Olfactory cytochrome *P*-450

Studies with suicide substrates of the haemoprotein

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1. The olfactory epithelium of male hamsters has been found to be extremely active in the cumene hydroperoxide-supported oxidation of tetramethylphenylenediamine, and this peroxidase activity has been shown to be cytochrome *P*-450-dependent. 2. The interaction of a series of suicide substrates of cytochrome *P*-450 with the hepatic and olfactory mono-oxygenase systems has been assessed by determination of peroxidase, 7-ethoxycoumarin *O*-de-ethylase (ECOD) and 7-ethoxyresorufin *O*-de-ethylase (EROD) activities after treatment *in vivo* with these compounds. Chloramphenicol, *OOS*-trimethylphosphorothiolate and two dihydropyridines [DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine) and 4-ethyl DDC (3,5-diethoxycarbonyl-4-ethyl-1,4-dihydro-2,6-dimethylpyridine)] all caused similar percentage inhibitions of hepatic and olfactory activities, but the absolute amounts of enzymic activity lost were considerably greater in the latter tissue. In contrast, halothane had little effect upon hepatic cytochrome *P*-450-dependent reactions, whereas it severely inhibited those of the olfactory epithelium. 3. The time course of loss and recovery of hepatic and olfactory peroxidase, ECOD and EROD activities after a single dose of 4-ethyl DDC was studied. The rates of loss of activity observed were very similar, irrespective of tissue or reaction examined. In the olfactory epithelium, all three activities recovered concurrently and at a rate similar to that of the hepatic peroxidase activity. In contrast, the hepatic de-ethylation of 7-ethoxycoumarin and 7-ethoxyresorufin recovered significantly more rapidly. 4. It is suggested that this behaviour is due to 4-ethyl DDC acting not only as a suicidal inhibitor but also as an inducer of certain forms of cytochrome *P*-450 in the liver; in the olfactory epithelium, however, inactivation, but not induction, occurs. Classical inducing agents were reported to have no effect upon olfactory cytochrome *P*-450, and in the present study neither phenobarbitone nor β -naphthoflavone treatment had any effect upon olfactory cytochrome *P*-450-dependent reactions, although it induced those of the liver.

INTRODUCTION

Recent work has demonstrated that olfactory cytochrome *P*-450 is extremely active in the metabolism of a wide spectrum of compounds (Hadley *et al.*, 1982; Dahl & Hadley, 1983; Brittebo & Brandt, 1984; Longo *et al.*, 1986; Reed *et al.*, 1986). In many cases the olfactory metabolism is more rapid than that observed in the liver (Reed *et al.*, 1986), and the reasons for this considerable activity are not yet clear. A role has been suggested for components of the microsomal electron-transport system (Reed *et al.*, 1986), but the possibility of there being differences in the intrinsic properties of the olfactory and hepatic cytochromes *P*-450 has not been discounted. We have now made use of a series of suicide substrates of cytochrome *P*-450 to investigate the catalytic properties of the haemoproteins in these two tissues. These compounds are mechanism-based inhibitors of cytochrome *P*-450, and thus can be useful molecular probes to investigate the mechanism of enzyme catalysis. Also, since these substrates only inhibit those isoenzymes of the cytochrome which are capable of metabolizing them, information on the composition and substrate specificity of a mixed population of cytochromes *P*-450 could, in theory, be derived from their effects.

We now report differences between the liver and olfactory epithelium in the extent of inactivation of cytochrome *P*-450 by different suicide substrates, presumably reflecting differences in the extent and type of metabolism of these compounds in the two tissues. In addition, the liver and olfactory epithelium differ markedly in the rates of recovery of cytochrome *P*-450-dependent reactions after a single dose of 3,5-diethoxycarbonyl-4-ethyl-1,4-dihydro-2,6-dimethylpyridine (4-ethyl DDC). Finally, we also report that the liver and olfactory cytochromes *P*-450 exhibit considerable differences in cumene hydroperoxide-supported peroxidase activity, a reaction that is independent of the satellite electron-transport system and which we therefore consider to be a reflection of the intrinsic properties of the cytochrome *P*-450.

