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INDUCTION OF HEPATIC MICROSOMAL CYTOCHROME P450 AND DRUG-METABOLIZING ENZYMES BY 4-BENZYLPYRIDINE AND ITS STRUCTURALLY RELATED COMPOUNDS IN RATS

DOSE- AND SEX-RELATED DIFFERENTIAL INDUCTION OF CYTOCHROME P450 SPECIES

YASUNA KOBAYASHI, YOICHI MATSUURA,* EIICHI KOTANI, TOSHIHIRO IIO, TERUO FUKUDA, TAKAAKI AOYAGI and SEISHO TOBINAGA

Showa College of Pharmaceutical Sciences, Tokyo 194, Japan

and

TAKEMI YOSHIDA and YUKIO KUROIWA

Department of Biochemical Toxicology, School of Pharmaceutical Sciences, Showa University, Tokyo 142, Japan

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Abstract—We examined the abilities of 4-, 3- and 2-benzylpyridine and 4-*tert*-butylpyridine to induce hepatic microsomal cytochrome P450 and drug-metabolizing enzymes in male and female rats in order to define the effects of pyridine-containing compounds on drug metabolism. 4-Benzylpyridine (0.4 mmol/kg, for 2 consecutive days) induced total cytochrome P450 to about three times that of the controls at 24 hr, and its inducing effect was sustained for 120 hr after the treatment in male and female rats. 4-Benzylpyridine was a more potent inducer of cytochrome P450 than 3- and 2-benzylpyridine, which induced the cytochrome to 71.4 and 43.9%, respectively, of that produced by the 4-substituted isomer. 4-*tert*-Butylpyridine also induced cytochrome P450. Immunoblot analysis revealed that a single treatment of male rats with 4-benzylpyridine at doses ranging from 0.05 to 0.80 mmol/kg induced cytochrome P450b/e and caused a maximum increase in the level of the isozyme at the 0.2 mmol/kg dose. 4-Benzylpyridine at doses from 0.40 to 0.80 mmol/kg also induced cytochrome P450c/d in male rats. In female rats, 4-benzylpyridine induced cytochrome P450b/e at doses ranging from 0.1 to 0.80 mmol/kg and produced a maximum increase in the level of this isozyme at 0.40 to 0.60 mmol/kg. Induction of cytochrome P450c/d by 4-benzylpyridine in female rats was observed at a dose of 0.20 mmol/kg, and the magnitude of the induction of the isozyme was increased in a dose-dependent manner. Both 3- and 2-benzylpyridine induced cytochrome P450b/e and/or c/d depending on the increase of total cytochrome P450 without changing the induction patterns of the isozymes. 4-*tert*-Butylpyridine induced cytochrome P450b/e at doses ranging from 0.20 to 0.60 mmol/kg and slightly induced P450c/d at doses ranging from 0.10 to 0.40 mmol/kg in male rats. These results and our previous report (Matsuura *et al.*, *Biochem Pharmacol* **41**: 1949–1956, 1991) clearly show that the pyridine compounds having lipophilic groups at the 4- or 3-position of the ring could be inducers of cytochrome P450. The present results also revealed that 4-benzylpyridine shows dose- and sex-related differences in the induction of cytochrome P450b/e and c/d in rats.

N-Substituted imidazole compounds, such as imidazole-containing antimycotics [1–7], 1-phenylimidazole [8] and 1-benzylimidazole [9, 10], have been shown to induce hepatic microsomal cytochrome P450 content and its associated drug-metabolizing enzyme activities in rats. The inducible isozyme(s) of cytochrome P450 by these imidazole compounds has been partially identified [5, 9–11]. It has also been shown that these imidazole compounds inhibit drug-metabolizing enzyme activities *in vitro* [2, 7, 12–16]. Pyridine has chemical properties similar to those of imidazole and is an important structural component of various drugs; however, the effects of pyridine-containing compounds on hepatic microsomal cytochrome P450 and drug metabolism are not well characterized. The inductive effects of nicotinamide [17], isoniazid [18], metyrapone [19] and 1,4-bis(2-(3,5-dichloropyridyloxy))benzene [20] on cytochrome P450 and/or drug-metabolizing enzyme activities have been reported. Metyrapone is also well-known as an inhibitor of cytochrome P450-mediated drug metabolism [21]. Structural aspects of these pyridine-containing compounds for the induction of cytochrome P450 have not been fully considered.

Recently, we demonstrated that both imidazole and its lipophilic group-substituted compounds, such as clotrimazole and 1-benzylimidazole, are important and indispensable structural components for the induction of cytochrome P450 [22]. We have also shown that 4-diphenylmethylpyridine and

* Corresponding author: Dr. Yoichi Matsuura, Showa College of Pharmaceutical Sciences, 3165, 3-chome, Higashitamagawagakuen, Machida-shi, Tokyo 194, Japan. Tel. 0427-21-1564; FAX 0427-21-1588.

