Biochemical Pharmacology, Vol. 29, pp. 3257–3263.

0006-2952/80/1215–3257 $02.00/0

ROLE OF HEPATIC MICROSOMAL CYTOCHROME P-450 IN THE TOXICITY OF FLUORINATED ETHER ANESTHETICS*

MICHAEL J. MURPHY, DEBORAH A. DUNBAR and LAURENCE S. KAMINSKY†
Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201, U.S.A.

(*Received* 26 *February* 1980; *accepted* 16 *June* 1980)

Abstract—To evaluate the role of cytochrome P-450 in anesthetic toxicity, we investigated the effects of hepatic microsomal cytochrome P-450 inducers [phenobarbital (PB), 3-methylcholanthrene (3-MC) and pregnenolone-16α-carbonitrile (PCN)] and inhibitors [SKF 525-A, metyrapone, and 2-allyl-2-isopropylacetamide (AIA)] on the potentiation of lethal effects to rats of i.p. administered 2,2,2-trifluoroethyl vinyl ether (TFVE), ethyl 2,2,2-trifluoroethyl ether (TFEE), allyl 2,2,2-trifluoroethyl ether (TFAE) and 2,3-epoxypropyl 2,2,2-trifluoroethyl ether (EPTFE). The time courses of tail-vein blood anesthetic concentrations and quantities of exhaled anesthetics together with the *in vitro* metabolism of the anesthetics and their binding to microsomal cytochromes P-450 were also determined. The results indicate that (1) the majority of the administered anesthetics make a single pass through the liver prior to exhalation and apparently are metabolized to toxic products, (2) the epoxide (EPTFE) exerts its lethal effects independently of cytochrome P-450 catalyzed metabolism and does not lie on the major path of TFAE metabolism, (3) all the anesthetics yield 2,2,2-trifluoroethanol (TFE) on metabolism *in vitro* but lethality does not always correlate with the rates of TFE formation, (4) PB induced cytochromes P-450 potentiate lethal effects of TFVE and TFEE but not of TFAE, and inhibitors differentiate mechanisms of TFVE and TFEE lethality, (5) PCN induced cytochromes P-450 potentiate the toxicity of TFVE, TFAE, and TFEE in a similar manner, and (6) 3-MC induction potentiates TFEE and TFAE lethality apparently independently of cytochrome P-450 catalyzed metabolism.

Nonlethal doses of the anesthetic fluroxene (2,2,2-trifluoroethyl vinyl ether) become lethal when administered to rats subsequent to the administration of certain hepatic cytochrome P-450 inducing agents. Those inducing agents that have been demonstrated to potentiate the toxic effects of fluroxene are phenobarbital (PB) [1], mephobarbital [2], pregnenolone-16α-carbonitrile (PCN) [2], and polychlorinated biphenyl mixtures [3]. Other known inducers of cytochromes P-450, including 3-methylcholanthrene (3-MC) and other polycyclic aromatics, mirex, kepone, 8-hydromirex, spironolactone, barbital, hexobarbital and pentobarbital, did not potentiate lethal effects of fluroxene in rats [2]. Based on these results, it was concluded that only certain forms of cytochrome P-450 are capable of catalyzing the metabolism of fluroxene to toxic metabolites, while for other forms fluroxene is either a poor substrate or not a substrate or non-toxic metabolites are formed [2]. Furthermore, since the non-fluorinated analog of fluroxene, ethyl vinyl ether, did not produce lethal effects at doses and conditions of induction similar to those that potentiate lethal effects with fluroxene, it was concluded that the trifluorinated moiety of fluroxene was associated with the toxic metabolite [4]. Trifluoroethanol (TFE), which has been demonstrated to be toxic [5], is the most likely toxic metabolite.

Fluroxene is metabolized by hepatic microsomal cytochrome P-450 to TFE and CO_2 in mice [5], dogs [6], and monkeys [7]. In man, the major urinary metabolite is 2,2,2-trifluoroacetic acid which, because it is less toxic than TFE, could account for the apparent lesser susceptibility of man to fluroxene toxicity [8].

Cytochrome P-450, the terminal oxidase of the hepatic microsomal mixed function oxidase system, has as its principal function the detoxification of xenobiotics by conversion of hydrophobic to more hydrophilic and, thus, excretable compounds. Compounds other than fluroxene, however, exhibit enhanced toxicity as a consequence of cytochrome P-450 catalyzed metabolism. For example, cytochrome P-450 plays a toxifying role in the mutagenicity and, presumably, carcinogenicity of polycyclic aromatic compounds [9] and in the hepatotoxicity of chloroform [10].

In the present study we have continued our investigations into the role of cytochrome P-450 in potentiating the toxicity of fluroxene by examining the effects of cytochrome P-450 inducers and inhibitors on the lethality of fluroxene and analogous compounds. The relationship of toxic effects to the extent of binding of the fluroxene analogs to differently induced forms of rat hepatic microsomal cytochrome P-450 and the *in vitro* metabolism of the compounds catalyzed by cytochrome P-450 have been studied.

