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METABOLIC ACTIVATION OF LIDOCAINE AND COVALENT BINDING TO RAT LIVER MICROSOMAL PROTEIN

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Abstract—Incubation of [^{14}C]lidocaine with rat liver microsomes in the presence of an NADPH-generating system resulted in covalent bindings of a ^{14}C-labelled material to microsomal protein. The covalent binding of radioactivity needed NADPH and atmospheric oxygen, and was diminished by purging of carbon monoxide and the addition of SKF-525A. Hence the covalent binding of a ^{14}C-labelled material resulting from a reactive metabolite of lidocaine formed by cytochrome P450-dependent monooxygenation. The covalent binding measured at various concentrations of lidocaine (2.5–30 μM) followed Michaelis–Menten kinetics, and the K_m value (4.52 μM) of the activation reaction was close to the K_m value (1.78 μM) of lidocaine 3-hydroxylation. The metabolism-dependent covalent binding of lidocaine to microsomal protein as well as lidocaine 3-hydroxylase activity was much lower in the Dark Agouti strain rat, which is known as a poor-metabolizer animal model of debrisoquine 4-hydroxylation, than in the Wistar rat for the corresponding sexes. The covalent binding in male rats was greater than that in females of both strains, but the extent of the sex difference in the binding was smaller than that of the lidocaine *N*-deethylase activity in Wistar rats. Propranolol and quinidine, specific inhibitors of debrisoquine 4-hydroxylase, markedly inhibited lidocaine 3-hydroxylase activity of Wistar male rats, but not *N*-deethylase activity. These compounds also inhibited the metabolism-dependent covalent binding of lidocaine to microsomal protein. These strain difference and inhibition studies showed that the reaction converting lidocaine to a reactive metabolite capable of binding covalently to microsomal protein was related to lidocaine 3-hydroxylation, and may be catalysed by cytochrome P450 isozyme(s) belonging to the CYP2D subfamily. The covalent binding of radioactivity to rat liver microsomal protein was diminished by nucleophiles, reduced glutathione and cysteine, indicating that the reactive metabolic intermediate of lidocaine is an electrophilic metabolite such as an arene oxide.

In patients receiving lidocaine infusions lasting more than 24 hr, plasma lidocaine concentrations continued to rise beyond the time that intravenous bolus data would predict a steady-state, and their terminal half-lives measured after stopping the infusion were 2–3-fold longer than those measured after a single bolus [1–4]. A pharmacokinetic study in healthy subjects given deuterium-labelled lidocaine as intravenous bolus doses during prolonged infusions of unlabelled lidocaine clearly demonstrated a decrease in lidocaine clearance and no change in its volume of distribution [5]. A decrease in lidocaine metabolism was confirmed by experiments [6] in dogs showing a marked reduction in the intrinsic hepatic clearance and hepatic extraction ratio during continuous intravenous infusion for 24 hr.

Time-dependent lidocaine metabolism has been observed in studies using the perfusion method through the rat liver [7–9]. Lennard *et al.* [7] studied time effects on lidocaine disposition in recirculating perfused rat liver preparations. They observed that lidocaine half-lives were prolonged when rat livers were previously exposed to the drug, suggesting that the intrinsic ability of the liver to remove lidocaine was reduced.

Tam and co-workers [8, 9] also studied the mechanisms of time-related reduction in lidocaine clearance using single-pass perfused rat liver preparations. The concentration of a primary metabolite, monoethylglycinexylidide (MEGX†), reached a maximum 5 min after the start of infusion, and then declined gradually to its steady-state level, whereas the concentration of its precursor lidocaine approached quite slowly and monotonically to its steady-state. In addition, *in vivo* preadministration of lidocaine eliminated these "abnormal" time-dependent kinetics of lidocaine and MEGX [9]. From the mathematical modelling of these data, they concluded that the deethylation pathway of lidocaine was partly inactivated by lidocaine pretreatment, and the inactivation could be the major determinant for the time-dependent effects on lidocaine elimination.

