DOSE-DEPENDENT UP-REGULATION OF RAT PULMONARY, RENAL, AND HEPATIC CYTOCHROME P-450 (CYP) 1A EXPRESSION BY NICOTINE FEEDING

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ABSTRACT:

In a previous study in which a single 2.5 mg/kg (15.4 μmol/kg) s.c. dose of nicotine effected a transient, lung-specific induction of cytochrome P-450 (CYP) 1A1 in the rat, a dose-response study and assessment of the lung specificity of the induction was limited by toxicity of the acute parenteral nicotine exposure. In the present study, we examined the dose–CYP1A1/2 induction response relationship and the tissue specificity of the induction by orally administered nicotine, which lacks the toxicity of the parenterally administered drug. Nicotine, administered in a nutritionally balanced liquid diet, at a level of 20 (low), 60 (medium), or 200 (high) mg/kg of diet, induced CYP1A1 in the lung and kidney in a dose-dependent manner and in the liver at the high nicotine dose only, whereas CYP1A2 was induced in the liver dose-dependently and in the kidney at the high nicotine dose only. The high nicotine dose up-regulated mRNA level in the three tissues examined, but with the lung being the most responsive to the up-regulation. Induction of the CYP1A1-preferential activity ethoxyresorufin O-deethylase by the low, medium, and high nicotine diets was 1.9-, 4.9-, and 21.6-fold, respectively, in the lung, 1.4-, 1.7-, and 15.9-fold, respectively, in the kidney, and 1.7-, 2.9-, and 5.1-fold, respectively, in the liver. Similarly, albeit to lower extents, the dietary alkaloid induced the CYP1A2-preferential activity methoxyresorufin O-demethylase in all three tissues dose-dependently. Plasma nicotine concentrations correlated neither with the dietary nor intake dose of the alkaloid nor with tissue levels of CYP1A1, especially with the high-dose diet. Plasma nicotine levels at which CYP1A induction was maximal were comparable to those reported in smokers, suggesting that nicotine may induce CYP1A1 in humans.

The pyridine alkaloid nicotine is a major constituent of tobacco (International Agency for Research on Cancer, 1986) and is the causative agent of tobacco addiction (Benowitz, 1996). The alkaloid is currently available in various formulations, such as chewing gum, topical patches, and nasal spray for the controlled cessation of the smoking habit (Rose, 1996). Nicotine modulates neurobehavioral, respiratory, cardiovascular, endocrinological, and several other functions, and it contributes to diseases in these functions commonly observed in smokers (U.S. Public Health Service, 1988).

Tobacco smoke exposure is also associated with increased lung cancer susceptibility (Doll and Hill, 1950; U.S. Public Health Service, 1988), an effect commonly attributed predominantly to the many potent procarcinogenic and promutagenic chemicals present in the smoke. These include the polyaromatic hydrocarbon (PAH)1 benzo[a]pyrene (Wynder and Hoffmann, 1967; International Agency for Research on Cancer, 1986) and tobacco-specific nitrosamines (Hecht and Hoffmann, 1988). However these chemicals, as exemplified by benzo[a]pyrene, are carcinogenic only after metabolic bioactivation, preferentially by cytochrome P-450 (CYP) 1A1 (Gautier et al., 1996), to electrophiles capable of reacting with genomic DNA to cause oncogenic mutations (Denissenko et al., 1996). Accordingly, an elevated pulmonary level of CYP1A1 constitutes a risk factor for lung cancer from cigarette smoke exposure (McLemore et al., 1990; Anttila et al., 1991). CYP1A1 inducibility is highly correlated with increased lung cancer susceptibility from tobacco smoke (Kellermann et al., 1973; Kouri et al., 1982). Tobacco smoke is also a potent inducer of pulmonary CYP1A1 in humans (Kouri et al., 1982; McLemore et al., 1990) and experimental animals (Godden et al., 1987; Gebremichael et al., 1996; Fung et al., 1998). Tobacco smoke also induces hepatic CYP1A2 (Pelkonen et al., 1986) and its associated catalytic activities (Kuntzmann et al., 1977). The induction is commonly attributed to the PAH constituents in tobacco smoke (Akin et al., 1975; Kuntzmann et al., 1977). However, non-PAH constituents of the smoke, such as pyridine (International Agency for Research on Cancer, 1986), also induce pulmonary CYP1A1 (Iba et al., 1993a).