MATERIALS AND METHODS

Materials

Chloramphenicol, propylene glycol, horse heart cytochrome *c*, NADPH, NADH, 7-ethoxycoumarin and TMPD were obtained from Sigma Chemical Co.; arachis oil and phenobarbitone were purchased from BDH Chemicals Ltd.; β -naphthoflavone, 7-hydroxycoumarin

Abbreviations used: TMPD, *NNN'*-tetramethylphenylenediamine; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; 4-ethyl DDC, 3,5-diethoxycarbonyl-4-ethyl-1,4-dihydro-2,6-dimethylpyridine; *OOS*-Me(O), *OOS*-trimethylphosphorothiolate; ECOD, 7-ethoxycoumarin *O*-de-ethylase; EROD, 7-ethoxyresorufin *O*-de-ethylase.

and resorufin were from Aldrich Chemical Co.; glucose 6-phosphate and glucose-6-phosphate dehydrogenase were from Boehringer-Mannheim; halothane was from May and Baker; 7-ethoxyresorufin was from Pierce; and cumene hydroperoxide was from Fluka A.G. *OOS*-Me(O) and the dihydropyridines (DDC and 4-ethyl DDC) were gifts from Mr. R. Verschoyle and Mr. A. H. Gibbs (MRC Toxicology Unit, Carshalton, Surrey, U.K.). Other chemicals used were of the highest quality available commercially.

Treatment of animals and preparation of tissue

Male Syrian hamsters were between 6 and 8 weeks old and were allowed food and water *ad libitum*. Unless specified otherwise in the text, the treatment of animals was as follows. In experiments with suicide substrates of cytochrome *P*-450, animals were given a single i.p. (intraperitoneal) injection of arachis oil (5 ml/kg), containing, where appropriate, DDC or 4-ethyl DDC (50 mg/kg), halothane (10 mmol/kg) or *OOS*-Me(O) (50 mg/kg) and killed after 2 h. Alternatively, animals were given a single i.p. injection of chloramphenicol (100 mg/kg) in propylene glycol (5 ml) and killed after 1 h. Control animals received the appropriate solvent alone. In experiments with inducing agents of cytochrome *P*-450, animals were given three i.p. injections of phenobarbitone (80 mg/kg) in saline (5 ml) or β -naphthoflavone (40 mg/kg) in arachis oil (5 ml) at 24 h intervals and killed 24 h after the final injection. Control animals received either saline or arachis oil alone (5 ml/kg) with a similar dosing regime. Animals were killed either by decapitation or by a dose of Epiral (sodium pentobarbitone; 200 mg/ml) given by the intraperitoneal route (0.3 ml/100 g) and followed by decapitation of the animals while they were in deep anaesthesia. Olfactory epithelium and liver were obtained and microsomes prepared as described previously (Reed *et al.*, 1986).

Assays

Cytochrome *P*-450 levels, NADPH:cytochrome *c* reductase, 7-ethoxycoumarin *O*-de-ethylase (ECOD) and

7-ethoxyresorufin *O*-de-ethylase (EROD) activities were measured as described previously (Reed *et al.*, 1986).

Cytochrome *P*-450-dependent peroxidase activity was assayed by the method of Hrycay & O'Brien (1971), except that the final volume (2.5 ml) contained 0.1 M-sodium/potassium phosphate buffer, pH 7.4, containing 1 mM-EDTA, 50 μ M-cumene hydroperoxide and either hepatic or olfactory microsomes (microsomal fractions) at final protein concentrations of less than 0.2 mg/ml and 0.03 mg/ml respectively.

All enzymic assays, including peroxidase, were performed under conditions of linearity with protein concentration and time.

Porphyrin levels were determined in homogenates of liver or olfactory epithelium by the method of Grandchamp *et al.* (1980) as described by Ferioli *et al.* (1984).

Protein concentrations were determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

The probability, *P*, of the significance of the difference between two sets of results was calculated by using the Student's *t* test. Values of *P* < 0.05 were considered to indicate that two sets of results were statistically significantly different.

RESULTS

Hepatic and olfactory cytochrome *P*-450-dependent peroxidase activity

Microsomal peroxidase activity, measured by using cumene hydroperoxide as the peroxide substrate and TMPD as the hydrogen donor, was found to be considerably greater in the hamster olfactory epithelium than in the liver (Table 1). This was so whether activity was related to microsomal protein or to cytochrome *P*-450 content, the difference between the two tissues being more marked in the latter case (Table 1). KCN (10 mM) inhibited this activity by approx. 60% in both tissues (results not shown), implicating a haemoprotein in the peroxidation reaction. After treatment *in vivo* with 4-ethyl DDC, a suicide substrate of cytochrome *P*-450, cytochrome levels and peroxidase activity were decreased

Table 1. Effect of 4-ethyl DDC treatment *in vivo* on hepatic and olfactory cytochrome *P*-450, peroxidase and NADPH:cytochrome *c* reductase activities

Assay conditions were as described in the Materials and methods section. Hamsters were given a single i.p. injection of arachis oil (5 ml/kg) or 4-ethyl DDC (50 mg/kg) in arachis oil (5 ml) and killed after 24 h. Results are means $\pm$ S.D. for four determinations or means (individual values in parentheses) of two determinations, each on tissue from a single animal or on tissue pooled from five animals.

