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METABOLISM OF LIDOCAINE BY PURIFIED RAT LIVER MICROSOMAL CYTOCHROME P-450 ISOZYMES

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Abstract—The metabolism of lidocaine was studied using rat liver microsomes or a reconstituted lidocaine monooxygenase system with one of eight forms of cytochrome P-450 purified from liver microsomes from untreated- (P450 UT-2 and UT-5), phenobarbital- (P450 PB-1, PB-2, PB-4, and PB-5) or 3-methylcholanthrene- (P450 MC-1 and MC-5) treated rats. A reverse phase high-performance liquid chromatography system capable of simultaneously assaying four major lidocaine metabolites, namely, monoethylglycinexylidide (MEGX), 3-hydroxylidocaine (3-OH LID), methylhydroxylidocaine (Me-OH LID) and glycinexylidide (GX), was employed to determine the rate of formation of each metabolite. Untreated microsomes generated MEGX, Me-OH LID, and 3-OH LID, but the formation of GX was not detected. In male rat liver microsomes, MEGX was the major metabolite of lidocaine when a concentration of 1 mM was employed. The formation of MEGX and Me-OH LID was increased significantly ($P < 0.01$) by microsomes from phenobarbital-treated rats, and the formation of 3-OH LID was increased with 3-methylcholanthrene. The study with the reconstituted system with purified cytochrome P-450 isozymes revealed that all eight forms of cytochrome P-450 used have an ability to *N*-deethylate lidocaine to form MEGX. Among these isozymes, cytochrome P450 PB-4 and P450 UT-2 showed a higher turnover number for the formation of MEGX. Me-OH LID was formed exclusively by P450 PB-5, and 3-OH LID exclusively by P450 MC-1. Selectivity of cytochrome P450 PB-5 for aromatic methyl hydroxylation of lidocaine was confirmed by an inhibition study; formation of Me-OH LID by microsomes of rats treated with phenobarbital was inhibited completely by antibody against P450 PB-5. It was concluded that different cytochrome P-450 isozymes metabolize lidocaine with a different rate and different position selectivities. Since a specific substrate of cytochrome P450 PB-5 (P-450e) is not known, lidocaine may be a useful substrate for the identification of P450 PB-5.

Lidocaine is the local anesthetic and antiarrhythmic drug most widely used in the clinic; its metabolites also have an antiarrhythmic effect [1] and central nervous system toxicity [2, 3]. The metabolic fate of lidocaine has been studied extensively in experimental animals [4–8] and humans [9–11]. In rat liver microsomes, lidocaine is N-deethylated to give monoethylglycinexylidide (MEGX) and glycinexylidide (GX), and the aromatic ring and methyl of lidocaine are hydroxylated to form 3-hydroxylidocaine (3-OH LID) and methylhydroxylidocaine (Me-OH LID) respectively [6–8, 12] (Fig. 1). These reactions are catalyzed with microsomal cytochrome P-450. The formation of MEGX and Me-OH LID is induced by treatment with phenobarbital (PB) [7]. Lidocaine metabolism alters position selectively with age and sex [8], suggesting that multiple forms of cytochrome P-450 are involved in lidocaine metabolism. However, there are no data on lidocaine metabolism by multiple forms of purified cytochrome P-450 in the reconstituted systems. We have purified many liver microsomal cytochrome P-450 isozymes from untreated rats or rats treated with PB or 3-methylcholanthrene (MC) [13, 14]. This paper was designed to identify the specific form of cytochrome P-450 in the metabolic pathways of lidocaine in a reconstituted system with purified cytochrome P-450 by the simultaneous measurement by HPLC of four lidocaine metabolites.

