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THE INTERACTION OF HEPATIC MICROSOMAL CYTOCHROME P-450 WITH FLUROXENE (2,2,2-TRIFLUOROETHYL VINYL ETHER) *IN VITRO**

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Abstract—The anaesthetic agent fluroxene (2,2,2-trifluoroethyl vinyl ether) and a closely related compound 2,2,2-trifluoroethyl ethyl ether (TFEE) interact with the cytochrome P-450 component of isolated rat hepatic microsomes to produce a type I difference spectrum. The extent of the absorbance difference (ΔA) between λ_{max} (390 nm) and λ_{min} (420 nm) produced with fluroxene or TFEE is dependent on the concentration of the anaesthetic agent and the extent and type of prior induction of the microsomes. Induction of cytochrome P-448 with 3-methylcholanthrene (MC) or 3,4-benzpyrene (BP) does not affect the magnitude of the maximal absorbance difference spectrum (ΔA_{max}) relative to uninduced microsomes. In contrast, phenobarbital (PB) induced microsomes exhibit ΔA_{max} values with either anaesthetic agent which, relative to controls, are increased approximately in proportion to the increase in the level of total type P-450 cytochromes. The K_s values for the binding of fluroxene and TFFE to all microsomal preparations are $9{\cdot}3 \times 10^{-4}$ M and $1{\cdot}7 \times 10^{-3}$ M respectively. Both anaesthetics are metabolized by hepatic microsomal cytochrome P-450 as evidenced by enhanced carbon monoxide-inhibitable NADPH oxidation in the presence of these compounds. The maximum velocities of NADPH consumption in the presence of either anaesthetic are unaffected by induction with BP or MC but are increased approximately 3-fold following induction of cytochrome P-450 with PB. For fluroxene metabolism by all microsomes K_m was determined to be $8{\cdot}4 \times 10^{-4}$ M. Determination of K_m values for TFEE metabolism is more complex as biphasic effects are observed with some systems. We conclude that fluroxene and TFEE bind to cytochrome P-450 and are metabolized but that TFEE is a poorer substrate. In contrast cytochrome P-448 neither binds nor metabolizes either anaesthetic. Since K_m and K_s values for fluroxene are the same we conclude that the rate-limiting step of its metabolism occurs at a step after the binding of fluroxene to ferricytochrome P-450.

Recent reports that the anaesthetic fluroxene, which has been safely used clinically since 1953 [1, 2, 3], can become toxic to animals [4, 5, 6] and man under certain circumstances [7, 8, 9, 10] prompted us to investigate the metabolism of the anaesthetic *in vivo* [11].

The results of our investigation indicated that fluroxene is metabolized in the rat with the rate-limiting step possibly being catalyzed by hepatic cytochrome P-450, and that the toxic effects of the anaesthetic arise from a metabolite of its trifluoroethyl moiety. A further effect of the anaesthetic is that it produces destruction of hepatic cytochrome P-450 *in vivo* [11] and *in vitro* [12], a phenomenon not observed with TFEE. In contrast hepatic cytochrome P-448, which is induced by 3-methylcholanthrene and 3,4-benzpyrene, is apparently unable to metabolize the anaesthetic or to potentiate the toxicity of fluroxene.

In the present paper we report on our investigations of the interaction of fluroxene with cytochrome P-450 in isolated rat hepatic microsomes which were performed in order to elucidate the mechanism by which the anaesthetic agent fluroxene becomes toxic and to provide an explanation on a biochemical level of our observations on the effects of fluroxene anaesthesia on the intact animal [11]. In this study we have utilized microsomes from uninduced rats and rats which have been induced with phenobarbital (PB), 3-methylcholanthrene (MC) or 3,4-benzpyrene (BP) and have studied the binding and metabolism of fluroxene and 2,2,2-trifluoroethyl ethyl ether (TFEE) by the type P-450 cytochromes of these microsomes.

