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THE ROLE OF CYTOCHROME P-450 IN THE TOXICITY OF FLUROXENE (2,2,2-TRIFLUOROETHYL VINYL ETHER) ANAESTHESIA *IN VIVO**

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Abstract—Induction of type P-450 cytochromes in rats by i.p. injections of phenobarbital potentiated the toxicity (100% mortality) of the normally non-toxic anaesthetic fluroxene (2,2,2-trifluoroethyl vinyl ether). The toxic effects were eliminated by administration of 2-allyl-2-*iso*propylacetamide prior to anaesthesia. Hepatic microsomal cytochrome P-450 levels of the dead rats were markedly diminished relative to unanaesthetised induced controls. Induction by 3-methylcholanthrene and 3,4-benzpyrene did not potentiate toxic effects of fluroxene but anaesthesia after mixed induction with 3-methylcholanthrene and phenobarbital manifested toxicity more rapidly than induction with phenobarbital alone. When 2,2,2-trifluoroethyl ethyl ether was used as the anaesthetic similar toxic effects were observed except that levels of type P-450 cytochromes were not depressed at the time of death and induction with 3-methylcholanthrene did potentiate toxic effects with this anaesthetic. We interpret these results to indicate that cytochrome P-450 catalyses an essential step in the production of toxic metabolites from fluroxene and that elevated concentrations of the enzyme are required to potentiate the toxicity. Apparently, cytochrome P-448 does not metabolize fluroxene and elevated levels of this enzyme therefore do not potentiate the toxicity of fluroxene anaesthesia. The ability of fluroxene to destroy cytochrome P-450 resides in its vinyl group while the toxic metabolite arises from the trifluoroethyl moiety.

Fluroxene (2,2,2-trifluoroethyl vinyl ether) is a volatile anaesthetic agent first introduced into clinical practice in 1953 [1]. Although it has accumulated a considerable record of safe clinical usage in man with no evidence of organ toxicity [2, 3], recent work has shown it to be toxic to many animal species [4, 5, 6] and even to man under certain circumstances [7, 8, 9, 10]. This toxicity, which includes hepatotoxicity [11], is markedly enhanced by pretreatment of the experimental animal with phenobarbital. The toxicity is considered to result from biotransformation of fluroxene into toxic metabolites such as trifluoroethanol glucuronide [12, 13] or trifluoroacetaldehyde [14, 15] rather than to be an effect of fluroxene itself [4, 16]. Since phenobarbital is known to induce the proliferation of the hepatic endoplasmic reticulum and the biosynthesis of the components of the cytochrome P-450 drug metabolizing pathway [17], it appeared possible that hepatic microsomal cytochrome P-450 could be involved in potentiating the toxicity of fluroxene anaesthesia [11], especially in view of the localization of fluroxene metabolism in the hepatic endoplasmic reticulum [12]. We will use the terms 'cytochromes P-450' or 'type P-450 cytochromes' to represent both cytochrome P-450 and cytochrome P-448 (see later). We have consequently investigated the effect of induction of cytochromes P-450 on the toxicity of fluroxene and 2,2,2-trifluoroethyl ethyl ether (TFEE) anaesthesia in rats with a view to investigating the role of the cytochromes P-450 in potentiating these toxic effects, and determining the mechanisms of production of toxic effects.

MATERIALS AND METHODS

Materials. Sodium phenobarbital (PB) and 3-methylcholanthrene (MC) were obtained from Maybaker, S.A. and Eastman-Kodak, respectively. 3,4-benzpyrene (BP) was from Sigma Chemicals. 2-Allyl-2-isopropylacetamide (AIA) was a generous gift from Hoffman-La Roche, Nutley, New Jersey. Ampoules of sterile saline (0·9%, w/v) were obtained from Petersen, Ltd., S.A. Fluroxene was supplied by Ohio-Medical Products, Madison, Wisconsin. 2,2,2-Trifluoroethyl ethyl ether (TFEE) was prepared by hydrogenation of fluroxene using our previously published method [18]. The TFEE was tested for the presence of peroxides with 5% aqueous KI (w/v) immediately before use. Cylinders of CO and O_2 were supplied by Afrox Ltd. All other chemicals were analytical grade reagents. Water was distilled and deionized.

