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EFFECTS OF ETHER ANAESTHESIA AND FASTING ON VARIOUS CYTOCHROMES P450 OF RAT LIVER AND KIDNEY

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Abstract—Fed and fasted, male, Wistar albino rats exposed to light ether anaesthesia and killed immediately or after 30 or 120 min recovery were compared with non-anaesthetized rats for changes in liver and kidney cytochrome P450 (CYP) activities. In fed rats, liver total CYP (nmol/mg protein) decreased by 30% immediately after ether, but was restored to normal levels after 30 min recovery; in fasted rats, liver total CYP increased by 20% by fasting alone, then decreased by 65% immediately after ether, and recovered to only 70% of control at 2 hr after ether. Rat liver cytochrome P4501A (CYP1A; 7-ethoxyresorufin *O*-deethylase or EROD activity) and cytochrome P4502B (CYP2B; 7-pentoxyresorufin *O*-dealkylase or PROD activity) were decreased after ether anaesthesia, similar to those for total CYP. In contrast, rat liver cytochrome P4502E1 (CYP2E1), determined by *p*-nitrophenol hydroxylation, *increased* by 40% by ether anaesthesia alone, 70% by fasting alone and 140% by ether plus fasting; these increases were confirmed by the CYP2E1-mediated activation of nitrosopyrrolidine and by immunoblot analysis using antibody to CYP2E1. In rat kidney, losses of total CYP, CYP1A and CYP2B, and *increases* of CYP2E1, induced by ether anaesthesia, were much more marked in fasted (90% loss in total CYP, 30% increase in CYP2E1) than in fed rats (slight loss in total cytochrome P450, 30% increase in CYP2E1). As maximum losses of total CYP in liver of fasted rats exposed to ether occurred at the time of maximum increase of CYP2E1 and maximum rate of generation of reactive oxygen species (ROS), it is suggested that the increase of CYP2E1, resulting from its stabilization by fasting and ether, leads to generation of ROS, increase in lipid peroxidation and consequent loss of total CYP, associated with the hepatic and renal necrosis seen in ether intoxication and surgical trauma.

In the earlier part of this century, post-surgical trauma resulting in hepatic dysfunction, hepatic and renal necrosis, and a high level of morbidity was a seriously limiting feature of operative surgery [1, 2]. More recently, the production of reactive oxygen species (ROS‡) associated with anaesthetic toxicity [3] and with haemorrhage-induced reperfusion injury [4] has been proposed to account for the tissue damage of surgical trauma. Exposure of rats to 20 hr of fasting followed by light ether anaesthesia, to mimic the anaesthetic phase of surgery earlier this century, revealed that extensive lipid peroxidation occurred, as monitored *in vivo* by alkane exhalation, and in tissues by the formation of malondialdehyde and other lipid peroxidation products that behave as thiobarbituric acid-reacting substances (TBAR) [5]. Simultaneously, ROS in liver and kidney was produced, as indicated by marked increases in luminol-activated chemiluminescence (LAC) [6], and the loss of total cytochrome P450 (CYP) from these tissues [5, 7].

Ether at high doses is know to result in ketosis and to cause pathological changes in liver and kidney [2, 8], to inhibit hepatic drug metabolism and to produce an immediate loss of hepatic CYP in mice [3]. Furthermore, fasting and ketosis are known to increase the activity of hepatic cytochrome P4502E1 (CYP2E1) [9, 10], and CYP2E1 is known to evoke the generation of ROS [11]. However, the contributions of the individual and combined effects on hepatic CYP, of pre-anaesthesia fasting and ether anaesthesia, which are routine procedures in biological experimentation and human surgical operations, have not been studied.

As the phenomenon of toxicity and tissue necrosis, resulting from the generation of ROS by fasting plus ether anaesthesia, is of major importance to the safety of operative surgery and to the success of a variety of experimental animal procedures in pharmacology/toxicology, it was important to ascertain if both fasting and ether anaesthesia were involved and, if so, what were the relative contributions of each. Furthermore, it was considered desirable to ascertain the temporal effects of fasting and ether, separately and combined, on the individual CYP of liver and kidney, and to elucidate whether CYP2E1 activation is the cause or effect of the ROS generation, the consequent lipid peroxidation of the membranes of the endoplasmic reticulum and the loss of CYP.

