smaller peak at −6 ppm, which comes from the difluoroethyl ^{19}F nuclei, represents separate NMR peaks that can be resolved *in vitro*.

It was chosen to emulate previous NMR experiments and recent invasive gas chromatography experiments. Anesthesia was induced in seven 3.0–4.0-kg New Zealand white rabbits with ketamine (35 $mg \cdot kg^{-1}$), xylazine 5 $mg \cdot kg^{-1}$), and oxygen. The trachea was intubated with a cuffed endotracheal tube, pancuronium was administered, and the rabbits were mechanically ventilated with a Harvard® ventilator to maintain the $P_{A_{CO_2}}$, between 35 and 40 mmHg. Anesthesia was maintained during surgery with 60% nitrous oxide and an intravenous infusion of ketamine (10 $mg \cdot kg^{-1} \cdot hr^{-1}$). Rectal temperature was monitored with a nonmagnetic thermocouple (Mon-a-Therm, Inc.) and maintained at 38.5 ± 1.0° C with a servocontrolled water-jacketed cradle. Intravascular volume was supported with a constant intravenous infusion of normal saline (4 $mg \cdot kg^{-1} \cdot hr^{-1}$), and pancuronium was administered as needed to maintain paralysis.

Arterial blood gas measurements were made with a Corning 178® analyzer that was calibrated prior to each use. Isoflurane washout was evaluated in four rabbits after reflecting the scalp and removing a 4-cm diameter circle of cranial bone. A 1.0 cm diameter, two-turn radiofrequency surface detection coil with a balanced matched circuit[16] was centered over the exposed dura, and situated at least 1 cm away from any surrounding bone or scalp. The coil was tuned to 80.49 MHz, the ^{19}F resonance frequency of our GE CSI-II, 2.0 Tesla spectrometer/imager. The magnetic field homogeneity was optimized by adjusting room temperature shimming coils until the water proton linewidth was less than 25 Hz. Just before collecting NMR spectra, the ketamine infusion was discontinued, and the inspired isoflurane concentration was set to 1.5% using a Fluotec 3® direct-reading vaporizer that was calibrated by gas chromatography. After 90 min of administration, isoflurane was discontinued, and the ^{19}F NMR washout from the brain was recorded for an additional 90 min.

^{19}F NMR spectra were obtained every 10 min by averaging 540 acquisitions that were separated by a 1.1-s recycle time. The spectral width was 5000 Hz, and the pulse width was chosen to give the maximum signal intensity. A representative series of washout spectra from one rabbit with an exposed brain are shown in figure 1. The cerebral metabolic integrity of each animal was confirmed at the end of the isoflurane washout by a ^{31}P spectrum which showed a normal brain intracellular *p*H and a normal brain phosphocreatine/ATP ratio (≈1.8). The isoflurane NMR signal intensity, obtained by numerical integration, was normalized to the maximum area that was observed during the final 10 min of the isoflurane administration. The data from four rabbits were averaged for each time point and the resulting values were then fit to a single exponential decay. A fifth rabbit was studied with a protocol that was similar to the one described by Wyrwicz *et al.*[4] Anesthesia was induced in it as in the first four rabbits, but no surgery was performed. A 3-cm diameter surface coil was placed on the intact scalp directly above the brain. After 90 min of 1.5% isoflurane administration, the ^{19}F washout was recorded until less then 10% of the original signal remained. At the end of this study, N_2O and pancuronium were discontinued, and the rabbit was allowed to recover. Behavior and gross neurological exam were normal. The signal intensity in each NMR spectrum was again normalized to the largest ^{19}F signal that occurred during the final 10 min of isoflurane administration. The decrease of the ^{19}F signal intensity with time was fit to a biexponential decay using a nonlinear least-squares fitting computer program.

Fluorine NMR relaxation times for isoflurane in brain and fat were measured in two additional rabbits.