Animals. Male Wistar rats weighing between 175 g and 300 g were used in all experiments; animals were permitted free access to Epol Laboratory Chow (protein min. 20%, fat 2·5%, fibre max. 6%, calcium 1·4%,

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phosphorus 0·7%) and water. Induction of cytochrome P-450 was by i.p. injection of sodium phenobarbital (80 mg/kg/day in 0·9% sterile saline), 3,4-benzpyrene (40 mg/kg/day in corn oil) or 3-methylcholanthrene (40 mg/kg/day in corn oil) for three consecutive days. One group of rats received sodium phenobarbital (80 mg/kg) for 1 day only. In the case of a simultaneous induction by sodium phenobarbital and 3-methylcholanthrene, the animals received the above doses in separate i.p. injections for 3 consecutive days. Control animals were injected i.p. with vehicle for 3 days. All animals were fasted overnight after the final injection, and where indicated, anaesthetised the following morning. Animals receiving AIA were injected subcutaneously with a dosage of 200 mg/kg (20 mg/ml in 0·9% sterile saline) in the loose skin of the neck 1 hr prior to anaesthesia. Rats were anaesthetised in groups of ten or less in a special perspex anaesthetic chamber (30 × 30 × 60 cm). Animals were placed on a fenestrated floor raised 8 cm from the base of the chamber, the space below the fenestrated floor being occupied by standard anaesthetic sodalime. The anaesthetic (fluroxene or TFEE), vaporised by a 5 l/min stream of oxygen through a Cyprane Fluoromatek vaporiser, was passed as a continuous stream into the anaesthetic chamber through an inlet port sited at one corner beneath the fenestrated floor and exhausted through an exhaust port sited at the diagonally opposite upper corner. A flow rate of this order was shown to result in CO_2 concentration of <0·1%. Animals were exposed to a 2 hr period of anaesthesia of a level approximately equivalent to that which would be attained by the minimum anaesthetic concentration of the respective agent, this being achieved by exposure of animals to a 3% concentration of fluroxene or TFEE. Unanaesthetised control animals in these experiments were exposed to air. In subsequent experiments in which unanaesthetised control rats were exposed to O_2 only, no differences in the parameters under consideration were noted. Toxicity was judged by death after anaesthesia. Animals not dying were sacrificed by cervical fracture. Liver weight was determined immediately after excision of the liver.

Preparation of microsomes. Livers were excised from animals within minutes of death. A portion of the liver was fixed in buffered aqueous formaldehyde (10% v/v) for histological investigation and the remainder was utilized for the preparation of microsomes by differential ultracentrifugation [19]. Protein concentration was determined by the method of Lowry *et al.* [20], as modified by Chaykin [21] using bovine serum albumin as a standard.

Spectrophotometry. Microsomal suspensions (protein concentration 2 mg/ml) were divided between two cuvettes of 1-cm optical path length. Difference absorbance spectra of microsomal suspensions were recorded at room temperature in a Unicam SP1800 recording spectrophotometer using the cell holder adjacent to the photomultiplier. Microsomal cytochrome P-450 content was determined by the CO difference spectral method of Omura and Sato [22]; it should be noted that total type P-450 cytochromes were measured, and that these values include the contributions of both cytochromes P-450 and P-448 to the total.

RESULTS

Toxicity of fluroxene anaesthesia. The effects of various inducing agents of cytochromes P-450 on the toxicity to rats of fluroxene anaesthesia are reported in Table 1. Injection of saline alone or in conjunction with exposure to 3% fluroxene anaesthesia for 2 hr did not produce toxic effects and did not affect cytochrome P-450 content per mg of microsomal protein or the ratio of liver to body weight relative to untreated control animals.

Phenobarbital treatment of animals for three days, as described in Methods, resulted in a 2·6-fold increase in cytochrome P-450 levels and a slight increase in liver weight but produced no toxic effects. All rats induced with phenobarbital and subsequently anaesthetised with fluroxene died between 1 and 3 hr after the termination of anaesthesia (100% mortality). Immediately after death, these animals were shown to have a 2·8-fold decrease in cytochromes P-450 content and markedly enlarged livers, relative to unanaesthetised phenobarbital induced controls.