The objectives of the present study were therefore: (i) to determine the separate and combined effects of 24 hr fasting and light ether anaesthesia on total and individual CYP of rat liver and kidney, and (ii) to determine the temporal changes in the CYP of

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‡ Abbreviations: CYP, cytochrome P450; ROS, reactive oxygen species; LAC, luminol-activated chemiluminescence; NADPH, reduced nicotinamide adenine dinucleotide phosphate; EROD, 7-ethoxyresorufin *O*-deethylase; PROD, 7-pentoxyresorufin *O*-dealkylase.

liver and kidney after fasting and ether anaesthesia, and to correlate these with previously determined temporal changes in ROS formation and lipid peroxidation resulting from this treatment.

MATERIALS AND METHODS

Materials. Diethyl ether (Pronalysis grade, May and Baker, Dagenham, U.K.), 7-ethoxyresorufin, 7-pentoxyresorufin and resorufin (Molecular Probes Inc., Junction City, OR, U.S.A.), NADPH, 4-nitrophenol, 4-nitrocatechol, donkey/anti-sheep immunoglobulin G (whole molecular) and ascorbic acid oxidase (Sigma Chemical Co., Poole, U.K.), cellulose nitrate (Anderman Co., Kingston-upon-Thames, U.K.), and acryl gel EC-810 and EC-820 (National Diagnostics, Aylesbury, U.K.) were purchased and other chemicals were of the highest purity available. The antibody to CYP2E1 was a gift from Dr D. R. Koop, (Case and Western School of Medicine, OH, U.S.A.).

Methods. CYP was determined spectrophotometrically as the carbon monoxide complex by the method of Omura and Sato [12], using an extinction coefficient of 91 $mM^{-1} cm^{-1}$. Cytochrome P4501A (CYP1A) enzymic activity was determined by the specific 7-ethoxyresorufin *O*-deethylase (EROD) method of Burke and Mayer [13], and cytochrome P4502B (CYP2B) was similarly determined using the 7-pentoxyresorufin *O*-deethylase (PROD) method of Lubet *et al.* [14]. CYP2E1 was determined by the specific 4-nitrophenol hydroxylase procedure of Koop and co-workers [15, 16], using 4-nitrocatechol as the standard oxidation product, and by activation of nitrosopyrrolidine to mutagenic intermediates [17] using the Ames test and a fresh overnight culture of *Salmonella typhimurium* TA1530 to monitor the mutagenic products [18]. Protein concentrations of microsomal preparations were determined by the method of Lowry *et al.* [19].

Immunoblot (western blot) analysis was carried out as described by Towbin *et al.* [20]. Liver and kidney microsomes from rats exposed to fasting and ether anaesthesia, or liver microsomes from rats pretreated with isoniazid (0.1% in drinking water for 10 days) to induce CYP2E1, were subjected to SDS–polyacrylamide gel electrophoresis [21], transferred to nitrocellulose paper and probed with antibody to CYP2E1 as previously described [20].

Animals. Male, Wistar albino rats (250–300 g body wt), purchased from the University of Surrey Experimental Biology Unit, were housed in polypropylene cages on wood shaving bedding, at

Table 1. Changes in the cytochrome P450 activities of (A) rat liver and (B) rat kidney following brief ether anaesthesia, with and without prior fasting