MATERIALS AND METHODS

Materials. NADPH was purchased from Miles Laboratories. SKF 525A was a generous gift from Smith, Kline & French, Ltd. Sepharose 2B was obtained from Pharmacia, Uppsala. All other materials were obtained or prepared as described previously [11, 12]. Induction of microsomal proteins by phenobarbital (PB), 3-methylcholanthrene (MC) and 3,4-benzpyrene (BP) was also performed as described [12].

Preparation of microsomes. Livers were removed from the animals immediately after sacrifice. Microsomes were prepared by gel filtration on Sepharose 2B equilibrated with 0·15 M KCl–0·02 M Tris–HCl buffer, pH 7·4 by the method of Tangen *et al.* [13]. All operations were performed at 4°. The microsomal

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fraction was collected, diluted to a concentration of 2 mg protein/ml with 0·02 M Tris HCl buffer, pH 7·4 and used immediately.

Difference spectra. Suspensions of hepatic microsomes were divided equally between two 1-cm path length cuvettes. Fluroxene or TFEE was introduced below the surface of the microsomal suspension in the sample cuvette by means of a Hamilton syringe. The cuvette was then stoppered and vortexed for 30 sec in order to disperse and solubilize the anaesthetic agent. The magnitude of the resultant difference spectrum was measured as the difference in absorbance between the peak at ca. 390 nm and the trough at ca. 420 nm and was corrected for the absorbance differences of control microsomal suspensions at these wavelengths and designated as ΔA. Vortexing of the microsomal suspension without added anaesthetic agent did not produce a difference spectrum. All difference spectra were recorded at room temperature (21–24°).

NADPH oxidation. The rates of metabolism of fluroxene or TFEE by hepatic microsomes were determined by monitoring NADPH consumption as follows: Equal quantities of microsomal suspension were divided between two 1-cm path length cuvettes. Varying quantities of fluroxene or TFEE were introduced into the sample cuvette as described for the difference spectra. The reaction mixtures were equilibrated at 28°C, and the reaction was initiated by the addition of 50–100 μl of NADPH solution (0·12–0·24 mM final concentration). NADPH oxidation was monitored spectrally at 340 nm and was corrected for background rates of endogenous NADPH oxidation in the presence of fluroxene or TFEE in an atmosphere of $CO:O_2$ (80:20; v/v) [14].

These rate studies on microsomal suspensions were performed in a Unicam SP1800 recording spectrophotometer, using a thermostatted cell compartment adjacent to the photomultiplier. All other methods are as described previously [11, 12].

Calculations. Binding constants were initially calculated from the intercepts of Lineweaver–Burk type double reciprocal plots of $1/\Delta A$ or $1/v$ versus 1/[anaesthetic agent]. In addition, plots of ΔA versus

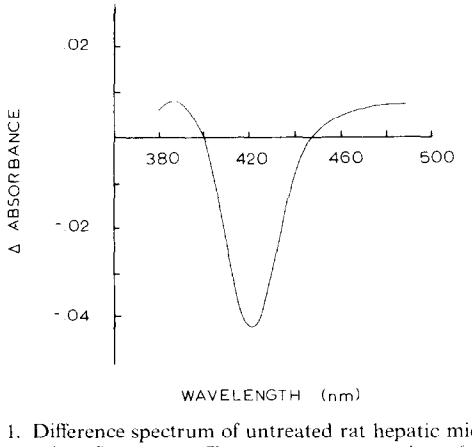

Fig. 1. Difference spectrum of untreated rat hepatic microsomes with fluroxene. Fluroxene concentration $6·0 \times 10^{-3}$ M, microsomal concentration 2·0 mg protein/ml, cytochrome P-450 concentration 1·08 nmol/mg protein, buffer 0·02 M Tris–HCl, pH 7·4.