The toxic effects of fluroxene anaesthesia observed in phenobarbital pretreated animals were prevented by treatment of the animals with AIA 1 hr prior to anaesthesia. No deaths were observed in this latter group. The animals appeared normal at the time of sacrifice and up to that time demonstrated none of the symptoms of the phenobarbital induced fluroxene anaesthetised animals (e.g. crouching, staring coat [23], glassy eyes, sluggishness, bleeding eyelids). Furthermore, fluroxene anaesthesia apparently does not elicit any marked effect on cytochromes P-450 content or liver weight in phenobarbital induced AIA treated rats, relative to unanaesthetised phenobarbital induced AIA treated animals.

Pretreatment of animals with phenobarbital for 1 day only, elevated cytochromes P-450 levels approximately 2-fold relative to control animals and was in itself non-toxic. Fluroxene anaesthesia of animals induced in this manner was less toxic (40% mortality), and the animals that did not die subsequent to anaesthesia appeared normal prior to sacrifice at 190 hr.

Injection with corn oil, the vehicle for induction with polycyclic hydrocarbons, does not affect the hepatic microsomal cytochromes P-450 levels but does increase liver weight relative to saline-injected or untreated controls. The above effects were not altered by subsequent fluroxene anaesthesia.

Injection of 3,4-benzpyrene or 3-methylcholanthrene suspended in corn oil increased levels of type P-450 cytochromes approximately 2-fold and increased liver weight relative to corn oil controls. Subsequent fluroxene anaesthesia was non-toxic and did not affect cytochrome levels and liver weight of 3,4-benzpyrene induced animals but did slightly reduce the cytochrome levels of those rats pretreated with 3-methylcholanthrene. After five days the cytochrome content and liver weight of the fluroxene anaesthetised rats decreased to within the range found for control animals. The appearance and behaviour of the anaesthetised animals was completely normal at the time of sacrifice.

Mixed induction by injection of 3-methylcholanthrene plus phenobarbital elevated levels of both cytochromes P-448 and P-450 and resulted in increased

Table 1. Toxicity of fluroxene anaesthesia

Pretreatment* (no. rats)	Anaesthetised	Time of death† (hr)	Toxicity	Cyt P-450 (nmol/mg microsomal protein)	wt. liver/ wt. body (%)
Untreated (2)	—			1·27 ± 0·12	2·76 ± 0·22
Saline (2)	—	K24	—	1·31 ± 0·10	3·07 ± 0·29
Saline (2)	+	K24	—	1·32 ± 0·02	3·47 ± 0·63
PB (4)	—	K4	—	3·42 ± 0·34	3·39 ± 0·05
PB (9)	+	1–3	+	1·23 ± 0·13	4·63 ± 0·44
PB + AIA (2)		K 2§		0·81 ± 0·02	3·83 ± 0·08
PB + AIA (2)	—	K24	—	0·91 ± 0·02	4·82 ± 0·01
PB + AIA (2)	—	K166	—	0·99 ± 0·04	3·20 ± 0·18
PB + AIA (2)	+	K24		0·75 ± 0·15	4·70 ± 0·10
PB + AIA (2)	+	K141	—	0·98 ± 0·07	2·69 ± 0·02
PB (3)‡	—	K0	—	2·33 ± 0·17	4·07 ± 0·16
PB (2)‡	+	18	+	1·41	4·26
PB (3)‡	+	K190	—	0·98 ± 0·14	4·14 ± 0·29
Corn Oil/saline (2)		K2	—	1·28 ± 0·12	3·84 ± 0·31
Corn oil/saline (2)	+	K2	—	1·16 ± 0·15	3·72 ± 0·08
PB + MC (2)	—	K2	—	4·87 ± 0·32	5·10 ± 0·38
PB + MC (5)	+	2	+	1·92 ± 0·17	6·96 ± 0·44
Corn Oil (2)	—	K24	—	1·27 ± 0·12	3·75 ± 0·16
Corn Oil (2)	+	K24	—	1·10 ± 0·20	3·97 ± 0·05
BP (2)	—	K24	—	1·86 ± 0·09	4·49 ± 0·02
BP (6)	+	K24	—	1·57 ± 0·21	4·31 ± 0·31
BP (2)	+	K140	—	1·31 ± 0·03	3·41 ± 0·21
MC (3)	—	K24	—	2·47 ± 0·12	4·81 ± 0·08
MC (2)	—	K190	—	1·34 ± 0·09	4·88 ± 0·15
MC (3)	+	K24	—	1·52 ± 0·29	4·89 ± 0·79
MC (2)	+	K190	—	1·51 ± 0·04	4·62 ± 0·32

