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Biochemistry

Human cytochrome P-450$_{PA}$ (P-450IA2), the phenacetin *O*-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines

(aromatic amines/heterocyclic amines/carcinogen metabolism)

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ABSTRACT **Aromatic amines are well known as occupational carcinogens and are found in cooked foods, tobacco smoke, synthetic fuels, and agricultural chemicals. For the primary arylamines, metabolic N-oxidation by hepatic cytochromes P-450 is generally regarded as an initial activation step leading to carcinogenesis. The metabolic activation of 4-aminobiphenyl, 2-naphthylamine, and several heterocyclic amines has been shown recently to be catalyzed by rat cytochrome P-450$_{ISF-G}$ and by its human ortholog, cytochrome P-450$_{PA}$. We now report that human hepatic microsomal caffeine 3-demethylation, the initial major step in caffeine biotransformation in humans, is selectively catalyzed by cytochrome P-450$_{PA}$. Caffeine 3-demethylation was highly correlated with 4-aminobiphenyl N-oxidation ($r = 0.99$; $P < 0.0005$) in hepatic microsomal preparations obtained from 22 human organ donors, and both activities were similarly decreased by the selective inhibitor, 7,8-benzoflavone. The rates of microsomal caffeine 3-demethylation, 4-aminobiphenyl N-oxidation, and phenacetin O-deethylation were also significantly correlated with each other and with the levels of immunoreactive human cytochrome P-450$_{PA}$. Moreover, a rabbit polyclonal antibody raised to human cytochrome P-450$_{PA}$ was shown to inhibit strongly all three of these activities and to inhibit the N-oxidation of the carcinogen 2-naphthylamine and the heterocyclic amines, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole and 2-amino-3-methylimidazo[4,5-*f*]quinoline. Human liver cytochrome P-450$_{PA}$ was also shown to catalyze caffeine 3-demethylation, 4-aminobiphenyl N-oxidation, and phenacetin O-deethylation. Thus, estimation of caffeine 3-demethylation activity in humans may be useful in the characterization of arylamine N-oxidation phenotypes and in the assessment of whether or not the hepatic levels of cytochrome P-450$_{PA}$, as affected by environmental or genetic factors, contribute to interindividual differences in susceptibility to arylamine-induced cancers.**

The carcinogenicity of arylamines has been well established in both humans and experimental animals (1). Humans are frequently exposed to arylamines such as 4-aminobiphenyl (ABP), 2-naphthylamine (2-NA), and *o*-toluidine in mainstream and sidestream cigarette smoke (2) and to mutagenic and carcinogenic heterocyclic arylamines in cooked foods (3). Arylamines are also found in coal- and shale-derived oils (4) and in agricultural chemicals (5), and they are used in a variety of industrial processes (1, 6, 7).

Metabolic N-oxidation of primary arylamines, catalyzed by hepatic cytochromes P-450 (P-450s), is a critical initial activation step leading to carcinogenesis (reviewed in refs. 8 and 9).¶ Studies with purified rat and rabbit P-450s have shown high specificity for the N-oxidation of 2-acetylaminofluorene, 2-NA, ABP, and several heterocyclic amines to their proximate carcinogenic and/or mutagenic forms by the P450IA2 gene products in various species (8–15). In humans, the orthologous P-450 (16, 17), termed P-450$_{PA}$ (also HLd), appears to be primarily responsible for ABP N-oxidation (15) and for the mutagenic activation of several heterocyclic amines (18). Recent studies have indicated that considerable interindividual variability in this metabolic activation step exists in human populations, since human hepatic microsomal preparations have been shown to vary from 30- to 60-fold in their ability to catalyze the N-oxidation of 2-NA (12), 2-acetylaminofluorene (19), and ABP (15); while total P-450 levels varied <4-fold. Differences have also been observed in the metabolic capacity of human hepatic microsomal preparations to activate the heterocyclic arylamines 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx; ref. 20) to bacterial mutagens. Similar variations in the amounts of immunoreactive P-450$_{PA}$ in human liver microsomes have also been reported (15, 21). This interindividual variation has been suggested to be due to environmental and/or genetic factors and may contribute to individual differences in susceptibility to arylamine-induced cancer. In this regard, cigarette smoking has been shown to induce hepatic P-450$_{PA}$ levels in human liver and to increase microsomal phenacetin *O*-deethylase activity (21), which is catalyzed selectively by human P-450$_{PA}$ (16). In addition, a genetic polymorphism for phenacetin O-deethylation has been described, with 5–10% of the population deficient in this activity (22).