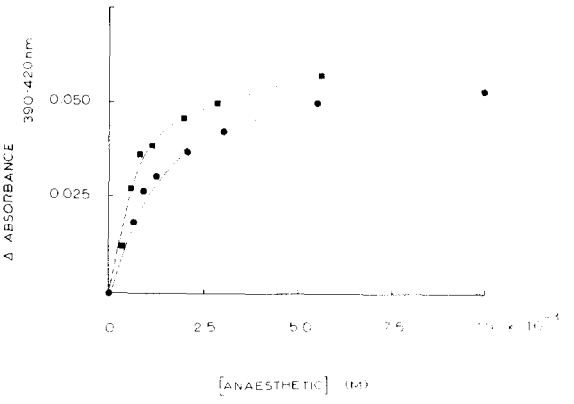

Fig. 2. Effect of anaesthetic concentration on the extent of the difference spectrum with untreated hepatic microsomes; (●), fluroxene with cytochrome P-450 concentration 1·08 nmol/mg protein; (■), TFEE with cytochrome P-450 concentration 1·31 nmol/mg protein. Microsomal concentration 2·0 mg protein/ml, buffer 0·02 M Tris HCl, pH 7·4.

ΔA/[anaesthetic agent] or v versus v/[anaesthetic agent] were prepared as a more sensitive index of linearity. In all plots ΔA represents the difference in absorbance and v represents the rate of consumption of NADPH measured as described above.

RESULTS

The binding of fluroxene and TFEE to hepatic microsomal cytochromes P-450 in vitro

Fluroxene and TFEE bind to hepatic microsomal cytochromes P-450 isolated from untreated male rats, resulting in the appearance of a "Type I" difference spectrum (e.g. Fig. 1). The extent of binding of fluroxene or TFEE to cytochrome P-450 with increasing concentrations of the anaesthetic agent follows a typical saturation curve (Fig. 2) with the maximum extent of binding at saturating substrate concentrations being a function of the type of anaesthetic agent and the pretreatment of the animal source of the microsomes (Table 1).

Pretreatment of animals with the polycyclic hydrocarbons 3-methylcholanthrene or 3,4-benzpyrene, which induce cytochrome P-448 and raise cytochrome levels to double that of controls, does not affect the extent of binding of either fluroxene or TFEE to hepatic microsomal cytochrome P-450 relative to control microsomes, as evidenced by the maximal spectral changes observed (Table 1). In contrast, pretreatment with phenobarbital, which elevates hepatic microsomal cytochrome P-450 content per mg microsomal protein approximately 2·6-fold relative to control animals, also increases the maximal extent of binding of fluroxene and TFEE approximately 2·2-fold. We have utilized a single extinction coefficient for both types of cytochrome P-450 in view of the discrepancy in the literature with regard to the differences in extinctions of the CO complexes of the two hemoproteins [15].

K_s for fluroxene and TFEE. Double reciprocal plots of the data from difference spectral studies are linear

Table 1. Effects of induction of hepatic microsomal cytochrome P-450 on the binding and metabolism of fluroxene (2,2,2-trifluoroethyl vinyl ether)

Induction*	Cyt.† P-450 (nmol/mg microsomal protein)	K_m‡ (M)	V_{max}§ (nmol NADPH/min·mg microsomal protein)	K_s¶ (M)	ΔA_{max}** (O.D.)	Toxicity‖
NONE	1·1	$7{\cdot}6 \times 10^{-4}$	4	$9{\cdot}3 \times 10^{-4}$	0·06	-
BP	2·3	10×10^{-4}	4	$9{\cdot}0 \times 10^{-4}$	0·06	—
PB	2·7	$6{\cdot}3 \times 10^{-4}$	13	$9{\cdot}5 \times 10^{-4}$	0·13	+
MC	2·1	$9{\cdot}5 \times 10^{-4}$	3	$9{\cdot}4 \times 10^{-4}$	0·07	-

* Abbreviations used are BP, 3,4-benzpyrene; MC, 3-methylcholanthrene; PB, Phenobarbital.
† Total type P-450 cytochromes, including cytochromes P-450 and P-448.
‡ Values calculated from plots of $1/v$ versus $1/[S]$.
§ S.D. ± 1 nmol/min·mg microsomal protein.
¶ Values calculated from plots of $1/\Delta A$ versus $1/[S]$.
** $\Delta A = A_{peak} - A_{trough}$ of difference spectrum, S.D. ± 0·02
‖ Reference [11].

for both anaesthetic agents for all types of induction and permit calculation of the spectral dissociation constants (K_s) [16] for the binding of these compounds to hepatic microsomal cytochrome P-450 (see e.g. Fig. 3). The more sensitive plots of ΔA versus ΔA/[anaesthetic agent] are also monophasic under all conditions (see e.g. Fig. 4) and confirm the K_s values calculated from the double reciprocal plots.