These reports [7–9] suggested impairment of drug-metabolizing enzyme activity in the N-deethylation pathway of lidocaine metabolism, but did not provide enzymatic evidence for metabolic impairment. Lidocaine metabolism is catalysed by cytochrome P450 (P450), and different P450 isozymes contribute to the formation of major lidocaine metabolites [10–13] (Scheme 1). Possible mechanisms for the

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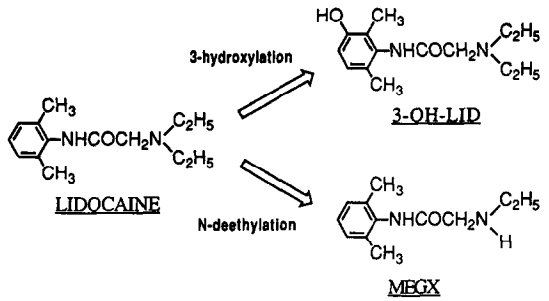
† Abbreviations: MEGX, monoethylglycinexylidide; P450, cytochrome P450; DA, Dark Agouti; GX, glycinexylidide; 3-OH LID, 3-hydroxylidocaine; GSH, reduced glutathione; G-6-P, glucose 6-phosphate; G-6-PDH, glucose 6-phosphate dehydrogenase; TCA, trichloroacetic acid.



Scheme 1. Major primary pathways of lidocaine metabolism in rat liver microsomes.

impairment of hepatic drug metabolism include the formation of stable intermediate complexes with a P450 isozyme(s) and the autocatalytic enzyme inactivation through covalent binding of a metabolic intermediate with a P450 isozyme(s). The former was demonstrated for SKF-525A and other amine drugs [14] and the latter was for chloramphenicol [15] and propranolol [16, 17]. In preliminary experiments, we observed that a metabolic product of lidocaine oxidation bound covalently to rat liver microsomal protein as previously reported for propranolol [16, 17]. It thus seems likely that a reactive metabolic intermediate formed during lidocaine metabolism interacts with microsomal protein.

The object of this work is to characterize a metabolic reaction converting lidocaine to a reactive metabolite. We investigated what isozyme(s) of P450 relates to covalent binding to rat liver microsomal protein using various enzyme sources and inhibitors. The *in vitro* covalent binding in liver microsomes obtained from Wistar and Dark Agouti (DA) rats were examined, since selective 3-hydroxylase deficiency of lidocaine was observed in the DA rat [13], which is an animal model for a human debrisoquine poor metabolizer [18, 19]. Further, effects of the addition of propranolol and quinidine, inhibitors of a P450 isozyme belonging to the CYP2D subfamily [20, 21], on the metabolism-dependent covalent binding in rat liver microsomes were investigated.

MATERIALS AND METHODS

Chemicals. Lidocaine, MEGX, glycinexylidide (GX), 3-hydroxylidocaine (3-OH LID) and 3-OH MEGX were used as hydrochloride derivatives. These were synthesized as described previously [22]. The sources of reagents were: [^{14}C]lidocaine hydrochloride labelled at the carbonyl position from New England Nuclear (Boston, MA, U.S.A.); SKF-525A from Research Biochemicals, Inc. (Natick, MA, U.S.A.); scopolamine hydrochloride, propranolol hydrochloride and quinidine sulfate from the Sigma Chemical Co. (St Louis, MO, U.S.A.); reduced glutathione (GSH) from the Wako Chemical Co. (Osaka, Japan); glucose 6-phosphate (G-6-P), glucose 6-phosphate dehydrogenase (G-6-PDH) and NADPH from the Oriental Yeast Co. (Tokyo, Japan). Other chemicals were of analytical grade.

Radiochemical purity. The radiochemical purity of [^{14}C]lidocaine hydrochloride was stated to be at least 99.0% when determined by the following methods: (a) HPLC using the mobile phase, 1% triethyl ammonium acetate pH 4.0: acetonitrile (85:15) and (b) TLC on silica gel using the following solvent systems: (1) chloroform: methanol (95:5), (2) chloroform: ethanol: diethylamine (95:5:1), (3) acetone: concentrated hydrochloric acid (4:1).