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MATERIALS AND METHODS

Materials. Lidocaine, MEGX, and GX were supplied by the Fujisawa Pharmaceuticals Co., Ltd (Osaka, Japan). 3-OH LID and Me-OH LID were synthesized as described previously [7, 15]. Dilauroylphosphatidylcholine (DLPC) was obtained from the Sigma Chemical Co. (St Louis, MO). NADPH was obtained from the Oriental Yeast Co. (Tokyo, Japan). Emulgen 911 was the gift of the Kao Corp. (Tokyo, Japan). Other reagents and organic solvents were obtained from Wako Pure Chemical Industries (Tokyo, Japan). A C_{18}-column (TSKgel ODS-120T) was obtained from the TOSOH Corp. (Tokyo, Japan).

Microsomes, purified cytochrome P-450, and P-450 antibody. Male Sprague–Dawley rats weighing 200–250 g were obtained from Nippon Clea (Tokyo, Japan). PB (80 mg/kg, dissolved in saline) or MC (40 mg/kg, dissolved in corn oil) was given intraperitoneally daily for 4 or 2 days respectively. Hepatic microsomes were prepared as reported elsewhere [13]. The specific content of cytochrome P-450 (nmol/mg) in the microsomes from untreated

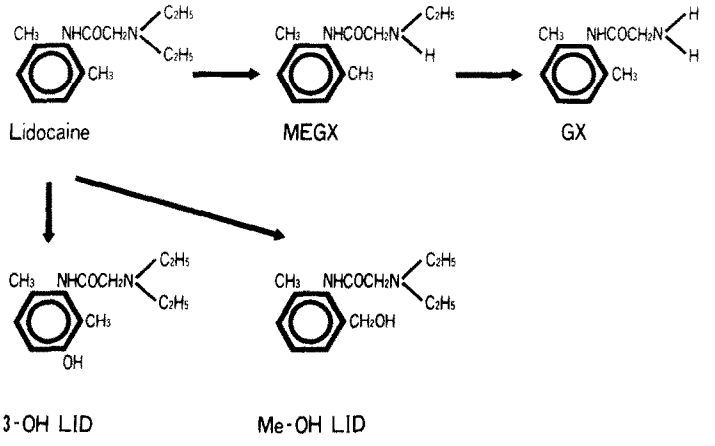

Fig. 1. Metabolic pathways of lidocaine.

male rats and rats treated with PB or MC was 0.74, 1.44, and 1.45 respectively. Cytochrome P-450s were purified from hepatic microsomes by ion-exchange HPLC followed by 7–15% polyethylene glycol fractionation and octylamino-Sepharose chromatography as described previously [13, 14]. Cytochrome P450 UT-2* and UT-5 were purified from untreated male rats, P450 PB-1, 2, 4, and 5, and P450 MC-1 and 5 were purified from rats treated with PB or MC as reported before [13].

Antibody against purified cytochrome P-450s (P450 UT-2 and P450 PB-5) was raised in a female Japanese white rabbit obtained from Biotech (Saga, Japan), and the IgG fraction was prepared by ammonium sulfate precipitation as described previously [26]. NADPH–cytochrome P-450 reductase and cytochrome b_5 were purified as previously reported [13]. The specific activity of the purified reductase was 38 units/mg protein. The specific content of purified cytochrome b_5 was 28 nmol/mg protein.

Formation and measurement of lidocaine metabolites. Lidocaine was metabolized in a microsomal incubation mixture (total volume, 0.5 ml) containing microsomes (200 μg protein), 0.5 μmol lidocaine, and 0.1 M potassium phosphate buffer (pH 7.4). The reaction was started by the addition of 20 μl of 10 mM NADPH, dissolved in aqueous solution, to the mixture. The reaction was carried out in air at 37° for 15 min. The substrate concentration (1 mM) was chosen according to previous studies [8, 15].

The rates of aromatic 3-hydroxylation, methyl hydroxylation, and N-deethylation in the reconstituted system were measured by the following method. Purified cytochrome P-450 (30 pmol), cytochrome P-450 reductase (0.3 units), DLPC (5 μg), and lidocaine (0.5 μmol), with or without cytochrome b_5 (30 pmol), were incubated in 0.5 ml of 0.1 M potassium phosphate buffer, pH 7.4. The reaction was started by the addition of NADPH (0.2 μmol) and was carried out in air at 37° for 10 min.