[The evidence that human P-450$_{PA}$, the phenacetin *O*-deethylase, is the product of the human P450IA2 gene is as follows: antibodies raised to rat P-450$_{ISF-G}$ (P-450IA2) or human P-450$_{PA}$ recognize a single polypeptide in human liver microsomes and inhibit microsomal phenacetin *O*-deethylase activity; the level of immunoreactive protein is highly cor-

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Abbreviations: P-450, cytochrome P-450; ABP, 4-aminobiphenyl; *N*-OH, *N*-hydroxy; 13X, 1,3-dimethylxanthine (theophylline); 17X, 1,7-dimethylxanthine (paraxanthine); 37X, 3,7-dimethylxanthine (theobromine); 137U, 1,3,7-trimethyluric acid; 2-NA, 2-naphthylamine; Glu-P-1, 2-amino-6-methyldipyrido[1,2-a:3′,2′-*d*]imidazole; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole.

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¶The nomenclature of the P-450 genes and gene products in different species has been described (10); P-450IA2 encodes P-450$_{ISF-G}$ = P-450d = P$_3$-450; and P-450IA1 encodes P-450$_{BNF-B}$ = P-450c = P$_1$-450.

related with phenacetin *O*-deethylase activity (15, 16, 21) but not aryl hydrocarbon hydroxylase activity (23); the rat and human P-450IA gene families appear to encode only two products, derived from P-450IA1 and P-450IA2 (10), the former of which is thought to be the aryl hydrocarbon hydroxylase (P_1-450); an antibody prepared to rat P-450d (P-450IA2), which did not recognize P-450c (P-450IA1), was used to isolate a protein that had an N-terminal sequence (17) matching that predicted by the human P-450IA2 (P_3-450) cDNA sequence (24); our own human $P\text{-}450_{PA}$ preparation, which exhibits phenacetin *O*-deethylase activity, did not yield an unambiguous N-terminal sequence, but major peaks were present for Ala, Leu, Gln, Val, and Pro at cycles 1, 2, 4, 6, and 7, as predicted (24); hepatic immunoreactive protein (anti-rat P-450d) and microsomal phenacetin *O*-deethylase activity are both increased in cigarette smokers, while aryl hydrocarbon hydroxylase activity is unaffected (21); the drug furafylline inhibits human liver microsomal phenacetin O-deethylation but stimulates aryl hydrocarbon hydroxylase (23).]

To describe the hepatic N-oxidation phenotype in humans, we sought to identify a safe drug that would show similar high specificity for oxidation by $P\text{-}450_{PA}$ and that could be used to provide an index of individual differences in carcinogen metabolism, as previously suggested (25, 26). Since *in vivo* and *in vitro* studies (27–31) have suggested a correlation between caffeine oxidation and the hydrocarbon-inducible P-450s (P450IA family), we examined the role of $P\text{-}450_{PA}$ (P450IA2) in caffeine metabolism. In this investigation, we report that 3-demethylation of caffeine is highly correlated with ABP N-oxidation in human hepatic microsomal preparations and that both activities are similarly inhibited by 7,8-benzoflavone and by an antibody to human $P\text{-}450_{PA}$. Metabolic N-oxidation of 2-NA and of the heterocyclic arylamines Glu-P-1 and IQ is also strongly inhibited by anti-human $P\text{-}450_{PA}$. In addition, microsomal caffeine 3-demethylation, ABP N-oxidation, and phenacetin O-deethylation activities are significantly correlated to levels of immunoreactive $P\text{-}450_{PA}$. Furthermore, we demonstrate that human $P\text{-}450_{PA}$, devoid of other P-450s, catalyzes both ABP N-oxidation and caffeine 3-demethylation.

