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Nicotine metabolism by rat hepatic cytochrome P450s

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Abstract—Many kinds of cytochrome P450s were purified from rat hepatic microsomes, and their role in the metabolization of nicotine in a reconstituted system examined. Of four phenobarbital-inducible P450s, P450 2B1 had the highest nicotine oxidation activity and P450 2B2 showed a low rate of nicotine oxidation, whereas P450 2C6 and 3A2 had no detectable activity toward nicotine. Among eleven other purified cytochrome P450s tested, P450 2C11 had high nicotine oxidation activity and P450 1A2 and 2D1 showed low catalytic activity toward nicotine. The other cytochrome P450s, P450 1A1, 2A1, 2A2, 2C7, 2C12, 2C13, 2E1 and 4A1, had no detectable nicotine oxidation activity. Based on these results, participation of cytochrome P450s in nicotine metabolism in human and animal livers is discussed.

Nicotine is a major constituent of tobacco and exerts a number of physiological effects involving the central and peripheral nervous systems. Nicotine dependence, in particular, has been given much attention. To evaluate these problems, it is necessary to understand better the pharmacokinetic and pharmacodynamic characteristics of nicotine. The metabolism of nicotine into cotinine is a major pathway of hepatic nicotine metabolism, and cytochrome P450s catalyze the first step of this pathway [1]. McCoy *et al.* [2] examined six kinds of cytochrome P450s purified from rabbit livers to catalyze nicotine oxidation in a reconstituted system and found that P450 2B4 and 2C3 have high nicotine oxidation activity. Using the expression system of human cytochrome P450 cDNA, Flammang *et al.* [3] have shown that of 12 human cytochrome P450s tested, P450 2B6 has the highest nicotine oxidation activity. However, the extrapolation of these *in vitro* findings to animals and humans has not been shown except for phenobarbital (PB*)-inducible cytochrome P450s (P450 2B4 and 2B6) because of the small amount of data on *in vivo* and *in vitro* microsomal nicotine metabolism associated with cytochrome P450s in rabbits and humans. On the other hand, nicotine metabolism in the rat has been studied extensively both *in vivo* and *in vitro* [1]. However, which cytochrome P450s participate in nicotine metabolism in the rat liver is not well characterized except for P450 1A1, 1A2, 2B1 and 2C6 [4–6]. In this study, we have determined nicotine oxidation activities of many rat cytochrome P450 forms in a reconstituted system. Based on these results, cytochrome P450-dependent nicotine metabolism in animals and humans is discussed.

Materials and Methods

Materials. *S*(−)-Nicotine was obtained from the Maruwaka Co. Ltd., Japan. NADPH and dilaroylphosphatidylcholine (DLPC) were purchased from the Kohjin Co. Ltd., Japan, and the Sigma Chemical Co., U.S.A., respectively. Other chemicals were obtained from Nacalai Tesque Inc., Japan, or Wako Pure Chemical Industries, Japan.

Assay of nicotine oxidation activity in a reconstituted system. Cytochrome P450s were purified from microsomes of rat livers as described previously [7–9]. The catalytic activities of purified cytochrome P450s toward nicotine in a reconstituted system were determined in the presence of saturating amounts of NADPH–cytochrome P450 reductase and DLPC by disappearance of nicotine as described elsewhere [6]. The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.4), 10 μg DLPC, 0.5 mM NADPH, 0.5 mM nicotine, 0.3 units of NADPH–cytochrome P450 reductase and 35–50 pmol of purified cytochrome P450s in a total volume of 0.1 mL. Cytochrome P450, NADPH–cytochrome P450 reductase and DLPC were incubated for 10 min at room temperature, followed by the addition of phosphate buffer and nicotine. Reaction was started by the addition of NADPH and continued for 30 min at 37°. Antibody against purified cytochrome P450 3A2 was raised in a Japanese White rabbit as described previously [8]. When inhibition of microsomal nicotine oxidation by anti-P450 3A2 was studied, 10–30 μL of anti-P450 3A2 serum was first incubated with 200–300 μg of hepatic microsomes prepared from PB-treated rats for 20 min at room temperature, and then nicotine and phosphate buffer were added, followed by an additional 2-min incubation at 37°. After this preincubation, reactions were started by the addition of NADPH. In the control experiments, preimmune rabbit serum was used instead of anti-P450 3A2 serum. Approximately 90% of cytochrome P450 3A2-dependent testosterone 6β-hydroxylation in 100 μg of microsomal protein of rat liver was inhibited by 5–10 μL of anti-P450 3A2 serum. Testosterone 6β-hydroxylation activity and anti-P450 3A2 inhibition of the hydroxylation were measured by methods described previously [10]. PB was injected intraperitoneally each day into 7-week-old male Wistar rats at 70 mg/kg for 5 days. The incubation was terminated by the addition of ice-cold 1 M Tris and 0.5 mL chloroform. After 10 μg quinoline was added as an internal standard, the mixture was vortex-mixed for 10 min at room temperature and then centrifuged at 10,000 *g* for 10 min. The lower chloroform layer was used for the determination of nicotine and quinoline. Gas chromatographic analysis of nicotine and quinoline was performed with a glass column (2 m × 3 mm, i.d.) filled with 10% Apiezon grease L + 10% KOH on 80/100 mesh Chromosorb W (Gasukuro Kogyo Inc., Japan), equipped with a nitrogen/phosphorus detector. The instrument settings were: column temperature 170°, injector 200° and detector 250°.