Treatment	Total CYP	EROD activity (CYP1A)	PROD activity (CYP2B)	*p*-Nitrophenol hydroxylase activity (CYP2E1)
(A) Liver				
Fed Rats				
Control (no ether)	0.71 ± 0.06	6.2 ± 0.6 (8.7)	3.2 ± 0.7 (4.5)	2.3 ± 0.2 (3.2)
0 min after ether	0.51 ± 0.06*	4.2 ± 0.4 (8.2)*	1.8 ± 0.5 (3.5)*	3.2 ± 0.3 (6.3)*
30 min after ether	0.70 ± 0.05	5.5 ± 1.3 (7.8)	2.9 ± 0.9 (4.1)	3.1 ± 0.5 (4.4)
120 min after ether	0.84 ± 0.14	6.9 ± 1.7 (8.2)	3.3 ± 0.9 (4.0)	3.7 ± 0.2 (4.4)*
Fasted rats				
Control (no ether)	0.84 ± 0.13	5.4 ± 0.4 (6.2)	1.9 ± 0.8 (2.3)	3.9 ± 1.1 (4.6)*
0 min after ether	0.29 ± 0.04*	1.3 ± 0.3 (4.5)*	1.1 ± 0.1 (3.8)*	5.6 ± 0.7 (19.3)*
30 min after ether	0.51 ± 0.09*	2.1 ± 0.2 (4.1)*	1.9 ± 0.2 (3.7)*	4.9 ± 0.6 (9.6)*
120 min after ether	0.58 ± 0.04*	2.7 ± 0.1 (4.7)*	2.1 ± 0.5 (3.6)	4.8 ± 0.2 (8.3)*
(B) Kidney				
Fed rats				
Control (no ether)	0.31 ± 0.09	2.0 ± 0.1 (6.5)	2.0 ± 0.3 (6.5)	2.4 ± 0.4 (7.7)*
0 min after ether	0.29 ± 0.08	2.2 ± 0.2 (7.6)	1.6 ± 0.5 (5.0)	3.1 ± 0.4 (10.7)*
30 min after ether	0.30 ± 0.10	3.4 ± 1.1 (11.3)*	1.6 ± 0.5 (5.0)	2.9 ± 0.4 (9.7)*
120 min after ether	0.32 ± 0.05	3.1 ± 0.7 (9.7)*	2.0 ± 0.3 (6.5)	3.2 ± 0.4 (10.0)*
Fasted rats				
Control (no ether)	0.35 ± 0.09	1.5 ± 0.8 (4.3)	1.2 ± 0.5 (3.4)*	2.9 ± 0.2 (8.0)
0 min after ether	0.05 ± 0.03*	0.8 ± 0.1 (16.0)*	0.8 ± 0.1 (16.0)*	3.7 ± 0.3 (74)*
30 min after ether	0.08 ± 0.01*	1.0 ± 0.1 (12.5)*	1.0 ± 0.1 (12.5)*	3.6 ± 0.5 (45)*
120 min after ether	0.13 ± 0.03*	1.4 ± 0.1 (10.8)*	1.4 ± 0.9 (10.8)*	3.4 ± 0.5 (26)*

Results are mean values ± SEM of four rats in each of the fed and fasted anaesthetized groups, and of six rats in each of the two control groups. Anaesthesia was for a total of 6 min. CYP1A was determined as EROD activity, CYP2B as PROD activity, and CYP2E1 as *p*-nitrophenol hydroxylase activity.

Units for total CYP are nmol/mg protein, for CYP1A and CYP2B are pmol resorufin/min/mg protein, and for CYP2E1 are pmol 4-nitrocatechol/min/mg protein.

Units in parenthesis for CYP1A, 2B and 2E1 are pmol/nmol total CYP/min.

* $P < 0.05$ compared with control fed rat.

22° and 50% humidity, with lighting from 7.00 a.m. to 7.00 p.m., and were given Spratts Laboratory Animal diet No. 1 (Spratts Betchworth, U.K.) and drinking water *ad lib.* Rats were divided randomly into four groups, namely;

Group 1, *fed, non-anaesthetized:* six rats were given access to diet and water *ad lib.*, without ether anaesthesia.

Group 2, *fasted, non-anaesthetized:* six rats were deprived of food for 24 hr, but given access to drinking water, without ether anaesthesia.

Group 3, *fed, plus ether anaesthesia:* 12 rats were given access to food and water *ad lib.*, and exposed to ether for a total of 6 min.

Group 4, *fasted, plus ether anaesthesia:* 12 rats were deprived of food for 24 hr, but given access to drinking water, and then exposed to ether for a total of 6 min.

The rats of Groups 3 and 4 were anaesthetized for 6 min (2 min to loss of righting reflexes, plus a further 4 min) with diethyl ether in a closed chamber, using the minimum amount of ether at a maximum dose of approx. 5 g/kg, as previously described [5]. Rats (six in each of Groups 1 and 2, and four in each of Groups 3 and 4) were killed by cervical dislocation immediately, and four more in each of Groups 3 and 4, after 30 and 120 min. The liver and kidneys were removed from all animals and microsomes prepared as previously described [5].

Results are reported as mean values ± SEM of four or six rats, and the significance was evaluated by Student's *t*-test.