The reaction was stopped by the addition of 50 μl of 1 N NaOH, and the mixture was immediately placed in ice, mixed with 1.5 ml of ethyl acetate and centrifuged at 3000 rpm for 5 min. One milliliter of the supernatant fraction was transferred to a different tube, and ethyl acetate was evaporated to dryness under reduced pressure at 40°. The residue was dissolved in 200 μl of the mobile phase of HPLC, and 140 μl of the sample was injected onto an HPLC apparatus equipped with a C_{18}-column. HPLC was carried out with a mobile phase (acetonitrile : 0.1 M potassium phosphate buffer, pH 3.0, 1:9) at a flow rate of 1.7 ml/min at 60°, and metabolites were detected at a wavelength of 214 nm. The amount of metabolites was calculated from the peak area with a data-processor. Catalytic activities of all enzyme preparations were assayed under conditions in which the metabolism was proportional to the cytochrome P-450 concentrations and time of incubation. The amounts of NADPH–cytochrome P-450 reductase and DLPC were optimum for the purified hemoproteins, and the NADPH concentration was a saturating one.

RESULTS

Separation of metabolites by HPLC. Lidocaine and four authentic metabolites were resolved clearly by HPLC equipped with a C_{18}-column (Fig. 2A) under conditions essentially the same as those described previously [7, 15], except that Ref. 7 did not show GX as a separately resolved metabolite and Ref. 15, Me-OH LID. Peaks 1–5 corresponded to 3-OH LID, Me-OH LID, GX, MEGX, and lidocaine, respectively, and the retention times were 6.6, 7.6, 10.7, 14.2, and 22.9 min. The HPLC profile of lidocaine metabolites generated by microsomes from rats treated with PB is shown in Fig. 2B. Peaks of Me-OH LID, MEGX, and lidocaine were observed. These peaks were identified by comparison of the

* The corresponding forms of cytochrome P-450 purified by other researchers include the following: P450 UT-2 = P-450h [16], P-450 male [17], UT-A [18], RLM_5 [19] and P450 IIC2 [20]; P450 UT-5 = P-450g [16] and RLM_3 [19]; P450 PB-2 = PB-C [18], P-450 PB-1 [21] and P450 IIC6 [20]; P450 PB-4 = P-450b [22], PB-B [18], P-450 PB-4 [23] and P450 IIB1 [20]; P450 PB-5 = P-450e [24], PB-D [18], P-450 PB-5 [23] and P450 IIB2 [20]; P450 MC-1 = P-450d [25], βNF/ISF-G [18] and P450 IA2 [20]; P450 MC-5 = P-450c [22], βNF-B [18] and P450 IA1 [20]. The purification of a cytochrome P-450 corresponding to cytochrome P450 PB-1 has not been reported by others.