Anesthesia was also induced with ketamine (35 mg/kg) and xylazine (5 mg/kg), and maintained with 1.5% isoflurane in oxygen. The scalp was retracted and a craniotomy performed as described earlier. Spin-lattice (T_1) and spin-spin (T_2) NMR relaxation times were measured for the brain and subcutaneous fat in studies where a 1.0-cm surface coil was placed on the dura, and then over the skin and subcutaneous fat at the back of the head. The measurements of brain T_1 were made after 6 h of isoflurane exposure, while measurements of fat T_1 were made after 11 h. T_1 was measured with the saturation-recovery technique by varying the delay between the saturation and observation pulse (t) from 50 to 2000 msec in a random order.[17] A modified Hahn spin-echo method[18] was employed to estimate T_2 values, using a composite 180° pulse (90(x)-180(y)-90(x)) to partially compensate for inhomogeneties that are present in the rf excitations that are generated by a surface coil. In the spin-echo experiments, the delay between the 90° and 180° pulses was varied from 1 to 700 msec.

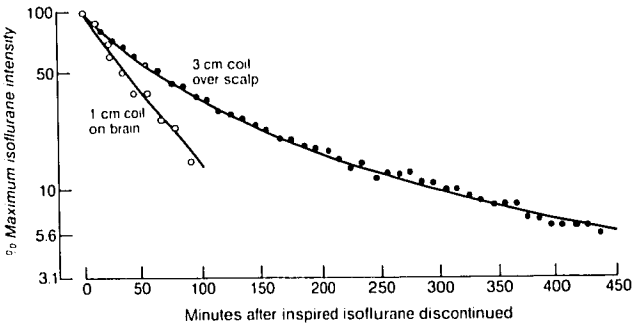

FIG. 2. ^{19}F NMR washout curves after 90 min of 1.5% isoflurane anesthesia. The data shown in this semi-log plot are from experiments done with two NMR surface coils of different diameters. Open circles (O) represent the ^{19}F NMR signals that were detected with a 1.0-cm diameter surface coil placed directly on the dura. Each open circle represents the average integrated NMR signal intensity (isoflurane concentration) for four rabbits. A straight line shows the single exponential decay curve that was fit to these points, as described in the text. The solid circles (●) represent the ^{19}F NMR signal intensities that were found during the isoflurane washout from one rabbit that had a 3.0-cm diameter NMR coil placed over the intact scalp. The line through the solid circle shows the optimum least-squares fit that was obtained with a biexponential decay, as described in the text.

Results

The time course of isoflurane elimination from the rabbit brain (N = 4; scalp and muscle excised) is shown in figure 2. After 90 min of washout, the isoflurane signal could not be distinguished from the noise. The linear decrease with time in the logarithmic plot of relative isoflurane concentrations in the rabbit brain (fig. 2) corresponds to a $t_{1/2}$ of 36 ± 5 min:

$$\%(^{19}\text{F signal}) = 100e^{(-0.020t)} \quad (1)$$

The isoflurane signal could still be detected after 7.5 h of washout in the one rabbit with a larger (3.0-cm diameter) surface coil placed directly on its intact head. In this rabbit, the kinetic data were fit best by a biexponential decay. The result,

$$\%(^{19}\text{F signal}) = 84e^{(-0.013t)} + 16e^{(-0.0024t)} \quad (2)$$

shows that $t_{1/2}$ is 55 min for the faster component and 290 min for the slower component. The coefficients of the biexponential decay reflect the relative volumes of fluorine in different tissue compartments sampled by the coil, and by the T_1 relaxation times of isoflurane in the various environments. We measured T_1 for both fat and brain, and found them to be identical. Thus, the coefficients of the biexponential fit are proportional to the number of fluorine nuclei in the two compartments being sampled.

A sagittal proton magnetic resonance image of a rabbit head is shown in figure 3. The rabbit brain is the central area. Bright areas repesenting fat can be seen in the regions anterior and posterior to the brain. The approximate volume in the rabbit head that was sampled by the 3-cm surface coil is schematically displayed in figure 3 by a scaled sketch from a 2D NMR imaging experiment.[19] The T_1 values for fat and brain, obtained from the slopes of log $(1 - I/I_\infty)$ versus t where I is the intensity of the peak at time t and I_∞ is the maximum intensity of the fluorine peak, were 81 ± 3 msec and 87 ± 8 msec, respectively. The T_2 values, obtained from the slopes of log (I/I_0) versus t where I_0 is the maximum fluorine signal, were 70 ± 10 msec and 3 ± 2 msec for fat and brain, respectively. Although the relaxation times were obtained by quantitating only the sharp downfield (trifluoromethyl) fluorine peak, the broad upfield (difluoroethyl) peak with a low signal-to-noise ratio qualitatively behaved the same.

