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Inhibition and induction of cytochrome P450 2B1 in rat liver by promazine and chlorpromazine

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Abstract—Phenothiazine tranquilizers have been associated with pharmacokinetic drug interactions in man. In this study the *in vivo* and *in vitro* effects of the clinically important phenothiazines promazine (PZ) and chlorpromazine (CPZ) on drug oxidations catalysed by specific cytochrome P450 (P450) enzymes were investigated in the rat. *In vitro*, the two drugs were relatively ineffective inhibitors of constitutive P450 activities, but were inhibitory toward the principal phenobarbital-inducible P450 2B1 and, to a lesser extent, P450 1A1. Administration of PZ and CPZ to male rats did not markedly influence the total microsomal P450 content of the liver. However, the quantitatively important male-specific P450 2C11 was down-regulated by CPZ and concomitant induction of P450 2B1 and associated 7-pentylresorufin *O*-dependentylase activity were noted. A small increase in the activity of microsomal 7-ethylresorufin *O*-deethylase was also observed following administration of both drugs to rats, suggesting induction of P450 1A1/2. Considered together, it is apparent that the two phenothiazines are preferential inhibitors and inducers of P450 2B1 in rat liver. Drug interactions in humans involving phenothiazines may reflect a combined effect of induction and inhibition processes as well as down-regulation of other P450s, such as that produced by CPZ on P450 2C11.

Numerous endogenous and exogenous lipophilic substances are converted to polar metabolites by the hepatic microsomal cytochrome P450 (P450†) superfamily of enzymes. Certain P450s are inducible by exposure to foreign compounds *in vivo*. Thus, in the male rat, the sedative–hypnotic phenobarbital (PB) increases the microsomal expression of the P450s 2B1, 2B2, 2C6, 3A1 and 3A2 and decreases the hepatic content of P450 2C11 [1, 2]. Induction processes may lead to drug interactions with co-administered drugs as a result of the enhanced oxidation capacity of the liver. However, perhaps the most common underlying mechanism of drug interactions involves inhibition of specific P450 reactions. Thus, many lipophilic chemicals interact with P450 enzymes to inhibit the biotransformation of coadministered drugs and cause the accumulation of these drugs in the body.

Phenothiazine neuroleptics have been associated with modulation of P450 activity; it has been suggested that these drugs elicit drug interactions by either P450 induction or inhibition [3–6]. The present study was undertaken to clarify the relationship between the *in vivo* and *in vitro* effects of the clinically important phenothiazines, promazine (PZ) and chlorpromazine (CPZ), on hepatic P450 in the rat. Thus, the relative importance of induction and inhibition of specific P450s was assessed directly by use of P450 specific substrates and protein immunoquantitation.

Materials and Methods

Chemicals. PZ HCl, CPZ HCl and biochemicals were from the Sigma Chemical Co. (St Louis, MO, U.S.A.). [4-^{14}C]Testosterone (sp. act. 54.5 mCi/mmol), ACSII scintillant and Hyperfilm MP for autoradiography were purchased from Amersham Australia (Sydney, NSW, Australia). [4-^{14}C]Progesterone (sp. act. 60 mCi/mmol) was obtained from New England Nuclear (Sydney, NSW, Australia). Reagents for electrophoresis and DEAE-Affigel Blue were obtained from Bio-Rad Laboratories and nitrocellulose sheets were from Hoefer Scientific Instruments (San Francisco, CA, U.S.A.). 7-Pentyl- and 7-ethylresorufin were purchased from Pierce Chemicals (Rockford, IL, U.S.A.) and resorufin was from Aldrich (Milwaukee, WI, U.S.A.). Solvents and other analytical reagents were obtained from Ajax Chemicals (Sydney, NSW, Australia).