The effects of inducers of cytochromes P-450 or P-448 on the spectral dissociation constants (K_s) for fluroxene and TFEE are presented in Tables 1 and 2. For control animals the spectral dissociation constant for fluroxene has a value of $9{\cdot}3 \times 10^{-4}$ M. Induction of cytochrome P-448 by polycyclic hydrocarbons or of cytochrome P-450 by phenobarbital does not affect K_s values for fluroxene binding to microsomal cytochrome P-450.

For TFEE K_s is found to have a value of $1{\cdot}5 \times 10^{-3}$ M for control microsomes (Table 2). As seen in the case of fluroxene, K_s is not markedly altered by induction with polycyclic hydrocarbons or phenobarbital. Again, the K_s values calculated from the ΔA versus ΔA/[anaesthetic agent] plots agree with those calculated from the double reciprocal plots.

The binding of both fluroxene (Fig. 5) and TFEE (Fig. 6) to hepatic microsomal cytochrome P-450 is competitively inhibited by 2-allyl-2-isopropylacetamide (AIA), a compound known to specifically interact with cytochrome P-450, but is unaffected by SKF 525A.

The metabolism of fluroxene and TFEE by cytochrome P-450

Fluroxene and TFEE stimulate NADPH consumption by hepatic microsomes. The enhanced NADPH consumption observed in the presence of either anaesthetic agent is inhibited by an atmosphere of CO–O_2 (80:20; v/v) or by 10 mM KCN but is not affected by 1 mM KCN indicating that NADPH consumption reflects cytochrome P-450 mediated metabolism of these halocarbon anaesthetics. The metabolism of fluroxene and TFEE by hepatic microsomes was

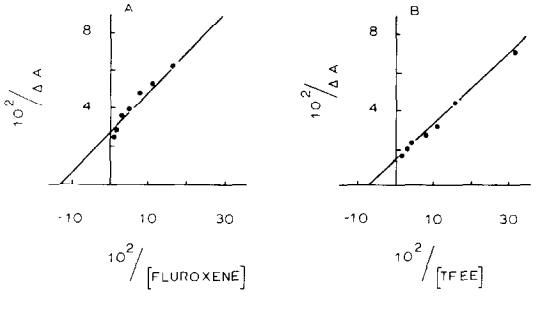

Fig. 3. Effect of (A) fluroxene concentration with cytochrome P-450 concentration 3·34 nmol/mg protein and (B) TFEE concentration with cytochrome P-450 concentration 2·23 nmol/mg protein on the extent of the difference spectrum with phenobarbital induced microsomes. The inverse of the absorbance difference, in arbitrary units, is plotted against the inverse of the anaesthetic concentration. Microsomal concentration 2·0 mg protein/ml, buffer 0·02 M Tris–HCl, pH 7·4.

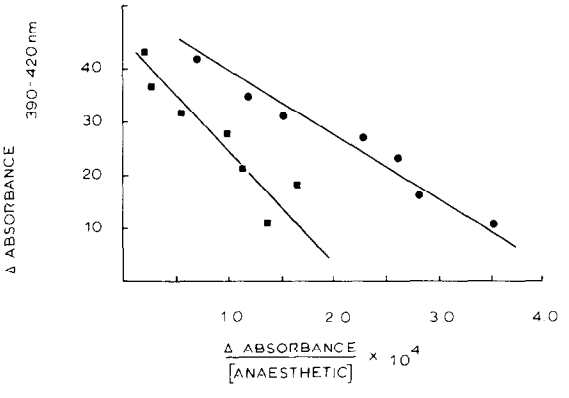

Fig. 4. Effect of anaesthetic concentration on the difference spectrum of 3-methylcholanthrene induced hepatic microsomes; (●), fluroxene with type P-450 cytochromes concentration 2·12 nmol/mg protein; (■), TFEE with type P-450 cytochromes 2·03 nmol/mg protein. The absorbance difference in arbitrary units is plotted against the absorbance difference divided by the anaesthetic concentration. Microsomal concentration 2·0 mg protein/ml, buffer 0·02 M Tris–HCl, pH 7·4.