Preparation of hepatic microsomes. Male and female Wistar rats were obtained from Takasugi Experimental Animal (Kasukabe, Japan). Male and female DA rats (2 months old) were obtained from Shizuoka Laboratory Co. (Shizuoka, Japan). Hepatic microsomal fractions were prepared according to the method of Omura and Sato [23]. Protein concentrations were assayed by the method of Lowry *et al.* [24].

Incubation of labelled or unlabelled lidocaine A 1 mL microsomal reaction mixture contained 1 mg microsomal protein, 5 mM G-6-P, 1 U/mL G-6-PDH, 1 mM NADPH, 5 mM $MgCl_2$, 5 μM $MnCl_2$ and 15 μM ^{14}C-labelled (0.5 μCi/mL) or unlabelled lidocaine in 50 mM Tris–HCl buffer (pH 7.4). After preincubation of the reaction mixture without NADPH for 5 min at 37°, reaction was started by the addition of NADPH. The reaction was carried out in air for 2.5 min when concentrations of lidocaine and its metabolites were determined, and for 15 min when covalent binding of radioactivity was determined. In the kinetic study of the covalent binding, lidocaine concentrations ranged from 2.5 to 100 μM. In the time course studies of the covalent binding and lidocaine metabolism, incubation was performed for several time periods up to 30 min. The reaction was stopped by the addition of 1 mL of 1 N NaOH when the concentrations of lidocaine and its metabolites were determined, and by the addition of 1 mL of 10% trichloroacetic acid (TCA) when the covalent binding of radioactivity to microsomal protein was determined.

Modifications of incubation conditions. In some experiments, the NADPH-generating system was omitted or the reaction mixture was preincubated at 50° for 5 min to denature microsomal protein. In other experiments, nitrogen or CO as a mixture of CO and oxygen (9:1, v/v) was purged in the reaction mixture for 5 min before preincubation, for 5 min during preincubation, and then for 15 min during incubation. The final concentrations of the compounds added to the reaction mixture were 1 mM (SKF-525A), 30 μM (propranolol and quinidine) and 0.1 or 1 mM (GSH and cysteine).

Assays of lidocaine and its metabolites. Lidocaine, MEGX, GX, 3-OH LID and 3-OH MEGX in the reaction mixture were assayed by the HPLC method as described previously [22]. After the termination of incubation with 1 N NaOH, lidocaine and its metabolites were extracted into ethyl acetate, and then back extracted into an acidic aqueous layer (0.01 N H_2SO_4) containing an internal standard, scopolamine hydrochloride (1.0 μg). The organic layer was removed by aspiration, followed by neutralization with 0.01 N NaOH. The aqueous layer

was evaporated to dryness, and the residue was dissolved in 0.1 mL of redistilled water. The sample was applied to a reversed-phase column (Inertsil ODS, Gasukuro Kogyo Ltd, Tokyo) of an HPLC equipment (a Shimadzu model LC-3A pump, Shimadzu, Kyoto, Japan). The HPLC column was kept at 40°, and the mobile phase (0.1 M potassium phosphate buffer, pH 3.0: acetonitrile, 85:15, v/v) was pumped through the column set at a flow rate of 1.8 mL/min. The absorption intensity was monitored at 210 nm with an HPLC UV detector (Shimadzu SPD-2A).

