Hepatic Metabolism of Halothane, Methoxyflurane, Cyclopropane, Ēthrane, and Forane* in Miniature Swine*

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Hepatic metabolism of halothane, methoxyflurane, cyclopropane, Ēthrane, and Forane was studied in miniature swine, using a modification of a technique which demonstrated that the liver extracts (metabolizes) a greater fraction of halothane from hepatic blood flow at lower than at higher anesthetic partial pressures. Animals were exposed to constant, low subanesthetic concentrations of each anesthetic for periods of 20 hours to one week and measurements of extraction made thereafter. We found considerable hepatic metabolism of halothane and methoxyflurane, little or none of Ēthrane, and none of cyclopropane or Forane. These results may be related to toxicity since toxic effects of inhalation anesthetics may be due to their metabolites. (Key words: Hepatic metabolism; Halothane; Methoxyflurane; Cyclopropane; Ēthrane; Forane.)

THE TOXIC SIDE-EFFECTS of an anesthetic may result from products of its metabolism rather than from the anesthetic itself. Halothane and methoxyflurane are metabolized both *in vivo* [1-5] and *in vitro* [6]; both appear to have toxic side-effects. Trifluoroacetic acid, a metabolite of halothane,[3] is thought to be formed via trifluoroethanol and trifluoroacetaldehyde,[7] and all these compounds are toxic.[8,9] Fluoride has been observed as a metabolite of methoxyflurane,[5,10] and its toxicology has been extensively studied.[11,12] A goal in the development of new anesthetic agents may be the absence of *in-vivo* biotransformation. Many recent studies have used the appearance of metabolites as an indication of metabolism.[7] Sawyer *et al.*[13,14] suggested a technique for the *in-vivo* measurement of the other side of this coin: the disappearance of anesthetic from the hepatic circulation. This group demonstrated that the fractional rate of hepatic metabolism of halothane in miniature swine was concentration-dependent. They measured the anesthetic concentration in samples of blood from the hepatic artery, portal vein, and hepatic vein, and determined the fraction of anesthetic removed by the liver. At high alveolar concentrations (*i.e.*, at MAC),[15] the liver extracted little or none of the anesthetic passing through it, but as alveolar concentration decreased the fraction extracted increased. At 1/100 of MAC the fraction extracted was 50 per cent; at still lower concentrations it approached 100 per cent. The most reasonable explanation for the extraction of the anesthetic was its metabolism by the liver—the arguments leading to this conclusion [13,14] are expanded in the discussion section of this paper.

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In this study, we modified the technique of Sawyer *et al.*[13, 14] to demonstrate the extent of hepatic metabolism of five inhalation anesthetics: halothane, methoxyflurane, cyclopropane, Ēthrane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether) and Forane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether). Since metabolism is more apparent at subanesthetic concentrations, we exposed miniature swine to approximately 1/100 MAC for prolonged periods. After establishment of equilibrium, the fraction of anesthetic removed by the liver was determined.

Methods

Twelve experiments were conducted, using three Hormel miniature swine (weights 35–45 kg). Nylon intravascular catheters were implanted in the femoral artery and the common hepatic vein of each pig as previously described.[13] These surgical procedures were carried out with the pig under either halothane or nitrous oxide/sodium thiopental anesthesia several months prior to the metabolic studies. The animal breathed a constant controlled atmosphere during all studies of metabolism except the study with methoxyflurane (see below). During these studies the pig lived in an enclosed metal cage (size approximately 2½ × 3 × 3½ feet) with a plexiglass top for periods of 20 hours to a week. In the Ēthrane and methoxyflurane studies which lasted more than a day the cage was cleaned every day and the food and water inside the cage replenished. This necessitated opening the cage for half an hour each day. The temperature of the chamber was maintained at 22–25 C and cooled if necessary with ice on top of the chamber. A gas flow of 50 l/min through the cage maintained the inspired anesthetic concentration constant and eliminated carbon dioxide. Anesthetic was added to the gas flow from a separate vaporizer. We studied halothane, cyclopropane, Ēthrane (enflurane: Compound 347, Ohio Medical Products), and Forane (Compound 469, Ohio Medical Products). Because of the difficulty in obtaining tissue equilibration with the very-soluble methoxyflurane, we initially anesthetized the pig used for this study with methoxyflurane for three hours. Anesthesia was discontinued and the animal subsequently exposed to a low constant inspired concentration in the same manner as in the studies of the other agents. Blood samples were obtained via coiled nylon extension tubes attached to the implanted catheters. These extensions passed through the top of the chamber, thereby permitting sampling without alteration of the inspired concentration. It was sometimes necessary to manipulate the animal to allow sample withdrawal. In these cases the investigator entered the cage but breathed through a tube connected to the outside of the chamber. Blood samples were taken at least a half hour after re-establishment of the inspired concentration to the level present prior to the investigator's entry into the chamber.