Table 2. Effects of induction of hepatic microsomal cytochrome P-450 on the binding and metabolism of 2,2,2-trifluoroethyl ethyl ether (TFEE)

Induction*	Cyt.† P-450 (nmol mg microsomal protein)	K_m (M)			V_{max} (nmol NADPH min·mg microsomal protein)	K_s (M)		ΔA_{max}** (O.D.)	Toxicity‡
		K_{m1}‡	K_{m2}‡	K_m§		K_{s1}¶	K_s		
NONE	1·3	$1·5 \times 10^{-3}$	$0·6 \times 10^{-3}$	$1·1 \times 10^{-2}$	3	$1·5 \times 10^{-4}$	$1·3 \times 10^{-3}$	0·07	
BP	1·8	$4·7 \times 10^{-3}$	$1·3 \times 10^{-3}$	$1·3 \times 10^{-2}$	3	$1·7 \times 10^{-4}$	$1·5 \times 10^{-3}$	0·06	
PB	2·7	$2·1 \times 10^{-3}$	$1·9 \times 10^{-3}$		10	$1·8 \times 10^{-4}$	$1·2 \times 10^{-3}$	0·17	+
MC	1·9	$3·7 \times 10^{-3}$	$0·9 \times 10^{-3}$	$1·1 \times 10^{-2}$	2	$2·5 \times 10^{-4}$	$2·1 \times 10^{-3}$	0·09	+

* Abbreviations used are BP, 3,4-benzpyrene; MC, 3-methylcholanthrene; PB, Phenobarbital.
† Total type P-450 cytochromes including cytochromes P-450 and P-448.
‡ Values calculated from plots of $1/v$ versus $1/[S]$.
§ Values calculated from v versus $v/[S]$ plots.
¶ S.D. ± 1 nmol/min·mg microsomal protein.
‖ Calculated from plots of $1/\Delta A$ versus $1/[S]$.
Calculated from plots of ΔA versus $\Delta A/[S]$.
** $\Delta A = A_{(-cyt)} - A_{(+cyt)}$ of difference spectrum, S.D. ± 0·02.
‡ Reference [11].

therefore monitored by NADPH oxidation and corrected for any non-cytochrome P-450 dependent NADPH oxidation according to the method of Stripp *et al.* [14]. The values of V_{max} for microsomes from all sources are given in Tables 1 and 2 for fluroxene and TFEE metabolism. For both anaesthetics, the induction of predominantly cytochrome P-448 with 3,4-benzpyrene or 3-methylcholanthrene did not alter V_{max} relative to control microsomes. However, phenobarbital induction elevated V_{max} relative to control animals approximately in proportion to the increase in cytochrome P-450 content.

Lineweaver–Burk plots of $1/v$ versus $1/$[anaesthetic agent] were linear for fluroxene and apparently for TFEE in all cases (see Fig. 7) and permitted calculation of the Michaelis constants, K_m, for the different inducing agents. However, although the more sensitive plot of Δv versus $v/$[anaesthetic agent] was linear for microsomes from all sources when metabolizing fluroxene (see Fig. 8), the equivalent plots for TFEE were not linear in all cases (Fig. 8). Some plots were biphasic, permitting the calculation of two different K_m values for uninduced, 3-methylcholanthrene and 3,4-benzpyrene induced microsomes. For the metabolism of TFEE by phenobarbital induced microsomes, the plot was nearly linear over the range investigated permitting calculation of only a single K_m.