MATERIALS AND METHODS

Chemicals. [2,2′-^{3}H]ABP (55 mCi/mmol; 1 Ci = 37 GBq), [9*b*-^{14}C]Glu-P-1 (27 mCi/mmol), [5-^{3}H]IQ (118 mCi/mmol), 3-[3-^{14}C]amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2; 46 mCi/mmol), [5,6,7,8-^{3}H]2-NA (47 mCi/mmol), and [*ring*-^{3}H]phenacetin (110 mCi/mmol) were obtained from Chemsyn Science Laboratories (Lenexa, KS). [2-^{14}C]Caffeine (5.35 mCi/mmol) was a gift from International Life Sciences Institute–Nutrition Foundation (Washington, DC). Diethylamine, NADPH, $NADP^+$, NAD^+, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, L-α-dilauroyl-*sn*-glycero-3-phosphocholine, 7,8-benzoflavone, caffeine, 1,7-dimethylxanthine (17X), 1,3-dimethylxanthine (13X), and 3,7-dimethylxanthine (37X) were purchased from Sigma. 1,3,7-Trimethyluric acid (137U) was supplied by Fluka. Antibodies were prepared in female New Zealand White rabbits by modifications of techniques described previously (32). Rabbit P-450 reductase was prepared according to published procedures (33). 2-[(2,4-Dichloro-6-phenyl)phenoxy]ethylamine was a gift from Eli Lilly.

Tissues, Enzyme Preparations, and Analyses. Human liver samples were obtained from organ donors immediately after death (34) and microsomal fractions were prepared and stored at −70°C as described (35). Protein concentrations were measured by the biuret reaction (36) and P-450 concentrations were determined by the method of Omura and Sato (37). Human $P\text{-}450_{PA}$ was purified as described and was judged to be free of other P-450s by SDS/PAGE (>95% purity; ref. 16). Immunochemical staining combined with gel electrophoresis was conducted by using the basic procedures described previously (15).

Instrumentation. To measure the rates of N-oxidation of ABP, 2-NA, Glu-P-1, and IQ, analyses were conducted by HPLC on a Beckman-Altex Model 100A instrument equipped with a Waters μBondapak C_{18} column (0.39 × 30 cm) and a Waters model 440 absorbance detector set at 280 nm (ABP, 2-NA) or 254 nm (Glu-P-1, IQ). Quantitation of metabolites was obtained with a Radiomatic Flo-One/Beta radioactivity flow detector. The solvent composition and elution program used were as described (15). Conditions for the HPLC analysis of Trp-P-2 metabolites were as described by Yamazoe *et al.* (38). The rate of metabolite formation was determined by measuring the percentage of total radioactivity eluting with the metabolite standard and by calculating nmol of product formed per min per mg of protein or nmol of P-450. Metabolites were identified by their UV spectra and by their retention times compared to the authentic standards. Because chemical oxidation of *N*-hydroxy (*N*-OH)-ABP and *N*-OH-2-NA sometimes occurred during the HPLC procedure, rates of N-oxidation were calculated from the total *N*-OH and nitroso/nitro products observed. Retention times (min) were as follows: *N*-OH-ABP, 14.7; ABP, 16.0; 4-nitrosobiphenyl/4-nitrobiphenyl, 22.4; *N*-OH-2-NA, 9.5; 2-NA, 11.0; 2-nitrosonaphthalenene/2-nitronaphthalene, 19.3; *N*-OH-Glu-P-1, 9.3; Glu-P-1, 12.2; *N*-OH-IQ, 7.6; IQ, 9.6; *N*-OH-Trp-P-2, 3.7; Trp-P-2, 4.9. Phenacetin reactions were analyzed as described (15, 39).