Determination of covalent binding of radioactivity to hepatic microsomes. The covalent binding of the radioactivity after incubation of [^{14}C]lidocaine with hepatic microsomes was measured as described by Nakagawa *et al.* [25]. After the termination of the incubation by the addition of 1 mL of 10% TCA, 4 mL of the same solvent was added to the reaction mixture. The resultant precipitate was collected by centrifugation (2000 *g*, 10 min) and was resuspended in 4 mL of 7.5% TCA. After centrifugation, the washed pellet was extracted with 4 mL of methanol–water (4:1, v/v, twice), hot methanol–water (4:1, v/v, twice), methanol–ether (1:1, v/v, twice) and methanol–water (4:1, v/v, twice). After the last extraction, no further radioactivity could be removed from the pellet. The thoroughly extracted precipitate was dissolved in 1 N NaOH, and was mixed with 10 mL of scintillation medium (1000 mL toluene including 4 g of 2,5-diphenyloxazole, 100 mg of 1,4-bis[2-(4-methyl-5-diphenyloxazolyl)]benzene and 500 mL of Triton X-100). The radioactivities in these samples were measured by a liquid scintillation counter (Beckman LS-1800).

Data analysis. Enzyme kinetic parameters (K_m and V_{max}) were analysed according to a non-linear least squares regression analysis based on a simplex method [26]. Best fittings of the data were performed by weighting them with the reciprocal of their square. Statistical significance was calculated by Student's *t*-test.

RESULTS

Time courses for in vitro *covalent binding of radioactivity and lidocaine metabolism*

Incubation of [^{14}C]lidocaine with rat liver microsomes in the presence of an NADPH-generating system resulted in *in vitro* covalent binding of a ^{14}C-labelled material to microsomal protein (Fig. 1A). The amount of the covalently bound material increased linearly with respect to incubation time up to 20 min. Therefore, incubations in further experiments were performed for 15 min. The linearity of the binding was also confirmed with respect to microsomal protein concentrations ranging up to 4 mg (data not shown). When unlabelled lidocaine was incubated under the same conditions, lidocaine concentration decreased almost linearly up to 15 min, and was close to a detection limit (0.05 nmol/mL) 25 min after the onset of incubation (Fig. 1B). Concentrations of two major primary metabolites of lidocaine, MEGX and 3-OH LID, increased up to 10 min and decreased after reaching their plateaux due to undergoing subsequent metabolism [22]. Significant amounts of two secondary metabolites, GX and 3-OH MEGX, appeared after the plateaux (data not shown).

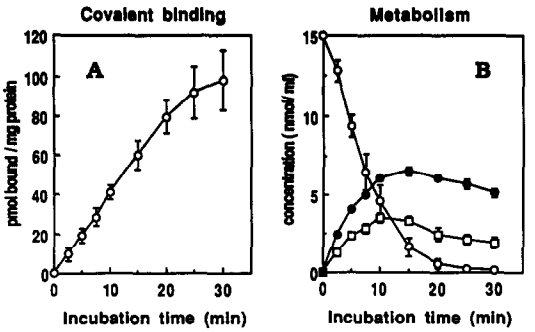


Fig. 1. Time courses for covalent binding of radioactivity to rat liver microsomal protein and *in vitro* metabolism of lidocaine. [^{14}C]Lidocaine or unlabelled lidocaine (15 μM each) was incubated with liver microsomes (1 mg/mL) of Wistar male rats in the presence of an NADPH-generating system for 30 min. The left panel shows the *in vitro* covalent binding of a ^{14}C-labelled material to rat liver microsomal protein after incubation of [^{14}C]lidocaine. The right panel shows the disappearance of lidocaine (○) and the appearance of 3-OH LID (□) and MEGX (●) in the reaction mixture. Results are means ± SE for three difference microsomes from three rats.