Effect of inducers on K_m *for fluroxene and TFEE.* The effects of induction of cytochromes P-450 on the K_m for fluroxene and TFEE are presented in Tables 1 and 2. The Michaelis constant for fluroxene is found to be $7·6 \times 10^{-4}$ M for control microsomes. Induction by any of the compounds utilized in this study was without marked effect on the K_m value for fluroxene. For TFEE, however, microsomes from all sources except phenobarbital induced animals, exhibit two

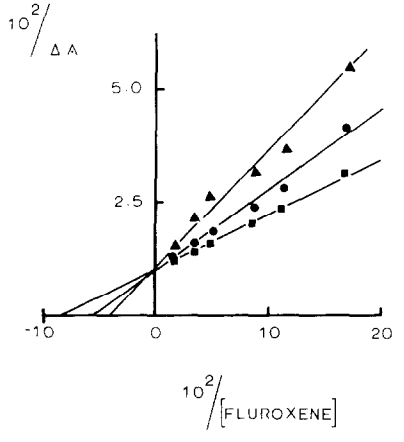

Fig. 5. Effect of fluroxene concentration on the extent of the difference spectrum with phenobarbital induced microsomes: (■), no 2-allyl-2-isopropylacetamide (AIA) added; (●), AIA (3·0 mM) added; (▲), AIA (6·0 mM) added. The inverse of the absorbance difference in arbitrary units, is plotted against the inverse of the fluroxene concentration. Cytochrome P-450 concentration 3·82 nmol/mg microsomal protein, microsomal concentration 2·0 mg protein/ml.

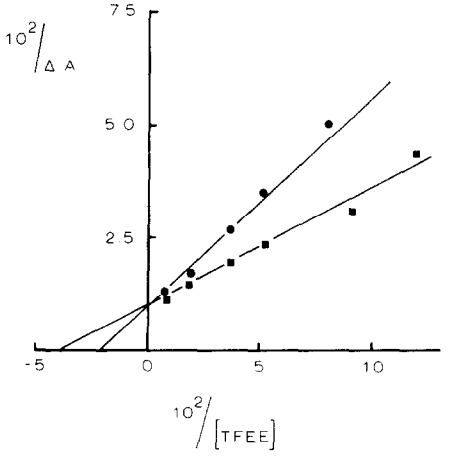

Fig. 6. Effect of TFEE concentration on the extent of the difference spectrum with phenobarbital induced microsomes; (■), no AIA added; (●), AIA (3·0 mM) added. The inverse of the absorbance difference in arbitrary units, is plotted against the inverse of the TFEE concentration. Cytochrome P-450 concentration 2·82 nmol/mg microsomal protein, microsomal concentration 2·0 mg protein/ml.

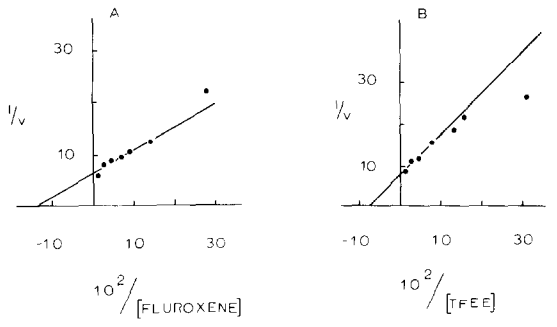

Fig. 7. Effect of (A) fluroxene concentration with cytochrome P-450 concentration 3·34 nmol/mg protein and (B) TFEE concentration with cytochrome P-450 concentration 2·23 nmol/mg protein on their rates of metabolism by cytochrome P-450 in phenobarbital-induced hepatic microsomes. The inverse of the rates, determined by monitoring the corrected rates of NADPH oxidation, are plotted against the inverse of the anaesthetic concentration. Microsomal concentration 2·0 mg protein/ml, buffer 0·02 M Tris·HCl, pH 7·4, temperature 28°.

separate K_m values of roughly $1{\cdot}0 \times 10^{-3}$ M and $1{\cdot}2 \times 10^{-2}$ M. Phenobarbital induced microsomes exhibit a single calculable K_m of $1{\cdot}9 \times 10^{-3}$ M.

