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Involvement of testosterone in the induction of hepatic microsomal cytochrome *P*-450 2B1/2 (*P*-450 2B1/2) by 1-benzylimidazole in male and female rats: sex-differentiated induction of *P*-450 2B1/2 species

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Abstract

We examined the effect of 1-benzylimidazole on the induction of cytochrome *P*-450 1A1/2 (*P*-450 1A1/2) and cytochrome *P*-450 2B1/2 (*P*-450 2B1/2) in normal, castrated, ovariectomized and hypophysectomized male rats, and in castrated rats treated with testosterone. 1-Benzylimidazole markedly increased *P*-450 content in male and female rats. Parallell to the dose-dependent increase in *P*-450 content, 1-benzylimidazole produced a significant increase in *P*-450 2B1/2 in male rats, but not in female rats. 1-Benzylimidazole failed to induce *P*-450 2B1/2 in castrated male and ovariectomized female rats. Treatment of castrated male rats with testosterone restored the induction of *P*-450 2B1/2 by 1-benzylimidazole. Treatment of ovariectomized female rats with 1-benzylimidazole or phenobarbital led to the increase in *P*-450 content, accompanying by the induction of *P*-450 2B1/2 by the latter treatment, but not the former. In hypophysectomized male rats, 1-benzylimidazole was able to induce *P*-450 2B1/2 in contrast to castrated male rats. Neonatal male and female rats responded well to the induction of *P*-450 2B1/2 by 1-benzylimidazole. The present findings suggest that *P*-450 2B1/2 induction by 1-benzylimidazole would be coupled with circulating testosterone regulated by hypophysis-testis axis. 1-Benzylimidazole produced sex-differentiated induction of *P*-450 2B1/2 in pubertal rats, but not in neonatal animals. The present findings would be provide information on a unique effect of 1-benzylimidazole on *P*-450 2B1/2 induction in rats.

Key words: Cytochrome *P*-450 2B1/2; 1-Benzylimidazole; Testosterone

1. Introduction

Some imidazole-, triazole- and pyridine-containing compounds have been therapeutically utilized for treating local and/or systemic mycosis. Generally, N-substituted imidazole- or triazole-containing antimycosis, such as clotrimazole and fluconazole, and their structural analogues, such as 1-benzylimidazole and 4-benzylpyridine, have been shown to induce and/or inhibit hepatic microsomal cytochrome *P*-450 (*P*-450) and its associated drug-metabolizing enzyme activities in animals [1–5]. These antifungal agents inhibit ergosterol biosynthesis [6,7] and thus leading to block cell wall integration. Furthermore, it has been also revealed that the N-substituted imidazole-containing compounds inhibit ergosterol synthesis by binding to the substrate binding site of the heme moiety of the *P*-450 molecule. Likewise, the N-substituted imidazole compounds have been shown to be potent inhibitors of *P*-450 presented in various internal organs of experimental animals [8–12].

We have already shown that imidazole and its N-substituted compounds having lipophilic groups are indispensable structural components for the induction of *P*-450 [13]. Additionally, we have revealed that pyridine-containing compounds having some lipophilic groups at 4 or 3 position of pyridine ring are essential for inducing *P*-450 like imidazole-containing compounds [14]. Of pyridine-compounds, 4-benzylpyridine showed dose- and sex-related differential induction of *P*-450 1A1/2 and *P*-450 2B1/2 in male and female rats [14]. Sex-differences in response to imidazole-con-

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taining antimycotic drugs and related compounds have been well documented in rats with respect to magnitudes of *P*-450 induction [4]. However, little information is available for the sex-differentiated induction of *P*-450 species by imidazole-containing compounds. Of imidazole-containing compounds, 1-benzylimidazole, which has similar chemical properties with 4-benzylpyridine, was a minimum structural element of the imidazole derivatives for the induction of *P*-450 [13]. Taking these facts into consideration, we examined whether 1-benzylimidazole produces a sex-differentiated effect on the induction of *P*-450 like 4-benzylpyridine in male and female rats.

In the present study, we employed normal male and female, castrated male, ovariectomized female, hypophysectomized male, and neonatal male and female rats. We found that 1-benzylimidazole clearly showed sex-related differential induction of *P*-450 2B1/2, possibly associating with testosterone levels regulated by hypophysis-testis axis.

2. Materials and methods

2.1. Chemicals

1-Benzylimidazole and 3-methylcholanthrene were purchased from the Aldrich Chemical Co. (Milwaukee, WI, USA). Testosterone and phenobarbital sodium were purchased from Wako Pure Chemical Industries, LTD (Tokyo, Japan). All other chemicals used were of the highest grade commercially available.