Results and Discussion

Several *in vivo* and *in vitro* studies on nicotine metabolism in rats have suggested that both PB-inducible and constitutive forms of cytochrome P450 have nicotine oxidation activities [1, 11–16]. PB induces four cytochrome P450s (P450 2B1, 2B2, 2C6 and 3A2) in hepatic microsomes of rats [17, 18]. The catalytic activities of these PB-inducible cytochrome P450s toward nicotine are shown in Table 1. The nicotine oxidation activities of P450 2B1, 2B2 and 2C6 were determined in a reconstituted system. Reconstitution with P450 3A2 was not performed, but the effect of antibody against P450 3A2 on microsomal nicotine oxidation was examined. Of the four PB-inducible cytochrome P450s, P450 2B1 has been found to catalyze

* Abbreviations: PB, phenobarbital; DLPC, dilaroylphosphatidylcholine.

Table 1. Nicotine oxidation activities of PB-inducible cytochrome P450s in a reconstituted system

P450	Nicotine oxidation activity (nmol/min/nmol P450) Present study*	Hammond *et al.*†	Williams *et al.*‡
2B1	5.31 ± 0.18 (3)	15.60	1.02 (iminium ion) 0.17 (*N'*-oxide)
2B2	1.44 ± 0.50 (6)	§	—
2C6	ND‖ (2)	ND	—
3A2¶	ND (2)	—	—

* Values are means ± SD; numbers in parentheses represent the number of experiments.

† Data are from Ref. 4, in which nicotine oxidation activities were determined as cotinine formation in the presence of cytosol.

‡ Data are from Ref. 5, in which nicotine oxidation activities were determined as the formation of Δnicotine 1'5'-iminium ion and nicotine -*N'*-oxide.

§ —, not determined.

‖ ND, not detectable.

¶ In the case of P450 3A2, the effect of antibody against P450 3A2 on hepatic microsomal nicotine oxidation activity was examined.

Table 2. Nicotine oxidation activities of rat cytochrome P450s in a reconstituted system

P450	Nicotine oxidation activity (nmol/min/nmol P450)	No. of experiments
1A1	ND*	4
1A2	1.68 ± 0.39†	4
2A1	ND	2
2A2	ND	2
2C7	ND	4
2C11	3.45 ± 0.48	4
2C12	ND	4
2C13	ND	2
2D1	1.26 ± 0.41	4
2E1	ND	2
4A1	ND	2

* ND, not detectable.