* This research was supported by grant GM 23029 from the National Institutes of Health, USPHS, DHEW.

† Author to whom all correspondence should be addressed: Toxicology Center, Division of Laboratories and Research, New York State Department of Health, Tower Buildings, Empire State Plaza, Albany, NY 12201, U.S.A.

The time courses of blood anesthetic concentrations and the quantities of exhaled anesthetic following single i.p. administrations of the anesthetics were also determined to aid interpretation of lethal effects.

MATERIALS AND METHODS

Reagents. Fluroxene (TFVE) was obtained from Ohio Medical Products, Madison, WI. Ethyl 2,2,2-trifluoroethyl ether (TFEE) was prepared from fluroxene by catalytic hydrogenation [11]. Allyl 2,2,2-trifluoroethyl ether (TFAE) was prepared by the condensation of 2,2,2-trifluoroethanol with allyl chloride [12], and 2,3-epoxypropyl 2,2,2-trifluoroethyl ether (EPTFE) was prepared by condensation of 2,2,2-trifluoroethanol with epicholorohydrin [13]. PB, 3-MC, and NADPH were purchased from the Mallinckrodt Chemical Works (St. Louis, MO), Pfaltz & Bauer (Stamford, CT), and the Sigma Chemical Co. (St. Louis, MO) respectively. PCN, 2-allyl-2-isopropylacetamide (AIA), SKF 525-A, and metyrapone were gifts of The Upjohn Co. (Kalamazoo, MI), Hoffmann-LaRoche (Nutley, NJ), Smith, Kline & French (Philadelphia, PA) and Ciba-Geigy (Summit, NJ) respectively. TFE was purchased from the Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were of the highest grades commercially available. Water was deionized and glass distilled.

Animals and tissue preparation. Male Wistar rats (250 ± 30 g) from a colony maintained by this Division were used throughout this study. The rats were housed at 22° with a 12-hr light cycle. They were allowed free access to food (Wayne Laboratory Animal Diet) and water. However, 24 hr before treatment with an anesthetic or before killing, the rats were allowed water only.

Rats were injected i.p. with PB (100 mg $PB \cdot kg^{-1} \cdot day^{-1}$ in 0.9% saline) for 2 days and treated experimentally on day 4. They were given 25 mg $3\text{-}MC \cdot kg^{-1} \cdot day^{-1}$ or 100 mg $PCN \cdot kg^{-1} \cdot day^{-1}$ for 3 days i.p. in corn oil, and treated experimentally on day 5. Control animals were injected with saline or corn oil using the experimental regimen. Those animals treated with the cytochrome P-450 inhibitors were injected as follows: AIA, 200 mg/kg in 0.9% saline, s.c. (neck), 8 hr before anesthesia or killing; SKF 525-A, 50 mg/kg, in saline, i.p., 60 min before anesthesia or killing, simultaneously with anesthesia, or 60 min after anesthesia; and metyrapone, 60 mg/kg in saline, i.p., 60 min before anesthesia or killing.

Rats were killed by cervical dislocation; the liver was immediately removed, washed in ice-cold 0.15 M KCl–0.02 M Tris–HCl (pH 7.4) and finely minced. Microsomes were prepared from individual animal livers, as described previously [14], by a modification of the gel filtration method of Tangen *et al.* [15]. Microsomal protein concentrations were determined by the method of Schacterle and Pollack [16]. Cytochrome P-450 concentrations were determined by the difference spectral method of Omura and Sato [17].

Methods. Blood concentrations of the anesthetics were determined by gas chromatographic analysis of a carbon tetrachloride extract of rat tail-vein blood. A rat, after being given the anesthetic i.p., was placed in a restraining cage. The tail was immersed in water maintained at 50° for approximately 30 sec, after which it was withdrawn and dried off; a sample of blood was taken from a tail vein using a 1-ml syringe fitted with a 20-gauge needle. Twenty μl of this blood was immediately transferred to a 300-μl Reacti-Vial (Pierce Chemical Co., Rockford, IL) containing 200 μl of CCl_4. After thorough vortexing, the mixture was allowed to separate. One μl of the CCl_4 layer was injected onto a 10 ft 5% OV-101 on 100–120 mesh Chromosorb WHP column for analysis. The column, injection port, and flame ionization detector temperatures were 100°, 100°, and 120° respectively. The anesthetics were quantitated by integration of the areas under their chromatographic peaks and comparison with the corresponding areas of standard solutions of the anesthetics in carbon tetrachloride.

The rate of anesthetic exhalation by a rat was determined by gas chromatographic analysis of the expired air trapped in CCl_4. We have previously described the method for trapping exhaled anesthetic gases [3]. Analysis of the CCl_4 extract of anesthetic was performed as described above. Recovery of anesthetics added to the trapping system was 100 per cent.