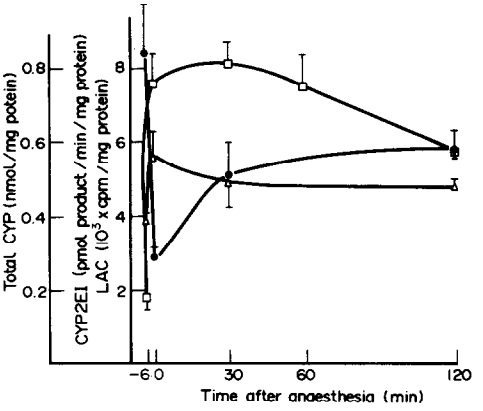

Fig. 1. Temporal response of rat liver cytochromes P450 to ether anaesthesia plus fasting. The total CYP (●) and CYP2E1 (△) data are from Table 1 of this paper, the LAC data (□) is from Ref. 5. Total CYP is expressed as nmol/mg protein, and CYP2E1 as pmol nitrocatechol/mg protein/min, the means of four rats; LAC is expressed as 10^3 cpm/mg protein.

RESULTS

As shown in Table 1, exposure of rats to brief (6 min) ether anaesthesia results in an immediate loss of liver total CYP, which is far greater in fasted rats. A similar loss occurs in kidney, but this is significant only in fasted rats. Examination of the changes in individual CYP activities following ether anaesthesia reveals that CYP1A (EROD activity) and CYP2B (PROD activity), like total P450, are decreased by both ether anaesthesia and fasting; CYP2E1 (*p*-nitrophenol hydroxylase activity) in contrast, is *increased* by both ether and fasting.

Liver

In fed rat liver, total CYP is initially decreased by 30% after ether anaesthesia but almost fully recovers within 30 min (see Table 1 and Fig. 1). In fasted rats, liver total CYP is initially slightly *increased* by fasting alone, then loses 65% immediately (0 min) after ether anaesthesia, and slowly recovers to 70% of control at 2 hr after ether. CYP1A and CYP2B change similarly to total CYP, and losses are generally much greater in fasted, anaesthetized rats (losses at 0 min after anaesthesia were 75% for CYP1A, 40% for CYP2B and 60% for total CYP) than in fed rats (losses at 0 min were 30% for CYP1A, 40% for CYP2B and 30% for total CYP). In contrast, liver CYP2E1 is *increased* by both fasting and ether anaesthesia, and the increases are additive.

When the activities of individual cytochromes are expressed in terms of the corresponding total CYP it can be seen that the activities of CYP1A and CYP2B are not greatly changed in the livers of fed or fasted rats, but that the level of CYP2E1 activity is markedly increased (5-fold) especially in anaesthetized, fasted rats (Table 1). This indicates that the relative proportions of hepatic CYP1A and CYP2B remain constant after fasting and ether anaesthesia, and that the increase in CYP2E1 activity makes this a dominant microsomal enzyme activity.

When CYP2E1 activity is quantified by 4-nitrophenol hydroxylation, ether anaesthesia alone results in a 60% increase, fasting alone results in a 70% increase, while fasting plus ether gives a 140% increase (Table 1). When CYP2E1 activity is quantified by the activation of nitrosopyrrolidine similar increases are obtained (Fig. 2); taking the net data for the 5 mg/plate of nitrosopyrrolidine, ether anaesthesia alone gives a 4-fold increase in the number of revertants, fasting alone gives a 3-fold increase, and fasting plus ether gives a 7-fold increase. Although the two methods are not strictly comparable on absolute values, as they measure distinctly different endpoints, they are complementary and supportive of each other, indicating at least a 1–2-fold increase in CYP2E1 activity.

Kidney

Losses in total CYP were observed in fasted rats (85% at 0 min), but no statistical differences were seen in fed animals; recovery in fasted rats is only 70% of control after 120 min. For individual cytochromes, losses of CYP1A and CYP2B are significant only in fasted rats, attaining 45 and 30%, respectively, at 0 min. Similarly, the increases in CYP2E1 are less than was found in liver, and amount to an increase of 30% by ether anaesthesia alone, about 20% by fasting alone, and a total of 50% after both fasting and ether anaesthesia. This shows that, for kidney also, fasting and ether anaesthesia both contribute to the increase in CYP2E1 activity, and that these effects are additive.