HPLC analyses for ^{14}C-labeled caffeine metabolites were conducted on a Waters Associates instrument consisting of a model 510 solvent delivery system equipped with a 5-μm Ultrasphere ODS (0.46 × 25 cm) column and a Hewlett–Packard 1040B high-speed spectrophotometric detector. The solvents used were aqueous 0.05% acetic acid and CH_3OH. The solvent program was 12% CH_3OH (0–8 min; 1.2 ml/min), 12–20% CH_3OH (8–18 min; curve 2, 1.2 ml/min); 20% CH_3OH (18–19 min; 1.2 ml/min), 20–50% CH_3OH (19–23 min; curve 2, 1.5 ml/min), and 50–12% CH_3OH (23–26 min; curve 2, 1.5 ml/min). Retention times (min) for unlabeled standards were as follows: 37X, 10.5; 17X, 16.0; 13X, 17.0; 137U, 19.5; caffeine, 23.2. The rate of metabolite formation was determined from the percentage of total radioactivity eluting with each standard, and calculating nmol of metabolite formed per min per mg of protein or nmol of P-450.

Enzyme Assays. The microsomal oxidations of ABP, 2-NA, Glu-P-1, IQ, or Trp-P-2 were carried out in duplicate in 100 mM potassium phosphate buffer (pH 7.6) containing 0.5 mM EDTA, 5 mM glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase per ml, 0.4 mM $NADP^+$, 0.3 mM NAD^+, 0.1 mM arylamine substrate, and 0.5–1.0 mg of microsomal protein per ml. Caffeine reactions were identical but contained 1 mM caffeine and 0.5–2.0 mg of microsomal protein per ml. Phenacetin reactions were conducted as detailed earlier (15, 39). After a 3-min preincubation in a shaking water bath at 37°C, the reactions were initiated by the addition of microsomes and were continued for up to 15 min. Where indicated, chemical inhibitors, dissolved in dimethyl sulfoxide/ethanol (4:1), were added to the preincubation mixtures (10 μl per ml of assay mixture). When immunochemical inhibition studies were conducted, rabbit preimmune immunoglobulin or anti-$P\text{-}450_{PA}$ was incubated with the microsomal protein for 30 min at 25°C prior to the 3-min preincubation at 37°C. These reactions were initiated by the addition of glucose-6-phosphate dehydrogenase. Reactions containing ABP and 2-NA were terminated by extraction of a 0.45-ml aliquot with 0.22 ml of cold, freshly prepared, water-saturated ethyl acetate. Reactions containing the heterocyclic amines were stopped by the addition of an equal

volume of ice-cold acetonitrile. After centrifugation, aliquots (0.05 ml) of these organic extracts were then analyzed by HPLC. Caffeine reactions were terminated by the addition of trichloroacetic acid to 0.1 M and then were adjusted to pH 7.0 with 3.6 M KOH; after addition of the unlabeled standards 17X, 13X, 37X, and 137U, an aliquot was analyzed by HPLC. Preliminary experiments indicated that these substrate concentrations were near saturation and that product formation was linear with time for at least 15 min.

Human hepatic P-450$_{PA}$ was reconstituted and the reactions with arylamines or phenacetin were carried out as described (15, 16, 39). Caffeine reactions were terminated and analyzed as described above.

RESULTS

Human Hepatic Microsomal N-Oxidation of Arylamines and 3-Demethylation of Caffeine. Human hepatic microsomal samples were prepared from liver tissue of 22 human organ donors and were used initially for *in vitro* reactions to determine the rates of N-oxidation of ABP and 3-demethylation of caffeine. The rates of ABP N-oxidation ranged from 0.04 to 5.00 nmol of *N*-OH-ABP formed per min per mg of protein and caffeine 3-demethylation varied from 0.008 to 0.43 nmol of product formed per min per mg of protein (Fig. 1). Excellent correlation between these two activities was observed ($r = 0.99$; $P < 0.0005$), suggesting that the same enzyme may catalyze both reactions. Microsomal caffeine 3-demethylation and ABP N-oxidation were also significantly correlated with the O-deethylation of phenacetin (Table 1), an activity associated with human P-450$_{PA}$ (16).