† Means ± SD.

nicotine oxidation efficiently in a reconstituted system [4, 5], whereas P450 2C6 has no detectable nicotine oxidation activity [4]; these observations were confirmed by the present study. In addition, the nicotine oxidation activity of P450 2B2 was lower than that of P450 2B1, and anti-P450 3A2 serum did not inhibit nicotine oxidation activity in hepatic microsomes of PB-treated rats. An increase in the amount of the reductase (up to 1.5-fold) resulted in no appreciable change in the P450-dependent nicotine oxidation. Table 2 shows nicotine oxidation activities of 11 other cytochrome P450 forms in the reconstituted system. Of the 11 cytochrome P450 forms tested, P450 2C11 had the highest nicotine oxidation activity and P450 2D1 showed low nicotine oxidation activity. P450 1A1 and 1A2 are 3-methylcholanthrene-inducible cytochrome P450s [17, 18]. P450 1A2 had low nicotine oxidation activity, whereas P450 1A1 had no detectable nicotine oxidation activity. P450 2A1, 2A2, 2C7, 2C12, 2C13, 2E1 and 4A1 also had no detectable nicotine oxidation activity.

Of five cytochrome P450s found to have nicotine oxidation activity in a reconstituted system, the level of P450 2B1 is the highest in hepatic microsomes of PB-treated rats [18]. These results strongly suggest that P450 2B1 plays the most important role in hepatic microsomal nicotine oxidation of PB-treated rats. Rabbit P450 2B4 and human P450 2B6 are PB-inducible cytochrome P450s and also have high nicotine oxidation activities [2, 3]. Of the constitutive cytochrome P450 forms examined, P450 2C11 was found to have the highest nicotine oxidation activity in a reconstitutive system. P450 2C11 is a major male-specific cytochrome P450 and its expression is developmentally regulated [19–21]. Male rats have been found to metabolize nicotine faster than females [22]. Castration of male rats results in a decrease of nicotine metabolism, and the effect of the castration is reversed by testosterone administration [22]. P450 2C11 is also induced by testosterone [23]. In hepatic microsomes of untreated rats, the level of P450 2C11 is much higher than those of other cytochrome P450s having nicotine oxidation activity [21]. The nicotine oxidation activity of P450 2B1 was higher than that of P450 2C11, but its level was very low in hepatic microsomes of untreated rats. Therefore, P450 2C11 probably plays an important role in hepatic microsomal nicotine oxidation in untreated rats. Rabbit P450 2C3 and human 2C9 are constitutive forms and have been reported to have nicotine oxidation activity [2, 3]. P450 2C3 and 2C9 show high degrees of sequence homology with P450 2C11 [24, 25]. Moreover, P450 2C3 and 2C11 catalyze efficiently testosterone hydroxylation [17, 18]. There seems to be a high degree of consistency across species with respect to the specific cytochrome P450 forms responsible for microsomal nicotine metabolism.

More recently, using a cDNA-directed expression system, McCraken *et al.* [26] have shown that human P450 2A6, 2B6 and 2D6 but not 2C8, 2C9, 2E1 or 3A4 catalyze nicotine oxidation. With respect to P450 2C9, 2D6 and 2E1, their findings are inconsistent with those of Flammang *et al.* [3]. Furthermore, some ambiguities in K_m values of cytochrome P450s for nicotine remain. The K_m values found by McCoy and his coworkers are two to three orders higher than those reported by other groups [2–5, 27]. This may depend not only on the forms of cytochrome P450 examined but also on the assay methods used.

Treatment of rats with PB increases the specific activity of nicotine oxidation in hepatic microsomes [12]. In contrast to the increase in the levels of P450 2B1 and 2B2, PB treatment decreases the P450 2C11 level [18]. Thus, the elevation of P450 2B1- and 2B2-dependent nicotine oxidation probably exceeds the decrease of the P450 2C11-dependent reaction. There are apparently conflicting reports concerning induction of nicotine metabolism by administration of nicotine and other drugs [1]. Nicotine releases many kinds of neurotransmitters, neuropeptides and steroid hormones, and administration of large doses of nicotine results in toxic effects or the production of stress. These complicated effects of nicotine may have hindered progress in the characterization of the regulation of nicotine metabolism. However, the present study, together with other results derived from rabbit and human nicotine oxidation by cytochrome P450 forms, suggests that only some forms of cytochrome P450 play an important role in nicotine metabolism, which may make it possible to relate the regulation of nicotine metabolism to that of cytochrome P450s having high nicotine oxidation activity. This means a new approach to studies on the regulation of nicotine metabolism. Knowledge about cytochrome P450s is helpful in considering the regulation of nicotine metabolism at molecular levels.

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