2.2. Animals and treatment

Male and female Wistar rats (7 wk of age) were used in this study. They were fed commercial solid diet (MF Oriental Yeast Co, LTD, Tokyo, Japan) and water ad libitum. Neonatal male and female rats were purchased at day 21 after birth and also used in this study. 1-Benzylimidazole dissolved or suspended in an appropriate volume of corn oil and injected intraperitoneally into rats at doses of 0.05 to 0.40 mmol/kg. Some of the castrated male and ovariectomized female rats were left 10 days with free access to food and water after the surgical operation, then given a subcutaneous injection of testosterone (in corn oil, 1 ml/kg) at the dose of 20 mg/kg once a day for 5 days. Some of the castrated male rats treated with testosterone, starting on the day 15 after the castration, were received by 1-benzylimidazole at the dose of 0.30 mmol/kg. Hypophysectomized male rats were kept for 10 days with free access to food and water after the operation and then given an intraperitoneal injection of 1-benzylimidazole at the dose of 0.30 mmol/kg. Control and sham-operated rats were injected intraperitoneally or subcutaneously with the vehicle only. All animals were kept in a light-, temperature- and moisture-controlled room in wire-bottomed cages and were fasted for 24 h before being killed.

2.3. Tissue preparation

All rats were killed by decapitation and their livers were perfused in situ with 0.9% NaCl solution. The liver was homogenized with 5 volumes of 1.15% KCl using a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at $9000 \times g$ for 15 min and the resulting supernatant was ultracentrifuged at $105\,000 \times g$ for 1 h. The resulting microsomal pellet was suspended with 0.1 M phosphate buffer (pH 7.4) and used for determining *P*-450 content, aminopyrine demethylase, aniline hydroxylase, dimethylnitrosamine demethylase activities and immunoblot analysis.

2.4. Enzyme assay

The *P*-450 content was determined by CO difference spectrum of dithionate-treated microsomes as described by Omura and Sato [15], except that microsomes had been bubbled for 5 min with CO gas as described by Kahl et al. [16].

2.5. Electrophoresis and immunoblot analysis

Microsomal proteins obtained from normal and surgically operated control and 1-benzylimidazole-treated male and female rats were solubilized in sodium dodecyl sulfate and resolved by polyacrylamide gel electrophoresis according to the method of Laemmli [17], and then transferred to nitrocellulose membrane. Antigenic components reactive with APF3 and APL2 monoclonal antibodies raised against *P*-450 2B1 and *P*-450 1A1, which were also reactive to *P*-450 2B2 and *P*-450 1A2, respectively, were visualized with 4-chloro-1-naphthol in the presence of 0.006% hydrogen peroxide and peroxidase-labelled anti-mouse IgG in 0.05 M phosphate buffer (pH 7.4). These monoclonal antibodies were kindly donated from Drs. T. Masuko and Y. Hashimoto, Pharmaceutical Institute, Tohoku University, Sendai, Miyagi, Japan [18].

3. Results

3.1. Effect of 1-benzylimidazole on the induction of P-450 and its associated drug-metabolizing enzyme activities in male and female rats

Firstly, we investigated dose- and time-dependent alterations of the induction of *P*-450 and its associated

Table 1
Dose-response effect of 1-benzylimidazole on cytochrome *P*-450 content in male and female rats
Rats were treated i.p. with 1-benzylimidazole at the doses indicated and the animals were killed 24 hr after the treatment. Each value is the mean ± S.D. of four rats

Dose (mmol/kg)	Cytochrome *P*-450 (nmol/mg protein)	
	Male	Female
Control	0.728 ± 0.036	0.555 ± 0.061
0.05	0.753 ± 0.027	0.814 ± 0.017 [a]
0.10	1.204 ± 0.017 [b]	0.910 ± 0.075 [a]
0.20	1.537 ± 0.036 [b]	1.237 ± 0.037 [b]
0.40	1.797 ± 0.048 [b]	1.248 ± 0.006 [b]

[a,b] Significantly different from control groups;
[a] $P < 0.01$ and [b] $P < 0.001$.

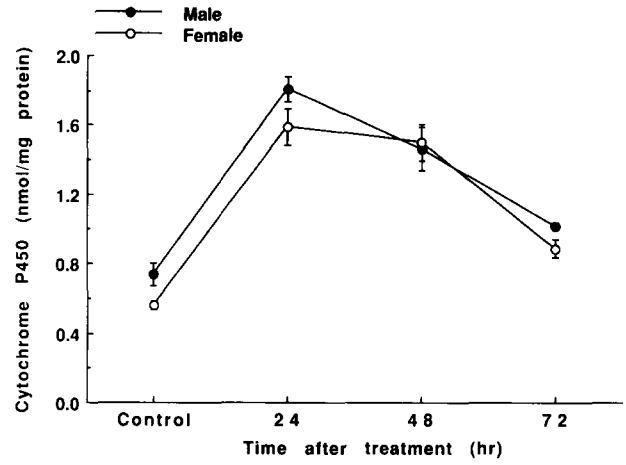

Fig. 1. Time-course of the effect of 1-benzylimidazole on hepatic microsomal *P*-450 content in male and female rats. Rats were treated i.p. with 1-benzylimidazole at the dose of 0.30 mmol/kg and were killed at the time indicated. Control rats were treated i.p. with an appropriate volume of the vehicle only. All rats were fasted for 24 h before being killed. Each value is the mean ± S.D. of four rats.