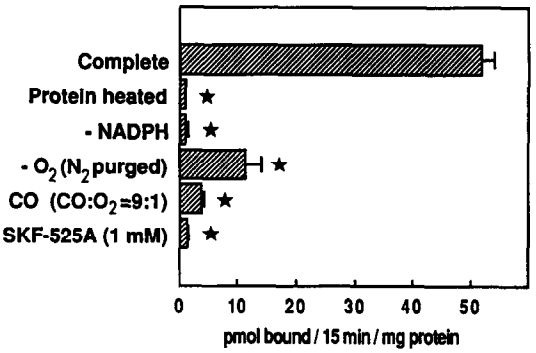


Fig. 2. Effects of various incubation conditions of [^{14}C]-lidocaine on *in vitro* covalent binding of a ^{14}C-labelled material to rat liver microsomal protein. In the "Complete" system, [^{14}C]lidocaine (15 μM) was incubated with liver microsomes (1 mg/mL) of Wistar male rats in the presence of an NADPH-generating system for 15 min. "Protein heated" and "-NADPH" denote microsomes preincubated at 50° for 5 min and without NADPH, respectively. "N_2" and "CO" were purged for 5 min, and then during preincubation (5 min) and incubation (15 min). "SKF-525A (1 mM)" was added to the "Complete" system. Results are means ± SE for three different microsomes from three rats. ★: Significantly different from mean values of "Complete" system ($P < 0.01$).

Cofactor requirements for covalent binding. Figure 2 shows effects of various incubation conditions of [^{14}C]lidocaine on covalent binding of a ^{14}C-labelled material to rat liver microsomes. The binding was

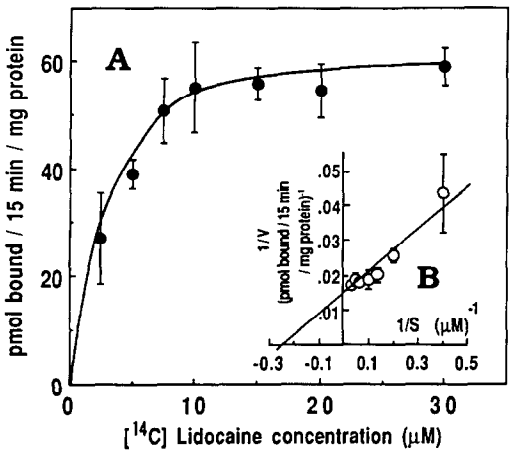


Fig. 3. Kinetics of *in vitro* covalent binding of a [^{14}C]-lidocaine metabolite to rat liver microsomal protein. [^{14}C]-Lidocaine (2.5–30 μM) was incubated with liver microsomes (1 mg/mL) of Wistar male rats in the presence of an NADPH-generating system for 15 min. Data are shown using normal plots (A) and Lineweaver–Burk plots (B). The V_{max} and K_m values for the covalent binding were 72.0 ± 4.6 pmol/15 min/mg protein and 4.52 ± 1.30 μM, respectively. Results are means ± SE for three different microsomes from three rats.

inhibited almost completely by heating microsomes and omitting an NADPH-generating system. A marked inhibition of the covalent binding occurred when the incubation tubes were purged with nitrogen to remove the oxygen present. CO and SKF-525A diminished the covalent binding of radioactivity to microsomal protein almost completely. Therefore, the ^{14}C-labelled material bound covalently to microsomal protein after incubation of [^{14}C]lidocaine is thought not to be lidocaine but to be a reactive metabolite of lidocaine.

Kinetic study for covalent binding. Figure 3 shows the effect of lidocaine concentration on the formation rate of a covalently bound material to microsomal protein. The covalent binding of radioactivity was saturated at a lidocaine concentration of 10 μM (Fig. 3A), and no significant increase of the binding was observed even at 100 μM. The inset (B) of Fig. 3 shows double reciprocal plots of the amounts of covalent binding per 15 min against substrate concentrations. The covalent binding followed Michaelis–Menten kinetics, and the V_{max} and K_m values (mean ± SE for three different microsomes from three rats) of the binding were 72.0 ± 4.6 pmol/15 min/mg protein and 4.52 ± 1.3 μM, respectively.