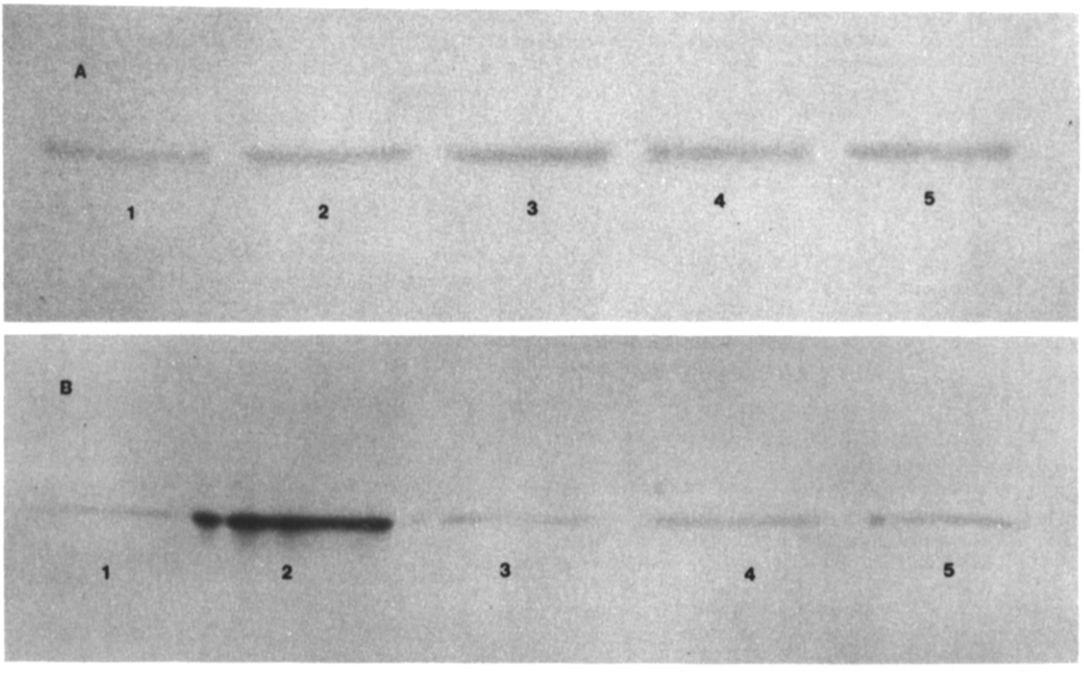

Fig. 2. Western blots of CYP2E1 in (A) liver and (B) kidney microsomes from rats exposed to fasting and brief ether anaesthesia. Rats, fed or fasted for 24 hr, were anaesthetized with ether for 6 min, killed immediately and liver and kidney microsomes prepared and subjected to western blot analysis. CYP2E1 standard was a liver microsomal preparation from rats induced by treatment with isoniazid. Microsomes were subjected to SDS–PAGE [21], transferred to nitrocellulose paper and probed by incubating with the monospecific CYP2E1 antibody at dilutions of 1:5000, after blocking with 1% bovine serum albumin in 10 mM phosphate-buffered saline, pH 7.4 [20]. The detecting antibody (donkey/anti-sheep) conjugated to horseradish peroxidase was used at a dilution of 1:2000 in 10 mM phosphate-buffered saline, pH 7.4, containing 1% bovine serum albumin. Immunopositive bands on the nitrocellulose were detected with aq. diaminobenzidine chloride (0.5 mg/mL) and H_2O_2 (30 vol. diluted 1:10,000) for 60 sec, then rinsing in water for 8 hr to develop. The kidney microsome bands were developed for 24 hr because of the 3-fold lower contents of CYP2E1, with consequent over development of the CYP2E1 standard. Key to lanes: (1) fed rat, (2) CYP2E1 liver standard, (3) fed plus ether rat, (4) fasted rat and (5) fasted plus ether rat.

When the activities of individual cytochromes of kidney are expressed in terms of total CYP it may be seen (Table 1) that even CYP1A and CYP2B are somewhat enhanced in the anaesthetized, fasted rats (2–3-fold), although CYP2E1 is much more markedly increased (3–9-fold). When CYP2E1 was quantified by the activation of nitrosopyrrolidine, the increases in kidney microsomes were much less than were seen with the liver microsomes (Fig. 2). However, bearing in mind that CYP levels in ether-treated fasted rats are only 15% of that of untreated fasted rats, the increase in CYP2E1 activation is much more pronounced.