The techniques for sampling, tonometry, analysis, and calculation have been described.[13] Five-ml blood samples were obtained in heparinized glass syringes and subsequently equilibrated with 15 ml of nitrogen at 37 C. The anesthetic concentration in the gas phase was measured using a gas chromatograph with a flame ionization detector and the concentration of anesthetic in the original blood sample calculated. The blood/gas and heparin/gas partition coefficients at 37 C for the anesthetics were assumed to be as follows[16–18]: halothane 2.3, 0.74; methoxyflurane 13.0, 4.5; cyclopropane 0.6, 0.2; Ēthrane 1.91, 0.775; Forane 1.4, 0.69.

Although several studies of each pig were done, a given animal was exposed to only one anesthetic at a time. A minimum of two weeks elapsed between exposure of pig to one anesthetic and exposure to another agent. In the Forane studies, experiments 1–4 were carried out on consecutive days. On each day this animal was exposed to the anesthetic for 20 hours and allowed to exercise outside of the cage for four hours.

Results

Results of the studies of five anesthetics are shown in table 1. The fraction of anesthetic removed from the blood after passage through the liver (*i.e.*, one minus the ratio of anesthetic concentration in the hepatic vein to that in the femoral artery) reflects hepatic metabolism of anesthetic. A ratio of "0" indicates no removal of the anesthetic, while a ratio of "1" indicates complete removal. All concentra-

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TABLE 1. Results of Hepatic Metabolism Studies

	Experiment Number	Exposure Time (Days)	Chamber Concentration (vol per cent × 10²)	Alveolar Concentration (vol per cent × 10²)	Ratio of Alveolar Concentration to MAC (× 10²)	Femoral Arterial Concentration (F.A.) (× 10²)	Hepatic Venous Concentration (H.V.) (× 10²)	Removal Fraction 1 minus H.V./F.A.
Halothane								
Pig 1	5	1	0.61	0.65	0.81	1.5	1.0	0.33
Pig 1	5	1	0.61	0.65	0.81	1.5	0.95	0.37
Methoxyflurane								
Pig 3	12	6.5	0.42	0.85	4.3	11	6.2	0.44
Pig 3	12	7	0.42	0.85	4.3	11	4.4	0.60
Cyclopropane								
Pig 1	9	1	3.7	3.8	0.38	2.3	2.3	0
Pig 3	8	1	3.2	3.0	0.30	1.8	1.7	0.06
Pig 3	8	1	3.2	3.2	0.32	1.9	2.0	−0.05
Ēthrane								
Pig 2	10	2	1.7	1.5	0.75	2.9	2.4	0.17
Pig 3	11	2	0.68	0.68	0.34	1.3	1.2	0.08
Pig 3	11	2.5	0.68	0.68	0.34	1.3	1.1	0.15
Pig 3	11	2.8	0.68	0.68	0.34	1.3	1.2	0.08
Forane								
Pig 1	6	1	0.89	1.3	1.0	1.8	1.9	−0.06
Pig 1	6	1	0.89	1.4	1.1	1.9	1.8	0.05
Pig 2	1	1	9.6	6.1	4.7	8.6	8.3	0.03
Pig 2	2	0.8	2.2	2.8	2.2	3.9	3.8	0.03
Pig 2	3	0.8	0.53	0.51	0.39	0.72	0.69	0.04
Pig 2	4	0.8	1.4	1.1	0.85	1.6	1.6	0
Pig 3	7	1	0.71	1.3	1.1	1.9	2.0	−0.05
Pig 3	7	1	0.76	1.3	1.1	1.9	2.1	−0.11

tions are volumes per cent at 1 atmosphere and 37 C. Alveolar concentrations are calculated from femoral arterial concentrations. The calculated alveolar concentration was sometimes higher than the chamber concentration, although (with the exception of the methoxyflurane experiments) the chamber concentration was not higher than the recorded value at any time. This discrepancy may reflect a) incorrect values of the oil/gas partition coefficient; b) a variable concentration of anesthetic delivered by the vaporizer (possibly due to temperature variations during the night); c) the difficulty of accurate measurements of very low anesthetic concentrations. The following concentrations were assumed for MAC[19-20]: halothane 0.8 per cent; methoxyflurane 0.2 per cent; cyclopropane 10 per cent; Ēthrane 2 per cent; Forane 1.3 per cent. Experiment numbers indicate separate studies, each with its own exposure time, and are arranged in chronological order. Sometimes more than one set of measurements were made during a particular experiment on a particular pig; the data from these are also recorded in table 1.