To further assess whether or not the N-oxidation of ABP and the 3-demethylation of caffeine are catalyzed by the same P-450, the effect of the selective inhibitor 7,8-benzoflavone (15, 18, 40–42) on both of these reactions was determined. 7,8-Benzoflavone had been reported to inhibit the demethylation of caffeine (43). When examined in the same preparation, inhibition of both activities was strikingly similar (Fig. 2). At 0.1 μM inhibitor, caffeine 3-demethylation (17X formation) and ABP N-oxidation were inhibited 40% and 23%, respectively, while at 1.0 μM, they were inhibited 85% and 84%. Both reactions were inhibited >90% at 10 μM 7,8-benzoflavone. Caffeine 1-demethylation (37X formation) was similarly inhibited, whereas 7-demethylation (13X formation) and 8-hydroxylation (137U formation) were not appreciably affected by 0.03–10 μM 7,8-benzoflavone.

To obtain additional evidence that one enzyme is primarily responsible for both arylamine N-oxidation and caffeine

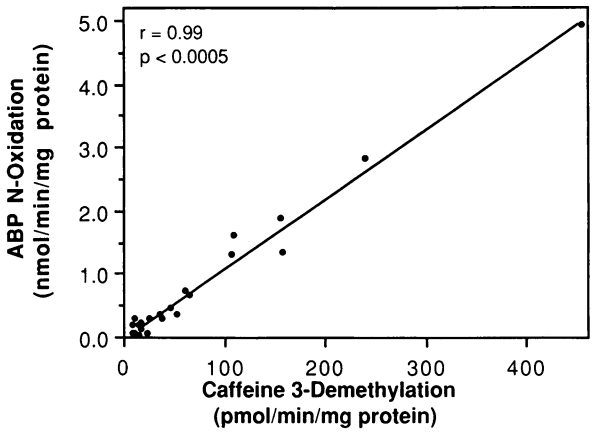

FIG. 1. Correlation between ABP N-oxidation and caffeine 3-demethylation in 22 human hepatic microsomal preparations. The correlation coefficient (r) was determined by least-squares linear regression analysis.

Table 1. Correlation (r) of P-450$_{PA}$ catalytic activities and immunoreactive P-450$_{PA}$ content in human hepatic microsomes

	Immunoreactive P-450$_{PA}$ content	Phenacetin O-deethylation	Caffeine 3-demethylation
ABP N-oxidation	0.85*	0.94†	0.99‡
Caffeine 3-demethylation	0.85*	0.92§	—
Phenacetin O-deethylation	0.77¶	—	0.92§

Correlation coefficients (r) were calculated by the least-squares linear regression method.
*$n = 15$; $P < 0.001$.
†$n = 8$; $P < 0.001$.
‡$n = 22$; $P < 0.0005$.
§$n = 8$; $P < 0.01$.
¶$n = 10$; $P < 0.01$.

3-demethylation, we examined several arylamines as substrates (Table 2) and also compared their sensitivity to inhibition by a rabbit antibody that was prepared against human P-450$_{PA}$ (18). Using the same human liver microsomal preparation, caffeine 3-demethylation was inhibited 72% with the addition of 2.5 mg of anti-P-450$_{PA}$ per nmol of P-450; while at 5 mg of anti-P-450$_{PA}$ per nmol of P-450, caffeine 3-demethylation was inhibited 87% (Fig. 3). Anti-P-450$_{PA}$ also inhibited ABP N-oxidation by 60% and 87% at 2.5 and 5 mg per nmol of P-450, respectively, and inhibited phenacetin O-deethylation as well as 2-NA N-oxidation to a similar extent. N-oxidation of IQ and Glu-P-1 was also significantly inhibited by both levels of the antibody. The rates of Trp-P-2 N-oxidation were judged too low for antibody inhibition studies. With two other human microsomal preparations, 5 mg of anti-P-450$_{PA}$ per nmol of P-450 inhibited ABP N-oxidation and caffeine 3-demethylation by 68–88% and 72–77%, respectively.

To demonstrate that only the P-450 monooxygenase is responsible for the N-oxidation of ABP and the 3-demethylation of caffeine, the specific P-450 inhibitor 2-[(2,4-dichloro-6-phenyl)phenoxy]ethylamine (44) was added at 0.5 mM to the incubation mixtures. The results showed that microsomal-catalyzed caffeine 3-demethylation and ABP N-oxidation were inhibited by >99%.