The increases in CYP2E1 activities in liver microsomes, and in kidney microsomes, were further confirmed by immunospecific precipitation by the western blot technique using a specific antibody to CYP2E1; fasted rats anaesthetized with ether showed stronger immunospecific bands in both liver and kidney preparations than seen in rats only fasted or anaesthetized, and stronger bands than in preparations from control fed and non-anaesthetized rats (see Fig. 3). In agreement with the nitrosopyrrolidine activation data, the CYP2E1 bands in kidney microsomes were less marked than corresponding bands in the liver microsomes. It must be emphasized that the immunoblot gels were loaded on the basis of protein contents, so that the effects would be more marked when total CYP contents are considered.

Correlation with ROS production

Figure 1 shows the temporal relationship between the liver concentration of total CYP, CYP2E1 activity and LAC (ROS production) in fasted rats exposed to ether anaesthesia. It can be seen that the maximum loss of total CYP occurs at about the time of maximum production of ROS, and that both of these coincide with the time of maximum activity of CYP2E1. The production of ROS and the resulting lipid peroxidation are undoubtedly associated with the loss of total CYP, which recovers to normal values as ROS production declines (Fig. 1). Furthermore, as CYP2E1 activity reaches its maximum at zero time after the 6 min of anaesthesia, and ROS production reaches its maximum at 30 min

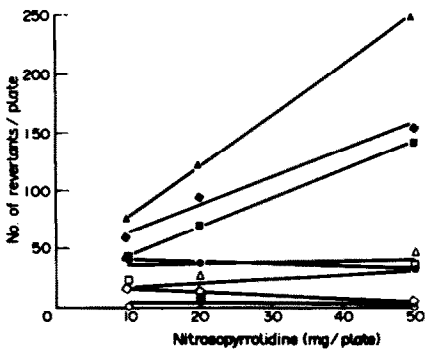

Fig. 3. Estimation of CYP2E1 activities in liver and kidney of rats exposed to brief ether anaesthesia with and without prior fasting, by activation of nitrosopyrrolidine to mutagens quantified by the Ames assay. Rats fed or fasted for 24 hr were anaesthetized with ether for 6 min, killed immediately and liver and kidney microsomes prepared and incubated (50 μg protein/plate) with nitosopyrrolidine (0, 1, 2 and 5 mg/plate) in a culture of *Salmonella typhimurium* TA1530 to quantify the mutagenic CYP2E1 metabolites of nitrosopyrroliine by determination of the number of revertants. Liver microsomes from fasted rats given ether (▲——▲); from fed rats given ether (◆——◆); from fasted rats without ether (■——■); and from fed rats without ether (●——●). Kidney microsomes from fasted rats given ether (△- - -△); from fed rats given ether (◇- - -◇); from fasted rats without ether (□- - -□); and from fed rats without ether (○- - -○).

after anaesthesia, it is probable that the anomalous increase in CYP2E1 activity is a cause of the increased ROS production, and is not merely the consequence of an enhanced stability of this isoform to lipid peroxidation.

DISCUSSION

In previous studies with rats to examine the possible adverse effects of ether anaesthesia it was shown unequivocally that the usual anaesthetic procedure of fasting followed by light ether anaesthesia resulted in ROS generation, oxidative stress, lipid peroxidation and loss of CYP in both liver and kidney [5]. The results of the present experiments to evaluate the separate effects of fasting and ether establish that both contribute, about equally, to the auto-oxidation-induced stress and loss of total CYP, and that these effects are additive. Furthermore, Mori *et al.* [22] have also shown that lipid peroxidation *in vitro* causes inactivation of liver microsomal mixed-function oxidases and decreases the content of CYP.

CYP

From the previous data of Liu *et al.* [5] the immediate cause of the loss of total CYP would appear to be lipid peroxidation of the membranes of the endoplasmic reticulum, with denaturation and degradation of the CYP and loss of haem, their characteristic spectral properties and enzymic functions. The losses of total CYP (65% maximum in fasted rats), CYP1A (75%) and CYP2B (40%) activities are all similar, although liver CYP1A in fasted rats appears to be the most vulnerable. In contrast, CYP2E1 activity is not decreased, but is actually enhanced, and fasting and anaesthesia both *increase* CYP2E1 activity to similar extents; once again, the two effects are additive. This agrees with previous work showing that CYP2E1 activity is increased by fasting [9] and by ether anaesthesia [23].