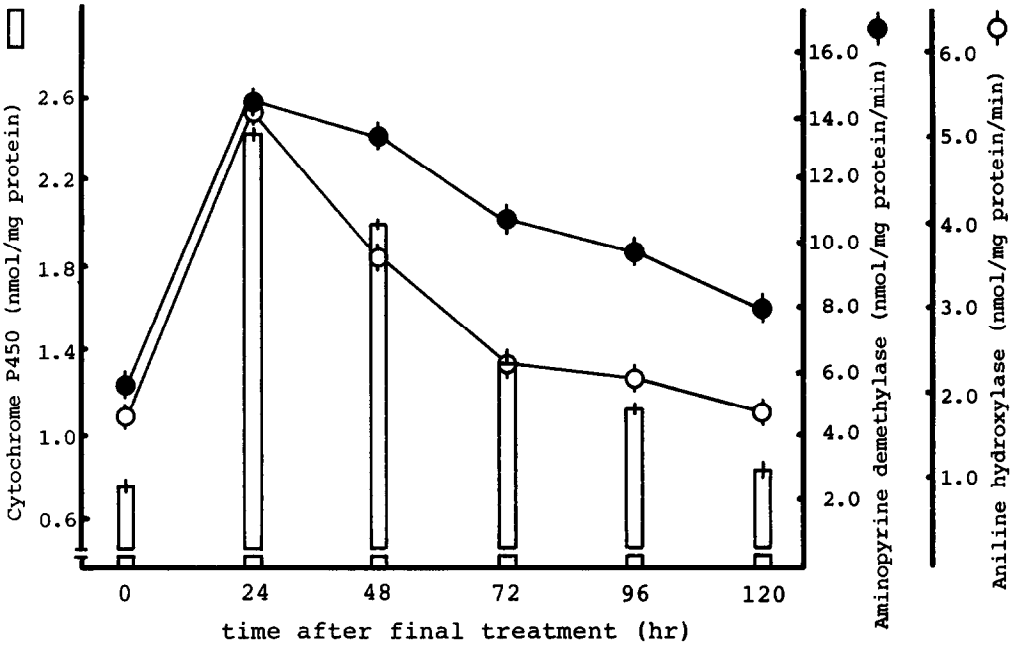

Fig. 1. Time course of the effect of 4-benzylpyridine on hepatic microsomal cytochrome P450 content and on aminopyrine demethylase and aniline hydroxylase activities in male rats. Rats were treated i.p. with 4-benzylpyridine at a dose of 0.40 mmol/kg for 2 consecutive days and were killed at the time indicated. Control rats were treated with an appropriate volume of vehicle. All rats were starved for 24 hr before being killed. The amount of CO-binding cytochrome P450 was determined by the method described in Materials and Methods. Each value is the mean ± SD of four rats.

4-benzylpyridine are inducers of cytochrome P450, whereas 4-benzylpiperidine is not. These findings suggest that pyridine-containing compounds may also be inducers of cytochrome P450, like imidazole compounds.

In the present study, we conducted experiments with pyridine-containing compounds (4-, 3- and 2-benzylpyridine and 4-*tert*-butylpyridine) to characterize their effects on hepatic microsomal cytochrome P450 and its associated drug-metabolizing enzyme activities, and on inducible isozymes of cytochrome P450 in male and female rats.

MATERIALS AND METHODS

Chemicals and treatment. 4-, 3- and 2-Benzylpyridine and 4-*tert*-butylpyridine were purchased from the Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). All other chemicals used were of the highest grade commercially available. Male and female Wistar rats, weighing 180–200 g, were used in this study. They were fed a commercial solid diet (MF, Oriental Yeast Co.) *ad lib.* 4-, 3- and 2-Benzylpyridine and 4-*tert*-butylpyridine were diluted with or dissolved in an appropriate volume of corn oil and injected i.p. into rats at a routine dose of 0.40 mmol/kg for 2 consecutive days as indicated in the figure and table legends. Control rats were injected with vehicle only. The rats were starved for 24 hr before being killed.

Tissue preparation. The rats were killed by decapitation and their livers were perfused *in situ* with 0.9% NaCl solution. The liver was homogenized with 5 vol. of 1.15% KCl solution using a Potter–Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 9,000 *g* for 15 min and the resulting supernatant was ultracentrifuged at 105,000 *g* for 1 hr. The microsomal pellet was suspended in 0.1 M phosphate buffer (pH 7.4) and used for determining cytochrome P450 content and aminopyrine demethylase and aniline hydroxylase activities.