We reported previously that nicotine induces CYP1A1 in rats after an s.c. [2.5 mg/kg (15.4 μmol/kg)] or inhalation (200 μg/m3) dose of the alkaloid, by mechanisms suggestive of transcriptional activation of the CYP1A1 gene (Iba et al., 1998). The induction by the acute parenteral nicotine exposure was transient and appeared to be lung-specific (Iba et al., 1998). Tremors and convulsions, the major toxic effects of rapid parenteral nicotine administration (Porchet et al., 1987), were observed even at the lowest s.c. doses of the alkaloid that...
induced CYP1A1 and precluded a dose-response study of the induction (Iba et al., 1998).

Earlier studies of CYP induction by nicotine in experimental animals examined only catalytic activities and were limited to the liver; the studies were also limited by the toxic effects of the compound (Ruddon and Cohen, 1970). Induction of CYP1A1-related activities was not observed in some of these studies (Ruddon and Cohen, 1970; Schmoldt et al., 1988) but was suggestive in others (Yamamoto et al., 1966), although one study reported the elevation of CYP1A1/2 immunoreactive proteins and their respective catalytic activities in specific brain regions but not in the liver of nicotine-treated rats (Anandatheerthavarada et al., 1993). Because the induction in these studies focused on the liver and was assessed most commonly 24 h after nicotine treatment, the failure of the studies to detect CYP1A1 induction in animals after acute nicotine treatment could have been due to the transience, as well as lung specificity, of the induction after acute nicotine treatment.

The objectives of the current study were to: 1) determine the relationship between administered dose and CYP1A1/2 induction by nicotine, 2) determine the tissue specificity of the induction response, and 3) assess the relationship between plasma nicotine concentration and the induction response, after a 30-day extended oral feeding of the compound, a route of administration free of the motor effects (tremor and convulsions) characteristic of parenterally administered nicotine. The data also suggest involvement of indirect factors, such as nicotine metabolites, or a threshold-dose effect of nicotine, in the induction response. This is the first reported induction of CYP1A1 by oral nicotine exposure and its relationship to tissue levels of the compound.

### Experimental Procedures

**Animals and Treatments.** Male Long-Evans rats (Charles River Laboratories, Wilmington, MA), weighing 121 to 138 g, were housed individually in suspended metal cages and were fed a complete and nutritionally balanced liquid diet, the composition of which was described previously (Fisher et al., 1996). The animals were divided into four groups (eight animals per group) and were fed the liquid diet either alone or supplemented with a low (20 mg), medium (60 mg), or high (200 mg) dose of nicotine bitartrate per kilogram of diet. Feeding was ad libitum and continuous for 30 days. Average diet consumption and nicotine intake (calculated as the free base) were recorded daily and body weight changes were recorded every 3 days. On day 30 of the diet regimen, the animals were decapitated, blood was collected from the trunk into heparinized tubes, and plasma was prepared and frozen at −70°C for nicotine and cotinine analysis. Protocols for the animal studies were approved by the Rutgers University Institutional Review Board for the Use and Care of Animals. The University has an Animal Welfare Assurance on file with the U.S. Public Health Service.

**Assay of Microsomal Catalytic Activities.** Microsomal O-dealkylation of methoxyresorufin or ethoxyresorufin was assayed fluorometrically as described by Pohl and Fouts (1980), at a substrate concentration of 5 μM. Each assay was linear within the duration (10 min) of the assay and at the microsomal protein concentrations used (50 μg of protein for liver microsomes and 100 μg of protein for lung or kidney microsomes per milliliter of incubation mixture).