Tissue	Treatment	Cytochrome <i>P</i> -450		Peroxidase		NADPH:cytochrome <i>c</i> reductase	
		(nmol/mg of protein)	(%)	(nmol/min per mg of protein)	(%)	(nmol/min per mg of protein)	(%)
Liver	Arachis oil	1.51 (1.31; 1.71)	100	17.26 (17.35; 17.16)	100	104 $\pm$ 30	100
	4-Ethyl DDC	0.53 (0.50; 0.55)	35	5.99 (6.77; 5.20)	35	86 $\pm$ 26	83
Olfactory epithelium	Arachis oil	0.62 (0.45; 0.78)	100	76.66 (81.06; 72.26)	100	358 $\pm$ 106	100
	4-Ethyl DDC	0.16 (0.10; 0.22)	26	19.85 (20.15; 19.55)	26	263 $\pm$ 108	73

Table 2. Effect of administration of suicide substrates of cytochrome *P*-450 *in vivo* on hepatic cytochrome *P*-450-dependent reactions

Assay conditions were as described in the Materials and methods section. Hamsters were given either a single i.p. injection of arachis oil (5 ml/kg), 4-ethyl DDC (50 mg/kg in 5 ml of arachis oil), DDC (50 mg/kg in 5 ml of arachis oil), halothane (10 mmol/kg in 5 ml of arachis oil) or *OOS*-Me(O) (50 mg/kg in 5 ml of arachis oil) and killed 2 h later, or a single i.p. injection of propylene glycol (5 ml/kg) or chloramphenicol (100 mg/kg in 5 ml of propylene glycol) and killed 1 h later. Results are either means $\pm$ s.d. of at least four determinations, or means of two determinations (individual values in parentheses). Each determination was on tissue from a single animal. * P < 0.05 or ** P < 0.01, when compared with corresponding value obtained with control animals.

Treatment	Peroxidase		ECOD		EROD	
	(nmol/min per mg of protein)	(%)	(nmol/min per mg of protein)	(%)	(nmol/min per mg of protein)	(%)
Arachis oil	19.64 $\pm$ 2.58	100	4.58 $\pm$ 0.73	100	0.26 (0.32; 0.19)	100
4-ethyl DDC	6.68 $\pm$ 1.17**	34	0.51 $\pm$ 0.06**	11	0.06 (0.08; 0.03)	23
Arachis oil	19.94 $\pm$ 2.39	100	4.08 $\pm$ 0.50	100	0.27 $\pm$ 0.03	100
DDC	18.64 $\pm$ 1.23	93	3.62 $\pm$ 0.46	89	0.20 $\pm$ 0.03**	74
Halothane	16.55 $\pm$ 3.19	83	3.32 $\pm$ 0.41*	81	0.33 $\pm$ 0.06	122
Arachis oil	18.12 $\pm$ 1.76	100	4.35 $\pm$ 0.66	100	0.21 $\pm$ 0.04	100
<i>OOS</i> -Me(O)	16.59 $\pm$ 1.20	92	2.66 $\pm$ 0.43**	61	0.15 $\pm$ 0.02	71
Propylene glycol	17.45 $\pm$ 5.60	100	4.46 $\pm$ 0.70	100	0.17 $\pm$ 0.02	100
Chloramphenicol	14.75 $\pm$ 2.28	85	1.93 $\pm$ 1.41*	43	0.14 $\pm$ 0.02*	82

to an equivalent extent in both the hamster liver (65 % loss) and the olfactory epithelium (74 % loss) (Table 1). A marginal decrease in NADPH:cytochrome *P*-450(*c*) reductase activity was observed in both tissues (Table 1), but this was not statistically significant. In one experiment hepatic cytochrome *b*₅ levels were measured and found to be unaffected by 4-ethyl DDC treatment. These results strongly suggest that cytochrome *P*-450 is catalysing the peroxidation reaction in both tissues.