Correlations of Caffeine 3-Demethylation, ABP N-Oxidation, and Phenacetin O-Deethylation Activities with Immunochemically Determined Levels of P-450$_{PA}$. The rates of caffeine 3-demethylation, ABP N-oxidation, and phenacetin O-deethylation in individual human hepatic microsomal preparations were compared to the immunochemically determined levels of P-450$_{PA}$ by immunoblotting with rabbit anti-human P-450$_{PA}$ IgG. A single polypeptide band was observed

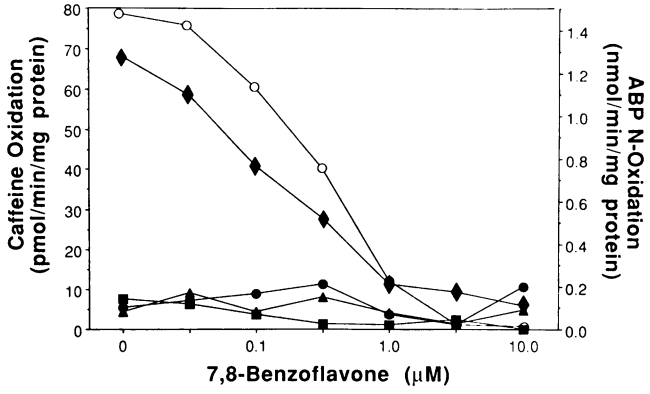

FIG. 2. Effects of 7,8-benzoflavone on caffeine oxidation and ABP N-oxidation in a human liver microsomal preparation. ○, *N*-OH-ABP; ◆, 17X; ■, 37X; ▲, 13X; ●, 137U.

Table 2. Rates of caffeine 3-demethylation and N-oxidation of carcinogenic arylamines by human liver microsomes

Substrate	Rates of oxidation, nmol per min per mg of protein
Caffeine	0.43 ± 0.03
ABP	5.00 ± 0.32
2-NA	2.49 ± 0.28
Glu-P-1	2.42 ± 0.14
IQ	2.30 ± 0.21
Trp-P-2	0.28 ± 0.14

Rates of caffeine 3-demethylation and arylamine N-oxidation were determined by HPLC analysis of reactions, which were conducted with hepatic microsomes obtained from the same individual; $n = 3$ or 4 (±SD).

at the same M_r as P-450$_{PA}$ in all preparations. The correlation of microsomal caffeine 3-demethylation activity with the levels of immunoreactive P-450$_{PA}$ (Fig. 4) was highly significant ($r = 0.85$; $P < 0.001$). Similarly, the N-oxidation of ABP and O-deethylation of phenacetin were also significantly correlated with the amount of P-450$_{PA}$ protein present (Table 1; $r = 0.85$, $P < 0.001$; $r = 0.77$, $P < 0.01$, respectively).

ABP N-Oxidation and Caffeine 3-Demethylation by Purified Human Liver P-450$_{PA}$. ABP is selectively N-oxidized by purified rat P-450$_{ISF-G}$ and its human ortholog P-450$_{PA}$, both of which are known to catalyze the O-deethylation of phenacetin (15, 16). To demonstrate conclusively that both phenacetin O-deethylation and caffeine 3-demethylation are catalyzed by the same human P-450, we obtained a P-450$_{PA}$ preparation from one of the liver samples used in this study. Although the specific activity of the purified enzyme was not increased over that of the microsomal preparation under the reconstitution conditions used, the enzyme was separated from other major human P-450s (16) and it catalyzed phenacetin O-deethylation, caffeine 3-demethylation, and ABP N-oxidation at rates of 0.33, 0.19, and 1.39 nmol per min per nmol of P-450, respectively. For comparison, the corresponding rates of 0.54, 0.35, and 4.18 nmol of product formed per min per nmol of P-450, respectively, were obtained in the microsomal preparation.