drug-metabolizing enzyme activities by 1-benzylimidazole in rats. 1-Benzylimidazole increased *P*-450 content even at the lowest dose employed (0.05 mmol/kg) in male and female rats when determined 24 h after the treatment and it produced dose-dependently the increase of the hemoprotein in both sexes (Table 1). Aminopyrine demethylase, aniline hydroxylase and dimethylnitrosamine demethylase activities were also increased proportionally with the increase of *P*-450 content in both sexes (data not shown). Then, we also examined time-dependent alteration of the induction of *P*-450 by 1-benzylimidazole in rats. As shown in Fig. 1, the administration of 1-benzylimidazole at the dose of 0.30 mmol/kg resulted in a marked increase of *P*-450 at 24 h in male rats (1.803 ± 0.072 nmol/mg protein). At 72 h after 1-benzylimidazole treatment, *P*-450 content had returned to the control levels (Fig. 1). Similarly, in female rats, *P*-450 content was increased to about 2.4 times of the controls at 24 h after a single administration of 1-benzylimidazole, remained this increased level up to 48 h, and returned to the control levels by 72 h (Fig. 1).

3.2. Induction of *P*-450 1A1/2 and *P*-450 2B1/2 by 1-benzylimidazole in male and female rats

Magdalou et al. [19] have already shown that 1-benzylimidazole induces two major *P*-450 species, *P*-450 2B1/2 and *P*-450 1A1/2, in male rats. However, it is not clear whether 1-benzylimidazole is able to induce *P*-450 1A1/2 and *P*-450 2B1/2 in female rats. Therefore, we examined the effect of 1-benzylimidazole on the induction of *P*-450 1A1/2 and *P*-450 2B1/2 in male and female rats. Fig. 2 shows immunoblot analysis

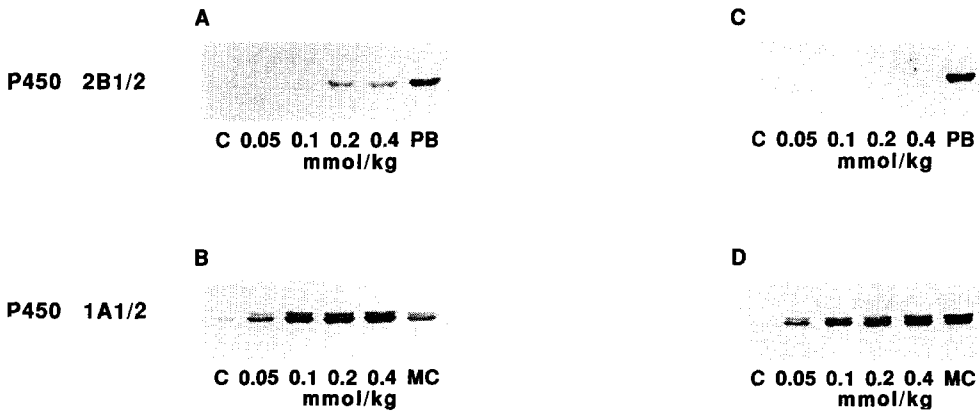

Fig. 2. Immunoblot analysis for *P*-450 1A1/2 and *P*-450 2B1/2 in microsomes from male and female rats treated with various doses of 1-benzylimidazole. Microsomal samples were obtained from male (A) and (B), and female (C) and (D) rats that had been treated with 1-benzylimidazole at doses of 0.05 to 0.40 mmol/kg; the rats were killed 24 h after the treatment. C; control, PB; phenobarbital sodium (80 mg/kg/day for 2 days), MC; 3-methylcholanthrene (30 mg/kg/day for 2 days) treated rats. Each numeral indicates the dose of 1-benzylimidazole.

for *P*-450 1A1/2 and *P*-450 2B1/2 in male and female rats treated with 1-benzylimidazole at doses of 0.05 to 0.40 mmol/kg. As shown in Fig. 2A, 1-benzylimidazole induced *P*-450 2B1/2 at doses ranging from 0.20 to 0.40 mmol/kg, with a maximum induction of this isozyme at the dose of 0.40 mmol/kg in male rats. 1-Benzylimidazole also induced *P*-450 1A1/2 (Fig. 2B). At the lowest dose of 0.05 mmol/kg, 1-benzylimidazole was able to induce *P*-450 1A1/2 without the induction of *P*-450 2B1/2.

