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INVESTIGATION OF THE MECHANISM OF DEFLUORINATION OF ENFLURANE IN RAT LIVER MICROSOMES WITH SPECIFICALLY DEUTERATED DERIVATIVES

Terrence R. Burke,Jr.[1], Jackie L. Martin, John W. George and Lance R. Pohl

Laboratory of Chemical Pharmacology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205

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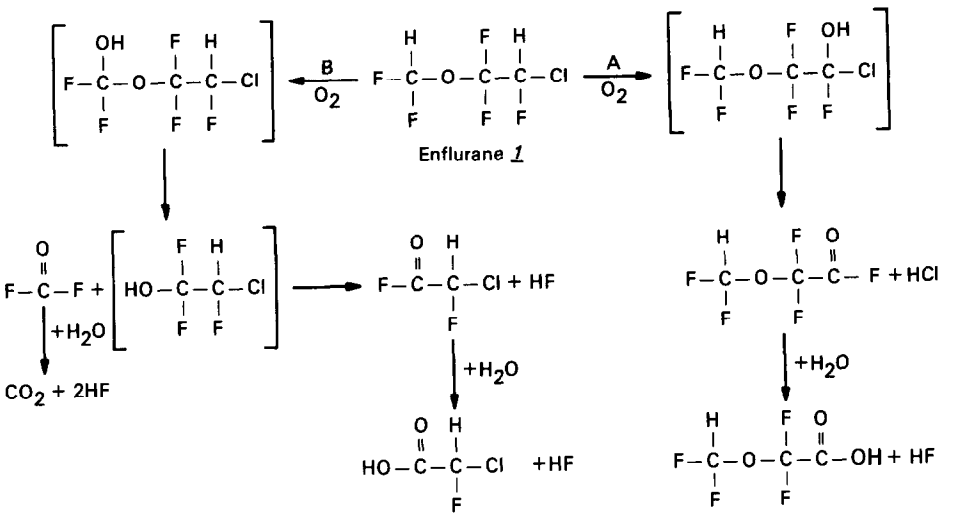
Since hepatic (1) and renal (1) toxicities have been associated with the inhalation anesthetics halothane ($CF_3CHBrCl$) and methoxyflurane ($CH_3OCF_2CHCl_2$) respectively, there has been increased interest in the development of safer alternative drugs. One compound that has become widely employed is the fluorinated ether, enflurane (CHF_2OCF_2CHClF, compound 1). Although this inhalation anesthetic appears to be safer than halothane and methoxyflurane, there have been recent reports indicating that it can produce renal changes in both man (2-4) and rat (5) similar to those seen with methoxyflurane.

Inorganic fluoride (F^-) appears to be responsible, at least in part, for the nephrotoxicity associated with the administration of methoxyflurane (1,5,6) and enflurane (4,5). The release of F^- is catalyzed predominantly by cytochrome P-450 in the microsomal fraction of liver (7). Pretreatment of rats with phenobarbital increases the rate of defluorination in liver microsomes by as much as two-fold (7-9), whereas treatment with 3-methylcholanthrene does not appreciably affect the rate of defluorination of enflurane (7,9). Pretreatment with isoniazid, however, has been reported to induce defluorination of enflurane in rat liver microsomes by approximately four-fold (10); this observation may be clinically important because it has been reported that a patient treated with isoniazid developed a very high serum F^- level and a transient urinary concentrating defect following enflurane anesthesia (11).

Although the pathway for formation of F^- from enflurane is not known, the results of metabolic studies with structurally related halogenated hydrocarbons such as chloroform (12,13), bromoform (14,15), chloramphenicol (16,17), halothane (18) and dihalomethanes (19,20) suggest that enflurane is likely metabolized to F^- by an oxidative dehalogenation mechanism (Fig.1). This reaction may occur by either initial oxidation of the C-H bond

[1]Staff Fellow in the Pharmacology-Toxicology Research Associate Program, NIGMS, Bethesda, Md.

Fig. 1. Potential pathways for metabolic oxidative defluorination of enflurane



of the chlorofluoromethyl carbon (Pathway A) or by oxidation of the C-H bond of the difluoromethyl carbon (Pathway B, Fig. 1). In both proposed pathways the alcohol intermediates would be expected to dehydrohalogenate spontaneously to produce acyl halides which upon hydrolysis would yield carboxylic acids and halide ion.

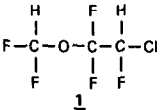
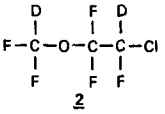
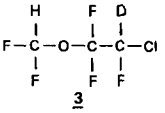
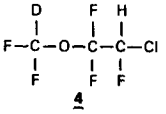
It seemed possible that the relative importance of each of these pathways of metabolism might be determined by comparing the relative rates of defluorination of specifically deuterated derivatives of enflurane. Since deuterium substitution decreases the oxidative dehalogenation of chloroform (21), bromoform (14,15), and dihalomethanes (20) in rat liver microsomes, substitution of deuterium at the chlorofluoromethyl carbon should inhibit Pathway A whereas substitution at the difluoromethyl carbon should inhibit Pathway B (Fig.1). According to this view, the predominant pathway by which enflurane is oxidized by liver microsomes from phenobarbital and isoniazid pretreated rats should be revealed by determining which deuterated form of enflurane manifests the greater isotope effect.

