Metabolism in Vitro of Enflurane, Isoflurane, and Methoxyflurane

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Specific activities of enflurane, isoflurane, and methoxyflurane defluorinases were measured in microsomes prepared from the livers of Fischer 344 rats; the ratio of these activities was 23:3:1. Pretreatment with phenobarbital significantly increased the defluorinase activities of all three agents. Factors that influence anesthetic drug metabolism are discussed; tissue solubility is considered to be the most important. (Key words: Anesthetics, volatile, methoxyflurane; Anesthetics, volatile, enflurane; Anesthetics, volatile, isoflurane; Biotransformation, volatile anesthetics.)

Enflurane, isoflurane, and methoxyflurane are metabolized in vivo to inorganic fluoride.¹⁻⁵ Enzyme induction with phenobarbital in rats enhances defluorination of methoxyflurane in vivo,⁶ but does not enhance defluorination of enflurane or isoflurane in vivo.²⁻⁷ This paradox may be explained by the large differences in tissue solubilities among these drugs. Enflurane and isoflurane are 5–10 times less soluble than methoxyflurane in most tissues, including fat. Therefore, the concentrations of enflurane and isoflurane in vivo may be of the order of the Kₛ or higher for binding to enzyme for only a few hours after anesthesia. Thus, substrate availability would be rate-limiting, and induction of defluorinase activity would not significantly alter the extent of defluorination. By contrast, methoxyflurane is likely to be present in sufficient concentrations to permit defluorination at maximum velocity for many hours, even days, into the postanesthetic period. Therefore, enzyme concentration would be rate-limiting and induction would be a significant factor in defluorination.

To test the hypothesis that substrate availability is rate-limiting in vivo, it must be demonstrated that enflurane and isoflurane metabolism can be enhanced by enzyme induction in vitro, where substrate availability is not rate-limiting. The present study compares the in-vitro metabolism of enflurane, isoflurane, and methoxyflurane by hepatic microsomes prepared from rats pretreated with phenobarbital and from untreated rats.

Methods

IN VITRO

Eighteen adult Fischer 344 rats that had been bedded on ground corn cob for 30 days were randomly assigned to two groups. One group received phenobarbital, 25 mg/kg, subcutaneously, twice a day for four days. The other group (control) was injected with an equivalent volume of 0.9 per cent NaCl. After the last injection, the animals were fasted overnight, then sacrificed by decapitation. The livers were excised and immediately placed in ice-cold Tris–HCl buffer (0.05M, pH 7.4). All subsequent procedures were carried out at 0–4°C. Seven grams of liver were minced in 40 ml of the Tris buffer and the tissue then homogenized using a Polytron homogenizer for 30 seconds at maximum velocity. The resulting homogenate was centrifuged at 10,000 × g for 30 minutes and the supernatant solution subsequently centrifuged at 105,000 × g for 1 hour. The 105,000 × g pellet was resus-

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Received from the Departments of Anesthesia Stanford University School of Medicine, Stanford, California 94305, and Veterans Administration Hospital, Palo Alto, California 94304. Accepted for publication August 28, 1974. Supported in part by VA Hospital, Palo Alto, California and USPHS Grants GM-18514 and GM-00862.

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Kₛ is the substrate (anesthetic) concentration at which the rate of defluorination is half the maximum.
pended in buffer and then centrifuged again at 105,000 × g for 1 hour. The washed pellet was suspended in 5 ml of buffer and immediately assayed for hepatic microsomal defluorinase activity. The assay was carried out in a 25-ml sealed reaction flask that contained: 1) 250 μmol Tris, pH 7.4; 2) 100 μmol MgCl₂; 3) an NADPH regenerating system consisting of 1 μmol NADP, 10 μmol glucose-6-phosphate and 0.5 ml of the first 105,000 × g supernate as a source of glucose-6-phosphate dehydrogenase. Substrate was added by bubbling 0.5 per cent methoxyflurane, 1.5 per cent enfurane, or 1.5 per cent isoflurane vapor in oxygen through the mixture for 2 minutes at a flow rate of 1 l/min. This resulted in an anesthetic concentration of approximately 1 mM for each agent, as determined by direct injection of the mixtures into a Varian 1440 gas chromatograph. Reactions were started by addition of 1.0 ml of the suspended 105,000 × g pellet to the flask. Total volume of the reaction mixture was 6.1 ml. Studies in our laboratory have demonstrated that these conditions result in maximum rates of defluorination. The mixture was incubated for 30 minutes at 37 C, with constant agitation, following which the reaction was stopped by unsealing the flasks and heating them to drive off anesthetic vapors. The reaction mixture was then evaporated to dryness and resuspended in 1 ml of 2.5 M acetate buffer, pH 4.8. Two control reaction mixtures were prepared as above, except that oxygen without anesthetic was bubbled through one and the other contained substrate (methoxyflurane), but no microsomal suspension. Inorganic fluoride activity of the final solutions was measured with an Orion ion-specific electrode.