These results indicate that 1-benzylimidazole produces dose-differentiated induction of *P*-450 1A1/2 and *P*-450 2B1/2 in the liver of rats, with the former *P*-450 species being more susceptible than the latter.

Fig. 2 also illustrates the dose-response effect of 1-benzylimidazole on *P*-450 1A1/2 and *P*-450 2B1/2 induction in female rats. Of interest was that 1-benzylimidazole failed to induce *P*-450 2B1/2 in female rats even at the highest dose (0.40 mmol/kg) employed (Fig. 2C), irrespect of the marked increase in *P*-450 content (Table 1). 1-Benzylimidazole dose-dependently induced *P*-450 1A1/2, with a maximum induction of this *P*-450 species at the dose of 0.40 mmol/kg (Fig. 2D).

3.3. Induction of P-450 1A1/2 and P-450 2B1/2 by 1-benzylimidazole in castrated male and ovariectomized female rats

In order to ascertain a possible sex-differentiated isozyme induction, we examined the effect of 1-benzylimidazole on *P*-450 1A1/2 and *P*-450 2B1/2 induction in castrated male and ovariectomized female rats. Fig. 3 shows the inductive effect of 1-benzylimidazole on *P*-450 1A1/2 and *P*-450 2B1/2 in normal male, female, castrated male and ovariectomized female rats. As shown in Fig. 3A, 1-benzylimidazole induced *P*-450 2B1/2 in all normal male rats. Interestingly, 1-benzylimidazole could not increase *P*-450 2B1/2 in all castrated male rats (Fig. 3A). Phenobarbital, a prototype inducer of *P*-450 2B1/2, did induce this *P*-450 species in all castrated male rats in contrast to the 1-benzylimidazole-treated animals (Fig. 4). 1-Benzylimidazole induced *P*-450 1A1/2 in both normal male, castrated male, normal female and ovariectomized female rats at the dose examined without any changes in the induced levels (Figs. 3C and 3D). However, 1-benzylimidazole again failed to induce *P*-450 2B1/2 in ovariectomized female rats (Fig. 3B).

3.4. Effect of testosterone on the induction of P-450 2B1/2 by 1-benzylimidazole in rats

To investigate further a detailed relationship between *P*-450 2B1/2 induction by 1-benzylimidazole and its mediation by sex hormone, we examined the effect of testosterone on the induction of *P*-450 2B1/2 by employing castrated male rats. Fig. 5 shows the effect of testosterone on the induction of *P*-450 2B1/2 by 1-benzylimidazole in castrated male rats. As shown in Fig. 5A, testosterone (20 mg/kg, once a day for 5 days) could not induce *P*-450 2B1/2. Of particular interest was that the *P*-450 2B1/2 induction by 1-benzylimidazole was restored to normal levels in castrated male rats treated with testosterone (Fig. 5B). On the other hand, the ability of 1-benzylimidazole to induce *P*-450 1A1/2 was not changed in all castrated male rats treated with testosterone at the same dose employed (data not shown).

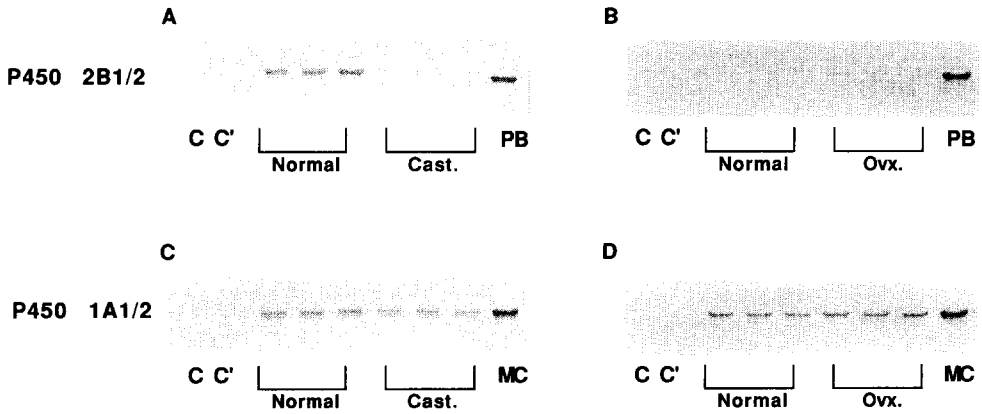

Fig. 3. Immunoblot analysis for *P*-450 1A1/2 and *P*-450 2B1/2 in microsomes from 1-benzylimidazole-treated normal male and female, castrated male and ovariectomized female rats. Microsomal samples were obtained from rats treated with 1-benzylimidazole at the dose of 0.30 mmol/kg; the rats were killed 24 h after the treatment. C; control, C'; sham-operated control, Normal; normal male and female rats, Cast; castrated male rats, Ovx; ovariectomized female rats. Microsomal samples obtained from three rats were immunoblotted individually in the case of normal, castrated and ovariectomized animals. Experimental details were identical to those described in the legend of Fig. 2.