Enzyme assays. Cytochrome P450 content was determined by CO-difference spectrum of dithionite-treated microsomes as described by Omura and Sato [23], except that microsomes had been bubbled for 5 or 10 min with CO gas as described by Kahl *et al.* [14]. Aminopyrine demethylase activity was measured by determining formaldehyde formation according to the method of Nash [24]. Aniline hydroxylase activity was determined by the method of Imai *et al.* [25]. To determine the inhibitory effects of 4-benzylpyridine and its two positional isomers on aminopyrine demethylase activity *in vitro*, these compounds were dissolved in a minimal volume of dimethyl sulfoxide and added to the assay medium at various concentrations. The final concentration of dimethyl sulfoxide in the assay medium did not exceed 0.05%.

Spectrophotometric measurements. Difference spectra were recorded with a Shimadzu dual wavelength spectrophotometer UV-3000. The cuvettes (10 mm quartz) contained 3 mL of microsomal suspension (about 2 mg protein/mL of 0.1 M

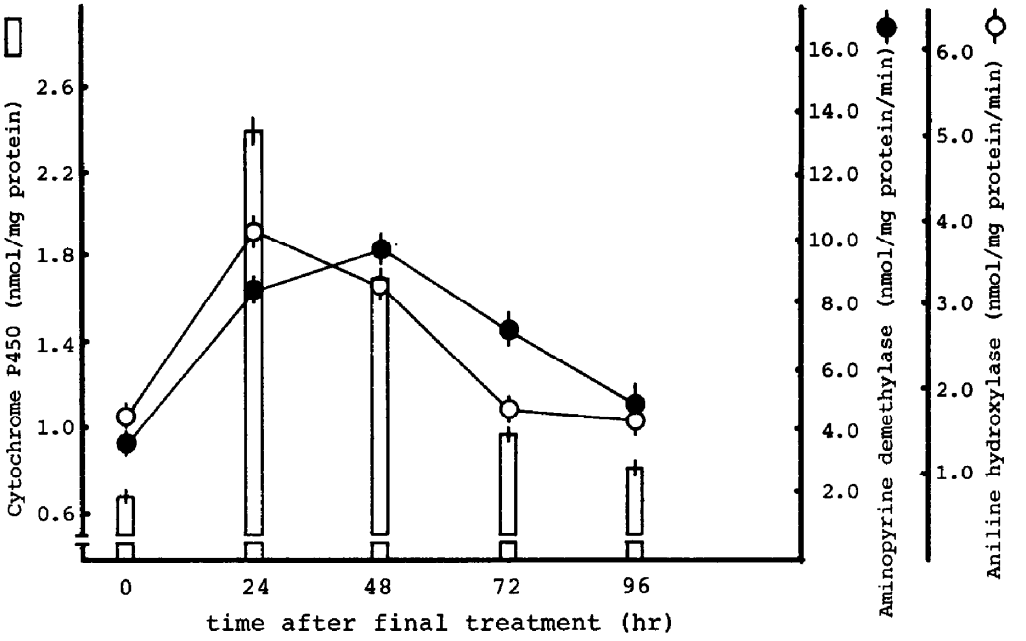

Fig. 2. Time course of the effect of 4-benzylpyridine on hepatic microsomal cytochrome P450 content and on aminopyrine demethylase and aniline hydroxylase activities in female rats. Experimental conditions and methods were identical to those described in the legend of Fig. 1. Each value is the mean ± SD of four rats.

phosphate buffer, pH 7.4) prepared from phenobarbital (80 mg/kg, daily for 2 days) pretreated rats. 4-, 3- and 2-Benzylpyridine, dissolved in a minimal volume of dimethyl sulfoxide and then diluted with water, were added in microliter volume to the sample cuvette and an equal volume of the dimethyl sulfoxide containing solvent was added to the reference cuvette. The concentration of dimethyl sulfoxide did not exceed 0.01%. Apparent spectral dissociation constants (K_s) were obtained by double-reciprocal plots of the absorbance difference between 426 and 394 nm as a function of ligand concentration.

Electrophoresis and immunoblot analysis. Microsomes obtained from control and drug-treated rats were solubilized in sodium dodecyl sulfate and resolved by polyacrylamide gel electrophoresis according to the method of Laemmli [26]. Antigen components reactive with APF3 and APL2 monoclonal antibodies raised against cytochrome P450b and P450c, which were also reactive to cytochrome P450e and P450d, respectively, were visualized with 4-chloro-1-naphthol in 0.006% hydrogen peroxide solution.

RESULTS

Induction of cytochrome P450 and drug-metabolizing enzymes by 4-benzylpyridine in male and female rats. A time course of the effects of 4-benzylpyridine (0.40 mmol/kg/day for 2 days) on cytochrome P450 content and on aminopyrine demethylase and aniline hydroxylase activities in male rats is shown in Fig. 1. Cytochrome P450 content was increased to about 3.0 times that of the controls at 24 hr after treatment with 4-benzylpyridine; then the cytochrome content gradually declined, but the content was increased markedly until 120 hr. Both aminopyrine demethylase and aniline hydroxylase activities were also increased proportionally to the increase of cytochrome P450 content. The results obtained in female rats under similar experimental conditions are shown in Fig. 2. Cytochrome P450 content was increased to about 3.4 times that of the controls at 24 hr after treatment with 4-benzylpyridine, then gradually declined and returned to control level 96 hr later. Interestingly, aniline hydroxylase activity in female rats was increased to a greater extent than aminopyrine demethylase activity at 24 hr. Further, the maximum wavelengths of CO-induced difference spectra of dithionite-treated microsomes from male and female rats were 450 and 448 nm, respectively (data not shown). These results suggest that there is a sex-related difference in the inducible cytochrome P450 isozymes.