Anaesthesia for 2 hr at 3% fluroxene. All values are means ± S.D. For experimental details see text.

* Abbreviations used are: BP, 3,4-benzpyrene; MC, 3-methylcholanthrene; PB, phenobarbital; AIA, allyl-*iso*-propylacetamide. The first two compounds are injected in corn oil, the remainder in saline.

† From the end of anaesthesia or, if not anaesthetized, from the equivalent time. K indicates that the animals were killed.

‡ Injected with only 1 dose of PB (80 mg/kg) 1 day prior to anaesthesia.

§ Killed at a time equivalent to the beginning of anaesthesia.

hepatic weight and cytochromes P-450 content in excess of that seen for either type of induction individually [24], but was in itself non-toxic (0% mortality). Fluroxene anaesthesia of these induced animals resulted in 100% mortality within 2 hr of the end of anaesthesia. A marked decrease in cytochrome levels and an increase in liver weight relative to the unanaesthetised but similarly induced animals were also noted.

Toxicity of TFEE anaesthesia. The effects of TFEE anaesthesia on rats with elevated levels of cytochrome P-450 and/or P-448 are reported in Table 2. Anaesthesia with TFEE did not affect animals injected with vehicle except for a slight increase in liver weight in the case of saline.

In phenobarbital-induced rats, anaesthesia with TFEE was toxic: 100% mortality was observed within 35 hr after termination of anaesthesia at which time a slight decrease in cytochromes P-450 content and an increase in liver weight were evident. Treatment of phenobarbital-induced rats with AIA prior to anaesthesia with TFEE completely protected the animals from the toxicity of anaesthesia (0% mortality). Cytochrome levels and liver weights in unanaesthetised and anaesthetised rats receiving phenobarbital and AIA were comparable.

TFEE anaesthesia of 3,4-benzpyrene induced animals was non-toxic (0% mortality) and did not alter the levels of type P-450 cytochromes but did diminish liver weight relative to that found for unanaesthetised benzpyrene treated controls. 3-Methylcholanthrene induction did potentiate the toxicity of TFEE anaesthesia (80% mortality) without markedly affecting cytochrome P-450 levels but decreased the liver weights in the animals that died. Increasing the duration of TFEE anaesthesia to 160 min for 3-methylcholanthrene induced rats increased mortality to 100%. Administration of AIA after 3-methycholanthrene induction did not noticeably diminish levels of type P-450 cytochromes but did overcome the toxic effects of TFEE anaesthesia.

Induction with phenobarbital and 3-methylcholanthrene together potentiated the toxicity of TFEE anaesthesia more than phenobarbital alone. TFEE lowered the levels of type P-450 cytochromes of these induced rats only very slightly.

DISCUSSION

Our experiments on the effects of various microsomal inducing agents on the toxicity of the anaesthetic fluroxene were initiated to explore the role of the cytochromes P-450 in potentiating this toxicity and