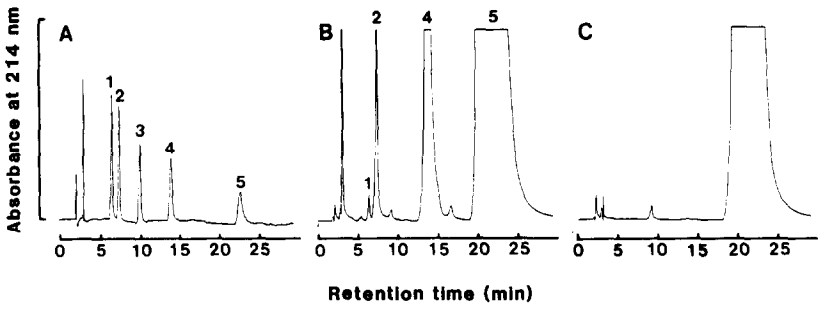

Fig. 2. Elution profiles of lidocaine and its metabolites by HPLC equipped with a C_{18}-column (4.6 × 250 mm). The chromatography was carried out at a flow rate of 1.7 ml/min at 60°. The mobile phase was potassium phosphate buffer: acetonitrile (90:10) adjusted to pH 3.0. (A) Chromatogram of authentic samples. Peaks 1–5 were 3-OH LID, Me-OH LID, GX, MEGX, and lidocaine respectively. (B) Chromatogram of lidocaine metabolites generated by microsomes from rats treated with phenobarbital. The reaction mixture, containing 200 μg of microsomes from phenobarbital-treated male rats, 0.2 μmol NADPH, and 0.5 μmol lidocaine, was incubated for 15 min at 37° in 0.1 M potassium phosphate buffer, pH 7.4. Lidocaine metabolites were extracted and analyzed by HPLC. (C) Chromatogram of blank assay. The incubation conditions were the same as in (B) except for the omission of NADPH.

Table 1. Lidocaine metabolic activity of rat liver microsomes

Microsomes	Lidocaine metabolites (nmol/min/mg)			
	MEGX	GX	3-OH LID	Me-OH LID
Untreated	4.84 ± 1.31	—	0.64 ± 0.19	0.24 ± 0.09
Phenobarbital	11.38 ± 1.54*	—	0.50 ± 0.13	2.13 ± 0.24*
3-Methylcholanthrene	4.45 ± 1.63	—	1.04 ± 0.21*	0.26 ± 0.10

The reaction mixture contained 200 μg of microsomal protein and 0.2 μmol of NADPH in a final volume of 0.5 ml. It was incubated for 15 min at 37° in 0.1 M potassium phosphate buffer, pH 7.4. Lidocaine metabolites were extracted with ethyl acetate and analyzed by HPLC. The values (N = 6–10) are expressed as nanomoles of product per minute per milligram of microsomal protein. Catalytic activities less than 0.2 nmol of product/min/mg protein are expressed as "—". Abbreviations: MEGX, monoethylglycinexylidide; GX, glycinexylidide; 3-OH LID, 3-hydroxylidocaine; and Me-OH-LID, methylhydroxylidocaine.
* $P < 0.01$.

retention time during HPLC with that of the authentic samples. When lidocaine was incubated with microsomes from rats treated with PB in the absence of NADPH, peaks corresponding to lidocaine metabolites were not observed. This result suggests that the two peaks shown in Fig. 2B corresponding to Me-OH LID and MEGX appeared because of the reaction of NADPH-dependent cytochrome P-450 monooxygenase. The identification of peaks was checked also by the amount of increase in the peak area when lidocaine was incubated with different amounts of microsomes. The peak areas for metabolites as calculated with the data-processor increased in proportion to the increase in the microsomes.

Metabolism of lidocaine by rat liver microsomes. The turnover rates of four metabolites formed by microsomes of untreated rats or rats treated with PB or MC are shown in Table 1. Microsomes of untreated rats produced MEGX, 3-OH LID, and Me-OH LID, but not GX. The production of MEGX was higher than that for 3-OH LID and Me-OH LID. PB treatment induced the formation of MEGX and Me-OH LID, while MC treatment induced the formation of 3-OH LID.

Metabolism of lidocaine by purified cytochrome P-450. The results above suggest that different forms of cytochrome P-450 have different metabolite patterns. To elucidate the specific metabolites formed by the specific cytochrome isozymes, the formation of MEGX, GX, 3-OH LID, and Me-OH LID by eight different purified cytochrome P-450s in a reconstituted system was studied (Table 2). The addition of cytochrome b_5 did not change the formation activity of any of these metabolites. MEGX was a major metabolite of lidocaine catalyzed by cytochrome P-450 in liver microsomes from rats at the substrate concentration used. Every purified cytochrome P-450 formed MEGX, and P450 UT-2, P450 PB-4, and P450 MC-1 were efficient in forming it. In the study of lidocaine metabolism in microsomes, PB induced the formation of MEGX. The result found in the reconstitution studies, that the PB-inducible form of P450 PB-4 formed MEGX with a high turnover number, was consistent with the results obtained in the microsomal study. P450 UT-2, which was the major form of untreated microsomes, appeared to play a major role in the formation of MEGX in untreated microsomes. GX formation from lidocaine was not observed in this study, except for those small amounts produced by UT-2. 3-OH LID was efficiently formed by MC-inducible P450 MC-1 but not by the other forms of P-450. This was consistent with the results obtained with microsomes. Me-OH LID was formed by P450 PB-5, but not by