**Electrophoresis and Western Blot Analysis.** SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis of microsomes were performed as described previously (Iba et al., 1998), using CDS, a monoclonal antibody raised in the mouse against rat liver CYP1A1, which also recognizes CYP1A2. Immunoreactive bands on the membranes were detected with an enhanced chemiluminescence system (Amersham Pharmacia Biotech, Arlington Heights, IL) and quantified by densitometry using a Bio Image IQ scanner (Bio Image, Ann Arbor, MI; Iba et al., 1998). Each densitometric (area under the curve) value was linear with respect to the protein concentration analyzed.

**Northern Blot Analysis.** This analysis was performed as described previously (Iba et al., 1998). Briefly, pooled total lung, kidney, or liver RNA from three control or nicotine-treated rats was fractionated on a denaturing formaldehyde/agarose (1%) gel and transferred to Zeta-Probe nylon membrane according to the manufacturer’s recommendation. UV-irradiation-fixed filters were incubated for 1 h at 45°C with 10 ml of hybridization solution: 3 × 0.15 M NaCl, 1 mM EDTA, 10 mM sodium phosphate buffer, pH 7.4 (3 × SSPE), containing 40% (v/v) deionized formamide, 7% (w/v) SDS, and 200 μg/ml denatured salmon sperm DNA. The filters were subsequently incubated with hybridization buffer containing the labeled DNA (CYP1A1 or glyceraldehyde-phosphate dehydrogenase (GAPDH) probe (specific activity: 1–2 × 106 cpm/μg, 1 × 107 cpm/ml of solution) for 20 h at 45°C. The rat CYP1A1 was prepared by random priming of the insert from plasmid p210 (Fagan et al., 1986). After hybridization, the filters were washed with a solution of 0.15 M NaCl, 15 mM sodium citrate, pH 7.4 (1 × SSC), containing 0.1% SDS, at room temperature for 30 min and three times with 0.1 × SSC, containing 0.1% SDS at 60°C for 15 min each. The blots were then exposed to X-ray film Biomax MR, with an intensifying screen at −70°C. After the exposure, the blots were stripped (Sambrook et al., 1989) and hybridized as described above but with 32P-labeled human GAPDH cDNA prepared by random priming, followed by autoradiography as described above for CYP1A1.

**Determination of Plasma Nicotine and Cotinine Concentrations.** Plasma nicotine concentrations were determined by gas chromatography-mass spectrometry, using the method described by Voncken et al. (1989). Briefly, a 0.5-ml sample of plasma was diluted to 4.0 ml with a saturated solution of potassium carbonate, followed by gentle mixing for 5 min. A 0.5-ml portion of methylene chloride was then added and the mixing was continued for an additional 10 min. After centrifugation of the mixture (5,000g for 10 min) to separate the organic and aqueous phases, a 2-μl aliquot of the organic phase was sampled with a gas-tight syringe (Hamilton 1750) and injected (in the splitless mode) into a Hewlett-Packard 5890 gas chromatograph equipped with a computer-controlled Hewlett-Packard 5971 mass-selective detector. The compounds were separated on a DB5, fused silica capillary column coated with methyl phenyl (5%)-silicone (30 m × 0.25 mm, film thickness 0.5 μm) with helium as carrier gas at a velocity of 0.8 ml/min. The oven temperature during injection was held at 80°C for 2 min and then increased at a rate of 50°C/min to 250°C. Injector and detector temperatures were 250 and 280°C, respectively. Detection was in the multiple ion mode, with nicotine and cotinine monitored at the selected masses of 84 and 98, respectively. Under these conditions, nicotine and cotinine were eluted at 5.76 and 7.55 min, respectively. Quantification of nicotine and cotinine in plasma was based on calibrations obtained by analysis of 0.5-ml samples of plasma from untreated rats spiked with various known amounts of nicotine and cotinine. Under these conditions, the recovery of nicotine and cotinine was 71 and 65%, respectively.