Next, we examined the dose–response effect of 4-benzylpyridine on cytochrome P450 content in male and female rats. As shown in Table 1, 4-benzylpyridine increased cytochrome P450 content even at the minimum dose (0.05 mmol/kg/day for 2 days) in male and female rats when determined 24 hr after the treatment and produced dose-dependently the increase of the hemoprotein in both sexes.

Induction of cytochrome P450 by 3- and 2-benzylpyridine and 4-tert-butylpyridine in rats. To clarify if there was a structural requirement for

Table 1. Dose–response of 4-benzylpyridine on cytochrome P450 content in male and female rats

Dose (mmol/kg)	Cytochrome P450 (nmol/mg protein)	
	Male	Female
None	0.804 ± 0.031	0.727 ± 0.044
0.05	1.062 ± 0.096*	0.846 ± 0.013*
0.10	1.376 ± 0.069†	0.976 ± 0.071†
0.20	2.102 ± 0.098†	1.522 ± 0.066†
0.40	2.599 ± 0.099†	2.168 ± 0.025†
0.60	2.756 ± 0.061†	2.313 ± 0.056†
0.80	2.985 ± 0.036†	2.270 ± 0.059†

Rats were treated i.p. with 4-benzylpyridine at doses ranging from 0.05 to 0.80 mmol/kg for 2 consecutive days, and the rats were killed 24 hr after the final treatment. Each value is the mean ± SD of four rats.

*† Significantly different from control groups: *$P < 0.05$ and †$P < 0.01$.

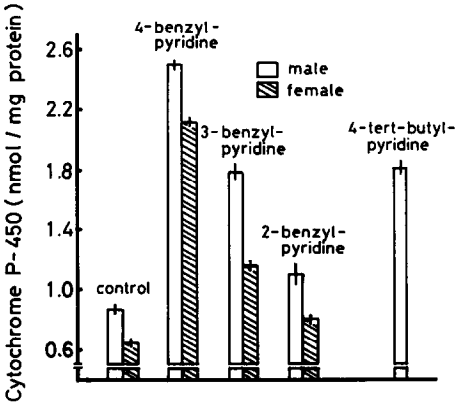

Fig. 3. Effects of 3- and 2-benzylpyridine and 4-*tert*-butylpyridine on hepatic microsomal cytochrome P450 content in rats. Rats were treated i.p. with 4-, 3- and 2-benzylpyridine and 4-*tert*-butylpyridine at a dose of 0.40 mmol/kg for 2 consecutive days and were killed 24 hr after the final treatment. Control rats were treated with an appropriate volume of vehicle. All rats were starved for 24 hr before being killed. Each value is the mean ± SD of four rats.

cytochrome P450 induction by 4-benzylpyridine, we examined the effects of 3- and 2-benzylpyridine and 4-*tert*-butylpyridine on the hemoprotein. The results of this experiment are shown in Fig. 3. Interestingly, 3- and 2-benzylpyridine (0.40 mmol/kg/day for 2 days) were also able to increase cytochrome P450 in male and female rats, but to a lesser extent than 4-benzylpyridine. The magnitudes of the increase of cytochrome P450 and 3- and 2-benzylpyridine were 71.4 and 43.9%, respectively, of that induced by 4-benzylpyridine. Figure 3 also shows that 4-*tert*-butylpyridine (0.40 mmol/kg/day for 2 days) increased cytochrome P450 content in male rats to 2.3 times that of the controls. These results suggest that the position of the benzyl group bound to the pyridine ring may affect the magnitude of cytochrome P450 induction.

Inhibitory effects of 4-, 3- *and* 2-*benzylpyridine on aminopyrine demethylase activity* in vitro *and the formation of difference spectra induced by these compounds.* It has been shown that many imidazole-containing compounds inhibit drug-metabolizing enzyme activities when added *in vitro* to the assay medium [2, 7, 12–16]. Therefore, we also examined the inhibitory effects of the pyridine-containing compounds on aminopyrine demethylase activity and the formation of difference spectra induced by these compounds. As shown in Fig. 4, the addition of 4- or 3-benzylpyridine to the assay medium at final concentrations of 10^{-6} to 10^{-4} M inhibited aminopyrine demethylase activity depending on the concentrations. 2-Benzylpyridine did not inhibit aminopyrine demethylase activity significantly. Figure 5 shows that 4- and 3-benzylpyridine induced typical type II difference spectra, and their spectral dissociation constants (K_s) obtained by double-reciprocal plots of the absorbance difference between 426 and 394 nm as a function of the compound concentration were 3.401 and 5.263 μM, respectively. 2-Benzylpyridine did not induce a type II difference spectrum.