Animals. Adult male Wistar rats were used in this study and received either PZ [0.25 mmol (80 mg)/kg i.p], CPZ [0.25 mmol (89 mg)/kg i.p], β-naphthoflavone (βNF; 40 mg/kg i.p) or PB (100 mg/kg i.p) on three consecutive days. Animals were killed 48 hr after the last dose of inducer and hepatic microsomal fractions were prepared by differential ultracentrifugation. After resuspension in 50 mM potassium phosphate buffer, pH 7.4, containing 20% glycerol and 1 mM EDTA, the microsomal fractions were stored at −70° until required in experiments.

Microsomal oxidase assays. Testosterone and progesterone hydroxylase activities were determined as before [7] except that 0.15 mg of microsomal protein was used in the incubations. Separation of testosterone metabolites was effected by TLC and sequential development in dichloromethane: acetone (4:1) followed by chloroform: ethyl acetate: ethanol (4:1:0.7) [8]. Progesterone metabolites were separated by TLC in the system toluene: ethyl acetate: acetone (10:1:1; three developments) [9]. TLC plates were autoradiographed for 48–72 hr and radioactive zones corresponding to authentic metabolites were located and transferred to vials for β-counting (ACSII scintillant). 7-Ethyl- and 7-pentylresorufin *O*-dealkylations were monitored by the procedure of Prough *et al.* [10] as described previously [6].

P450s and immunoquantitation. The isolation of the P450s 2B1 and 2C6 from PB-induced rat hepatic microsomes has been described previously [11, 12] and P450 2C11 was purified from untreated adult male rat liver [13]. Antisera to the P450s were raised in rabbit and IgG fractions were obtained after chromatography on DEAE-affigel blue [13]. SDS–PAGE of hepatic microsomes from differently pretreated rats was conducted as described previously [14]. Electrophoretic transfer of microsomal proteins to nitrocellulose followed by immunochemical blotting was performed as described by Towbin *et al.* [15]. A combination of peroxidase- and ^{125}I-labelled donkey-anti-rabbit IgG was used as the secondary antibody preparation [13]. Radioactivity localized in immunoblots was detected by autoradiography (12–24 hr) and quantitated by laser densitometry.

† Abbreviations: βNF, β-naphthoflavone; CPZ, chlorpromazine; P450, cytochrome P450; PB, phenobarbital; PZ, promazine.

Table 1. Inhibition of P450 reactions by CPZ and PZ in rat liver microsomes *in vitro*

Drug concentration (μM)	P450 Reaction (% of control activity) 7-Pentylresorufin O-dependentylation	7-Ethylresorufin O-deethylation	Testosterone hydroxylation AD*	2α	6β	7α	16α	Progesterone hydroxylation 2α	6β	21
PZ										
1	60	—†	102	94	82	94	115	91	90	93
2.5	47	—	—	—	—	—	—	—	—	—
25	4	72	—	—	—	—	—	—	—	—
50	—	62	82	63	80	99	87	70	89	90
100	—	45	71	56	77	44	76	34	87	106
CPZ										
1	80	—	89	75	84	66	116	98	80	128
5	26	72	—	—	—	—	—	—	—	—
25	0‡	16	—	—	—	—	—	—	—	—
50	—	5	74	59	70	50	78	67	66	116
100	—	—	57	39	58	41	51	54	71	92

Data are means of 2–3 separate estimates obtained in untreated (testosterone and progesterone hydroxylations), PB-induced (7-pentylresorufin O-dependentylation) or βNF-induced (7-ethoxyresorufin O-deethylation) rat hepatic microsomes. Mean variations from the stated average values were 3% for the O-dealkylations and 8% for steroid hydroxylations.

*AD, androst-4-ene-3,17-dione.

†Indicates that inhibition was not assessed at this concentration.

‡Inhibition essentially complete at this concentration.

Table 2. Effect of *in vivo* administration of PZ and CPZ on the microsomal content of cytochromes P450

Treatment	Holocytochrome P450	P450 2B1	P450 2C11 (nmol/mg protein)	P450 2C6
None (control)	1.33 ± 0.12	0.021 ± 0.004	0.47 ± 0.07	0.29 ± 0.16
PZ	1.29 ± 0.18	0.145 ± 0.015*	0.39 ± 0.14	0.30 ± 0.08
CPZ	1.15 ± 0.09	0.210 ± 0.040*	0.18 ± 0.048*	0.21 ± 0.03

Values are means ± SD of five individual observations (holocytochrome P450 data) or three individual observations (P450 2B1, 2C11 or 2C6 content) per group.