Partitioning of the anesthetics between water and the microsomal suspensions was determined by gas chromatographic analysis of the aqueous phase of a microsomal suspension in the presence of anesthetic. Ultracentrifuge tubes were completely filled with a known volume of microsomal suspension (2 mg protein/ml). A known quantity of anesthetic was added and the tubes were immediately sealed. After the tubes were shaken vigorously they equilibrated at 30° for 1 hr, after which they were centrifuged at 105,000 *g* for 1 hr at 30°. The aqueous phase was analyzed on a 6 ft 10% XE-60 on 80–100 mesh Chromosorb WHP column. The column, injection port, and flame ionization detector temperatures were 80°, 200°, and 200°, respectively, except during EPTFE analysis when temperatures were 150°, 200°, and 200° respectively. The concentration of the anesthetic in the aqueous phase was determined by comparison of integrated peak areas with corresponding areas of standard anesthetic solutions. By using the relation 0.4 μl phospholipid/mg protein [18], the concentration of the anesthetic in the lipid phase and, hence, the partition coefficient of the anesthetics between lipid and water (PC_{lw}) were calculated.

The *in vitro* rates of production of TFE by microsomal metabolism of the anesthetics were determined by gas chromatographic analysis of the aqueous phase of a microsomal incubation. Metabolic incubations were performed in a 2 ml vial with the following components: 500 μl microsomes (8 mg/ml) and 400 μl buffer [25 mM *N*-tris (hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES) buffer, pH 7.4, 0.5 mM Na_2 EDTA and 12.5 mM $MgCl_2$]. The vial was closed with a serum cap and anesthetic (3.0 μl) was introduced by injection through the cap. After equilibration at 30° for 5 min with vigorous shaking, 1.5% NADPH solution (100 μl) was added to initiate the reaction. The reaction was terminated after 10 min by plunging the vial into a boiling water bath to denature the enzymes. When

Table 1. Effects of cytochrome P-450 inducers and inhibitors on the per cent mortality of rats following administration of fluorinated anesthetic agents*

Inducer	Inhibitor	Per cent mortality† TFVE	TFEE	TFAE	EPTFE
None (control)	None	0	0	0	100
	AIA	0	5	0	100
	SKF 525-A	0	15	7	100
	Metyrapone	0	0	0	85
Phenobarbital	None	89	100	7	95
	AIA	0	5	10	100
	SKF 525-A	0	100	0	95
	Metyrapone	40	95	11	75
Pregnenolone-16α-carbonitrile	None	63	70	100	100
	AIA	0	0	0	100
	SKF 525-A	0	45	57	85
	Metyrapone	0	15	25	50
3-Methylcholanthrene	None	0	100	100	100
	AIA	0	0	17	100
	SKF 525-A	0	100	100	100
	Metyrapone	0	75	85	100

* Abbreviations: TFVE, fluroxene; TFEE, ethyl 2,2,2-trifluoroethyl ether; TFAE, allyl 2,2,2-trifluoroethyl ether; EPTFE, 2,3-epoxypropyl 2,2,2-trifluoroethyl ether; and AIA, 2-allyl-2-isopropyl acetamide. Doses of anesthetics administered i.p. were: TFVE, 3.0 g/kg; TFEE, 4.0 g/kg; TFAE, 3.0 g/kg; and EPTFE, 1.5 g/kg.

† Death occurring between 2 and 168 hr post-anesthetic administration. The number of animals in each group was seventeen to twenty.

the inhibitors SKF 525-A (2 mM) and metyrapone (2 mM) were included, they were added in the same buffer prior to addition of substrate. AIA (2 mM) was added with the NADPH and incubated for 10 min; then the substrate and more NADPH were added. The tubes were then centrifuged at 2000 *g* for 10 min. The supernatant layer was analyzed on an XE-60 column in a manner similar to that for the PC_{lw} determinations described above.

EPTFE in the microsomal incubations of TFAE was assayed by gas chromatography; the gas chromatographic system is described above. Microsomal suspensions were injected directly into the injection port. Standard curves of EPTFE concentrations in aqueous solutions and microsomal suspensions were constructed.

The interaction of the anesthetics with microsomal cytochromes P-450 was investigated using the difference spectral method [19] with an Aminco DW-2 spectrophotometer. Microsomes were diluted to 2 mg protein/ml with 0.02 M Tris-HCl–0.15 M KCl buffer (pH 7.4). Equal volumes of the diluted microsomes were placed in two cuvettes which were then sealed with rubber serum caps and equilibrated at 25°. A spectral baseline was determined. Various concentrations of the anesthetic were introduced through the serum cap of the sample cuvette, the cuvette was shaken for 1 min, and the spectrum was recorded between 340 and 490 nm. The absorbance difference between the peak and trough was determined and plotted against the corresponding anesthetic concentration, using Eadie–Hofstee plots. The binding constants, K_s, were determined from these plots.

The mortality data were compared using simplified pairwise comparisons [20] and the Bonferroni technique of multiple comparisons [21]. The limit of significance when mentioned in the text was $P < 0.05$.