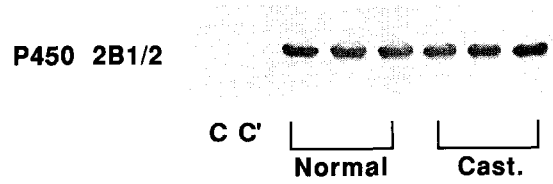

Fig. 4. Immunoblot analysis for *P*-450 2B1/2 in microsomes from phenobarbital-treated normal and castrated male rats. Microsomal samples were obtained from normal and castrated male rats treated with phenobarbital at the dose of 80 mg/kg for 2 consecutive days. Experimental details were identical to those described in the legend of Fig. 3.

3.5. Effect of ovariectomy on the induction of P-450 2B1 / 2 by 1-benzylimidazole in female rats

Some of the ovariectomized female rats were given intraperitoneal injection of both 1-benzylimidazole (0.20 mmol/kg) and phenobarbital (0.20 mmol/kg), respectively, for two consecutive days. As shown in Fig. 6A, the administration of both 1-benzylimidazole and phenobarbital also induced *P*-450 2B1/2 in normal and ovariectomized female rats. Fig. 6B also illustrates that phenobarbital alone (80 mg/kg, twice) led to the induction of *P*-450 2B1/2 in all ovariectomized female

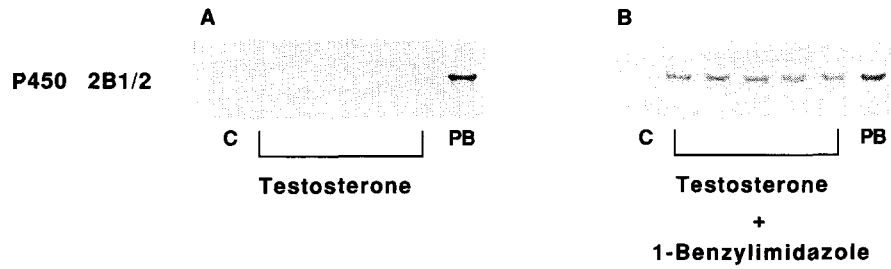

Fig. 5. Immunoblot analysis for *P*-450 2B1/2 in microsomes from testosterone- and 1-benzylimidazole-treated castrated male rats. Microsomal samples were obtained from castrated male rats treated with testosterone at the dose of 20 mg/kg for 5 consecutive days and with 1-benzylimidazole at the dose of 0.30 mmol/kg. The *P*-450 contents of testosterone- and testosterone plus 1-benzylimidazole-treated castrated rats were 0.778 ± 0.020 nmol/mg protein and 1.881 ± 0.053 nmol/mg protein, resprctively. Each value is the mean ± S.D. of five rats. Experimental details were identical to those described in the legend of Fig. 2.