Specific enzymic activity was calculated as the increase in the amount of inorganic fluoride present compared with that measured in the control reaction vessels. Protein determination was by the method of Lowry et al.\(^{a}\)

Results

Hepatic microsomes from control rats defluorinated methoxyflurane more readily than enfurane or isoflurane; the specific activities were 3.27 ± 0.35 nmol F⁻/30 min/mg protein for methoxyflurane defluorinase, 0.48 ± 0.10 for enfurane defluorinase, and 0.14 ± 0.05 for isoflurane defluorinase, a ratio of 23.3:1, respectively (fig. 1). Enzyme induction by phenobarbital significantly enhanced the specific activities of methoxyflurane defluorinase 7.3 times (P < 0.01), enfurane defluorinase 1.6 times (P < 0.01) and isoflurane defluorinase 4.3 times (P < 0.02), compared with activity measured in microsomes from control rats. The individual activities for methoxyflurane, enfurane, and isoflurane defluorinases after enzyme induction were in the ratio 39:1.3:1, respectively.

Discussion

In discussing the results of the present study, it is important to consider the several
factors involved in anesthetic drug metabolism. They are: A) substrate (anesthetic) factors; 1) solubility in tissue, particularly fat, 2) biologic stability, 3) concentration and duration of administration (dose). B) enzymic factors: 1) quantity, 2) affinity for substrate, 3) availability of cofactors, such as oxygen, NADPH, etc.

This study demonstrates that methylxyl ether defluorinase activity is inducible, as measured in an in-vitro system. The inability to measure enhanced enfurane and isofurane defluorinase activity, in vito, while enhanced methoxyfurane defluorinase can be demonstrated in vito emphasizes the importance of the factors listed above. Agents relatively insoluble in fat, e.g., enfurane (oil/gas partition coefficient, 98) and isofurane (oil/gas partition coefficient, 98), are more rapidly excreted from the lung than is an agent highly soluble in fat, such as methoxyfurane (oil/gas partition coefficient, 970). Following anesthesia, concentrations of enfurane and isofurane will rapidly fall to the order of the Kᵢ for binding to enzyme, or even lower. Although induction by phenobarbital results in production of additional enzyme, the rate of defluorination is more dependent on the amount of substrate than on the amount of enzyme once the anesthetic concentration has fallen to the Kᵢ. Therefore, the hypothesis of this study is tenable: substrate availability is rate-limiting for metabolism in vito. This hypothesis is in agreement with the work of Cohen and Hood,\(^\text{10}\) who studied the metabolism of \(^{14}\text{C}-\) chloroform using low-temperature whole-body autoradiography.