Table 2. Lidocaine metabolic activity of purified cytochrome P-450s

Purified P-450	Lidocaine metabolites (nmol/min/nmol cytochrome P-450)			
	MEGX	GX	3-OH LID	Me-OH LID
UT-2	28.8	1.4	—	—
UT-5	0.8	—	—	—
PB-1	0.6	—	—	—
PB-2	6.6	—	—	—
PB-4	45.8	—	—	—
PB-5	6.8	—	—	2.0
MC-1	14.9	—	3.4	—
MC-5	7.9	—	—	—

For licodaine metabolism, the reaction mixture was the same as in the footnote of Table 1, except for a reconstituted system containing 30 pmol of purified cytochrome P-450, 0.3 units of NADPH–cytochrome P-450 reductase, and 5 μg of dilauroylphosphatidylcholine being used in place of the microsomes. The values are expressed as nanomoles of product formed per minute per nanomole of cytochrome P-450. Metabolites were assayed by comparison of peak areas monitored at 214 nm to those of authentic samples. Catalytic activities less than 0.2 nmol of product/min/nmol of cytochrome P-450 are expressed as "—".

the other forms of P-450. PB-inducible P450 PB-5 formed Me-OH LID in microsomes from rats treated with PB.

Inhibition studies using antibodies. Lidocaine was metabolized to MEGX, GX, 3-OH LID and Me-OH LID by different forms of cytochrome P-450. We also confirmed this result with inhibition studies that used antibody against purified cytochrome P-450 isozymes. The effects of antibody prepared against P450 PB-5 and P450 UT-2 on the formation of MEGX, 3-OH LID and Me-OH LID in microsomes from untreated or PB-treated rats are shown in Fig. 3. Antibody against P450 PB-5 completely inhibited the formation of Me-OH LID by microsomes of rats treated with PB at a concentration of 10 mg/nmol cytochrome P-450 but had no effect on the formation of 3-OH LID (Fig. 3A). This result also suggested that P450 PB-5 was the specific form for the formation of Me-OH LID, as shown in the study of a reconstituted system. On the other hand, antibody against P450 PB-5 inhibited the formation of Me-OH LID by about 50% when 20 mg/nmol P-450 of antibody was added to microsomes of untreated rats (Fig. 3B). This finding suggested that there are forms of cytochrome P-450 that produce Me-OH LID in the microsomes of untreated rats other than P450 PB-5. Antibody against P450 PB-5 inhibited the formation of MEGX by about 50% at a concentration of 4 mg/nmol P-450 and the inhibition was not greater at higher concentrations (10 or 20 mg/nmol P-450, Fig. 3A). Antibody against P450 PB-5 was cross-reactive with P450 PB-4; the homology of P450 PB-5 and P450 PB-4 is high [27]. Therefore, antibody against P450 PB-5 inhibited the formation of MEGX by P450 PB-4. Antibody against P450 UT-2 inhibited the formation of MEGX by 30–40%, but did not inhibit the formation of Me-OH LID or 3-OH LID (Fig. 3C). That the formation of MEGX was not inhibited completely by the antibody against P450 UT-2 suggested that this reaction was also catalyzed by other forms of P-450 in liver microsomes.