RESULTS

In vivo *toxicity*. The effects of the cytochrome P-450 inducers PB, 3-MC, and PCN and the inhibitors AIA, SKF 525-A, and metyrapone on the lethal effects of the fluorinated ether anesthetics are shown in Table 1. Only those deaths that were judged to be a consequence of anesthetic metabolism, i.e. which occurred between 2 and 168 hr after anesthetic administration, were taken into consideration. Animals that were alive 168 hr after anesthetic administration were considered to be survivors; those dying between 0 and 2 hr post-anesthesia were

Table 2. LD_{50} values for fluorinated anesthetics in rats administered single doses i.p.*

Anesthetic	LD_{50} (g/kg) Control rats	Phenobarbital induced rats
TFVE	5.7	1.4
TFEE	6.2	2.5
TFAE	†	3.9
EPTFE	0.9	1.1

* Abbreviations: TFVE, fluroxene; TFEE, ethyl 2,2,2-trifluorethyl ether; TFAE, allyl 2,2,2-trifluoethyl ether; and ETPFE, 2,3-epoxypropyl 2,2,2-trifluoroethyl ether. Only deaths occurring between 2 and 168 hr post-anesthetic administration were considered, i.e. metabolic death.

† A large number of deaths before 2 hr precluded determination of an LD_{50} for anesthetic metabolite related toxicity.

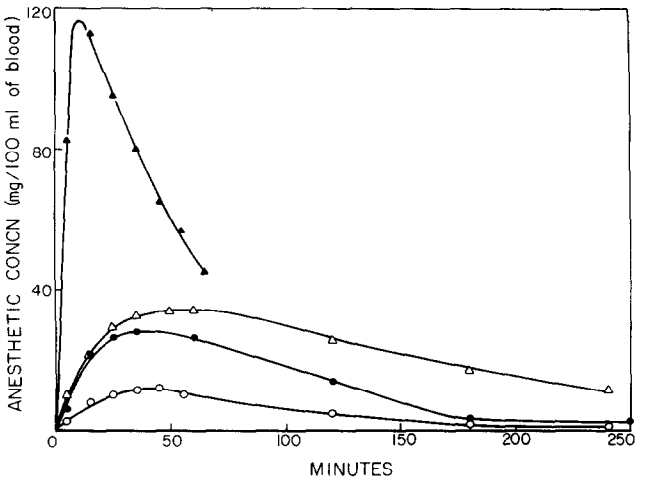


Fig. 1. Tail vein blood concentrations of the anesthetic agents as a function of time after administration of a single, i.p. dose. Key: (○) 2,2,2-trifluoroethyl vinyl ether (2.5 g/kg), (●) ethyl 2,2,2-trifluoroethyl ether (4.0 g/kg), (△) allyl 2,2,2-trifluoroethyl ether (3.0 g/kg), and (▲) 2,3-epoxypropyl 2,2,2-trifluorethyl ether (1.5 g/kg).

considered to have succumbed to the effects of the anesthesia rather than to anesthetic metabolites. Although the exact cause of death has not been determined, the clinical symptoms—lacrimation, tremors, labored respiration, and erythema about the face—were similar for all treated groups (except with EPTFE) and resembled those reported for TFE [5]. The doses of anesthetics are provided in Table 1 and were based on the LD_{50} values for single i.p. administrations of the anesthetics (Table 2). Doses of anesthetics, except in the case of EPTFE, were chosen to produce no deaths in control animals and significant numbers of deaths after induction with at least one inducing agent.

At doses of EPTFE where few or no deaths occurred in control animals, induction by any of the agents used in this study did not potentiate a lethal effect. A dose of EPTFE was accordingly chosen which produced large numbers of deaths of both control and all induced rats, to determine whether the inhibitors would diminish the lethal effects. Only metyrapone produced any significant decrease in EPTFE related deaths, and only with PCN induced rats (Table 1).

PB induction very markedly enhanced the lethal effects of TFVE and TFEE but not of TFAE. AIA significantly overcame the PB potentiated toxicities of both anesthetics, whereas SKF 525-A and metyrapone were only effective in significantly diminishing the toxicity of TFVE but not of TFEE. When SKF 525-A was administered 60 min after the TFVE to PB induced rats, the protective effect of the SKF 525-A was markedly diminished, and 66 per cent of the rats died vs 0 per cent when SKF 525-A was administered 60 min before the TFVE. PCN induction significantly enhanced the toxicities of TFVE, TFEE and TFAE, and all three inhibitors significantly diminished the toxic effects except in the case of SKF 525-A with TFEE, where the effect was not significant. 3-MC induction potentiated the toxicity of TFEE and TFAE but not of TFVE. AIA but not SKF 525-A or metyrapone significantly overcame the toxic effects (Table 1).

The PC_{lw} values were determined from studies with aqueous microsomal suspensions. The values obtained were in order of increasing lipophilicity: EPTFE, $7.2 \pm 0.8 \times 10^2$; TFVE, $14.1 \pm 4.7 \times 10^2$; TFAE, $19.4 \pm 2.0 \times 10^2$; and TFEE, $21.0 \pm 6.7 \times 10^2$. The epoxide EPTFE was significantly less lipophilic than TFVE, which was significantly less lipophilic than TFEE and TFAE, which were not significantly different at $P < 0.01$ using Student's *t*-test.