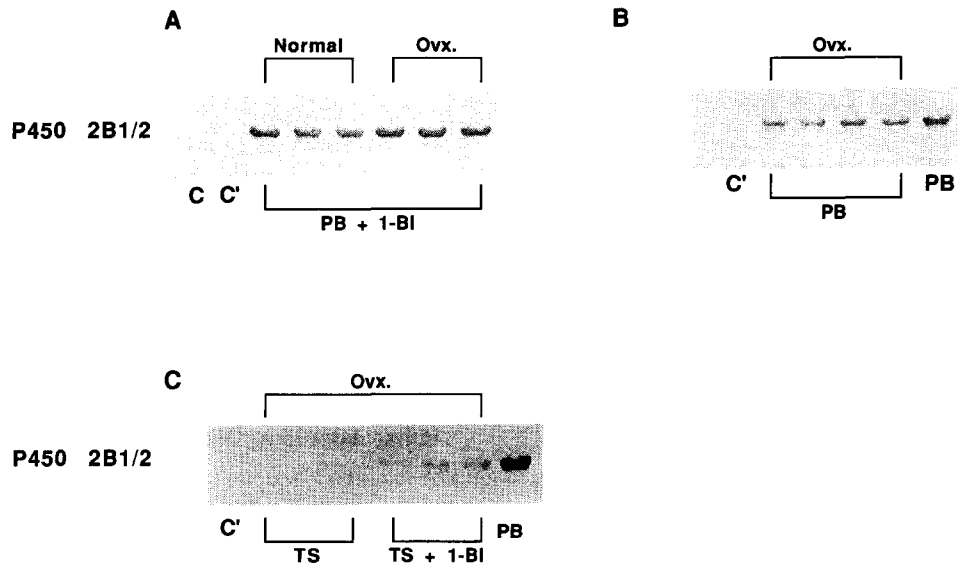

Fig. 6. Immunoblot analysis for *P*-450 2B1/2 in microsomes from normal and ovariectomized female rats treated with various conditions. Microsomal samples were obtained from normal and ovariectomized female rats. (A); rats were treated with phenobarbital (PB, 0.20 mmol/kg per day for 2 days) plus 1-benzylimidazole (1-BI, 0.20 mmol/kg/day for 2 days) in normal and ovariectomized female rats. (B); ovariectomized female rats were treated with PB at the dose of 80 mg/kg for 2 consecutive days. (C); rats were treated with testosterone (TS, 20 mg/kg/day for 5 days) alone and TS (20 mg/kg/day for 5 days) plus 1-BI (0.30 mmol/kg). The *P*-450 contents of normal and ovariectomized female rats treated with PB plus 1-BI shown in (A) were 2.217 ± 0.041 nmol/mg protein and 2.089 ± 0.056 nmol/mg protein, respectively, and those of ovariectomized female rats treated with phenobarbital shown in (B) were 1.313 ± 0.049 nmol/mg protein and those of ovariectomized female rats treated with testosterone alone and testosterone plus 1-benzylimidazole shown in (C) were 0.993 ± 0.061 nmol/mg protein and 1.210 ± 0.077 nmol/mg protein, respectively. These values are the mean ± S.D. of three or four rats. Experimental details were identical to those described in the legend of Figs. 2 and 3.

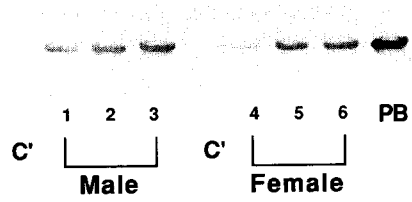

Fig. 7. Dose-dependent effect of testosterone on the induction of *P*-450 2B1/2 by 1-benzylimidazole in castrated male and ovariectomized female rats. Microsomal samples were obtained from castrated male and ovariectomized female rats treated with testosterone at the doses ranged from 1, 10 and 40 mg/kg for 5 consecutive days. 1-Benzylimidazole was injected to castrated male and ovariectomized female rats at the dose of 0.30 mmol/kg. The *P*-450 contents of castrated male rats treated with 1-benzylimidazole were 1.597±0.094 nmol/mg protein, 1.696±0.113 nmol/mg protein and 1.867±0.102 nmol/mg protein, at doses of 1 (lane 1), 10 (lane 2) and 40 (lane 3) mg/kg, respectively, and those of ovariectomized female rats treated with the compound were 1.293±0.061 nmol/mg protein, 1.363±0.079 nmol/mg protein and 1.515±0.102 nmol/mg protein, at doses of 1 (lane 4), 10 (lane 5) and 40 (lane 6) mg/kg, respectively. These values are the mean±S.D. of three rats. Experimental details were identical to those described in the legend of Figs. 2 and 3.

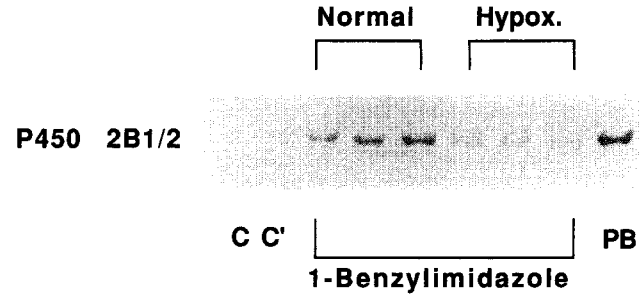

Fig. 8. Immunoblot analysis for *P*-450 2B1/2 in microsomes from normal and hypophysectomized male rats treated with 1-benzylimidazole. Microsomal samples were obtained from normal and hypophysectomized male rats treated with 1-benzylimidazole at the dose of 0.30 mmol/kg. The *P*-450 contents of normal and hypophysectomized male rats treated with 1-benzylimidazole were 1.958±0.098 nmol/mg protein and 1.373±0.112 nmol/mg protein, respectively. These values are the mean±S.D. of three rats. Hypox; hypophysectomized male rats. Experimental details were identical to those described in the legend of Figs. 2 and 3.

rats. Since, there was no inhibition of phenobarbital-mediated induction of *P*-450 2B1/2 by 1-benzylimidazole, both compounds could induce *P*-450 2B1/2 in a different manner.

As shown in Fig. 6C, simultaneous injection of testosterone and 1-benzylimidazole produced the induction of *P*-450 2B1/2 in ovariectomized female rats, but not by separate treatment (Figs. 3B and 6C).