DISCUSSION

Microsomes contain multiple forms of cytochrome P-450 and produce multiple metabolites by reaction with lidocaine. Therefore, we used HPLC to resolve and assay several metabolites simultaneously. The reconstitution study showed the MEGX was formed mainly by P450 PB-4 and P450 UT-2, and 3-OH LID

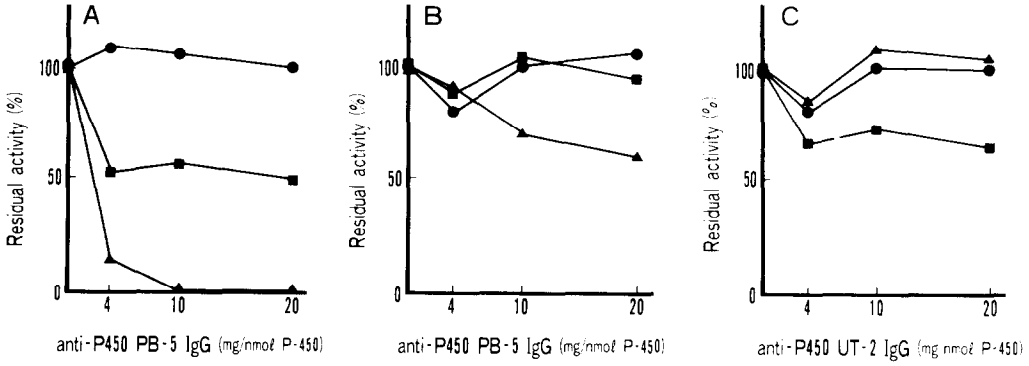

Fig. 3. Effect of antibody against P450 PB-5 or P450 UT-2 on the formation of MEGX, 3-OH LID and Me-OH LID in the microsomes. The designated amount of IgG was preincubated with the microsomes containing 50 pmol of cytochrome P-450 for 10 min at room temperature. (A) Effect of anti-P450 PB-5 IgG in microsomes from rats treated with phenobarbital. (B) Effect of anti-P450 PB-5 IgG in microsomes of untreated rats. (C) Effect of anti-P450 UT-2 IgG in microsomes of untreated rats. Residual activity was expressed as a percentage of the value measured with rabbit IgG instead of anti-P450 PB-5 or UT-2 IgG. Turnover rates of MEGX, 3-OH LID and Me-OH LID were 5.15, 0.36 and 1.51 nmol/min/nmol cytochrome P-450, respectively, by microsomes from phenobarbital-treated rats, and 5.72, 1.06 and 0.29 nmol/min/nmol cytochrome P-450, respectively, by microsomes from untreated rats. Key: (■) MEGX; (▲) Me-OH LID; and (●) 3-OH LID.

and Me-OH LID were formed selectively by P450 MC-1 and P450 PB-5 respectively.

The formation of Me-OH LID was induced strongly by PB treatment. We purified four different forms of cytochrome P-450 from rats treated with PB [13]. Among the four P-450s, as mentioned above, P450 PB-5 selectively formed Me-OH LID in the reconstituted study. Antibody against P450 PB-5 completely inhibited the formation of Me-OH LID in microsomes from rats treated with PB, suggesting that P450 PB-5 was a specific P-450 for the formation of Me-OH LID in such microsomes. Microsomes from untreated rats also formed Me-OH LID. A small amount of P450 PB-5 is contained in microsomes from untreated rats [28], but antibody against P450 PB-5 inhibited the formation of Me-OH LID about 50%. This finding suggests that there are forms of cytochrome P-450 which produce Me-OH LID other than P450 PB-5 in the microsomes from untreated rats. P450 MC-1 formed two metabolites, MEGX and 3-OH LID, simultaneously. Such position-specific reaction was also detected in the hydroxylation of testosterone at the 2α- and 16α-positions by P450 UT-2 [14]. In the reconstitution study, 3-OH LID was formed only by P450 MC-1, and the other forms of cytochrome P-450 did not catalyze formation of 3-OH LID. P450 UT-2 and P450 UT-5, which are the major male-specific forms of cytochrome P-450 in untreated male rats, did not form 3-OH LID. Microsomes from female rats also formed 3-OH LID (data not shown). Therefore, forms other than P450 UT-2 and P450 UT-5 may take part in the formation of 3-OH LID in microsomes from untreated male rats. Selective substrates for individual cytochrome P-450 isozymes are extremely useful for the identification of isozymes [18]. P450 UT-2 is the specific form of cytochrome P-450 for the hydroxylation of testosterone at the 2α- and 16α-positions and P450 PB-4 is specific for 16β-hydroxylation [29, 30], but the specific reactions for P450 PB-5 have not been characterized. Aromatic 3-hydroxylation (3-OH LID) and methyl hydroxylation (Me-OH LID) of lidocaine are the relevant reactions for P450 MC-1 and P450 PB-5, respectively, to characterize these cytochrome P-450 isozymes.