In Fig. 1, the blood concentrations of the anesthetic agents are shown as a function of the time after i.p. administration to rats. For TFVE, TFEE and TFAE, blood concentrations increased slowly with time to achieve maximal concentrations at 40–60 min, followed by a slow decline over the following 4 hr. For EPTFE, blood concentrations increased

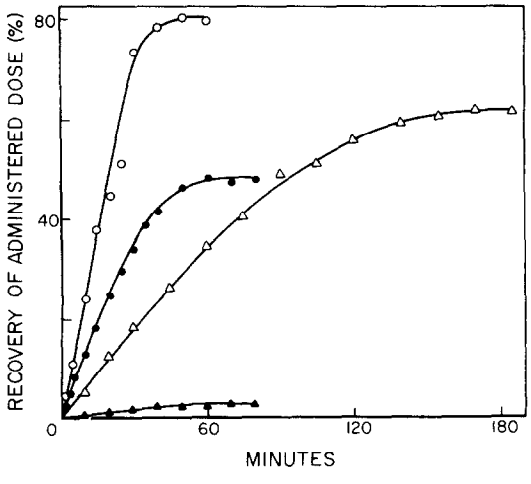


Fig. 2. Rates of exhalation of anesthetics following administration of a single, i.p. dose. Key: (○) 2,2,2-trifluoroethyl vinyl ether (2.0 g/kg), (●) ethyl 2,2,2-trifluoroethyl ether (4.0 g/kg), (△) allyl 2,2,2-trifluoroethyl ether (2.0 g/kg), and (▲) 2,3-epoxypropyl 2,2,2-trifluoroethyl ether (1.5 g/kg).

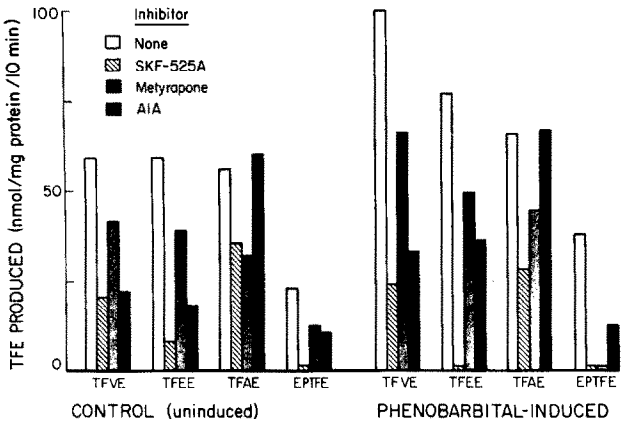


Fig. 3. Rates of formation of trifluorethanol (TFE) from 2,2,2-trifluoroethyl vinyl ether (TFVE), ethyl 2,2,2-trifluorethyl ether (TFEE), allyl 2,2,2-trifluoroethyl ether (TFAE) and 2,3-epoxypropyl 2,2,2-trifluoroethyl ether (EPTFE) catalyzed by microsomes from control and PB induced rats. Microsomal concentrations were 4 mg protein/ml; anesthetic concentrations were: TFVE, 26.8 mM; TFEE, 25.6 mM; TFAE, 23.3 mM; and EPTFE, 24.3 mM. The inhibitory effects of AIA (2 mM), SKF 525-A (2 mM), and metyrapone (2 mM) are shown. Incubation temperature was 30°; incubation time was 10 min. Results are the means of six to eight experiments.

much more rapidly with time after administration, reaching a maximum concentration, which was more than 3-fold greater than those of the other anesthetic agents, at approximately 10 min. The blood concentrations of EPTFE also declined at a much greater rate than was the case for the other anesthetic agents. At 24 hr post-administration, all of the anesthetic concentrations were below the detectible level in rat tail vein blood.

The rates of exhalation of single doses of anesthetics administered i.p. to rats are presented in Fig. 2. The order of the initial rates of exhalation of the anesthetics correlates with their volatilities (evidenced by the boiling points: TFVE, 43.5°; TFEE, 50–52°; TFAE, 74–76°; and EPTFE, 139–141°). Major portions of the administered doses of TFVE, TFEE and TFAE were exhaled within 1–2 hr post-administration; the proportion of the administered dose exhaled was inversely proportional to the partition coefficients for these anesthetics. In the case of EPTFE, markedly lower proportions of the administered doses were exhaled, which could be a consequence of its low volatility or chemical reactivity. The relatively low lipophilicity of EPTFE would tend to preclude the possibility of its deposition into body fat based on affinity for the fat.

Since EPTFE caused destruction of ferricytochrome P-450 (L. S. Kaminsky and M. J. Murphy, unpublished results), no spectral binding studies could be performed. TFVE, TFEE and TFAE all gave type I binding spectra with control rat liver microsomes, indicating that they were interacting at the substrate binding site. Eadie–Hofstee plots of the extent of absorbance change versus anesthetic concentration were linear for the three anesthetics and yielded the following binding constants, K_s: TFVE, 2.54 mM; TFEE, 2.68 mM; and TFAE, 1.73 mM. With PB induced microsomes the K_s values were decreased to similar extents with TFVE, 0.51 mM; TFEE, 0.87 mM; and TFAE, 0.38 mM.