Induction of cytochrome P450b/e and P450c/d by 4-benzylpyridine in male and female rats. Male and female rats were treated with 4-benzylpyridine at doses ranging from 0.05 to 0.80 mmol/kg for 2 consecutive days and the animals were killed 24 hr after the final treatment. Cytochrome P450b/e and P450c/d were determined by immunoblot analysis. Figure 6A shows that 4-benzylpyridine induced cytochrome P450b/e in male rats at doses ranging from 0.05 to 0.80 mmol/kg, and produced maximum induction of the isozyme at a dose of 0.20 mmol/kg. The magnitude of the increase in cytochrome P450b/e gradually declined when the dose of 4-benzylpyridine was increased up to 0.40 mmol/kg. In female rats, 4-benzylpyridine also induced cytochrome P450b/e with doses ranging from 0.40 to 0.60 mmol/kg, but lacked such an effect at a dose of 0.05 mmol/kg (Fig. 6C). Likewise, 4-benzylpyridine caused cytochrome P450c/d induction in male rats at doses ranging from 0.40 to 0.80 mmol/kg, but did not increase the isozyme at dose levels lower than 0.40 mmol/kg (Fig. 6B). In female rats, as shown in Fig. 6D, 4-benzylpyridine elicited an increase of cytochrome P450c/d at a dose of 0.20 mmol/kg, and increased the isozyme dose dependently up to 0.80 mmol/kg. These results show that only cytochrome P450b/e was induced in male rats treated with 4-benzylpyridine at doses lower than 0.40 mmol/kg, whereas cytochrome P450b/e and P450c/d were induced in rats treated with the compound at doses higher than 0.40 mmol/kg. In female rats, only cytochrome P450b/e was induced at doses lower than 0.20 mmol/kg, whereas cytochrome P450b/e and P450c/d were induced at doses higher than 0.20 mmol/kg.

Induction of cytochrome P450b/e and P450c/d by 3- *and* 2-*benzylpyridine and* 4-tert-*butylpyridine in rats.* Rats were treated with 4-, 3- and 2-benzylpyridine and 4-*tert*-butylpyridine at a dose of 0.40 mmol/kg for 2 consecutive days and killed

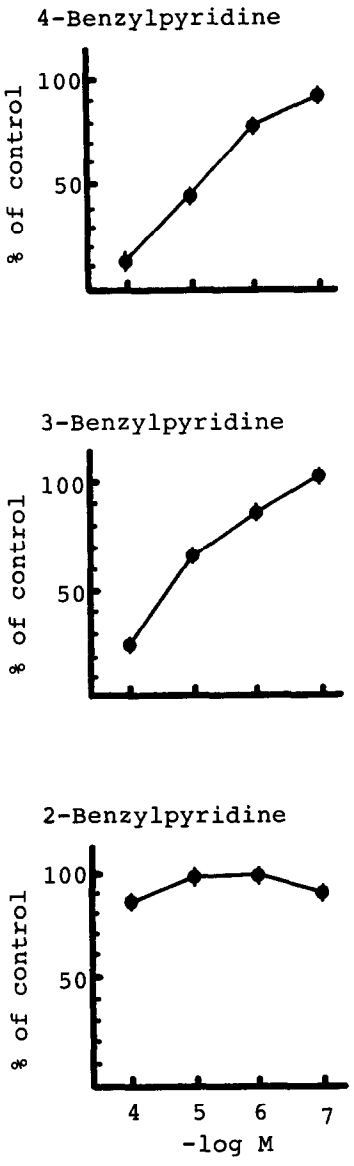

Fig. 4. Inhibitory effects of 4-, 3- and 2-benzylpyridine on aminopyrine demethylase activity *in vitro*. 4-Benzylpyridine and its two positional isomers were dissolved in a minimal volume of dimethyl sulfoxide and diluted with water. The final concentration of dimethyl sulfoxide in the assay medium did not exceed 0.05%. Solutions of the compounds were added to the aminopyrine demethylase assay at the final concentrations indicated. The microsomal suspension used was prepared from phenobarbital-pretreated (80 mg/kg/day for 2 days) male rats, and the cytochrome P450 content of the suspension was 1.591 nmol/mg protein. Each value represents the percent of control aminopyrine demethylase activity (16.479 ± 0.079 nmol/mg protein/min) obtained from three separate determinations.