In a typical experiment, male Fischer 344 rats (340-380 g, Charles River) were allowed free access to water and food (Purina Lab. Rat Chow) and pretreated either with phenobarbital (80 mg/kg, i.p.) 72, 48 and 24 hr before the beginning of the study or with isoniazid (50 mg/kg, i.p.) once daily for 7 days before the rats were killed by decapitation. The rat livers were homogenized in 3 volumes of 0.02 M Tris 1.15% KCl buffer, pH 7.4. The homogenate was centrifuged at 10,000 x g for 20 min and the supernatant then recentrifuged for 60 min at 100,000 x g. The resultant microsomal pellet was resuspended in 0.02 M Tris 1.15% KCl buffer and recentrifuged at 100,000 x g for 60 min. The washed microsomal pellet was resuspended in 0.02 M Tris 1.15% KCl buffer and diluted to a final protein concentration of 5 mg/4.5 ml. Incubations (5 ml) were run in sealed polyethylene vials containing 5 mg of microsomal protein, 5 μmoles of substrate and 0.5 ml of a cofactor mixture containing 1 μmole of NADP (Sigma), 10 μmoles of glucose-6-phosphate (Sigma) and 1 unit of glucose-6-phosphate dehydrogenase (Calbiochem) in 0.02 M Tris 1.15% KCl buffer. After incubation for 30 min at 37° the mixture was immediately frozen (dry ice-acetone bath) and evaporated to dryness by lyophilization. The residue was resuspended in 0.5 ml of 2.5 M sodium acetate buffer (pH 5.6) and the amount of F^- measured with an ion specific F^- electrode (Orion). A standard curve was prepared by incubating known amounts of NaF in reaction vials containing all constituents of the reaction mixture except enflurane. Fluoride ion production was determined by subtracting the amount produced in control reactions

(minus NADPH generating system) from that of the complete reaction mixture. Deuterated enfluranes were synthesized as described elsewhere (22) and were purified by GC with a Perkin Elmer 900 gas chromatograph, equipped with a 6' x 4 mm (i.d.) glass column, packed with Porapak Q 100/120 mesh. Enflurane had a retention time of 19 min (column temperature 130° and carrier gas (N_2) approximately 20 ml/min). Deuterium incorporations at the chlorofluoromethyl and difluoromethyl carbons respectively of the various derivatives are as follows: enflurane-d_2 (compound 2, Table 1),99%, 98%; enflurane-d_1 (compound 3, Table 1), 97%, 10%; enflurane-d_1 (compound 4, Table 1), 4%, 89%.

The results of incubations with liver microsomes from phenobarbital pretreated rats show that the levels of F^- produced from the dideuterated derivative (compound 2) and the monodeuterated chlorofluoromethyl derivative (compound 3) of enflurane are nearly identical, but significantly lower than that produced from enflurane (compound 1, Table 1). In contrast, the monodeuterated difluoromethyl derivative (compound 4) was metabolized to F^- to the same extent as enflurane (compound 1, Table 1). The primary isotope effect of approximately 2.8 in F^- formation for compounds 2 and 3 demonstrates that the breakage of the C-H bond of the chlorofluoromethyl carbon is the rate determining step in the metabolism of enflurane to F^- in liver microsomes from phenobarbital pretreated rats. The lack of an isotope effect for compound 4 suggests that oxidation of the difluoromethyl C-H bond does not occur in these microsomal preparations to any significant extent. These results, therefore, indicate that Pathway A is the major route of oxidative defluorination of enflurane in liver microsomes from phenobarbital pretreated rats. This conclusion is in agreement with the previous finding that F^- and nonvolatile radioactive metabolites are formed in approximately equimolar amounts when [^{14}C-chlorofluoromethyl]enflurane is incubated with liver microsomes from control, 3-methylcholanthrane and phenobarabital pretreated rats (23).

Table 1. Comparative metabolism of enflurane and various deuterated derivatives to F^- by liver microsomes from rats pretreated with phenobarbital or isoniazid.[1]

Substrate	Fluoride Produced (nmoles/mg protein/30 min)	
	Phenobarbital Pretreated	Isoniazid Pretreated
 1	1.1 ± 0.1	12.3 ± 0.7
 2	0.4 ± <0.1	2.5 ± 0.2
 3	0.4 ± <0.1	2.9 ± 0.2
 4	1.0 ± 0.1	12.4 ± 0.6

[1]Results represent the mean ± S.E. of at least 5 incubations.

The results in Table 1 confirm an earlier observation that isoniazid is a more potent inducer of enflurane defluorination than phenobarbital (10). In the present study, the metabolism of enflurane by liver microsomes from rats treated with isoniazid was approximately 12 times as rapid as that by rats treated with phenobarbital. At first it seemed plausible that isoniazid might induce a form of cytochrome P-450 that regioselectively oxidizes enflurane at the difluoromethyl carbon (Pathway B, Fig.1) because this route of metabolism would result in the formation of 4 times as much F^- per oxidation than would Pathway A. The results obtained with the deuterated derivatives of enflurane, however, show that the breakage of the C-H bond of the chlorofluoromethyl group rather than the difluoromethyl group is the predominant route of defluorination of enflurane in microsomes from isoniazid pretreated rats. Consequently, Pathway A appears to be the major and perhaps the only route of oxidative defluorination of enflurane in these microsomes, as was found with the liver microsomes from phenobarbital pretreated rats. A recent study employing compound 3 in untreated rats indicates that this is also the major pathway of defluorination of enflurane in vivo (24).

The results of this paper have demonstrated the use of specifically deuterated derivatives for readily determining the major pathways of defluorination of enflurane in various microsomal preparations. These results indicate that the C-H bond of the difluoromethyl group of enflurane is not significantly susceptible to metabolic oxidative defluorination. This finding can be used for the rational design of new inhalation anesthetics which will not be appreciably metabolized to fluoride ion.

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