Table 2. Toxicity of 2,2,2-trifluoroethyl ethyl ether (TFEE) anaesthesia

Pretreatment* (no. rats)	Anaesthetised	Time of death† (hr)	Toxicity	Cyt P-450 (nmol/mg microsomal protein)	wt. liver wt. body (%)
Untreated (2)				1·27 ± 0·12	2·76 ± 0·22
Saline (2)	—	K 24		1·31 ± 0·10	3·07 ± 0·29
Saline (3)	+	K 24		1·25 ± 0·05	3·79 ± 0·25
PB (4)		K 24		3·41 ± 1·08	4·82 ± 0·42
PB (8)	+	0–35	+	2·49 ± 0·63	5·33 ± 0·58
PB + AIA (2)	—	K 2§		0·81 ± 0·02	3·82 ± 0·08
PB + AIA (2)		K 46		0·97 ± 0·08	4·13 ± 0·22
PB (2)		K 46		2·13 ± 0·08	3·84 ± 0·14
PB + AIA (3)	+	K 46		1·22 ± 0·14	3·83 ± 0·01
Corn Oil/Saline (2)	+	K 24		1·32 ± 0·08	3·82 ± 0·28
Corn Oil/Saline (2)		K 2		1·28 ± 0·12	3·84 ± 0·31
PB + MC (2)	—	K 26		4·80 ± 0·69	5·69 ± 0·39
PB + MC (5)	+	17–26	+	4·09 ± 0·29	5·19 ± 0·22
Corn Oil (2)	—	K 24		1·27 ± 0·12	3·75 ± 0·16
Corn Oil (2)	+	K 24		1·31 ± 0·14	3·81 ± 0·22
BP (2)	—	K 24		2·25 ± 0·05	4·75 ± 0·05
BP (3)	+	K 24		2·13 ± 0·43	3·27 ± 0·23
BP (2)	+	K 145		1·38 ± 0·11	4·15 ± 0·25
MC (5)		K 48		1·72 ± 0·09	5·55 ± 0·35
MC (4)	+	27–76	+	1·96 ± 0·44	3·56 ± 0·40
MC (1)	+	K 144		1·03	5·05
MC + AIA (3)	—	K 2§		1·74 ± 0·32	4·85 ± 0·33
MC + AIA (3)	—	K 164		1·92 ± 0·16	5·20 ± 0·48
MC + AIA (3)	+	K 164		1·94 ± 0·09	5·53 ± 0·37
MC (5)‡	+	17–24	+	3·24 ± 0·34	4·19 ± 0·20

Anaesthesia for 2 hr at 3% TFEE. All values are means ± S.D. For experimental details see text.

* Abbreviations used are: BP, 3,4-benzpyrene; MC, 3-methylcholanthrene; PB, phenobarbital; AIA, allyl-*iso*-propylacetamide. (The first two compounds are injected in corn oil, the remainder in saline.) TFEE, 2,2,2-trifluoroethyl ethyl ether.

† From the end of anaesthesia or if not anaesthetized from the equivalent time. K indicates animals were killed.

‡ Anaesthesia for 2 hr 40 min at 3% TFEE.

§ Killed at a time equivalent to the beginning of anaesthesia.

to determine the mechanism whereby the toxicity is generated.

Type P-450 cytochromes are a heterogeneous group of enzymes that metabolize an extremely wide variety of substrates. In general, this class of enzymes catalyses the detoxification of hydrophobic xenobiotics by converting them to relatively more hydrophilic water soluble compounds which are more readily excreted from the body, although in some instances the metabolites are more toxic than the parent compound [25]. A variety of compounds have been demonstrated to induce the biosynthesis of cytochromes P-450. These inducing agents are classified into two major groups: (1) those which induce cytochrome P-450 and (2) those which induce a similar enzyme, cytochrome P-448 (also called cytochrome P_1-450) [26]. Phenobarbital is an example of the first type of agent. This compound also enhances the proliferation of the endoplasmic reticulum and elevates the levels of NADPH-cytochrome *c* reductase and cytochrome b_5 per mg of microsomal protein [17]. The second class of inducers includes polycyclic aromatic compounds such as 3,4-benzpyrene and 3-methylcholanthrene which do not increase the proliferation of the endoplasmic reticulum or the synthesis of microsomal NADPH-cytochrome *c* reductase or cytochrome b_5. These polycyclic hydrocarbons apparently only induce the synthesis of cytochrome P-448 which differs from cytochrome P-450 with regard to substrate specificity, spectral properties and sensitivity to inhibitors [26]. Mixtures of these two (or more) type P-450 cytochromes are apparently always present in hepatic microsomes, regardless of the pretreatment of the source animal. In phenobarbital induced and uninduced animals, cytochrome P-450 predominates, while in polycyclic hydrocarbon treated animals, cytochrome P-448 predominates [26]. We will use the terminology 'cytochromes P-450' or 'type P-450 cytochromes' to refer to both cytochrome P-450 and P-448, whereas 'cytochrome P-450' or 'cytochrome P-448' refers to the specific enzyme mentioned.