Significant difference from control: *$P < 0.01$.

Results

In vitro *effects of CPZ and PZ on microsomal drug oxidation.* The two phenothiazine tranquilizers were found to be effective inhibitors of 7-pentylresorufin *O*-dependentylase from PB-induced rat liver microsomes (Table 1). Thus, 1 μM PZ and CPZ inhibited the P450 2B1-mediated activity by 40 and 20%, respectively, and even more substantial inhibition was observed at higher concentrations of the drugs. P450 1A1-dependent 7-ethylresorufin *O*-deethylation in βNF-induced microsomes was somewhat less susceptible to inhibition. As shown in Table 1, 25 μM PZ and CPZ elicited 28 and 84% inhibition of this activity. Studies of regioselective testosterone and progesterone hydroxylation in hepatic microsomes from untreated male rats, however, indicated that the constitutive P450s active in these pathways were considerably less susceptible to inhibition than either P450 2B1 or P450 1A1 (Table 1). Thus, 10 μM PZ and CPZ elicited less than 25% inhibition of several pathways of testosterone and progesterone hydroxylation. At 100 μM concentrations, PZ produced 40–50% inhibition of steroid 2α- and 7α-hydroxylation (catalysed principally by P450 2C11 and 2A1, respectively [8]). CPZ was somewhat more effective than PZ against all reactions but progesterone 21-hydroxylation activity (usually attributed to P450 2C6 [16]) was refractory to inhibition.

In vivo *effects of phenothiazine tranquilizers on hepatic drug metabolism.* At the dose employed in this study (0.25 mmol/kg), PZ and CPZ had no effect on the liver to body weight ratio in male rats and did not increase either alkaline phosphatase or alanine aminotransferase activity in rat serum (not shown). Thus, this dose of the test drugs did not appear to elicit cholestasis or other types of hepatocellular injury. Total P450 content in rat liver was not altered significantly by either drug but, as indicated in Table 2, the apparent microsomal contents of certain P450 forms were influenced by drug treatment. Most notable were the 6.7- and 10-fold increases in P450 2B1-immunoreactive protein produced by PZ and CPZ, respectively. P450 2C11 immunoreactive protein content was decreased by CPZ, but not PZ, to approximately 40% of levels in untreated rat liver (Table 2); P450 2C6 content was not altered significantly by either drug.

In order to supplement these observations the microsomal oxidation of several P450-specific substrates was measured in fractions from untreated and phenothiazine-pretreated liver. In agreement with immunoquantitation experiments, signficant decreases were noted in the activity of P450 2C11, as reflected by steroid 2α- and 16α-hydroxylation measurements, to 71–81% of control (Table 3). Other steroid hydroxylations mediated by the P450s 3A2 (6β-hydroxylation) and 2A1 (testosterone 7α-hydroxylation)

were apparently unaltered by either PZ or CPZ. Thus, it seems likely that the expression of these P450s was not affected by either phenothiazine drug. Similarly, 2C6-mediated progesterone 21-hydroxylation was not decreased by either PZ or CPZ, a finding in keeping with data from immunoquantitation experiments.

The most pronounced increase in microsomal oxidation was noted with 7-pentylresorufin. O-dependentylation of this substrate, a reaction catalysed extensively by P450 2B1 [17], was increased 10- and 12-fold by CPZ and PZ, respectively. This is in accord with the estimates of P450 2B1-immunoreactive protein in these fractions. 7-Ethoxyresorufin O-deethylation activity was also measured in these fractions and PZ and CPZ were found to increase the activity to 4.2- and 2.6-fold of control, respectively. This is consistent with earlier reports of induction of P450 1A1 and 1A2 in rat liver following exposure to phenothiazine tranquilizers [18].