When the four anesthetic agents were incubated with microsomes from control or PB induced rats and NADPH, a single metabolite was detected in all cases (not necessarily the only metabolite) which was shown by gas chromatography–mass spectrometry to be TFE. In Fig. 3 the rates of formation of TFE from the four anesthetics and the effect of the cytochrome P-450 inhibitors on these rates of formation are shown. PB induction significantly ($P < 0.05$) enhanced the rates of metabolism of all four anesthetics, with TFAE metabolism being enhanced the least. In all cases, the inhibitors AIA, SKF 525-A, and metyrapone significantly inhibited the metabolism, except in the case of AIA with TFAE metabolism, catalyzed by microsomes from control and PB induced rats, where there was no effect.

We were unable to detect EPTFE as an intermediate metabolite of TFAE.

DISCUSSION

Although our previous investigations [1–3] have implicated the metabolism of fluroxene, catalyzed by specific forms of cytochrome P-450, in the lethal effects of the anesthetic, many details of the mechanism of toxicity are still unclear. An outstanding question concerns the role of an intermediate epoxide metabolite in the toxicity. All our attempts to synthesize 2,3-epoxyethyl 2,2,2-trifluoroethyl ether (the epoxide derived from fluroxene) for use in mechanistic studies have failed, however, possibly because of the inherent instability of the molecule. We have thus used compounds similar to fluroxene, TFAE and its epoxide EPTFE in these studies to aid in resolving the mechanism of toxicity of fluorinated ether anesthetics.

The selection of anesthetic doses, which is based on the requirement for lethal effects in induced rats and no lethal effects with control rats, results in the use of relatively high doses of anesthetics. The major portion of these doses, however, was exhaled within approximately 2 hr except in the case of the epoxide, EPTFE (Fig. 2). The failure of EPTFE to be exhaled is consistent with the initially relative high blood

concentrations of the EPTFE observed (Fig. 1). If the lethal effects of TFVE, TFEE and TFAE arise from hepatic metabolism, this metabolism must occur within approximately 2 hr of administration of the anesthetic because after this time the blood concentrations are too low to yield toxic concentrations of the metabolites. This is supported by the results which indicate that inhibition of PB induced cytochrome P-450 prior to administration of anesthetic prevents lethal effects, whereas inhibition of cytochrome P-450, at times exceeding 50 min post-anesthesia, is very much less effective in preventing mortality. It is thus apparent that, at least for TFVE, metabolism of the high concentrations of TFVE on the first passage through the liver after being absorbed in the peritoneium is ultimately responsible for the lethal effects.

The various inducers and inhibitors of cytochrome P-450 were used in this study to determine the role of cytochrome P-450 in potentiating anesthetic toxicity. The inducing agents were selected for ability to induce different forms of cytochrome P-450. We have demonstrated, by using the drug warfarin as an *in vitro* metabolic probe, that the livers of PB induced rats contain primarily the cytochrome P-450 forms PB-B and PB-C, those from 3-MC induced rats contain primarily form MC-B [22], whereas livers from PCN induced rats contain a different form of cytochrome P-450, as defined by the warfarin metabolite patterns, which has not yet been identified [23]. There have been numerous other reports on the ability of these agents to induce different forms of cytochrome P-450 (e.g. Refs. 24 and 25). We have previously reported the hepatic microsomal cytochrome P-450 concentrations following induction by the various agents [2].

Metyrapone and SKF 525-A have been demonstrated to inhibit cytochrome P-450 *in vitro* and *in vivo* and, depending on the substrates used, to act as competitive inhibitors [26, 27]. Control, PB or 3-MC induced forms of cytochrome P-450 are inhibited by both inhibitors [28]. AIA is not an inhibitor of cytochrome P-450 but effectively acts to diminish cytochrome P-450 catalyzed rates of metabolism by destroying cytochrome P-450 *in vivo* and *in vitro* as a consequence of its own metabolism [29, 30]. AIA is, however, most effective in its destruction of PB induced forms of cytochrome P-450 and is virtually ineffective in destroying 3-MC induced cytochromes P-450 [31, 32].