**Other Assays.** Washed microsomes were prepared by differential centrifugation as described previously (Iba et al., 1993a). Total RNA was prepared by the acid guanidinium thiocyanate-phenol-chloroform extraction method of Chomczynski and Sacchi (1987). Protein was determined by the method of Lowry et al. (1961).

**Statistical Analysis.** Differences between group means in catalytic activities and in plasma nicotine and cotinine concentrations were analyzed by one-way ANOVA and paired multiple comparisons, using the Student-Newman-Keuls test, with the level of significance set at p < .05. Differences between groups in total diet consumption and nicotine intake were analyzed by factorial ANOVA. Differences in body weight gains were analyzed by using a repeated measures two-way ANOVA.

**Materials.** The nicotine diets were prepared to our specifications by Research Diets, New Brunswick, NJ. (−)-Nicotine hydrogen tartrate salt, (−)-
nicotine (free base, 98–100% pure), (−)-cotinine (98% pure), NADPH, and NADP⁺ were obtained from Sigma Chemical Company, St. Louis, MO. The following were obtained from the commercial sources indicated in parentheses: ethoxyresorufin (Pierce Chemical Company, Rockford, IL); methoxyresorufin and resorufin (Molecular Probes, Eugene, OR); alkaline phosphatase-conjugated anti-mouse IgG (Biosource International, Camarillo, CA); alkaline phosphatase substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD); Zeta-Probe nylon membrane (Bio-Rad Laboratories, Hercules, CA); nitrocellulose membranes (Schleicher and Schuell, Keene, NH); [α-32P]ATP, 300 Ci/mmol (DuPont-NEN, Boston, MA); Kodak Biomax MR imaging film (Fisher Scientific, Springfield, NJ); DB5 fused silica capillary GC column (J and W Scientific, Folsom, CA). All other reagents and supplies used were of the highest grade of purity. Plasmid p210 and human GAPDH cDNA were kindly provided by Dr. John Fagan (Maharishi International University, Fairfield, IA) and Dr. Suzie Chen (Rutgers University, Piscataway, NJ), respectively.

Results

General Observations. The liquid diet supplying the low, medium, and high levels of nicotine was well tolerated by the animals, and the intake dose of nicotine base by the animals from the 20, 60, and 200mg nicotine/kg diets was estimated to be 1.7 ± 0.1, 4.9 ± 0.2, and 15.4 ± 0.4 mg/kg/day, respectively. Thus, the intake dose of the alkaloid differed between the three dietary groups (F3,28 = 863.7, p < .0001) and was proportionally related to the dietary concentrations of the compound used in the present study. No intergroup differences were observed in the consumption of the liquid diet on day 30 of the study (F3,28 = 1.82, p = .16). Significant differences between the groups were observed in body weight gains, with the differences in the order: control (210.4 ± 5.2) > low dose (206.5 ± 12.2) > medium dose (172.1 ± 9.0) > high dose (153.3 ± 5.0); (F3,22 = 31.3, p < .0001). Thus, although diet consumption did not differ between the groups, body weight gains were lower in the high- and medium-dose groups than in the control and low-dose groups. This observation suggests that nicotine-induced metabolic changes rather than insufficient dietary intake were the cause of the lowered body weight gains. Nevertheless, all the animals appeared healthy, with adequate body weight gains over the 30-day duration of the study.

Effect of Nicotine Administration on Tissue Microsomal Ethoxyresorufin-O-deethylase (EROD) and Methoxyresorufin-O-demethylase (MROD) Activities. (EROD) and (MROD) activities, which are catalyzed preferentially by CYP1A1 and CYP1A2, respectively (Yang et al., 1988), were detected in lung, kidney, and liver microsomes from control animals. Each activity was induced by nicotine in a dose-dependent manner but to various magnitudes in the three tissues examined. In the lung, EROD activity was induced 1.9-, 4.9-, and 21.6-fold, whereas MROD activity was induced 2.1-, 3.2-, and 4.4-fold by the low, medium, and high dietary doses of nicotine, respectively (Table 1). In the kidney, EROD and MROD activities were induced significantly (15.9- and 8.7-fold, respectively) only by the high nicotine dose (Table 1). In the liver, EROD activity was induced 1.7-, 2.9-, and 5.1-fold, whereas MROD activity was induced 1.6-, 3.6-, and 8.7-fold by the low, medium, and high nicotine dose, respectively (Fig. 1).