Discussion

The fluorine signal obtained with the 1.0-cm diameter surface coil located directly over the dura rapidly decayed. The rapid decline of the brain isoflurane concentration to ≈15% of the maximum intensity after 90 min is consistent with earlier invasive studies, and with perfusion-limited models of anesthetic compartmentation.[1-2] In recent experiments by Wyrwicz *et al.*,[20] a rapid drop in the brain anesthetic signal to ≈50% was seen during the first 30 min. Immediately thereafter, the rapid drop became a slow decline ($t_{1/2}$ ≈ 175 min) that reached its 15% reading after 6 h of washout. The $t_{1/2}$ for the rapid drop found by Wyrwicz *et al.* does not differ significantly from that found in this study (36 ± 5 min), and it is close to the value found in rats (34 ± 8

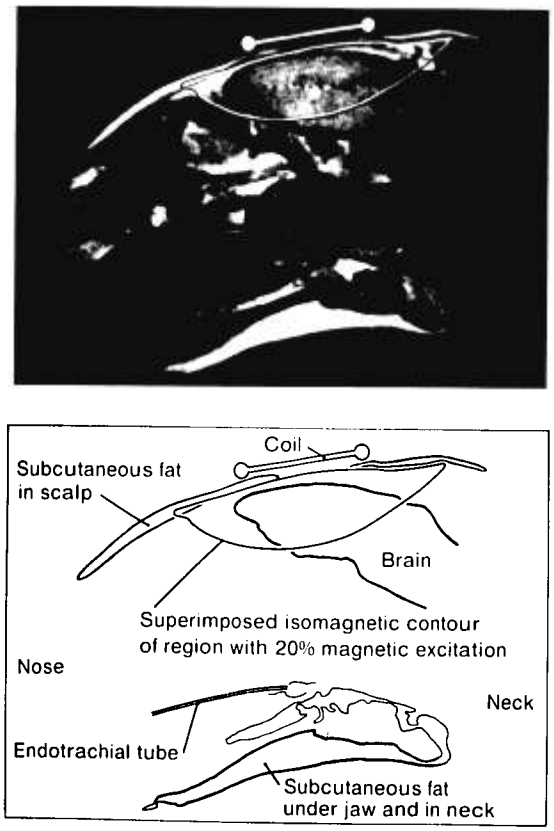

FIG. 3. Superposition of the measured NMR surface coil detection volume and a mid-sagittal proton NMR image of a rabbit's head. The animal is facing towards the left and the top of the head is at the top of the image. In the upper half of the figure, the schematic outline of a 3.0-cm diameter surface coil and its associated detection volume are drawn to scale and superimposed at the approximate location that the coil has in the NMR experiment. In the lower half of the figure, structures from the proton image are traced and labelled to show the location of the surface coil, the detection volume, the brain, and regions of fat. The contour delineates a cross section of the spatial region where the NMR detection efficiency is exactly 20%. The figure demonstrates that, for a 3.0-cm diameter coil, one must be concerned about the possibility of tissue outside the brain contributing to the detected NMR signal.

min) for halothane.[15] All *in vivo* NMR values of $t_{1/2}$ are greater than the value estimated from the invasive study that used gas chromatography[7] (18 ± 5 min).

The data we obtained with the 3.0-cm diameter surface coil have a substantial ^{19}F NMR signal 7.5 h after discontinuing the administration of isoflurane. The slow elimination of fluorine in the large coil data is characterized by a $t_{1/2}$ that is five times larger than that of the fast component. This slow washout contrasts with the rapid isoflurane washout from the brain that we observed with the 1-cm coil. The contrast between the washout behavior in the large and small coil data sets implies that the large coil ^{19}F NMR signal does not arise from brain tissue alone. The large $t_{1/2}$ from the 3-cm coil washout is consistent with the detection of isoflurane washout from fat, which has a slow elimination because it is poorly perfused and has a higher tissue/blood partition coefficient than brain.