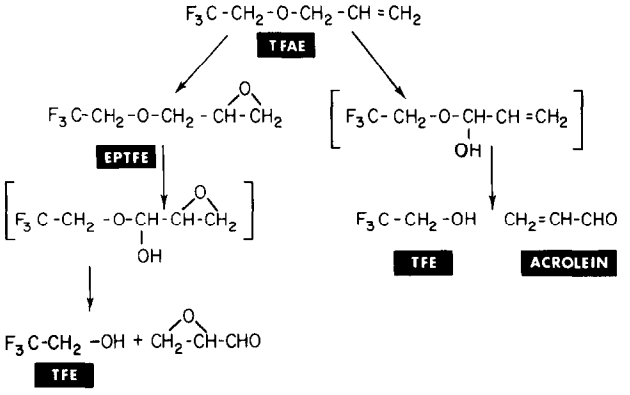
An analysis of the effects of cytochrome P-450 inhibitors and inducers on anesthetic-induced mortalities (Table 1) indicates that the role of cytochrome P-450 is probably more complex than simply providing toxic concentrations of TFE as was previously presumed [33, 34]. Thus, the epoxide EPTFE, which is possibly an intermediate of the metabolism of TFAE, by analogy with proposed mechanisms of fluroxene metabolism [34], exhibits lethal effects that are predominantly independent of its cytochrome P-450 catalyzed metabolism to TFE. This follows from the failure of the cytochrome P-450 inducing agents to potentiate EPTFE toxicity or, in the majority of cases, of cytochrome P-450 inhibitors to overcome EPTFE toxicity. Thus, if EPTFE were a metabolite on the major pathway of TFAE metabolism, then the toxicity of TFAE could be a consequence of the formation of EPTFE and not TFE. In fact, the rate of metabolism of EPTFE to TFE is significantly slower than the rate of metabolism of TFAE to TFE (Fig. 3), which implies that the epoxide is not on the major pathway of TFAE metabolism. Mechanistic aspects will be discussed later.

We have previously demonstrated that PB induction potentiates lethal effects of TFVE and TFEE [1]. The enhanced rates of formation of TFE from both of these compounds *in vitro*, and their enhanced affinity for cytochrome P-450 as a consequence of PB induction, are consistent with proposals that the potentiation of toxicity by PB induction is a consequence of increased concentrations of TFE arising from increased rates of formation. The present results, however, indicate that the mechanisms of formation of toxic metabolites from TFVE and TFEE differ since SKF 525-A and metyrapone inhibition overcomes the ability of PB to potentiate TFVE-but not TFEE-related mortalities. These inhibitors do, however, inhibit the cytochrome P-450 catalyzed formation of TFE from TFEE as effectively as from TFVE *in vitro*, which implies that factors apart from TFE concentrations may play a role in TFEE-related mortalities.

Although PB induction of hepatic microsomal cytochrome P-450 enhances rates of formation of TFE from TFAE and the affinity of cytochromes P-450 for TFAE *in vitro*, there is no significant increase in mortalities following TFAE administration to PB induced rats. Since blood levels of TFAE were similar to those of TFVE and TFEE, and with other inducing agents lethal effects of TFAE were potentiated, it appears probable that the TFAE must have been available for metabolism *in vivo*. Thus, other unknown factors must differentiate the mechanisms of toxicity of TFAE from that of TFVE under conditions of PB induction.

PCN induction of rats potentiates the lethal effects of TFVE, TFEE and TFAE that are overcome by administration of all three inhibitors of cytochrome P-450. These results indicate that, for the PCN induced cytochrome P-450 system, a common mechanism for metabolizing the three anesthetics to toxic metabolities is operative. With 3-MC induced rats, TFVE is clearly differentiated from TFEE and TFAE with respect to its toxicity. Although the absence of any potentiation of TFVE toxicity is consistent with our previous results which indicate that fluroxene is not a substrate for 3-MC induced cytochrome P-450 [35], 3-MC potentiation of the toxicity of TFEE and TFAE is difficult to explain. In particular, the effect of AIA, which does not eliminate hepatic 3-MC induced cytochrome P-450, in overcoming these lethal effects, SKF 525-A and metyrapone, which are known to inhibit this enzyme, are without effect, is indicative of the complexity of the role of cytochrome P-450. These results indicate that cytochrome P-450 does not function in 3-MC induced rats to potentiate the toxic effects and that other unknown factors are involved.

Scheme 1 shows pathways postulated for mixed function oxidase catalyzed metabolism of TFAE; they incorporate possible intermediates based on known cytochrome P-450 function. TFE has been



Scheme 1

demonstrated to be a metabolite of both TEAE and EPTFE, which supports EPTFE as an intermediate of TFAE metabolism. However, as previously discussed, the relative rates of formation of TFE from TFAE and EPTFE preclude the epoxide from being on the major pathway of TFAE metabolism. In no case was EPTFE detected as an intermediate in TFAE metabolism, indicating that either the rate of TFAE → EPTFE was slower than the rate of EPTFE → TFE, thus preventing any build-up of ETPFE intermediate, or EPTFE is not an intermediate in TFAE metabolism. We are currently developing an assay for acrolein in an effort to determine whether the alternative pathway of metabolism from TFAE is possible.

We have thus demonstrated that fluroxene and analogous fluorinated ethers become toxic through relatively rapid metabolism catalyzed by a variety of cytochromes P-450. The mechanisms of metabolism are varied and complex, and epoxidation across a vinyl group is apparently not a major factor in the potentiation of the toxicity in the unsaturated compounds.

Acknowledgements—We wish to thank Tom Turiel for performing the statistical analyses.