In the present study the peroxidase activity has merely been utilized as a marker for cytochrome *P*-450 levels; such a marker was necessary, since spectral measurement of olfactory cytochrome *P*-450 after treatment *in vivo* with suicide substrates is problematic, owing to the small volume of tissue and the extremely low cytochrome *P*-450 levels.

Interaction of suicide substrates with cytochrome *P*-450: comparison between liver and olfactory epithelium

Compounds acting as suicide substrates of cytochrome *P*-450 can be divided into two main classes according to the target, within the cytochrome, which is attacked during the inactivation reaction: (A) those, such as 4-ethyl DDC, that attack the haem prosthetic group, and (B) those that covalently modify the apoprotein moiety. It was decided to investigate the effect of administration of (i) a second compound belonging to group A (DDC) (De Matteis *et al.*, 1981, 1983; Ortiz de Montellano *et al.*, 1981; Tephly *et al.*, 1981), (ii) two compounds of the B type [*OOS*-Me(O) (Aldridge *et al.*, 1985) and chloramphenicol (Halpert *et al.*, 1985)], and (iii) halothane, the oxidative metabolism of which results in no loss of cytochrome *P*-450 but the reductive metabolism of which depletes both cytochrome *P*-450 and microsomal haem (Krieter & Van Dyke, 1983). These studies were designed to investigate the following points. (1) Are there qualitative and/or quantitative differences between the liver and olfactory epithelium in the susceptibility of their cytochrome *P*-450-dependent activities to inhibition

by suicide substrates? (2) Can the cumene hydroperoxide-supported peroxidase reaction be used to discriminate between those suicide substrates that attack the haem prosthetic group and those that cause modification of the apoprotein moiety of cytochrome *P*-450?

In addition to the marked loss of cytochrome *P*-450 and peroxidase activity already noted in both the hamster liver and olfactory epithelium (Table 1), 4-ethyl DDC treatment also resulted in a loss of hepatic and olfactory ECOD and EROD activities (Tables 2 and 3). In contrast, DDC had little or no effect upon any of these cytochrome *P*-450-dependent reactions in either the liver or olfactory epithelium (Tables 2 and 3). However, cellular porphyrin levels were raised in both tissues (results not shown), indicating the formation of *N*-methylprotoporphyrin, a selective inhibitor of ferrochelatase arising from alkylation of the cytochrome *P*-450 haem prosthetic group (De Matteis *et al.*, 1980). Thus, although DDC caused little observable inactivation of the mono-oxygenase systems of the liver and olfactory epithelium, it was apparently metabolized in both tissues.

The liver and olfactory epithelium differed markedly in their response to administration of halothane *in vivo*. In the liver there was little or no effect upon either peroxidase, ECOD or EROD activities (Table 2). In contrast, all three olfactory activities were decreased by more than 50 % after halothane treatment (Table 3). This implies that, if the anaesthetic is metabolized in the liver, it must be predominantly by the oxidative pathway, reductive metabolism being the major route in the olfactory epithelium.

Administration of *OOS*-Me(O) to hamsters had little effect upon the peroxidase activity of either the liver (Table 2) or the olfactory epithelium (Table 3). However, ECOD and EROD activities were both inhibited by approx. 35 % in these two tissues. Similarly, chloramphenicol treatment caused little inhibition of either hepatic or olfactory peroxidase activity, whereas it decreased rates of 7-ethoxycoumarin and 7-ethoxy-

Table 3. Effect of administration of suicide substrates of cytochrome *P*-450 *in vivo* on olfactory cytochrome *P*-450-dependent reactions

Assay conditions were as described in the Materials and methods section. Hamsters were given either a single i.p. injection of arachis oil (5 ml/kg), 4-ethyl DDC (50 mg/kg in 5 ml of arachis oil), DDC (50 mg/kg in 5 ml of arachis oil), halothane (10 mmol/kg in 5 ml of arachis oil) or *OOS*-Me(O) (50 mg/kg in 5 ml of arachis oil) and killed 2 h later, or a single i.p. injection of propylene glycol (5 ml/kg) or chloramphenicol (100 mg/kg in 5 ml of propylene glycol) and killed 1 h later. Results are either means $\pm$ s.d. of at least four determinations, or means of two determinations (individual values in parentheses). Each determination was on tissue from a single animal. * P < 0.05 or ** P < 0.01, when compared with corresponding value obtained with control animals.