Discussion

Phenothiazine tranquilizers are important in the management of schizophrenia and other neurological disorders. The treatment of these conditions commonly involves multidrug therapy. Since P450s have a crucial role in the oxidations of numerous drugs, agents that modulate P450 function or expression may well elicit pharmacokinetic interactions that result in drug toxicity. There have been several reports of P450 induction or inhibition by phenothiazines such as CPZ [3–6]. However, the two processes have not been considered together as potential contributors to altered drug pharmacokinetics caused by phenothiazines. Documentation that P450 2B1 is both induced and inhibited by these drugs is presented in this study. Thus, CPZ and PZ possess inhibitory properties toward P450 that are similar to those of the closely related thioridazine [6]. The present finding that major constitutive P450s are not markedly inhibited by the phenothiazines suggests that pharmacokinetic interactions observed in rats are probably unlikely to involve constitutive forms of the enzyme. However, another factor to emerge from this study is that certain phenothiazines may down-regulate the expression of specific P450s in liver. An analogous process in humans would give rise to impaired drug oxidation capacity that would appear similar to reversible inhibition, but that would be more protracted.

It has been suggested that several P450s are PB-inducible and are regulated similarly [19, 20]. However, this assertion could be an over-generalization since evidence of weak induction of only two of the four PB-inducible P450s studied has been reported with 2,4,5,3′,4′-pentabromobiphenyl [19]. This chemical increased the content of P450 2B1 and 2B2 in male rat liver but did not alter the P450 2C6- or P450 3A1/2-immunoreactive protein content. In this regard, CPZ is an inducer with properties that are similar to those of the biphenyl derivative. It is tempting to speculate a structural relationship between these compounds but it has been shown clearly that structurally dissimilar agents may well increase the hepatic expression of P450s 2B1 and 2B2 [20].

In summary, the present report has identified P450 2B1 as the P450 most susceptible to inhibition by PZ and CPZ. P450 2B1 is also induced in rat liver after exposure to these agents *in vivo*. Aside from down-regulation of P450 2C11 and apparent slight induction of P450 1A1, other P450s were unaffected by the drugs. From these findings it is likely that pharmacokinetic interactions due to phenothiazines are a combination of specific inhibition and induction phenomena. A relatively narrow range of P450s is apparently involved in these processes and it may be for this reason that previous studies have reported variable effects of phenothiazines on hepatic drug metabolism. It remains to be established whether the clinical use of phenothiazine tranquilizers leads to induction and inhibition

Table 3. Effect of *in vivo* administration of PZ and CPZ on microsomal P450 activities

Treatment	AD‡	P450 activity (nmol/min/mg protein)							7-Pentylresorufin O-dependentylation	7-Ethylresorufin O-deethylation
		Testosterone hydroxylation			Progesterone hydroxylation					
		2α	6β	7α	16α	2α	21	6β		
None (control)	1.43 ± 0.16	1.60 ± 0.09	2.48 ± 1.02	0.54 ± 0.10	1.85 ± 0.20	1.06 ± 0.14	0.55 ± 0.12	2.39 ± 0.73	4 ± 2	62 ± 9
PZ	1.28 ± 0.13	1.56 ± 0.11	2.69 ± 0.77	0.54 ± 0.14	1.97 ± 0.43	1.04 ± 0.11	0.54 ± 0.04	2.61 ± 0.69	47 ± 20*	260 ± 9*
CPZ	1.22 ± 0.13	1.14 ± 0.23†	1.98 ± 0.87	0.56 ± 0.10	1.50 ± 0.17†	0.82 ± 0.07	0.51 ± 0.08	2.00 ± 0.88	41 ± 9*	160 ± 70†

Data are means ± SD of measurements obtained in five individual microsomal preparations per group.
Significant difference from control: *$P < 0.01$, †$P < 0.05$.
‡AD, androst-4-ene-3,17-dione.

of the human protein analogous to rat 2B1, P450 2B6 [21], or other P450s in the human liver.

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