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SHORT COMMUNICATIONS

The effect of vesicle lipid composition on the metabolism of lignocaine by a male-specific isozyme of cytochrome P-450 from rat liver

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The early work on the mixed function oxidase system in the rat liver indicated that there were three essential components for full activity, i.e. cytochrome P-450, NADPH-cytochrome P-450 reductase and a heat stable factor [1], subsequently found to be phospholipid [2]. The requirement for phospholipid was further refined when it was found that dilauroylphosphatidylcholine (DLPC) could stimulate drug metabolism in a reconstituted enzyme system [3] although Parke [4] suggested that this was an oversimplification of the situation *in vivo* as DLPC did not exist in the endoplasmic reticulum. Indeed, Ingelman-Sundberg and Johansson [5] have demonstrated that phosphatidylcholine (PC) can inhibit the activity of cytochrome P-450 LM_2 and that this inhibition can be overcome by microsomal lipids.

We have shown, in earlier studies [6], that lipid, added back to a delipidated microsomal preparation, can affect the metabolism of lignocaine in that only male-derived microsomal lipid can fully reconstitute the male-specific *N*-deethylase activity. This type of sex specificity has also been seen using partially purified enzymes reconstituted into phospholipid vesicles [7]. We have, in this study, extended this work and present data on the effect of the lipid environment on the activity of a purified male-specific cytochrome P-450 isozyme, lignocaine *N*-deethylase.

Methods

Adult male Wistar rats (body weight 250–300 g) were used throughout the study. The microsomal fraction of the liver was prepared by the method of Berg and Gustafsson [8] and haemoglobin removed as described by Mihara and Sato [9]. The male-specific isozyme of cytochrome P-450 was prepared by the method of Waxman [10] and detergent removed by treatment with hydroxylapatite as described by Cheng and Schenkman [11]. Microsomal lipids were prepared by the method of Overturf and Dyer [12]. The NADPH-cytochrome P-450 reductase was prepared from phenobarbital-treated male rats as described by Shephard *et al.* [13]. Cytochrome P-450 was assayed by the method of Omura and Sato [14], NADPH-cytochrome P-450 reductase by the method of Phillips and Langdon [15] and phospholipid phosphorus by the method of Rouser *et al.* [16]. Total protein was assayed according to Lowry *et al.* [17] using bovine serum albumin as standard. The purity of the enzymes was assessed by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli [18].

Purified enzymes were recombined using the vesicle reconstitution method of Ingelman-Sundberg and Glaumann [19] and enzyme activity assayed using lignocaine as substrate in a modification of the method of Skett *et al.* [20]. A reaction mixture (1 ml) containing 0.1 nmole cytochrome P-450, 0.025 nmole NADPH-cytochrome P-450 reductase, 1 mg lipid, 0.1 μCi [carbonyl-^{14}C]lignocaine (100 μg) and 0.5 mM NADPH was incubated at 37° for 30 min and subsequently treated as described by Skett *et al.* [20]. The results were expressed as nmoles product/min/nmole cytochrome P-450 and as mean ± SD of at least three different preparations. Statistical significance was assessed using Student's *t*-test and the level of significance set at $P < 0.05$.

Results and discussion

An isozyme of cytochrome P-450 has been isolated from male rat liver which is homogenous (at least 95% in a single protein band) as assayed by SDS-PAGE. The isozyme has a minimum molecular weight of approximately 52,000 and a specific content of 12.8 nmoles cytochrome P-450/mg protein. The protein was found to have a specific activity for the 16α-hydroxylation of androst-4-ene-3,17-dione of 10 nmoles/min/nmole cytochrome P-450. These data suggest that the isozyme under study is the male-specific isozyme 2c of Waxman [10] and RLM5 of Morgan *et al.* [21]. The isozyme was found to have the highest lignocaine *N*-deethylase activity of the fractions tested and no 3-hydroxylase activity.

It is seen (Table 1) that, under the assay conditions used, there is an increase in the *N*-deethylation of lignocaine when the enzymes are incorporated into a membrane consisting of DLPC and dilauroylphosphatidylethanolamine (DLPE) in the proportions found in the endoplasmic reticulum (3:1—ref. 22) compared to DLPC alone. An effect of incorporation of phosphatidylethanolamine (PE) on cytochrome P-450-dependent drug metabolism is well documented; Ingelman-Sundberg and Glaumann [23], working with the rabbit liver cytochrome P-450 LM_2, showed that PE was better than PC in maintaining 7-ethoxycoumarin *O*-dealkylation. These authors suggested that this effect was due to a change in surface charge on the membrane. This data is also consistent with our own findings [6] that, using delipidated microsomes, DLPE had an effect only on the male-specific enzyme activity in the male-derived system. This may indicate that the male-specific isozyme(s) of cytochrome P-450 [10, 21] are more susceptible to modulation of enzyme activity by lipid environment.