Finally, the formation of lidocaine metabolites was induced by PB or MC treatment. MEGX has central nervous systems toxicity [2, 3], and the pharmacological actions of Me-OH LID and 3-OH LID are unknown. MEGX and 3-OH LID have been isolated from the reaction mixture of lidocaine and human liver microsomes [11]. Therefore, care must be taken in the clinical use of lidocaine when other drugs that induce PB-types of cytochrome P-450 are also given.

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REFERENCES

1. Burney RG, DiFazio CA, Peach MJ, Petrie KA and Silvester MJ, Anti-arrhythmic effects of lidocaine metabolites. *Am Heart J* **88**: 765–769, 1974.
2. Strong JM, Parker M and Atkinson AJ Jr, Identification of glycinexylidide in patients treated with intravenous lidocaine. *Clin Pharmacol Ther* **14**: 67–72, 1972.
3. Blumer J, Strong JM and Atkinson AJ Jr, The convulsant potency of lidocaine and its N-dealkylated metabolites. *J Pharmacol Exp Ther* **186**: 31–36, 1973.
4. Keenaghan JB and Boyes RN, The tissue distribution, metabolism and excretion of lidocaine in rats, guinea pigs, dogs and man. *J Pharmacol Exp Ther* **180**: 454–463, 1972.
5. von Bahr C, Hedlund I, Karlen B, Backstrom D and Grasdalen H, Evidence for two catalytically different binding sites of liver microsomal cytochrome P-450: importance for species and sex differences in oxidation pattern of lidocaine. *Acta Pharmacol Toxicol* **41**: 39–48, 1977.
6. Suzuki T, Fujita S and Kawai R, Precursor-metabolite interaction in the metabolism of lidocaine. *J Pharm Sci* **73**: 136–138, 1984.
7. Kawai R, Fujita S and Suzuki T, A new lidocaine metabolite, ω-diethylamino-2-hydroxymethyl-6-methylacetanilide. *Drug Metab Dispos* **14**: 277–279, 1986.
8. Fujita S, Tatsuno J, Kawai R, Kitagawa H, Suzuki T and Kitani K, Age associated alteration of lidocaine metabolism is position selective. *Biochem Biophys Res Commun* **126**: 117–122, 1985.
9. Breck GD and Trager WF, Oxidative N-dealkylation: a Mannich intermediate in the formation of a new metabolite of lidocaine in man. *Science* **173**: 544–546, 1971.
10. Blankenbaker WL, DiFazio CA and Berry FA Jr, Lidocaine and its metabolites in the newborn. *Anesthesiology* **42**: 325–330, 1975.
11. Hermansson J, Glaumann H, Karlen B and von Bahr C, Metabolism of lidocaine in human liver *in vitro*. *Acta Pharmacol Toxicol* **47**: 49–52, 1980.
12. Nyberg G, Karlen B, Hedlund I, Grundin R and von Bahr C, Extraction and metabolism of lidocaine in rat liver. *Acta Pharmacol Toxicol* **40**: 337–346, 1977.
13. Funac Y and Imaoka S, Simultaneous purification of multiple forms of rat liver microsomal cytochrome P-450 by high-performance liquid chromatography. *Biochim Biophys Acta* **842**: 119–132, 1985.
14. Funae Y and Imaoka S, Purification and characterization of liver microsomal cytochrome P-450 from untreated male rats. *Biochim Biophys Acta* **926**: 349–358, 1987.
15. Kawai R, Fujita S and Suzuki T, Simultaneous quantitation of lidocaine and its four metabolites by high-performance liquid chromatography: application to studies on *in vitro* and *in vivo* metabolism of lidocaine in rats. *J Pharm Sci* **74**: 1219–1224, 1985.
16. Ryan DE, Iida S, Wood AW, Thomas PE, Lieber CS and Levin W, Characterization of three highly purified cytochromes P-450 from hepatic microsomes of adult male rats. *J Biol Chem* **259**: 1239–1250, 1984.
17. Kamataki T, Maeda K, Yamazoe Y, Nagai T and Kato R, Sex difference of cytochrome P-450 in the rat: purification, characterization, and quantitation of constitutive forms of cytochrome P-450 from liver microsomes of male and female rats. *Arch Biochem Biophys* **225**: 758–770, 1983.
18. Guengerich FP, Dannan GA, Wright ST, Martin MV and Kaminsky LS, Purification and characterization of liver microsomal cytochromes P-450: electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or β-naphthoflavone. *Biochemistry* **21**: 6019–6030, 1982.
19. Cheng K-C and Schenkman JB, Testosterone metabolism by cytochrome P-450 isozymes RLM_3 and RLM_5 and by microsomes. *J Biol Chem* **258**: 11738–11744, 1983.