Treatment	Peroxidase		ECOD		EROD	
	(nmol/min per mg of protein)	(%)	(nmol/min per mg of protein)	(%)	(nmol/min per mg of protein)	(%)
Arachis oil	90.26 $\pm$ 33.37	100	32.17 $\pm$ 7.44	100	0.65 (0.72; 0.49)	100
4-ethyl DDC	27.57 $\pm$ 8.36**	31	5.19 $\pm$ 3.74**	16	0.22 (0.34; 0.10)	36
Arachis oil	126.77 $\pm$ 29.26	100	38.45 $\pm$ 6.07	100	0.51 $\pm$ 0.15	100
DDC	131.29 $\pm$ 10.75	104	39.08 $\pm$ 4.89	102	0.39 $\pm$ 0.08	76
Halothane	50.81 $\pm$ 22.81**	40	11.48 $\pm$ 2.02**	30	0.28 $\pm$ 0.04**	55
Arachis oil	146.58 $\pm$ 8.32	100	40.33 $\pm$ 3.11	100	0.24 $\pm$ 0.03	100
<i>OOS</i> -Me(O)	125.13 $\pm$ 11.89*	85	26.04 $\pm$ 2.03**	65	0.15 $\pm$ 0.03**	63
Propylene glycol	115.43 $\pm$ 24.63	100	34.98 $\pm$ 1.03	100	0.19 $\pm$ 0.02	100
Chloramphenicol	99.24 $\pm$ 26.14	86	26.04 $\pm$ 3.81**	74	0.16 $\pm$ 0.03	84

Fig. 1. Effect of administration of increasing concentrations of 4-ethyl DDC *in vivo* on (a) peroxidase, (b) ECOD and (c) EROD activities of liver (●) and olfactory epithelium (○)

Assay conditions were as described in the Materials and methods section. Hamsters were injected with arachis oil (5 ml/kg) or 4-ethyl DDC (2, 5, 10, 25 or 50 mg/kg) in 5 ml of arachis oil and killed 2 h later. Results in (a) and (b) are means $\pm$ s.d. of three determinations, results in (c) are means, with error bars indicating the individual values of two determinations. Each determination was on tissue obtained from a single animal. Control values (nmol/min per mg of protein) were as follows: in the liver: peroxidase, 22.09 $\pm$ 2.01; ECOD, 4.29 (4.41; 4.17); EROD, 0.26 (0.32; 0.19); in the olfactory epithelium: peroxidase 125.60 $\pm$ 3.59; ECOD, 41.79 (35.33; 48.24); EROD, 0.61 (0.72; 0.49).

resorufin de-ethylation in both tissues (Tables 2 and 3). With this compound the loss of ECOD activity was greater than that of EROD, and this was particularly marked in the liver (Table 2).

Comparison of liver and olfactory epithelium in sensitivity to 4-ethyl DDC, and recovery of cytochrome *P*-450-dependent activities after a single dose of this dihydropyridine

Hamsters were administered increasing doses of 4-ethyl DDC, killed after 2 h, and hepatic and olfactory

microsomes compared with regard to peroxidase, ECOD and EROD activities. Although some effect was noted at the lower doses, significant loss of these activities occurred only with the 25 and 50 mg/kg doses (Fig. 1). The responses of the liver and olfactory epithelium were found to be virtually identical, irrespective of the dose of 4-ethyl DDC or the particular cytochrome *P*-450-dependent reaction studied (Fig. 1).

The time course of loss and recovery of hepatic cytochrome *P*-450 and of hepatic and olfactory peroxidase, ECOD and EROD activities was also investi-

Fig. 2. Time course of loss and recovery of (a) peroxidase, (b) ECOD and (c) EROD activities in the liver (●) and olfactory epithelium (○) after 4-ethyl DDC treatment

Assay conditions were as described in the Materials and methods section. Hamsters were given a single i.p. injection of 4-ethyl DDC (50 mg/kg in 5 ml of arachis oil) and killed at 0, 2, 4, 24, 48 or 96 h. Results are means $\pm$ S.D. of three determinations, each on tissue obtained from a single animal. Control values (nmoles/min per mg of protein) were as follows: in the liver: peroxidase, 19.10 ± 3.57 ; ECOD, 4.32 ± 0.69 ; EROD 0.15 ± 0.03 ; in the olfactory epithelium: peroxidase, 135.35 ± 14.73 ; ECOD, 44.97 ± 5.70 ; EROD, 0.30 ± 0.02 .