Discussion

Results of this study demonstrate that halothane and methoxyflurane are metabolized at low subanesthetic concentrations. There is little or no metabolism of Ēthrane, and no detectable metabolism of cyclopropane or Forane. These findings are subject to the criticism that equilibrium between the arterial anesthetic concentration and that in portal venous blood may not have been established. If so, an arterial-to-hepatic vein concentration difference might be due to anesthetic uptake by the splanchnic bed rather than metabolism. Although it was not possible to obtain blood

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samples from the portal vein, we previously demonstrated that the anesthetic concentrations in the portal vein and hepatic artery are similar in shorter periods of exposure time than those used in these experiments.[13, 14] Therefore, we assumed equilibrium between arterial and portal vein blood. Furthermore, the criticism of incomplete equilibration cannot apply to the results showing no arterial-to-venous concentration difference (cyclopropane, Forane, and probably Ēthrane), because in no experiment (except with methoxyflurane) did the inspired anesthetic partial pressure exceed the equilibrium alveolar partial pressure and, therefore, the portal blood anesthetic partial pressure could not exceed that in hepatic (or femoral) arterial blood. Thus, a small or undetectable arterial-to-hepatic venous difference (i.e., with cyclopropane, Ēthrane, Forane) cannot be explained by masking of any metabolism of the anesthetic by the anesthetic output.

One further criticism may be applied to these studies: the prolonged and repeated exposure to low anesthetic concentrations may have induced increased enzymatic degradation of the anesthetics. It has also been suggested that the preservatives in volatile anesthetics may have a similar effect.[21] This criticism cannot apply to the studies of cyclopropane, Forane, and probably Ēthrane, where we found no significant metabolism. In addition, the metabolism found with halothane was of the same order as that previously found in more acute experiments.[13] The fraction of halothane removed (0.35 ± 0.02 [SE]) was lower (*i.e.*, less metabolism) than that found by Sawyer *et al.*[13] but nevertheless indicated significant metabolic degradation. This conclusion is supported by the work of Van Dyke,[1, 2] Cohen,[22] and their co-workers, who detected significant amounts of metabolic products of halothane.

We calculated that the high tissue solubility of methoxyflurane would necessitate two weeks or longer for attainment of equilibrium. Therefore, Pig 3 was anesthetized with methoxyflurane for three hours before he was put in the chamber at constant low inspired concentration. Thus, the intestine was initially equilibrated with an anesthetic partial pressure greatly in excess of that eventually achieved, resulting in a hepatic venous concentration higher than the arterial concentration during days 1 through 5. However, as exposure time increased, equilibrium was approached and the hepatic venous concentration fell below that of the femoral artery. The final removal fraction (0.52 ± 0.08) indicates significant hepatic metabolism, as has also been concluded from the experiments of Van Dyke *et al.*[1, 2, 7] Since release of methoxyflurane from the intestine probably continued during this time (6–7 days), our data probably underestimate methoxyflurane metabolism.

The fraction of cyclopropane removed was 0 ± 0.03, indicating no hepatic metabolism of the anesthetic. Van Dyke and Chenoweth[7] suggested that cyclopropane might be metabolized to $^{14}CO_2$ in rats, but they felt that their data were inconclusive. Sawyer *et al.*[14] found no significant metabolism of cyclopropane at 0.9 vol per cent.

Removal fractions for Ēthrane all lay above 0, with a mean of 0.12 ± 0.02. However, since portal vein concentrations were not measured we cannot say whether this small extraction (12 per cent) was the result of metabolism, although the indication is that only a small amount of metabolism may occur. This conclusion is of interest since Ēthrane (CF_2H–0–CF_2–CFHCl) and Forane (CF_2H–0–CHCl–CF_3) are isomers.

Removal fractions of Forane equalled 0 ± 0.02, indicating no metabolism of this agent. Hepatic metabolism of halothane and methoxyflurane at equivalent fractions of MAC has been demonstrated in this study. No previous studies have measured Forane metabolism, except that in our studies of human volunteers given five to seven hours of Forane anesthesia there was no significant elevation of serum inorganic fluoride.[23]

Previous workers have suggested that toxic effects of anesthetics may be related to their metabolites.[7, 10, 22] Convincing evidence for this has been obtained in studies of halothane,[3, 7, 9] methoxyflurane,[5, 10–12] and chloroform.[24, 25] If this is so, and if metabolism in miniature swine is similar to that in man, then the present study indicates that Forane, cyclopropane, and probably Ēthrane may be less toxic than halothane or methoxyflurane.

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Drugs

TOXICITY OF AEROSOLS The fluoroalkane gases used to propel aerosols were toxic to the hearts of 34 mice, sensitizing them to sinus bradycardia, atrioventricular block, and T-wave depression induced by asphyxia. Cardiac sensitization was rapid, long-lasting, and lethal. It also occurred in rats and dogs. The propellants are postulated to possess a spectrum of cardiotoxic effects capable of causing bradyarrhythmias, tachyarrhythmias, or myocardial depression. In humans the cardiac toxicity of aerosol propellants, particularly during asphyxia, may be a cause of sudden death in youths who "turn on" by inhaling propellants and in patients with asthma who use bronchodilator aerosols excessively. Cardiac toxicity due to propellent inhalation may be a potential hazard to frequent users of pressurized aerosol dispensers. *(Taylor, G. J., and Harris, W. S.: Cardiac Toxicity of Aerosal Propellants, J.A.M.A. 214: 81 (Oct.) 1970.)*

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