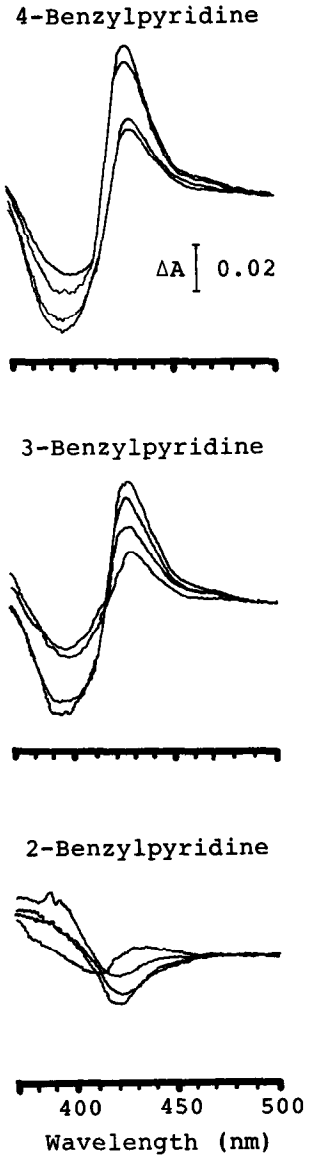

Fig. 5. 4-, 3- and 2-Benzylpyridine-induced difference spectra. Each cuvette contained 3 mL of microsomal suspension (2 mg protein/mL) in phosphate buffer (pH 7.4) from phenobarbital-pretreated (80 mg/kg/day for 2 days) male rats, and the cytochrome P450 content of this suspension was 1.478 nmol/mg protein. The compound solutions were prepared by the method described in the legend of Fig. 4, except that the final concentration of dimethyl sulfoxide in the cuvette did not exceed 0.01%. Spectra were recorded after the addition of a small volume of the compound solution to the sample cuvette and the same volume of solvent to the reference cuvette. The final concentrations of these compounds in the cuvette were as follows: 66 μM; 33 μM; 6 μM and 3 μM (not shown).

24 hr after the final treatment. The results are shown in Fig. 7. Cytochrome P450b/e was induced by 4- and 3-benzylpyridine in both male and female rats (Fig. 7A). The induction of this isozyme by 3-benzylpyridine was more potent in male than in female rats. Cytochrome P450c/d was also induced by 4- and 3-benzylpyridine in male and female rats, whereas 2-benzylpyridine did not induce this isozyme in either sex (Fig. 7B). The magnitudes of induction of these isozymes by these pyridine compounds were

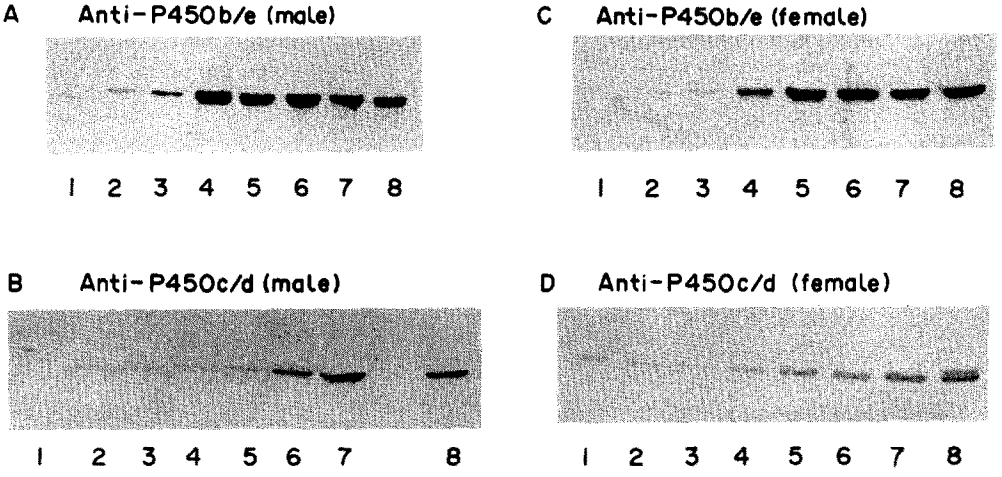

Fig. 6. Immunoblot analysis for cytochrome P450b/e and P450c/d in microsomes from male and female rats treated with various doses of 4-benzylpyridine. Microsomal samples were obtained from male (A) and (B), and female (C) and (D), rats that had been treated with 4-benzylpyridine at doses of 0.05 mmol/kg (lane 2), 0.10 mmol/kg (lane 3), 0.20 mmol/kg (lane 4), 0.40 mmol/kg (lane 5), 0.60 mmol/kg (lane 6) and 0.80 mmol/kg (lane 7) for 2 consecutive days; the rats were killed 24 hr after the final treatment. Lane 1, control; lane 8 in (A) and (C), phenobarbital (80 mg/kg/day for 2 days) treated rats.