gated after a single 50 mg/kg dose of 4-ethyl DDC. In the liver, all three cytochrome *P*-450-dependent activities declined rapidly, reaching a minimum value at 4 h (Fig. 2). The peroxidase activity then increased gradually, reaching control values at only 96 h. In contrast, the two drug-metabolizing reactions recovered at a significantly higher rate, reaching control values by 24 and 48 h for 7-ethoxycoumarin and 7-ethoxyresorufin de-ethylation respectively. The loss and recovery of hepatic cytochrome *P*-450 was similar to that of the peroxidase activity, with levels declining to $32 \pm 9\%$ of control values at 4 h and then increasing gradually to $68 \pm 7\%$ and $73 \pm 2\%$ of control values at 24 and 48 h respectively. In the olfactory epithelium the three cytochrome *P*-450-dependent activities also declined rapidly, reaching a minimum value at 4 h, as observed in the liver (Fig. 2). The rates of recovery of olfactory and hepatic peroxidase activity were also virtually identical. However, profound differences between the liver and olfactory epithelium in the recovery of drug metabolism were observed, as in the nasal tissue the rates of recovery of ECOD and EROD were similar to that of the peroxidase reaction, reaching control values only at 96 h.

Effect of treatment with phenobarbitone or β -naphthoflavone

One possible explanation for these differences is that, in the liver, 4-ethyl DDC has a dual role, acting not only as a suicide substrate of cytochrome *P*-450 but also as an inducer of specific cytochrome *P*-450 isoenzymes. Classical hepatic inducing agents apparently have little effect upon the nasal mono-oxygenase system (Hadley *et al.*, 1982; Bond, 1983), and thus, in the olfactory epithelium, 4-ethyl DDC may act solely as a suicide substrate.

Table 4 shows the effect of phenobarbitone and β -naphthoflavone on the hepatic and olfactory cytochrome *P*-450-dependent mono-oxygenase systems. In the liver, phenobarbitone treatment increased both cytochrome *P*-450 levels and peroxidase activity by approx. 65%; in addition there was a stimulation of NADPH:cyto-

chrome *c* reductase and ECOD activities (Table 4). 7-Ethoxyresorufin de-ethylation was not affected by this inducing agent. Administration of β -naphthoflavone also induced cytochrome *P*-450, ECOD and EROD activities in the liver, whereas peroxidase activity was unchanged and there was only a marginal decrease in NADPH:cytochrome *c* reductase activity (Table 4). It will be noted that the degree of induction obtained with these compounds in the hamster was considerably less extensive than that generally observed in the rat, especially the β -naphthoflavone-dependent induction of EROD activity. However, these levels of induction are compatible with those found by Chiang & Steggle (1983) and by Smith *et al.* (1986). In addition, Burke & Mayer (1974) reported that EROD activity is increased only 8-fold in the hamster, as compared with 70-fold in the rat, after treatment with 3-methylcholanthrene, a compound reported to induce species of rat liver cytochrome *P*-450 identical with those induced by β -naphthoflavone (Lau *et al.*, 1982).

In contrast with the situation in the liver, neither phenobarbitone nor β -naphthoflavone had any effect upon the olfactory peroxidase, NADPH:cytochrome *c* reductase, ECOD or EROD activities.

DISCUSSION

Cytochrome *P*-450-dependent peroxidase activity

Several laboratories have found the olfactory cytochrome(s) *P*-450 to be more active than the hepatic in the metabolism of a variety of drug substrates [see Reed *et al.* (1986) and references therein], and we have previously presented results suggesting that facilitated electron flow may be an important factor in the high specific activity of the olfactory cytochrome *P*-450 (Reed *et al.*, 1986). In the present work we have demonstrated that the olfactory epithelium is also extremely active in a reaction that does not involve the microsomal electron-transport system, namely the peroxidase activity of cytochrome *P*-450 (Table 1). The high olfactory peroxidase activity appears to be cytochrome *P*-450-dependent; it is inhibited by

Table 4. Effect of inducing agents on the cytochrome *P*-450-dependent mono-oxygenase system of liver and olfactory epithelium

Assay conditions were as described in the Materials and methods section. Hamsters were given three i.p. injections of saline (5 ml/kg), phenobarbitone (80 mg/kg in 5 ml of saline), arachis oil (5 ml/kg) or β -naphthoflavone (40 mg/kg in 5 ml of arachis oil) at 24 h intervals and killed 24 h after the final injection. Results are means $\pm$ s.d. of four determinations, each on tissue obtained from a single animal. * $P < 0.05$ or ** $P < 0.01$, when compared with corresponding value from control animals. Abbreviation used: ND, not determined.