DISCUSSION

The results of our previous *in vivo* investigations [11] revealed that the anaesthetic fluroxene becomes toxic to rats only when the levels of hepatic cytochrome P-450 are sufficiently high to catalyse the production of effective concentrations of toxic metabolite(s). These investigations also indicated that cytochrome P-448 either metabolized fluroxene very slowly or was unable to produce such toxic metabolites, possibly as a consequence of its inability to metabolize fluroxene at all. It was also clear that while TFEE, the chemically reduced form of fluroxene, became toxic under conditions of elevated cytochrome P-450 levels the toxic effects were produced more slowly than following fluroxene anaesthesia. It became apparent to us that the explanations for some of these results could only be provided by *in vitro* investigations of the interactions of cytochrome P-450 with the anaesthetics.

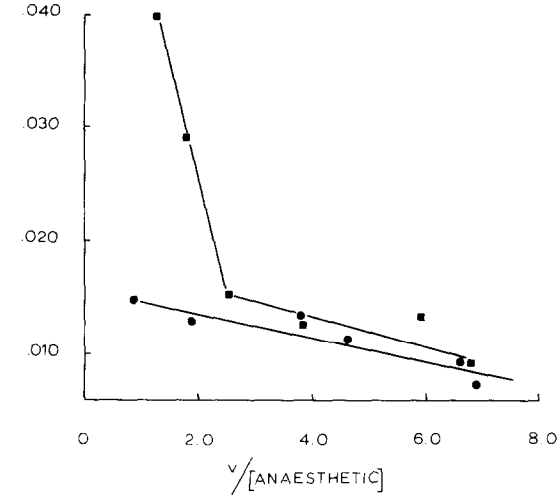

Fig. 8. Effect of (●), fluroxene concentration with type P-450 cytochromes concentration 2·32 nmol/mg protein and (■), TFEE concentration with type P-450 cytochromes concentration 1·52 nmol/mg protein on their rates of metabolism by cytochrome P-450 in 3,4-benzpyrene induced hepatic microsomes. The rates of metabolism, determined by monitoring the corrected rates of NADPH oxidation, are plotted against the rates divided by the anaesthetic concentration. Microsomal concentration 2·0 mg protein/ml, buffer 0·02 M Tris·HCl, pH 7·4, temperature 28°.

The appearance of a type I difference spectrum on the addition of certain compounds to isolated hepatic microsomes has clearly been demonstrated to be a consequence of the binding of those compounds to the catalytic site of ferricytochrome P-450[16]. In the present study the formation of such difference spectra (Fig. 1) and the saturating effect of anaesthetic concentrations on the magnitude of the difference spectra (Fig. 2), following addition of fluroxene or TFEE to isolated hepatic microsomes thus demonstrate that both anaesthetics bind to the catalytic site of cytochrome P-450. The fact that the binding of fluroxene or TFEE to cytochrome P-450 is competitively inhibited by 2-allyl-2-isopropylacetamide (AIA) (Figs. 5 and 6), is further proof that the two anaesthetics interact with the catalytic site of hepatic cytochrome P-450 since AIA has been shown to bind to the substrate binding site [17] and to be metabolized by cytochrome P-450 [18].

The results of investigations with various inducing agents reported here have clearly demonstrated that there is no binding of fluroxene or TFEE to hepatic microsomal cytochrome P-448. This conclusion follows from a comparison of (a) phenobarbital induced microsomes, which have elevated cytochrome P-450 levels, and exhibit difference spectra with fluroxene or TFEE which are markedly enhanced relative to uninduced microsomes with (b) 3-methylcholanthrene or 3,4-benzpyrene induced microsomes, with markedly enhanced cytochrome P-448 levels which do not exhibit enhanced difference spectra relative to uninduced controls (Table 1). This failure of fluroxene and TFEE to bind to cytochrome P-448 *in vitro* provides an explanation for the failure of 3-methylcholanthrene induction to potentiate the toxicity of fluroxene anaesthesia *in vivo* [11]. Apparently, although the levels of total hepatic type P-450 cytochromes in 3-methylcholanthrene induced rats are sufficient to potentiate fluroxene toxicity, since the major component of the cytochromes in this case is cytochrome P-448, which does not bind or metabolize fluroxene, no toxic effects are observed.