Strain and sex differences in covalent binding and lidocaine metabolism. Figure 4A shows the metabolism-dependent covalent binding of lidocaine to rat liver microsomes obtained from male and female rats of Wistar and DA strains. The DA strain rat has been known as an animal model for a debrisoquine poor metabolizer [18, 19]. The extent of covalent binding of radioactivity for the corresponding sexes was much higher in Wistar rats than in DA rats (Fig. 4A). Marked strain differences in both sexes were observed also in lidocaine 3-hydroxylase activity (Fig. 4B), as previously observed [13]. On the other hand, no strain difference in lidocaine *N*-deethylase activity was observed in either sex, and the activity in male rats was much higher than that in females for both strains (Fig. 4C). Sex differences (male > female) in the covalent binding were also observed in both strains (Fig. 4A). The extent of the sex difference in the covalent binding was smaller than that in lidocaine *N*-deethylase activity in Wistar rats (Fig. 4A and C).

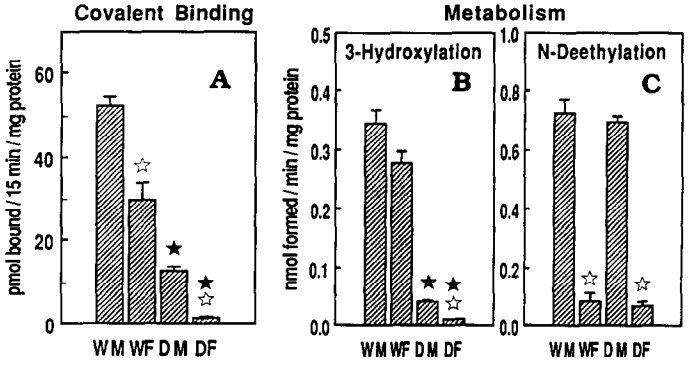


Fig. 4. Strain and sex differences in *in vitro* lidocaine metabolism and covalent binding of a [^{14}C]-lidocaine metabolite to rat liver microsomal protein. WM, WF, DM and DF indicate enzyme sources which were obtained from Wistar male, Wistar female, DA male and DA female, respectively. [^{14}C]-Lidocaine or unlabelled lidocaine (15 μM each) was incubated with microsomes (1 mg/mL) in the presence of an NADPH-generating system. The left panel shows the *in vitro* covalent binding of a ^{14}C-labelled material at 15-min incubation of [^{14}C]lidocaine. The right panels show lidocaine 3-hydroxylase and *N*-deethylase activities in rat liver microsomes. These activities were obtained using 2.5-min incubations of unlabelled lidocaine. Results are means ± SE for four different microsomes from four rats. ★: Significantly different from mean values of Wistar rats for the corresponding sexes ($P < 0.01$). ☆: Significantly different from mean values of male rats for the corresponding strains ($P < 0.01$).

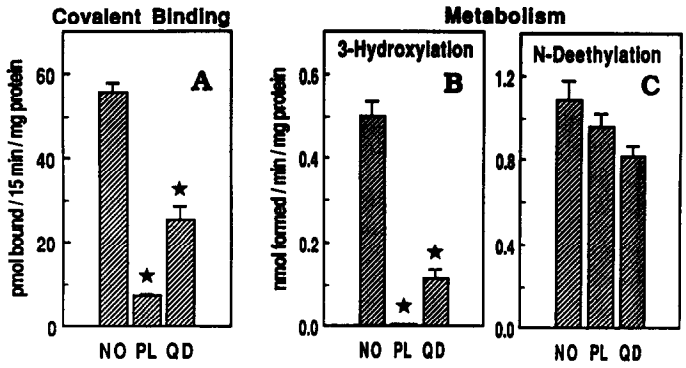


Fig. 5. Effects of propranolol and quinidine on *in vitro* metabolism of lidocaine and covalent binding of a [^{14}C]lidocaine metabolite to rat liver microsomal protein. [^{14}C]Lidocaine or unlabelled lidocaine (15 μM each) was incubated with liver microsomes (1 mg/mL) of Wistar male rats in the presence of an NADPH-generating system. The incubation conditions were the same as those shown in Fig. 4. PL and QD denote propranolol and quinidine, respectively, which were added to the incubation mixture as inhibitors at a concentration of 30 μM. NO denotes no inhibitor added. Results are means ± SE for three different microsomes from three rats. ★: Significantly different from mean values of "NO" ($P < 0.01$).