3.6. Dose-dependent effect of testosterone on the induction of P-450 2B1/2 by 1-benzylimidazole in castrated male and ovariectomized female rats

As previously shown in Fig. 5, the induction of *P*-450 2B1/2 by 1-benzylimidazole seemed to require a presence of testosterone in castrated rats. If testosterone is necessary for the induction of *P*-450 2B1/2 by 1-benzylimidazole treatment, the inducible levels of this species might be changed by the increasing doses of testosterone. Fig. 7 shows dose-dependent effect of testosterone on the induction of *P*-450 2B1/2 by 1-benzylimidazole in castrated male and ovariectomized female rats. As expected, *P*-450 2B1/2 induction by 1-benzylimidazole was proportionally occured with the increasing dose of testosterone in both castrated male and ovariectomized female rats. In addition, the magnitudes of the increase in *P*-450 content produced by 1-benzylimidazole tended to augment with the increasing dose of testosterone as described in the legend.

3.7. Induction of P-450 2B1/2 by 1-benzylimidazole in hypophysectomized male rats

In general, growth hormone secretion is regulated by the pituitary gland, and this hormone stimulates testosterone secretion. Therefore, we used hypophysectomized male rats in order to clarify a regulatory mechanism(s) involved in the induction of *P*-450 2B1/2 by 1-benzylimidazole. As shown in Fig. 8, 1-benzylimidazole induced *P*-450 2B1/2 in hypophysectomized male rats, but to a lesser extent than that seen in normal male rats. Likewise, the magnitude of *P*-450 content increased after the administration of 1-benzylimidazole was to less extensive in hypophysectomized rats than that seen in normal animals as described in the legend.

3.8. Effect of 1-benzylimidazole on the induction of P-450 2B1/2 in neonatal male and female rats

Finally, we examined an ability of 1-benzylimidazole to induce *P*-450 2B1/2 in neonatal male and female rats. 1-Benzylimidazole injected to neonatal male and female rats at the dose of 0.30 mmol/kg. Of particular interest was that 1-benzylimidazole was able to induce *P*-450 2B1/2 in neonatal male and female rats to similar extents to that seen in male adult animals (Fig. 9).

4. Discussion

The present study has dealt with sex-differentiated isozyme induction of hepatic microsomal *P*-450 species by 1-benzylimidazole, especially *P*-450 2B1/2, in male and female rats. We found dose-dependent increase in *P*-450 content after the injection of 1-benzylimidazole at doses of 0.05 to 0.40 mmol/kg in male and female rats without any toxic manifestation (Table 1). Dose-

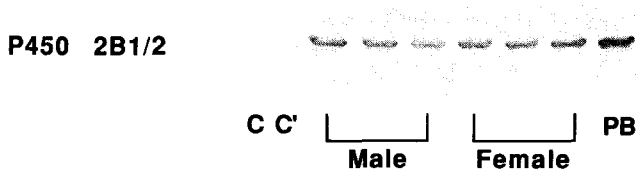

Fig. 9. Immunoblot analysis for *P*-450 2B1/2 in microsomes from neonatal male and female rats. Ages of neonatal male and female rats were 21 days after birth. Microsomal samples were obtained from neonatal male and female rats treated with 1-benzylimidazole at the dose of 0.30 mmol/kg. The contents of *P*-450 in control and 1-benzylimidazole-treated male and female rats were 0.873 ± 0.044 nmol/mg protein and 0.733 ± 0.019 nmol/mg protein, and 1.813 ± 0.049 nmol/mg protein and 1.652 ± 0.067 nmol/mg protein, respectively. These values are the mean $\pm$ S.D. of three rats. C; neonatal male control, C'; neonatal female control. Experimental details were identical to those described in the legend of Fig. 2.

dependent increase in *P*-450 of rats treated with 1-benzylimidazole was in goood agreement with those reported by Papac and Franklin [20]. Magdalou et al. [19] have shown that 1-benzylimidazole induces both *P*-450 1A1/2 and *P*-450 2B1/2 in male rats at doses of 25, 75 and 100 mg/kg for five consecutive days. However, they did not observe the induction of *P*-450 1A1/2 and *P*-450 2B1/2 in female rats. Of interest was that 1-benzylimidazole failed to induce *P*-450 2B1/2 at the every doses employed in normal female rats (Fig. 2C), while it was able to induce *P*-450 1A1/2 in both sexes in a similar manner (Figs. 2B and 2D). All of these findings suggest that there exists sex-related differential induction of *P*-450 species by 1-benzylimidazole, male rats being responsible to this compound with respect to *P*-450 2B1/2 induction, but not females. Because of the lack of antibodies for other *P*-450 species in our laboratory, we could not examine whether there exists sex-differential induction of other *P*-450 species in male and female rats. So far suspected from the increased dimethylnitrosamine demethylase activity (data not shown), 1-benzylimidazole might be able to induce *P*-450 2E1 in male and female rats, as reported by various imidazole-containing compounds [21,22].