Tissue	Treatment	Cytochrome <i>P</i> -450 (nmol/mg of protein)	NADPH:cytochrome <i>c</i> reductase (nmol of cytochrome <i>c</i> reduced/min per mg of protein)	Peroxidase (nmol/min per mg of protein)	ECOD (nmol/min per mg of protein)	EROD (nmol/min per mg of protein)
Liver	Saline	1.40 $\pm$ 0.10	136.13 $\pm$ 11.67	20.01 $\pm$ 2.93	4.58 $\pm$ 0.50	0.13 $\pm$ 0.01
	Phenobarbitone	2.37 $\pm$ 0.25	165.38 $\pm$ 19.71*	32.79 $\pm$ 3.63**	9.37 $\pm$ 2.16**	0.14 $\pm$ 0.02
	Arachis oil	1.25 $\pm$ 0.06	123.15 $\pm$ 10.40	14.98 $\pm$ 2.52	5.62 $\pm$ 1.40	0.14 $\pm$ 0.02
Olfactory epithelium	β -Naphthoflavone	1.91 $\pm$ 0.12	93.77 $\pm$ 10.47*	12.74 $\pm$ 1.48	10.31 $\pm$ 0.56**	0.56 $\pm$ 0.19**
	Saline	ND	352.91 $\pm$ 51.86	118.99 $\pm$ 32.35	34.41 $\pm$ 4.88	0.18 $\pm$ 0.04
	Phenobarbitone	ND	346.55 $\pm$ 36.94	105.21 $\pm$ 28.43	32.12 $\pm$ 4.87	0.17 $\pm$ 0.03
	Arachis oil	ND	251.62 $\pm$ 66.39	80.53 $\pm$ 18.34	21.36 $\pm$ 4.42	0.17 $\pm$ 0.04
	β -Naphthoflavone	ND	222.36 $\pm$ 42.24	84.02 $\pm$ 19.94	17.95 $\pm$ 7.99	0.17 $\pm$ 0.02

KCN, thus implicating a haemoprotein, and treatment with 4-ethyl DDC *in vivo* caused an equivalent loss of olfactory cytochrome *P*-450, measured spectrally, and peroxidase activity (Table 1). Since the provision of reducing equivalents is not required during the peroxidase reaction, intrinsic differences between the hepatic and olfactory cytochromes *P*-450 are the most probable explanation for the differences in peroxidase activity between the two tissues.

Inactivation of hepatic and olfactory cytochrome *P*-450 by suicide substrates

A variety of suicide substrates were investigated in order to explore differences in the inactivation of hepatic and olfactory cytochrome *P*-450, and in an attempt to utilize the inhibition (or lack thereof) of the cumene hydroperoxide-supported peroxidase activity to identify the target, within the cytochrome, of suicidal attack.

With the exception of halothane (see below), similar percentage losses of the three reactions studied were observed in the two tissues. However, on account of the higher control activity in the olfactory epithelium, as compared with the liver, the absolute amount of enzyme activity lost after treatment with these suicide substrates was significantly greater in the former tissue.

Two basic models may be used to describe the differences in rates of hepatic and olfactory drug metabolism: (A) the two tissues have similar numbers of relevant cytochrome *P*-450 molecules, which have different catalytic-centre activities: (B) the two tissues contain different amounts of the relevant isoenzymes, which turn over catalytically at similar rates. The greater absolute loss of activity in the olfactory epithelium, as compared with the liver, is compatible with either model. However, if the assumption is made that the ratio between non-lethal and suicidal metabolic events is the same in the two tissues, then it can be anticipated from model A that the olfactory cytochrome *P*-450 should be inactivated considerably more rapidly than the hepatic. This was not observed; we have not measured initial rates of inactivation after treatment with 4-ethyl DDC, but, from the time points examined, the rates at which cytochrome *P*-450-dependent activities were lost in the two tissues did not appear to differ significantly (Fig. 2). If this were also so for initial rates of inactivation, the results would be compatible with model B above. Unfortunately no definitive conclusions with regard to the two models can be drawn from this work, since initial rates of inactivation were not determined, and such studies *in vivo* require that additional assumptions be made regarding delivery of the drug to the two tissues.