CYP2E1

p-Nitrophenol hydroxylation is one of only a few P450-catalysed hydroxylations that is effected almost exclusively by CYP2E1 [24]; furthermore, as >90% of the *p*-nitrophenol hydroxylase activity of liver microsomes from acetone/ethanol-treated rabbits is attributable to CYP2E1 this is a relatively specific and sensitive assay for this isoform [16]. Similarly, the activation of nitrosopyrrolidine to mutagens is selectively catalysed by CYP2E1 [17]. Although the nitrosopyrrolidine assay for CYP2E1 activity gives much greater estimates (3–7-fold increases) of the enzyme than the *p*-nitrophenol hydroxylase method (60–140% increases) these are relative, and both indicate that the effects of fasting and ether are similar, and are also additive. The increases in CYP2E1 activity seen in the liver and kidney microsomes of fasted and ether anaesthetized rats were confirmed in the western blot analyses; fasted rats, and fed and fasted rats anaesthetized with ether, showed somewhat stronger immunospecific bands for CYP2E1 than did control fed/non-anaesthetized rats. This confirmed the presence of increased concentrations of the specific CYP2E1 apoprotein, which supports the evidence of increases in enzymic activity, and results from substrate-induced stabilization of the cytochrome [25–29].

The *p*-nitrophenol hydroxylase assay and the nitrosopyrrolidine activation assay both indicate that the increase of CYP2E1 activity in liver microsomes is 3–5 times greater than that in kidney microsomes, and this is also seen in the western blot analysis which had to be developed for a longer period of time in the case of the kidney microsomes in order to detect the immunospecific bands, with the consequent over-development of standard isoniazid-induced liver microsomes.

Mechanism of cytochrome loss

CYP2E1 is unusual, if not unique, among the numerous CYP isoforms in: (i) being enhanced (substrate-stabilized) by both fasting and ether, (ii) existing normally in the high spin state with a high potential for generating ROS [11], and (iii) being relatively resistant to lipid peroxidation (Fig. 1). The cytochromes P450s are normally regulated by a selective post-translational destabilization of individual isoforms involving their phosphorylation by specific protein kinases [25, 26] and degradation to cytochrome P420 [27]. During fasting or ether anaesthesia, CYP2E1 interacts with the substrates, acetone and ether, respectively, effecting stabilization of this isoform and inhibiting its phosphorylation and subsequent rapid proteolysis [28, 29]. The other CYP isoforms, in contrast, are continuously

being phosphorylated and spontaneously degraded, and are also degraded by the CYP2E1-induced lipid peroxidation and haem loss [22, 29].

The depletion of tissue glutathione, and natural antioxidants and radical scavengers, produced by the fasting, and mentioned as a possible cause of the lipid peroxidation by Liu *et al.* [5], is obviously a further contributory factor. However, the possible competitive binding of ether as a ligand to CYP, thereby decreasing its quantification as the carbon monoxide complex, is not likely, since varying concentrations of diethyl ether *in vitro* were not found to affect significantly either the absorption maximum or peak height of the CO-binding spectrum of normal rat liver microsomes [30].

From close examination of the present results, and data in Fig. 1, it would appear that CYP2E1 is increased just prior to ROS production and that, like total CYP but to a much lesser extent, it may also be subject to limited destruction by the ensuing lipid peroxidation of the microsomal membranes. CYP2E1 is highest in fasted rats at 0 min after ether anaesthesia (5.6 pmol/min/mg protein) and falls at 30 and 120 min (4.8 pmol/min/mg protein) after anaesthesia, whereas ROS production (LAC) is maximal at 30 min and remains high from 0 to 60 min after ether anaesthesia. Hence, we suggest that the stabilization-induced increase of CYP2E1 activity, due to fasting and exposure to ether, results in increased formation of ROS which, because of the depletion of tissue glutathione and radical scavengers by the fasting, result in lipid peroxidation and increased destruction of total CYP and other CYP isoforms [30].

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