It is noteworthy that the $t_{1/2}$ for the fast component of the biexponential fit to the large coil data was greater than the $t_{1/2}$ for the small coil data, where the signal originated from brain tissue only. We believe that, although the fluorine signal for the fast component in the large coil data comes primarily from brain tissue, these data also contain some contribution from muscle tissue. Because $t_{1/2}$ is longer for muscle than for brain, the $t_{1/2}$ of a muscle and brain mixture is greater than the $t_{1/2}$ for brain alone.

We recently performed a spin-echo magnetic resonance imaging study that demonstrated the significant extracerebral spatial distribution of halothane in the rabbit head.[21] No halothane was detected from the brain in that study, primarily because the spin-echo method that we used could not accommodate the short T_2 value (≈3 msec) for brain halothane. Because the T_2 of isoflurane in fat (70 msec) and brain (3 msec) are similar to those for halothane, and because the distribution volumes are also similar, we did not obtain spin-echo isoflurane images of the rabbit head. We also recently used two-dimensional NMR images of the surface coil detection volume to investigate the origin of the signal that washes out slowly in the large coil data. Using 2D-NMR display software, we demonstrated that surface coils have a zone of 20% detection efficiency that extends to approximately one radius on each side of the coil and to a depth of one radius.[19] This is schematically indicated on a proton image of the rabbit head (fig. 3), in which a substantial amount of fat can be seen surrounding the brain but within the detection volume of a 3-cm coil. The ratio of detected brain tissue to contaminating extracerebral tissue, such as fat or muscle, is a sensitive function of the placement of the coil on the rabbit's head. Our 3-cm surface coil was placed over the head in such a way as to maximize the signal from the brain. Even a small change in the surface coil position would increase the percentage of detected isoflurane that comes from fat, and alter the coefficients in the biexponential decay while leaving the time constants unaffected.

It is important to note that, while the detection of a substantial NMR signal from fat would result in the observation of a slow washout, the converse is not necessarily true. The observation of a slow washout does not require that the detected signal come from fat. The elimination of isoflurane from the brain is limited by the rate of decrease of the isoflurane concentration in arterial blood. Because the lungs do not remove all of the isoflurane from the pulmonary blood, the isoflurane concentration in the arterial blood, and hence the isoflurane concentration in the brain, is limited by the

multicompartmented behavior that one expects from standard pharmacokinetic calculations.

Using similar protocols of exposure and elimination, invasive studies find that the slowly falling exponential contribution to the brain washout curve first becomes visible after the washout has caused a 90% reduction in the brain isoflurane concentration. Our 1-cm coil data did not exhibit any evidence of the known second exponential because we did not pursue the brain washout curve beyond the 85% intensity reduction that occurs at 90 min. A better signal-to-noise ratio for the 1-cm coil experiment would have to be obtained in order to study the period beyond 90 min.

Because a large coil can be used to detect a signal that comes from both the brain and surrounding tissues, it is natural to ask if an accurate and unique resolution of the detected brain, fat, and muscle compartments could be obtained by decomposing the washout curve into sums of exponentials that are assignable to these different tissues. The pharmacokinetic issues raised in the preceding paragraph make this general question quite challenging. Under certain circumstances, it might be possible to resolve different tissue compartments, if their clearance half-times were to differ substantially and in a known way. However, to be certain that accurate pharmacokinetic data are being obtained with NMR spectroscopy from a specific tissue, it is necessary either to perform an invasive procedure that isolates the target organ from surrounding tissue, or to use NMR localization schemes, such as depth-pulsing[22] or volume-selective spectroscopy.[23]

In summary, the isoflurane washout that is observed with *in vivo* ^{19}F NMR spectroscopy using a 1.0-cm surface coil located directly over the brain differs significantly from the washout observed with a 3.0-cm coil located over the intact head. This difference arises because the large coil detects tissues outside the brain, but in close proximity to it. The isoflurane washout kinetics that we measured with the small coil agrees with previous invasive studies.

The authors wish to thank Professors Edmond Eger II and Alice Wyrwicz for stimulating and helpful discussions and for sharing their data from manuscripts that are in press. They also wish to thank Mon-a-Therm, Inc., for a gift of thermometers and non-magnetic thermocouple probes.

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