In marked contrast with the other suicide substrates studied, halothane treatment caused a differential effect in the two tissues. Whereas hepatic cytochrome *P*-450-dependent reactions were unaffected by administration of this anaesthetic, those of the olfactory epithelium were significantly inhibited. Halothane is metabolized by hepatic cytochrome *P*-450 both oxidatively and reductively (Sipes *et al.*, 1980), but it is only the latter pathway that leads to suicidal inactivation of the cytochrome (Krieter & Van Dyke, 1983), accompanied by a loss of microsomal haem (Krieter & Van Dyke, 1983), thus suggesting that the haem prosthetic group of cytochrome *P*-450 is the target of attack. In the liver the balance between the two pathways is dependent upon both oxygen partial pressure, and the induction state of the

animal reductive metabolism is favoured under hypoxic conditions and after phenobarbitone treatment (Maiorino *et al.*, 1978; Cousins *et al.*, 1979). The loss of cytochrome *P*-450-dependent activity observed in the olfactory epithelium suggests that the reductive pathway is a major route of halothane metabolism in this tissue. Whether this is due to cytochrome *P*-450 isoenzymes of the hepatic phenobarbital-inducible type being active in the olfactory epithelium, or to hypoxic conditions occurring in this tissue during anaesthesia, or to some intrinsic differences in the catalytic properties of the hepatic and olfactory cytochrome *P*-450, requires further elucidation.

Since the peroxidase reaction involves direct interaction of a hydroperoxide substrate with the haem prosthetic group of cytochrome *P*-450, thus by-passing the satellite components of microsomal electron transport, it was considered that this activity might prove a useful tool to discriminate between (1) suicide substrates, such as DDC and 4-ethyl DDC, that destroy the haem of cytochrome *P*-450 (De Matteis *et al.*, 1981, 1983; Ortiz de Montellano *et al.*, 1981; Tephly *et al.*, 1981) and (2) compounds that suicidally inhibit cytochrome *P*-450 via modification of the apoprotein, for instance chloramphenicol and, possibly, *OOS*-Me(O) (Halpert *et al.*, 1985; Aldridge *et al.*, 1985). It was found that chloramphenicol and *OOS*-Me(O) had little or no effect upon either hepatic or olfactory peroxidase activity. However, in both tissues there were significant losses of EROD and/or ECOD activities. In contrast, the losses of peroxidase and drug-metabolizing activities were generally fairly similar after administration of DDC, 4-ethyl DDC or halothane. Thus investigation of peroxidase activity might prove useful in elucidating the mode of inactivation of cytochrome *P*-450 by novel suicide substrates, particularly in extrahepatic tissues in which spectral measurement of cytochrome *P*-450 can be problematic. However more work is necessary to determine whether this concept is generally applicable.

Recovery of hepatic and olfactory cytochrome *P*-450-dependent activities after 4-ethyl DDC treatment

An important finding of this work is the extremely rapid recovery of hepatic ECOD and EROD activity, compared with total hepatic cytochrome *P*-450, after a single dose of 4-ethyl DDC. DDC has been shown to induce cytochrome *P*-450 and its dependent mono-oxygenase activity in chick-embryo liver (Rifkind, 1979; Brooker *et al.*, 1983). 4-Ethyl DDC may also have a dual role, acting not only as a suicide substrate, but also as an inducer of specific cytochrome *P*-450 isoenzymes, with those cytochromes capable of recognizing 4-ethyl DDC as a substrate (and therefore suffering suicidal inactivation) being those most readily induced by this compound. Compatible with this is the finding that of the two substrates investigated, 7-ethoxycoumarin and 7-ethoxyresorufin, the de-ethylation of the former was more significantly depressed in the early stages of the treatment and exhibited a more rapid recovery at later time points.

Several laboratories have demonstrated that nasal cytochrome *P*-450 does not respond to conventional hepatic inducing agents (Bond, 1983; Hadley *et al.*, 1982; Foster *et al.*, 1986), and in the present study we have shown that the hamster olfactory mono-oxygenase system

is apparently unaffected by administration of either phenobarbitone or β -naphthoflavone (Table 4). Thus the relatively slow and concurrent recovery of peroxidase, ECOD and EROD activities in the olfactory epithelium may be due to a lack of induction in this tissue, with rates of recovery merely reflecting resynthesis of cytochrome *P*-450 at its basal turnover rate.

In summary, we have presented evidence suggesting that there are intrinsic differences between the hepatic and olfactory cytochromes *P*-450 with regard to their cumene hydroperoxide-supported peroxidase activity, metabolic activation of halothane and recovery after a single dose of a suicide substrate. The significance of several of these observations requires further investigation.

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