Among drugs of similar tissue solubilities, e.g., enfurane and isofurane, biologic stability is important. We have demonstrated, in vito, that at similar anesthetic dosages enfurane metabolism yields about three times more inorganic fluoride than does isofurane\(^{12}\); in vitro, this ratio is approximately the same. An explanation for the difference in biologic stability between enfurane and isofurane may be found in the chemical properties of the fluorinated anesthetics. The structures of these agents are shown in figure 2. Both hydrogen and fluorine are univalent; while fluorine atoms are larger than hydrogen atoms, they are still small enough to occupy all four valence
electrons of carbon. The somewhat greater volume of fluorine compared with hydrogen and the greater stability of the C–F bond (rupture energy = 114 kcal) compared with the C–H bond (rupture energy = 93 kcal) provides a protective integument for the carbon chain. It follows, then, that the greater the number of fluorine atoms in a particular area of the carbon skeleton, the more resistant that part of the molecule will be to defluorination.\textsuperscript{12} Thus, it is not surprising that halothane and fluoroxyne are not defluorinated and that isoflurane is minimally defluorinated, the CF$_3$- portion of the molecules being very stable.\textsuperscript{4,5,11,12,13,14} It is likely that the inorganic fluoride resulting from metabolism of isoflurane originates from the difluoromethyl moiety after breakage of the ether linkage. The finding that trifluoroacetic acid is a metabolite of isoflurane supports this hypothesis.\textsuperscript{9} Breaking of the ether linkage of enflurane would result in defluorination of the difluoromethyl group, with additional fluoride ion originating from the trifluoroethyl portion of the enflurane molecule. Methoxyflurane is a difluorinated compound and, so, is more susceptible to enzymic attack than either of the pentafluorinated methylethyl ethers.

Finally, if drugs possess similar solubilities and chemical stability, then anesthetic dosage, \textit{i.e.}, concentration times duration of administration, will govern the amount of metabolism.\textsuperscript{5,15}

The preceding discussion assumes that enzymic factors are constant. However, enzymic factors differ among and within species and, from time to time, within an individual. The condition of the subject, particularly hepatocellular function and concurrent drug treatment, can affect enzyme factors within an individual. Drug treatment includes not only enzyme-inducing drugs but inhibitory agents as well.\textsuperscript{6,14} Anesthetic agents, when administered in the usual clinical manner, belong in the latter category. Inhibition of mitochondrial respiration during anesthesia\textsuperscript{17} will ultimately lead to depletion of NADPH, an essential cofactor for anesthetic metabolism,\textsuperscript{18} thus explaining why maximum rates of anesthetic metabolism would not occur during surgical anesthesia.

As noted above, the present study confirms the order of biologic stability of the fluorinated methylethyl ethers. This information, in conjunction with the solubilities of these drugs, should allow prediction of the extent of their metabolism at a given anesthetic dosage. Since nephrotoxicity is directly related to defluorination of these compounds,\textsuperscript{6} it should also be possible to predict the potential toxicity of fluorinated methylethyl ethers. Thus, methoxyflurane, the most fat-soluble and least biologically stable agent of the three, would be expected to have the greatest potential toxicity; isoflurane, the least soluble and most stable agent, would be expected to have the lowest potential toxicity. This has been shown to be the case in clinical studies.\textsuperscript{11,14}

An unexplained finding in this study was the difference in the amounts of induction of the three defluorinase activities. This might suggest that different enzymes, which are inducible to different extents, are responsible for the defluorination of these agents. Another possibility is that two enzymes are responsible for the metabolism of this class of compound; one attacks the molecule at the ether linkage (alpha carbon) and is induced by phenobarbital treatment, the second attacks at the beta carbon and is not induced. Methoxyflurane is known to undergo reaction at both alpha and beta carbon atoms,\textsuperscript{19} so its metabolism should be catalyzed by both enzymes and could be induced. Isoflurane has a relatively stable trifluoro group on the beta carbon, so it should undergo reaction only at the ether linkage; thus, its metabolism also could be induced. Enflurane, because it has four fluorine atoms located on the carbon atoms adjacent to the ether linkage, should be relatively stable at this position, so that induction of its metabolism should be minimal. Although this hypothesis appears to fit the experimental data it is not in keeping with the current concept that the mixed-function oxidase system is a single enzyme system, which alone is responsible for metabolism of most drugs,\textsuperscript{20} particularly compounds with such similar structures as the fluorinated methylethyl ethers. At the moment, this problem is unresolved. Finally we are unable to explain the
difference between our results and those of Van Dyke and Wood. They studied inorganic fluoride production in hepatic microsomes prepared from Fischer 344 and Sprague-Dawley rats and demonstrated defluorination of methoxyflurane but not defluorination of enfurane or isoflurane.

The authors thank Dr. Ellis Cohen for help in preparing the manuscript and Ms. Susan Scoggins for skillful technical assistance.

References


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