The linearity of double reciprocal and ΔA versus ΔA/[anaesthetic] plots of the difference spectra data produced by the addition of varying concentrations of fluroxene or TFEE to variously induced microsomes indicates that these compounds bind to a single site on the enzyme (Figs. 3 and 4). The constancy of the spectrally determined binding constants, K_s, for fluroxene binding to untreated and induced microsomes (Table 1) is probably a consequence of the fact that a single type P-450 cytochrome is binding fluroxene in all the microsomal preparations, i.e. cyto-

chrome P-450. In the case of 3-methylcholanthrene- and 3,4-benzpyrene-induced microsomes this cytochrome P-450 constitutes only approximately half of the total microsomal mixed function cytochromes present [19].

Stripp *et al.* [14] have thoroughly investigated the stoichiometric relationship between the rates of cytochrome P-450 catalyzed metabolism of drugs and the rates of oxidation of NADPH and concluded that an approximately 1:1 relationship existed if the NADPH oxidation rate was corrected for CO-insensitive endogenous NADPH oxidation. This method has been utilized in this present investigation to demonstrate that fluroxene and TFEE are metabolized by the cytochrome P-450 of hepatic microsomes while the results of inhibition studies with KCN indicate that the hepatic stearate desaturase system [20] is not involved in the metabolism of fluroxene or TFEE. Furthermore since phenobarbital-induced microsomes show markedly enhanced maximum rates of metabolism of fluroxene and TFEE relative to uninduced controls while 3-methylcholanthrene and 3,4-benzpyrene-induced microsomes show no change in maximum rates relative to control microsomes (Table 1) it can be concluded that cytochrome P-448 does not catalyze the metabolism of fluroxene or TFEE. This is consistent with our results which demonstrated that cytochrome P-448 does not bind fluroxene. The linear Lineweaver-Burke (Fig. 7A) and r versus r [anaesthetic] plots (Fig. 8) of the rates of fluroxene metabolism by all microsomes implies that Michaelis Menten kinetics are being followed and that fluroxene cytochrome P-450 complex formation is necessary for the metabolism of fluroxene.

The close agreement between the average values of K_m (0·8 mM) and K_s (0·9 mM) for the interaction of fluroxene with all microsomes investigated indicates that the type I difference spectrum observed is probably indicative of substrate cytochrome P-450 complex formation and that the rate-limiting step for the metabolism of fluroxene is subsequent to the binding of fluroxene to ferricytochrome P-450.

In the case of the metabolism of TFEE, however, the situation is more complex. The sensitive r versus r [anaesthetic] plots are biphasic in all cases except for that of phenobarbital induced microsomes (where the range of concentrations examined was not extensive enough to ascertain if more than one K_m could be observed). Two K_m values can thus be calculated. In all cases even the lower value of K_m is greater than the corresponding K_m value for fluroxene metabolism which demonstrates that TFEE is a poorer substrate for cytochrome P-450 than is fluroxene. This is consistent with the results of our *in vivo* experiments which showed that TFEE was less toxic than fluroxene, as measured by the time of death subsequent to anaesthesia [11]. Since fluroxene and TFEE yield the same toxic metabolite (Ivanetich, Marsh and Kaminsky, manuscript in preparation) then the enhanced effectiveness of fluroxene in potentiating toxicity must follow from its enhanced rate of metabolism as compared to that of TFEE.

In conclusion these results demonstrate that the toxicity of fluroxene anaesthesia potentiated by phenobarbital induction follows from the enhanced rates of metabolism of the anaesthetic by cytochrome P-450. In contrast the failure of 3-methylcholanthrene induction to potentiate a similar toxic effect is a consequence of the inability of cytochrome P-448 to metabolize fluroxene. TFEE elicits toxic effects *in vivo* more slowly than fluroxene because it is a poorer substrate of cytochrome P-450 than is fluroxene.

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