Effects of propranolol and quinidine on covalent binding and lidocaine metabolism. Figure 5A shows effects of the addition of propranolol and quinidine, inhibitors of a P450 isozyme belonging to the CYP2D subfamily [20, 21], on the metabolism-dependent covalent binding in rat liver microsomes. Both propranolol and quinidine at twice the lidocaine concentration significantly inhibited the covalent binding of radioactivity to microsomal protein (Fig. 5A). These two inhibitors also inhibited lidocaine 3-hydroxylase activity (Fig. 5B), but not *N*-deethylase activity (Fig. 5C). Inhibitory effects of propranolol on both the covalent binding and lidocaine 3-hydroxylase activity were more potent than those of quinidine (Fig. 5A and B).

Effects of nucleophiles on covalent binding. The covalent binding of radioactivity to microsomal protein was inhibited significantly and dose-dependently by GSH (0.1 or 1 mM), a typical nucleophile (Fig. 6). Similar extents of inhibition for the binding were observed when another thiol-compound, cysteine (0.1 or 1 mM), was added as an inhibitor.

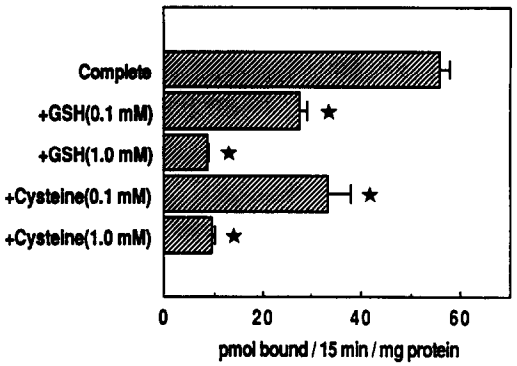


Fig. 6. Effects of nucleophiles on *in vitro* covalent binding of a [^{14}C]lidocaine metabolite to rat liver microsomal protein. The incubation conditions of the "Complete" system are the same as those shown in Fig. 2, and GSH or cysteine (0.1 and 1.0 mM each) was added to the incubation mixture. Results are means ± SE for three different microsomes from three rats. ★: Significantly different from mean values of "Complete" system ($P < 0.01$).

DISCUSSION

The present study showed that lidocaine was activated in rat liver microsomes to a chemically reactive metabolite binding covalently to microsomal protein as demonstrated previously in several compounds such as chloramphenicol [15] and propranolol [16, 17]. Covalent binding to rat liver microsomes was completely suppressed by heating the microsomes (Fig. 2). Since the covalent binding required NADPH and atmospheric oxygen, and was markedly inhibited by CO and SKF-525A (Fig. 2), the metabolic activation of lidocaine was thought to be catalysed by microsomal P450.

The formation of 3-hydroxylated and N-deethylated metabolites almost accounts for *in vitro* metabolic elimination of lidocaine in rat liver microsomes [22]. The kinetics of lidocaine metabolism in rat liver microsomes indicated that the K_m and V_{max} values for 3-hydroxylation were much smaller than those for N-deethylation [27]. The covalent binding obtained in the present study followed single Michaelis–Menten kinetics (Fig. 3B). The K_m value of the binding (4.52 μM), probably corresponding to the rate of the metabolic activation of lidocaine, was much smaller than that of lidocaine N-deethylation (595 μM [27]), and was close to that of lidocaine 3-hydroxylation (1.78 μM [27]).