REFERENCES

1. K. M. Ivanetich, J. J. Bradshaw, J. A. Marsh, G. G. Harrison and L. S. Kaminsky, *Biochem. Pharmac.* **25**, 773 (1976).
2. M. J. Murphy, L. J. Piper, D. N. McMartin and L. S. Kaminsky, *Toxic. appl. Pharmac.* **52**, 69 (1980).
3. M. J. Murphy, L. J. Piper, M. J. Fasco, M. J. Cashin, D. N. McMartin and L. S. Kaminsky, *Toxic. appl. Pharmac.* **48**, 87 (1979).
4. G. G. Harrison, K. M. Ivanetich, L. S. Kaminsky and M. J. Halsey, *Anesth. Analg.* **55**, 529 (1976).
5. D. A. Blake, H. F. Cascorbi, R. S. Rozman and F. J. Meyer, *Toxic. appl. Pharmac.* **15**, 83 (1969).
6. R. R. Johnston, E. I. Eger, W. C. Stevens and P. F. White, *Anesth. Analg.* **53**, 998 (1974).
7. E. S. Munson, M. H. Malagodi, R. P. Shields, M. K. Tham, V. Fiserova-Bergerova, D. A. Holaday, J. C. Perry and W. J. Embro, *Clin. Pharmac. Ther.* **18**, 687 (1975).
8. H. Gion, N. Yoshimura, D. A. Holaday, V. Fiserova-Bergerova and R. E. Chase, *Anesthesiology* **40**, 533 (1974).
9. C. Heidelberger, *A. Rev. Biochem.* **44**, 79 (1975).
10. I. G. Sipes, G. Krishna and J. R. Gillette, *Life Sci.* **20**, 1541 (1977).
11. K. M. Ivanetich, J. A. Marsh, J. J. Bradshaw and L. S. Kaminsky, *Biochem. Pharmac.* **24**, 1933 (1975).
12. O. W. Steward and O. R. Pierce, *J. org. Chem.* **26**, 2943 (1961).
13. M. L. Brey and P. Tarrant, *J. Am. chem. Soc.* **79**, 6533 (1957).
14. M. J. Fasco, L. J. Piper and L. S. Kaminsky, *J. Chromat.* **131**, 365 (1977).
15. O. Tangen, J. Jonsson and S. Orrenius, *Analyt. Biochem.* **54**, 597 (1973).
16. G. R. Schacterle and R. L. Pollack, *Analyt. Biochem.* **51**, 654 (1973).
17. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
18. N. Borgese, W. Mok, G. Kreibick and D. D. Sabatini, *J. molec. Biol.* **88**, 559 (1974).
19. J. B. Schenkman, H. Remmer and R. W. Estabrook, *Molec. Pharmac.* **3**, 113 (1967).
20. D. R. Cox, *Analysis of Binary Data*, p. 33. Methuen, London (1970).
21. R. G. Miller, *Simultaneous Statistical Inference*, p. 67. McGraw-Hill, New York (1966).
22. L. S. Kaminsky, M. J. Fasco and F. P. Guengerich, *J. biol. Chem.* **255**, 85 (1980).
23. M. J. Fasco, L. J. Piper and L. S. Kaminsky, *Biochem. Pharmac.* **28**, 97 (1979).
24. A. Y. H. Lu, A. Somogyi, S. West, R. Kuntzman and A. Conney, *Archs Biochem. Biophys.* **152**, 457 (1972).
25. A. P. Alvares, G. Schilling, W. Levin and R. Kuntzman, *Biochem. biophys. Res. Commun.* **29**, 521 (1967).
26. R. E. Stitzel, M. W. Anders and G. J. Mannering, *Molec. Pharmac.* **2**, 335 (1966).
27. G. F. Kahl and K. J. Netter, *Biochem. Pharmac.* **19**, 27 (1970).
28. R. Kahl, M. Buecker and K. J. Netter, in *Microsomes and Drug Oxidations* (Eds. V. Ullrich, I. Roots, A. Hildebrandt, R. W. Estabrook and A. H. Conney), p. 177. Pergamon Press, New York (1977).
29. F. DeMatteis, *Biochem. J.* **124**, 767 (1971).
30. W. Levin, M. Jacobson, E. Sernatinger and R. Kuntzman, *Drug Metab. Dispos.* **1**, 275 (1973).
31. M. B. Baird, L. S. Birnbaum, H. V. Samis, H. R. Massie and J. A. Zimmerman, *Biochem. Pharmac.* **25**, 2415 (1976).
32. J. J. Bradshaw, M. R. Ziman and K. M. Ivanetich, *Biochem. biophys. Res. Commun.* **85**, 859 (1978).
33. J. A. Marsh, J. J. Bradshaw, G. A. Sapeika, S. A. Lucas, L. S. Kaminsky and K. M. Ivanetich, *Biochem. Pharmac.* **26**, 1601 (1977).
34. V. Fiserova-Bergerova, *Envir. Hlth Perspect.* **21**, 225 (1977).
35. K. M. Ivanetich, J. J. Bradshaw, J. A. Marsh and L. S. Kaminsky, *Biochem. Pharmac.* **25**, 779 (1976).