The observed effects of the various inducing agents in potentiating the toxicity of fluroxene anaesthesia can now be considered in relation to the mechanism of toxicity. The fact that uninduced rats, with levels of hepatic microsomal cytochromes P-450 of approximately 1 nmol/mg microsomal protein, are not susceptible to the toxic effects of fluroxene anaesthesia, while phenobarbital pretreated rats, with approximately a three fold elevation of cytochromes P-450 levels are highly susceptible (100% mortality), (Table 1) implies that the metabolism of fluroxene is enhanced as a consequence of the inductive properties

of phenobarbital. Since phenobarbital induces a number of microsomal proteins, the enzyme(s) catylyzing the rate-limiting step of this metabolism is (are) not determined by this experiment. The effect of AIA, however, in overcoming the toxic potentiating effects of phenobarbital (Table 1) on fluroxene anaesthesia unequivocally demonstrated an essential role for cytochrome P-450, since AIA specifically degrades cytochrome P-450 while not affecting other microsomal proteins (e.g. cytochrome b_5 or NADPH–cytochrome *c* reductase) or reversing other non-microsomal effects of phenobarbital [27, 28]. Thus, while other microsomal enzymes definitely play a role in the metabolism of fluroxene, elevated levels of cytochrome P-450 are essential in potentiating the toxicity of fluroxene. Apparently, cytochrome P-450 is involved in an essential step of the metabolism producing the toxic metabolite and mechanisms such as those involving the effect of phenobarbital on other subcellular components can be excluded.

The failure of induction by 3-methylcholanthrene or 3,4-benzpyrene, which produces elevated levels of cytochrome P-448, to potentiate the toxicity of fluroxene (Table 1), is apparently not a consequence of insufficient concentrations of type P-450 cytochromes. In these animals the total type P-450 cytochrome levels are approximately 2 nmole/mg microsomal protein which is similar to the level obtained following phenobarbital induction for one day, a treatment which does produce toxic effects subsequent to anaesthesia (Table 1). There are several possible explanations for the toxicity differences observed between induction by phenobarbital or by 3-methylcholanthrene and 3,4-benzpyrene, which are still consistent with an essential role for cytochrome P-450:

(a) Cytochrome P-448, in contrast to cytochrome P-450, may be protecting the organism by converting fluroxene to non-toxic metabolites.

(b) In view of the different specificities of cytochromes P-448 and P-450, cytochrome P-448 may metabolize fluroxene slowly compared to cytochrome P-450, or not at all.

(c) 3-Methylcholanthrene may produce physiological or biochemical changes which are not directly involved with the microsomal system but which prevent fluroxene anaesthesia from mediating toxic effects.

The first possibility involving the production of different and non-toxic metabolites by cytochrome P-448 appears to be highly unlikely in view of the results of studies with mixed induction by phenobarbital and 3-methylcholanthrene (Table 1). The double induction potentiates the toxic effects of fluroxene anaesthesia even more rapidly than induction by phenobarbital alone (time of death subsequent to anaesthesia is used as an index of toxicity). In contrast, in view of the competition between cytochromes P-450 and P-448 for the substrate fluroxene in the doubly induced animals, it would be expected that a reduction of toxicity would occur if scheme (a) were operative. We can suggest no situation, where the suggestion outlined in scheme (c) could arise, to explain the results observed. Confirmation of scheme (b) requires establishing whether cytochrome P-448 binds to and metabolizes fluroxene. Our studies *in vitro* of the interaction of fluroxene with microsomes induced for cytochrome P-448 or P-450 indicate that although cytochrome P-450 forms a spectrally detectable complex with and metabolizes fluroxene, cytochrome P-448 does neither [29]. Apparently, then, the failure of the induction of rats with 3-methylcholanthrene or benzpyrene to potentiate the toxicity of fluroxene follows from the failure of cytochrome P-448 to metabolize the fluroxene.