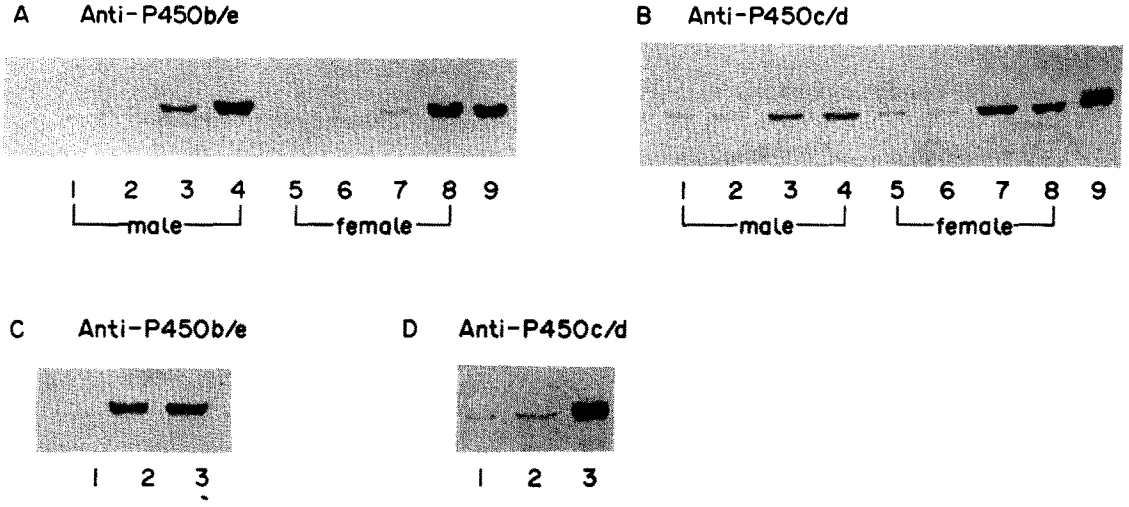

Fig. 7. Immunoblot analysis for cytochrome P450b/e and P450c/d in microsomes from 4-, 3- and 2-benzylpyridine and 4-*tert*-butylpyridine treated rats. Microsomal samples were obtained from rats treated with 4-, 3- and 2-benzylpyridine and 4-*tert*-butylpyridine at a dose of 0.40 mmol/kg for 2 consecutive days; the rats were killed 24 hr after the final treatment. (A) and (B), lanes 1 and 5, control; lanes 2 and 6, 2-benzylpyridine; lanes 3 and 7, 3-benzylpyridine; lanes 4 and 8, 4-benzylpyridine; lane 9 in (A), phenobarbital-treated rats; lane 9 in (B), 3-methylcholanthrene-treated rats. (C) and (D), lane 1, control; lane 2, 4-*tert*-butylpyridine; lane 3 in (C), phenobarbital-treated rats; lane 3 in (D), 3-methylcholanthrene-treated rats.

proportional to the increased amounts of cytochrome P450 content. 4-*tert*-Butylpyridine also induced cytochrome P450b/e and P450c/d in male rats, but the magnitude of cytochrome P450c/d induction was very low (Fig. 7, C and D).

DISCUSSION

The present study has revealed that 4-benzylpyridine is a potent inducer of hepatic microsomal cytochrome P450 and its associated drug-metabolizing enzyme activities in male and female rats. This compound induced cytochrome P450 at doses ranging from 0.05 to 0.80 mmol/kg without any toxic manifestations in either sex (Table 1). Additionally, we have also shown that two positional isomers of 4-benzylpyridine and 4-*tert*-butylpyridine are inducers of cytochrome P450, but to a lesser extent

than 4-benzylpyridine (Fig. 3). Because both benzyl and *tert*-butyl groups are lipophilic, these results suggest that the pyridine compounds which have somewhat bulky lipophilic groups at the 4-position of the ring would be inducers of cytochrome P450. Further, we also found that the magnitude of cytochrome P450 induction was gradually decreased by replacing the position of the benzyl group bound to the pyridine ring in the order of the 4-, 3- and 2-position (Fig. 3). The findings suggest that the magnitude of cytochrome P450 induction by the pyridine-containing compounds would depend on the position of the lipophilic groups bound to the pyridine ring. In contrast to the *in vivo* induction of cytochrome P450 by 4-, 3- and 2-benzylpyridine, the former two compounds, but not the latter, inhibited aminopyrine demethylase activity, depending on the concentrations added to the assay medium (Fig. 4). Further, 4- and 3-benzylpyridine showed typical type II difference spectra, whereas 2-benzylpyridine did not (Fig. 5). When the extent of cytochrome P450 induction was compared with the inhibitory potency or K_s values, these two opposite effects produced by the pyridine compounds seemed to be related to each other. The inhibitory mechanism on drug metabolism is believed to be the result of high-affinity binding of the compound to the heme moiety of the cytochrome P450 molecule [1, 2]. The results of the present study and our previous report [22] may lead to the conclusion that the characteristics of pyridine-containing compounds are similar to those of imidazole-containing compounds with respect to their effects on cytochrome P450 induction and drug-metabolizing enzymes.