20. Nebert DW, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips IR, Sato R and Waterman MR, The P450 gene superfamily: recommended nomenclature. *DNA* **6**: 1–11, 1987.
21. Waxman DJ and Walsh C, Cytochrome P-450 isozyme 1 from phenobarbital-induced rat liver: purification, characterization, and interactions with metyrapone and cytochrome b_5. *Biochemistry* **22**: 4846–4855, 1983.
22. Ryan DE, Thomas PE, Korzeniowski D and Levin W, Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital, and 3-methylcholanthrene. *J Biol Chem* **254**: 1365–1374, 1979.
23. Waxman DJ and Walsh C, Phenobarbital-induced rat liver cytochrome P-450. *J Biol Chem* **257**: 10446–10457, 1982.
24. Ryan DE, Thomas PE and Levin W, Purification and characterization of a minor form of hepatic microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls. *Arch Biochem Biophys* **216**: 272–288, 1982.
25. Ryan DE, Thomas PE and Levin W, Hepatic microsomal cytochrome P-450 from rats treated with isosafrole. *J Biol Chem* **255**: 7941–7955, 1980.
26. Imaoka S, Terano Y and Funae Y, Purification and characterization of two constitutive cytochromes P-450 (F-1 and F-2) from adult female rats: identification of P-450F-1 as the phenobarbital-inducible cytochrome P-450 in male rat liver. *Biochim Biophys Acta* **916**: 358–367, 1987.
27. Fujii-Kuriyama Y, Mizukami Y, Kawajiri K, Sogawa K and Muramatsu M, Primary structure of a cytochrome P-450: coding nucleotide sequence of phenobarbital-inducible cytochrome P-450 cDNA from rat liver. *Proc Natl Acad Sci USA* **79**: 2793–2797, 1982.
28. Wilson NM, Christou M and Jefcoate CR, Differential expression and function of three closely related phenobarbital-inducible cytochrome P-450 isozymes in untreated rat liver. *Arch Biochem Biophys* **256**: 407–420, 1987.
29. Waxman DJ, Ko A and Walsh C, Regioselectivitity and stereoselectivity of androgen hydroxylations catalyzed by cytochrome P-450 isozymes purified from phenobarbital-induced rat liver. *J Biol Chem* **258**: 11937–11947, 1983.
30. Wood AW, Ryan DE, Thomas PE and Levin W, Regio- and stereoselective metabolism of two C_{19} steroids by five highly purified and reconstituted rat hepatic cytochrome P-450 isozymes. *J Biol Chem* **258**: 8839–8847, 1983.