The results of preanaesthetic induction of hepatic microsomal cytochromes on the toxicity to rats of TFEE anaesthesia were compared with the results of the investigations of fluroxene anaesthesia, to identify the portion of the fluroxene molecule in which the potential toxicity resides (Tables 1 and 2). Since TFEE and fluroxene anaesthesia exhibited similar effects under conditions of no induction, benzpyrene induction, phenobarbital induction or mixed induction with phenobarbital and 3-methylcholanthrene (Tables 1 and 2), it is clear that the potential toxicity resides in the trifluoroethyl moiety of fluroxene rather than in the vinyl group which is not present in TFEE. There is ample support for the metabolism of the fluroxene molecule to trifluoroethanol glucuronide or trifluoroacetaldehyde *in vivo*, and the toxicity of trifluoroethanol and physiological derivatives thereof has been demonstrated [13]. However, although our results indicate that the toxicity arises from a metabolite of the trifluoroethyl portion of the fluroxene molecule, we have no evidence that the toxic effects observed in this study arise directly from a particular metabolite such as trifluoroethanol or its glucuronide or trifluoroacetaldehyde as proposed elsewhere [4, 14, 15].

The unexpected observation that induction with 3-methylcholanthrene potentiates the toxicity of TFEE is difficult to explain especially in view of the failure of 3,4-benzpyrene to produce a similar effect. We have concluded from *in vitro* studies that hepatic microsomal cytochrome P-448 does not bind or metabolize TFEE [29]. It could be postulated that the cytochrome P-450 component of the type P-450 cytochromes present after induction with 3-methylcholanthrene in these experiments is of a high enough concentration to potentiate the toxicity of TFEE. This simple explanation is, however, unlikely in view of the failure of 3-methylcholanthrene induction to potentiate fluroxene toxicity. This aspect is undergoing further investigation.

Comparison of the toxicity of fluroxene and TFEE indicates that since equivalently-pretreated animals expire earlier after fluroxene than after TFEE anaesthesia, the former agent is probably metabolised more rapidly by cytochrome P-450. In confirmation, we have demonstrated that fluroxene is a better substrate for hepatic microsomal cytochrome P-450 *in vitro* [29]. Even though fluroxene is metabolized by microsomal enzymes it does not induce elevated levels of cytochromes P-450 [12].

Although the vinyl group of the fluroxene molecule has no apparent role in the observed toxicity of fluroxene anaesthesia, this moiety appears to be necessary for the fluroxene induced destruction of cytochrome P-450 observed *in vivo* (c.f. Table 1 and Table 2), since TFEE, without the vinyl group, does not produce any similar destruction. Fluroxene-mediated destruction of cytochrome P-450 is of the greatest magnitude in phenobarbital or phenobarbital-plus-3-

methylcholanthrene-induced animals. It is, however, not clear from the *in vivo* studies whether this destruction arises from elevated concentrations of cytochrome P-450 or other microsomal enzymes (e.g. NADPH–cytochrome *c* reductase) in these animals prior to anaesthesia. We have, however, duplicated this phenomenon in isolated hepatic microsomes and have concluded that the destruction results from the interaction of fluroxene with ferrocytochrome P-450 [18]. The levels of other microsomal enzymes (e.g. NADPH–cytochrome *c* reductase and cytochrome b_5) are unaffected by fluroxene *in vitro*.

Previous reports concerning compounds capable of destroying cytochrome P-450 *in vivo* and *in vitro* confirm the ability of a monounsaturated carbon-carbon bond to function in this manner [30, 31]. Similarly, the vinyl portion of fluroxene is also essential for the destruction of cytochrome P-450 *in vitro* and *in vivo* since TFEE elicits no effect on cytochrome P-450 levels [18]. Cytochrome P-450 is apparently more susceptible to the destructive effects of fluroxene than is cytochrome P-448.

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