Also in Table 1 is shown the effect of incorporating the enzymes into vesicles prepared from hepatic microsomal lipids derived from male and female rats. The vesicles prepared from the female-derived lipids gave a lower *N*-deethylase activity than those prepared from the male-derived lipid. This is similar to the effect seen by Barr and Skett [7] using partially purified enzymes and our earlier work [6] using delipidated microsomes. It is noteworthy

Table 1. The effect of reconstituting a male-specific isozyme of cytochrome P-450 (lignocaine *N*-deethylase) in dilauroylphosphatidylcholine (DLPC), DLPC and dilauroylphosphatidylethanolamine (DLPE) (3:1 ratio) or microsomal lipids derived from male and female rat livers on the activity of the enzyme

Lipid	Activity (nmoles/min/nmole P-450)
DLPC	0.97 ± 0.17
DLPC/DLPE	1.39 ± 0.11
Male	2.37 ± 0.30
Female	1.61 ± 0.07

Results are expressed as mean ± SD of three separate experiments. All values are significantly different from each other.

that the sex difference seen for lignocaine *N*-deethylase in microsomes [20] is only fully expressed if the correct lipid mixture (i.e. lipids derived from male microsomes) is used with the male-derived enzymes. The data presented in this paper do not agree, therefore, with the view that all sex differences in drug metabolism are a consequence of differences in cytochrome P-450 isozyme content as expressed by Kamataki *et al.* [24] and others (for review, see ref. 25).

In all experiments it was seen that microsomal lipids are more efficient than DLPC in reconstituting *N*-deethylase activity and male-derived lipids gave a higher activity than DLPC:DLPE mixtures. This agrees with earlier findings using partially purified enzymes [7] and cytochrome P-450LM$_2$ [26]. The microsomal lipids as prepared contain a complex mixture of phospholipids, as well as triglycerides and cholesterol and any of these components could account for the effects seen. Indeed, cholesterol, added to DLPC vesicles, has been shown to influence the metabolism of drugs [27]. Sex differences also exist in the composition and fatty acid content of phospholipids in hepatic endoplasmic reticulum [28] and these could lead to the effects seen.

Further work is in progress to separate and analyse the microsomal lipids used in these experiments and to use the separated fractions in reconstitution experiments to ascertain the exact nature of the protein–lipid interaction seen in this study.

In summary, the *N*-deethylation of lignocaine catalysed by a purified isozyme of cytochrome P-450 from male rat liver is greater when reconstituted into microsomal lipids than either DLPC or DLPC:DLPE mixtures. Microsomal lipids derived from the male are more efficient than female-derived lipids in reconstituting this male-specific activity. Sex differences in lignocaine metabolism [20] may, thus, be partially due to protein–lipid interactions in the endoplasmic reticulum and not solely to the presence of sex-specific cytochrome P-450 isozymes.

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Molecular Pharmacology Laboratory
Department of Pharmacology
The University,
Glasgow G12 8QQ, Scotland, U.K.

NURI MUSA MEFTAH
PAUL SKETT*

* To whom all correspondence should be sent.

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Role of the intestinal flora in the acetylation of sulfasalazine metabolites

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A "phase II" reaction of xenobiotic metabolism [1] is known to occur in the liver as well as at such mammalian sites as the intestinal mucosa [2, 3]. Sulfapyridine (SP)* and 5-aminosalicylate (5-ASA), the two primary metabolites of sulfasalazine, are typical substrates for the acetylation reaction; the acetylated derivatives of these metabolites (Ac-SP and Ac-5-ASA) are found in the excreta of animals dosed with sulfasalazine [4, 5]. In this communication, we describe experiments which indicate that the intestinal bacteria of rats, guinea pigs, dogs and humans are capable of acetylating both 5-ASA and SP.

Male Hartley guinea pigs and conventional and germfree male CD rats (Charles River Breeding Laboratories, Wilmington, MA) were maintained in metabolism cages on, respectively, either guinea pig chow (Ralston Purina Co., St. Louis, MO) or germfree rat diet (Charles River Breed-

* Abbreviations: 5-ASA, 5-aminosalicylate; Ac-5-ASA, 5-acetamidosalicylate; Ac-SP, *N*-acetylsulfapyridine; and SP, sulfapyridine.