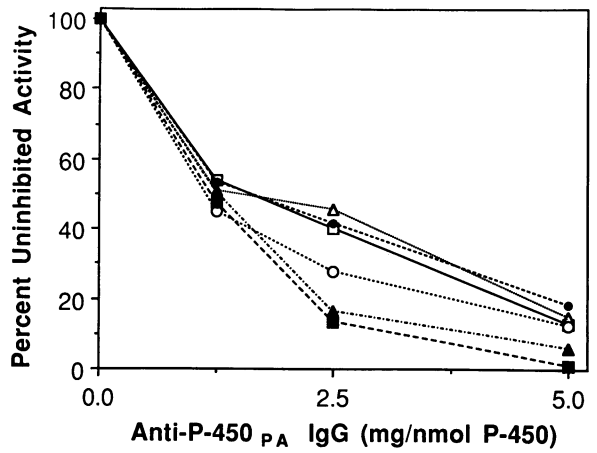

FIG. 3. Inhibition of caffeine 3-demethylation, phenacetin O-deethylation, and N-oxidation of several carcinogenic arylamines by rabbit anti-human P-450$_{PA}$ IgG. Reaction mixtures contained 0.49 nmol of P-450 per ml, radiolabeled substrate, and the indicated amounts of anti-human P-450$_{PA}$ IgG. In the absence of antibodies, rates of product formation (nmol per min per nmol of P-450) were as follows: 17X (○), 0.44; *N*-OH-ABP (□), 5.15; acetaminophen (△), 2.07; *N*-OH-2-NA (●), 2.78; *N*-OH-Glu-P-1 (■), 2.40; *N*-OH-IQ (▲), 2.46. In the presence of 5 mg of preimmune IgG per nmol of P-450, rates of oxidation were affected <10%.

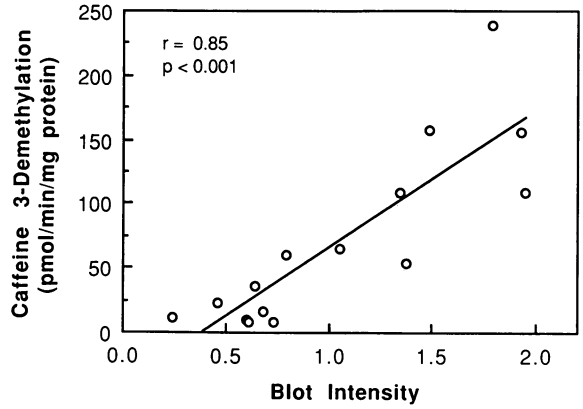

FIG. 4. Correlation of caffeine 3-demethylation activity and immunochemically determined content of human P-450$_{PA}$. Microsomal preparations from 15 human liver samples were analyzed for caffeine 3-demethylation activity and for P-450$_{PA}$ content by immunoblot analysis of 100 μg of microsomal protein using anti-human P-450$_{PA}$. Intensity units are arbitrary values determined by densitometry.

DISCUSSION

The 22 human liver microsomal preparations examined in this *in vitro* study varied widely in their ability to catalyze the 3-demethylation of caffeine and the N-oxidation of ABP. Caffeine 3-demethylation varied 57-fold and ABP N-oxidation varied 130-fold when expressed as nmol of product formed per min per mg of protein. However, there was an excellent correlation between both caffeine 3-demethylation and ABP N-oxidation ($r = 0.99$; $P < 0.0005$) in the 22 preparations. Since the rates of metabolism were not related to the total microsomal P-450 concentration (data not shown), the variations are presumed to have resulted from differences in the specific P-450s present. Both activities were inhibited to the same extent by anti-human P-450$_{PA}$ and by the specific P-450 inhibitors 7,8-benzoflavone and 2-[(2,4-dichloro-6-phenyl)phenoxy]ethylamine. Human liver microsomes also catalyzed the N-oxidation of 2-NA (12), Glu-P-1 (20), and IQ; each of these was strongly inhibited by anti-human P-450$_{PA}$. Interestingly, the rate of N-oxidation of these arylamines was about one-half the rate of that observed with ABP, which is regarded as the most potent of the arylamine human carcinogens (6); Trp-P-2 was poorly N-oxidized by human liver microsomes.