The present study has also revealed that, depending on the dose, 4-benzylpyridine induces cytochrome P450b/e and/or P450c/d in male and female rats (Fig. 6). 4-Benzylpyridine induced cytochrome P450b/e in male and female rats at doses ranging from 0.1 to 0.80 mmol/kg, but its ability to maximally induce this isozyme was different in both sexes. At the higher doses, 4-benzylpyridine also induced cytochrome P450c/d in male and female rats. Ritter and Franklin [27] reported similar dose-differentiated induction of cytochrome P450 isozymes, but not cytochrome P450b/e and P450c/d, by clotrimazole which is an imidazole-containing antimycotic. Further, we also observed that the inducing patterns of cytochrome P450b/e and P450c/d by treatment with various doses of 4-benzylpyridine in male rats differed from those of female rats (Fig. 6). Although it is not clear at present whether the phenomena are commonly observable after treatment with heteroaromatic compounds, the findings may provide information on dose- and sex-related differences in the induction of cytochrome P450 isozymes by these compounds.

It has been generally accepted that the induction of cytochrome P450 is controlled at transcriptional and posttranscriptional levels. With respect to cytochrome P450c/d induction, it has been accepted that the binding of an inducer to the Ah receptor is a prerequisite for initiating the induction of the isozyme. Magdalou *et al.* [9] have shown that 1-benzylimidazole induces both cytochrome P450b/e and P450c/d in male rats. They have also shown that 1-benzylimidazole fails to displace benzo[*a*]pyrene from the Ah receptor; however, they suggest that the compound could bind to the receptor with low efficiency, if indeed binding to the receptor is a prerequisite for inducing cytochrome P450. 4-Benzylpyridine was able to induce cytochrome P450c/d. The fact that both 4-benzylpyridine and 1-benzylimidazole have similarities in structure and inducible isozymes suggests that both compounds may induce cytochrome P450c/d by interacting with the Ah receptor even though they have low affinities for this receptor. In this respect, however, further detailed studies will be required.

It has been shown that some cytochrome P450 isozymes are controlled posttranscriptionally by their substrates and hormones. For example, Song *et al.* [28] revealed that cytochrome P450j is stabilized by acetone, which is an inducer of this isozyme. Schuetz *et al.* [29] showed that the half-life of cytochrome P450p was prolonged significantly by treating hepatocytes with γ-chlordane. Further, Eliasson *et al.* [30] revealed that stabilization and protection from destruction of cytochrome P450j were provided by its inducers, imidazole, 2-propanol, ethanol and acetone, whereas these compounds did not have the same effects on cytochrome P450b. With respect to the mechanism for this effect, they also showed that phosphorylation of the cytochrome P450 molecule by some protein kinases regulated by hormones, such as cAMP-dependent protein kinase, is a degradative signal, whereas the cytochrome P450 molecule complexed with its substrate is not phosphorylated by the enzyme in *in vitro* experiments [31]. We found that 4-benzylpyridine and its two positional isomers tended to induce cytochrome P450 content in proportion to their inhibitory potencies *in vitro* on aminopyrine demethylase activity. Further, it may be possible that the increases of cytochrome P450 content induced by 4-benzylpyridine and its two positional isomers were ascribed to the inhibition of cytochrome P450 degradation due to the complex formation. However, there were some inconsistent results excluding this possibility. Namely, cytochrome P450 content was proportionally increased in male and female rats by treatment with 4-benzylpyridine at doses ranging from 0.05 to 0.80 mmol/kg. Cytochrome P450b/e was also increased in a dose-dependent manner up to a dose of 0.20 mmol/kg in males and 0.40 mmol/kg in females, but no further increase of this cytochrome P450 species could be seen at higher doses in either sex (Fig. 6). Further, the dose- and sex-differentiated responses to 4-benzylpyridine were not compatible with the above possibility. In this respect, further study will be required.

In the present study, we observed sex-differentiated induction of cytochrome P450 content and its isoenzymes by 4- and 3-benzylpyridine. The reason for this differentiation may basically depend on sex hormones. Hormones may affect the magnitude of cytochrome P450c/d induction so that it is increased by 4- and 3-benzylpyridine more in female than in male rats. It may also be possible that inducible species of cytochrome P450 by the pyridine compounds are different in both sexes of

rats. Further study is required to clarify this matter.

In conclusion, the present study has revealed that pyridine derivatives with lipophilic groups at the 4- or 3-position of the heteroaromatic ring, such as 4- and 3-benzylpyridine and 4-*tert*-butylpyridine, are inducers of cytochrome P450, with inducing patterns of the enzyme species similar to those observed with imidazole compounds. Furthermore, 4-benzylpyridine induced cytochrome P450 isozymes in a dose- and sex-differentiated manner. Hence, attention should be paid to the dose of a compound and the sex of the experimental animal when using pyridine-containing compounds, such as 4-benzylpyridine, as a cytochrome P450 inducer.

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