A sex difference in N-deethylation [10, 11, 13] and a strain difference in 3-hydroxylation [13] have been observed in lidocaine metabolism. The covalent

binding of radioactivity to microsomal protein in DA rats as well as lidocaine 3-hydroxylase activity was much lower than that in Wistar rats for the corresponding sexes (Fig. 4A and B). In addition, a significant sex difference (male > female) was observed in the covalent binding in both Wistar and DA rats (Fig. 4A). However, the extent of sex difference in the binding (Fig. 4A) was much smaller than that in lidocaine *N*-deethylase activity in Wistar rats (Fig. 4C). These findings suggest that the activation process of lidocaine relates to the 3-hydroxylation pathway rather than the N-deethylation pathway. The sex difference in the binding of radioactivity in DA rats could be explained by the sex difference of lidocaine 3-hydroxylation. However, a small but significant sex difference of the binding in Wistar rats (Fig. 4A) could not be explained by lidocaine 3-hydroxylase activity, which shows no sex difference in this strain. It seems likely that the N-deethylation pathway also partially contributes to the metabolic activation of lidocaine.

DA rats have an impaired ability to metabolize debrisoquine [18, 19] and bufuralol [28], and female DA rats are particularly deficient in debrisoquine 4-hydroxylase [19, 29]. Recently, we have observed that DA rats are deficient in propranolol metabolism forming a reactive metabolite that binds covalently to microsomal protein [17]. Observations in the present study suggest that the metabolic activation of lidocaine, as well as propranolol, is mainly catalysed by a P450 isozyme(s) catalysing debrisoquine 4-hydroxylation and belonging to the CYP2D subfamily [29].

Two potent inhibitors of P450 isozyme(s) belonging to the CYP2D subfamily, propranolol and quinidine [20, 21], inhibited lidocaine 3-hydroxylase, but not *N*-deethylase (Fig. 5B and C), as expected from the regiospecific strain difference in lidocaine metabolism described above (Fig. 4B and C). These two inhibitors also inhibited the metabolism-dependent covalent binding of lidocaine to microsomal protein (Fig. 5A). In addition, the order of magnitude in the inhibition of binding for propranolol and quinidine was similar to that found for the inhibition of lidocaine 3-hydroxylase. Therefore, the activation pathway of lidocaine may be closely related to 3-hydroxylation, being consistent with the strain difference in the metabolic activation of lidocaine.

The present study showed that the metabolic activation of lidocaine might be related to 3-hydroxylation, while our study design did not permit clarification of the P450 isozyme(s) impaired, i.e. the reaction pathway. We think that a P450 isozyme(s) forming a reactive metabolite of lidocaine might be impaired as proposed previously in propranolol [17]. This presumption is at variance with reported results of the liver perfusion systems [7–9] suggesting that impairment of the N-deethylation pathway was the cause of the apparent time-dependent kinetics of lidocaine. It is necessary for elucidation of this variance to clarify that a reactive intermediate of lidocaine impairs the P450 isozyme(s) catalysing the reaction forming the reactive intermediate.

The chemical structure of a reactive metabolite of lidocaine remains unknown. The present study demonstrated that the reactive metabolic intermediate was related to the 3-hydroxylation pathway. It is probably a metabolic intermediate preceding 3-OH LID and/or a further metabolite of 3-OH LID. In addition, the activation of lidocaine was markedly suppressed by the addition of nucleophiles, GSH and cysteine. This finding indicates that the reactive metabolite of lidocaine is an electrophile. Since electrophilic arene oxides have been proposed as reactive metabolic intermediates of imipramine and another tricyclic anti-depressant [30, 31], it seems likely that 3,4-arene oxide is a possible candidate for a reactive intermediate of lidocaine. But it has not been demonstrated whether lidocaine 3-hydroxylation proceeds via an arene oxide intermediate. Further studies are required to clarify the formation process of 3-OH LID and to delineate the chemical structure of the reactive metabolite of lidocaine.

In summary, lidocaine was metabolically activated to a reactive intermediate capable of binding covalently to hepatic microsomal protein. This reaction was catalysed by a P450(s), which might be involved in debrisoquine 4-hydroxylation. The reactive metabolite of lidocaine was electrophilic and closely related to the lidocaine 3-hydroxylation pathway, probably being an intermediate before the formation of 3-OH LID.

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