Caffeine 3-demethylation and ABP N-oxidation activities were also strongly correlated with phenacetin O-deethylation in the human liver preparations. Furthermore, all three activities were significantly correlated with the levels of immunoreactive human P-450$_{PA}$. In addition, a human P-450$_{PA}$ preparation, although low in specific activity, had ratios of phenacetin O-deethylation/caffeine 3-demethylation that were similar to the activities measured in the human liver microsomal preparations. Thus, we conclude that all three of these activities are catalyzed predominantly by the same human P-450—namely, P-450$_{PA}$.

Since hepatic N-oxidation is believed to be a critical step in the activation of arylamine carcinogens, individuals in the human population might be expected to vary in their susceptibility to arylamine-induced cancers as a consequence of different levels of hepatic P-450$_{PA}$, as determined by genotype or by enzyme induction from dietary factors, drugs, environmental exposures to hydrocarbon inducers, etc. A genetic polymorphism for phenacetin *O*-deethylase has already been reported to exist in the human population (22) and individuals who are homozygous dominant for that gene would be expected to have higher levels of P-450$_{PA}$. Exposure to polycyclic aromatic hydrocarbon inducers, which are

found in cigarette smoke and in charcoal-broiled meats and are known to increase levels of P-450$_{ISF-G}$ in rats, have also been demonstrated to increase phenacetin O-deethylation rates in humans (21, 25, 45, 46). Smokers have also been demonstrated to have increased rates of caffeine disposition, with plasma half-lives one-half that of nonsmokers (47) and a labeled CO_2 elimination rate, measured in the $^{13}C/^{14}C$ caffeine breath test, twice that of nonsmokers (29). Furthermore, rates of caffeine metabolism vary between individuals, as caffeine half-life values have been reported to range from 1.5 to 9.5 hr (48).

Interindividual variation in the levels of hepatic P-450$_{PA}$ is suggested from the results of several earlier studies. 2-NA N-oxidation proficiency varied >30-fold in human liver microsomal preparations (12), while N-oxidation of ABP varied >44-fold in a different set of human liver microsomes (15), even when expressed per nmol of P-450. We recently demonstrated a good correlation between ABP N-oxidation activity and protein levels recognized by anti-P-450$_{ISF-G}$ (15). The content of human P-450HLd (P-450$_{PA}$), detected with an antibody raised to the orthologous rat P-450d (P-450$_{ISF-G}$), varied >10-fold in another set of 14 human hepatic microsomal preparations (21). In addition, a 180-fold interindividual variation in phenacetin O-deethylation rates (18) has been reported in 28 human liver samples, which varied in the amount of P-450d-immunoreactive protein by >60-fold. Finally, immunoreactive P-450d content was reported to be 3.5-fold higher, and phenacetin *O*-deethylase activity was 4-fold greater in hepatic microsomes from smokers than from nonsmokers (21).

Caffeine is a popular, self-administered stimulant considered to be relatively innocuous. In the United States, 7 billion kg are ingested yearly and the average daily intake in adults is 200 mg (49). In humans, the predominant caffeine metabolic pathway is through 17X formation (29, 48); 17X, the major metabolite in the bloodstream, is formed at a rate ≈7 times that of 13X and 37X (48). Determination of urinary caffeine metabolite ratios in humans consuming caffeine (31, 43, 50, 51), as well as the caffeine breath test (29, 52), have been proposed as methods for characterizing hydrocarbon-inducible P-450 levels in humans. Our work links human hepatic P-450$_{PA}$-catalyzed 3-demethylation of caffeine with the metabolic activation of arylamines known or suspected to be human carcinogens. We propose that an individual's ability to 3-demethylate caffeine be characterized by using caffeine metabolite ratios selected to be a reliable measure of 3-demethylation. Thus, this relatively safe and widely used drug may be used to describe the arylamine N-oxidation phenotype of individuals in order to assess the role of P-450 in determining their relative susceptibilities to arylamine-induced cancers.

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