In order to elucidate further this sex-differentiated isozyme induction by 1-benzylimidazole, we employed castrated male and ovariectomized female rats. Of interest was that all castrated male rats resulted in a dissapearance of the induction of *P*-450 2B1/2 by 1-benzylimidazole (Fig. 3A), but not by phenobarbital as shown in Fig. 4. These results and the findings with female rats suggest that the induction of *P*-450 2B1/2 by 1-benzylimidazole would be mediated by sex hormone(s), such as testosterone.

On this hypothesis, we examined further the effect of testosterone on the induction of *P*-450 2B1/2 by 1-benzylimidazole. The administration of testosterone to normal male rats did not cause any increase in *P*-450 2B1/2 (Kobayashi, Y. and Yoshida, T., unpublished data). Irrespective of the lack of exogenous testosterone to induce *P*-450 2B1/2 in normal rats, pretreatment of castrated male rats with this hormone led to the restoration of 1-benzylimidazole-mediated induction of this *P*-450 species (Figs. 5A and 5B). Likewise, simultaneous administration of 1-benzylimidazole and testosterone resulted in the slight induction of *P*-450 2B1/2 in ovariectomized female rats (Fig. 6C). Additionally, the ability of 1-benzylimidazole to induce *P*-450 2B1/2 was dependent on the dose of testosterone when examined in castrated male and ovariectomized female rats (Fig. 7). Furthermore, treatment of normal male rats with estradiol benzoate did not inhibit the induction of *P*-450 2B1/2 by 1-benzylimidazole (Kobayashi, Y. and Yoshida, T., unpublished data). All of these findings suggest that *P*-450 2B1/2 induction by 1-benzylimidazole would be mediated through the presence of testosterone; however, it is not clear how this hormone involved in the induction of *P*-450 2B1/2. In this respect, further detailed study will be required.

In contrast to 1-benzylimidazole, phenobarbital alone or together with 1-benzylimidazole induced *P*-450 2B1/2 in normal and ovariectomized female rats (Figs. 6A, 6B and 6C).

It is generally accepted that secretion pattern of growth hormone is regulated by pituitary gland. In addition, secretion of testosterone is regulated by growth hormone. Kamataki et al. [23,24] and other investigators [25–27] have shown that the induction of *P*-450 species, such as *P*-450 2C11 (*P*-450-male) and *P*-450 2C12 (*P*-450-female), are regulated by sex steroid. Based on these findings, we employed hypophysectomized male rats. We found that 1-benzylimidazole induced *P*-450 2B1/2, but to a lesser extent to that seen in normal male rats (Fig. 8). These results suggest that induction of *P*-450 by 1-benzylimidazole would be interrelated to pituitary-testis axis yet unknown manner. Thus, our present findings would provide information on a unique ability of 1-benzylimidazole to induce *P*-450 2B1/2 with respect to sex hormone dependency.

It has been well known that the expression of various *P*-450 species can be regulated by pituitary growth hormone and sex steroid hormones [23–28]. Therefore, pituitary growth hormone and liver somatogenic receptors have been shown to play key roles for the expression and maintenance of rat hepatic *P*-450s. Further, Yamazoe et al. [29,30] have shown that there are ontogenic differences in hepatic *P*-450 species regulating from developmental and functional differences in the growth hormone-somatogenic receptor system. On the basis of these findings together with the inducibility of *P*-450s, Kato et al. [31] have divided *P*-450s into the two major classes, neonatal type and pubertal type. The *P*-450 species concerned with the present study is

classified into neonatal type. Thus, we examined the effect of 1-benzylimidazole on *P*-450 2B1/2 induction in neonatal male and female rats (Fig. 9). We found that 1-benzylimidazole could induce *P*-450 2B1/2 in neonatal male and female rats. Loss of the sex-dependent induction of *P*-450 2B1/2 by 1-benzylimidazole in neonatal rats suggest that this compound could induce *P*-450 2B1/2 through somatogenic type receptor, which may be coupled to a physiological activity of testosterone in pubertal animals. Such a possible requirement for a presence of endogenous testosterone in the induction of *P*-450 2B1/2 by 1-benzylimidazole could lead to a proposal of a novel mechanism for *P*-450 induction by a chemical. Therefore, further detailed studies will be required as to whether possible involvement of testosterone in *P*-450 2B1/2 induction is only the case of 1-benzylimidazole or this phenomenon could be cases of various chemicals which produce sex-differentiated induction of the hemoprotein.

In conclusion, we have revealed that 1-benzylimidazole induces *P*-450 2B1/2 in sex-differentiated manner in pubertal male and female rats. We have also shown that testosterone plays an important role in the induction of *P*-450 2B1/2 by 1-benzylimidazole in male rats. In this respect, this study provides information on a novel effect of 1-benzylimidazole on *P*-450 2B1/2 induction.

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