Effect of Nicotine Administration on Tissue Microsomal CYP1A1 and CYP1A2 Immunoreactive Protein Levels. In control rats, CYP1A1 was detected only in lung and kidney microsomes, whereas CYP1A2 was detected only in liver microsomes, at the microsomal protein levels that we examined. CYP1A1 was induced in the lung and kidney in a dose-dependent manner by nicotine, but it was induced in the liver only at the high nicotine dose (Fig. 1). Conversely, CYP1A2 was induced by nicotine in the liver in a dose-dependent manner and was detected in the kidney, but not lung, only after the high nicotine dose (Fig. 1).

Effect of Nicotine Administration on Tissue CYP1A1 mRNA Levels. We determined CYP1A1 mRNA levels in tissues from the high nicotine dose-exposed animals, in which CYP1A1 protein level was most pronounced, to assess involvement of the transcript in the induction. The data from Northern blot analysis of CYP1A1 in lung, kidney, and liver of rats fed the high-nicotine diet are presented in Fig. 2. At the level of total RNA that we analyzed, CYP1A1 mRNA was present at low but detectable levels in all three tissues from untreated animals. The high-nicotine diet caused significant up-regulation of the level of the transcript in all three tissues, with the up-regulation being more pronounced in the lung (36-fold) than in the kidney (12-fold) or liver (18-fold) (Fig. 2).

Plasma Nicotine and Cotinine Concentrations after Nicotine Treatment and Their Relationships to Tissue CYP1A Levels and Activities. The plasma concentrations of nicotine, as well as those of cotinine, the major, long-lived nicotine metabolite (Benowitz and Jacob, 1984), that resulted from the dietary doses of nicotine in the current study are presented in Fig. 3. We observed no significant differences between animals from the three nicotine dietary groups in plasma nicotine concentrations. Only at the intermediate nicotine dose was the plasma level of cotinine greater than that at the low but not high nicotine dose (Fig. 3). There was wide interanimal variability in the plasma nicotine and cotinine concentrations, which most likely resulted from interanimal variability in the pharmacokinetics of the two compounds, as would be expected in outbred animal strains.

Discussion

The results of the present study confirm our previous findings that nicotine induces CYP1A1 and extends to oral administration of the alkaloid the dose-dependent induction of CYP1A1 in the lung, kidney,

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### TABLE 1

<table>
<thead>
<tr>
<th>Dietary Nicotine</th>
<th>EROD</th>
<th>MROD</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg diet</td>
<td>Lung</td>
<td>Kidney</td>
</tr>
<tr>
<td>0</td>
<td>8.3 ± 1.8</td>
<td>9.3 ± 4.4</td>
</tr>
<tr>
<td>20</td>
<td>15.6 ± 6.2</td>
<td>13.3 ± 7.4</td>
</tr>
<tr>
<td>60</td>
<td>40.5 ± 8.2</td>
<td>15.6 ± 6.2</td>
</tr>
<tr>
<td>200</td>
<td>178.9 ± 39.5</td>
<td>147.7 ± 39.3</td>
</tr>
</tbody>
</table>

Each value is the mean (±S.D.) of determinations in four to six animals. Each value in parentheses is the fold-increase over the control (zero-nicotine dietary group) of the same column. a, b, and c: Values bearing different superscripts within the same column are significantly different from each other, from values bearing no superscripts, and from the control (p < .05).
and liver. Similar to our earlier observation with a bolus, s.c. 2.5 mg/kg dose of nicotine, CYP1A1 induction by the low dietary dose of the alkaloid, which yielded a daily intake dose of 2.1 mg/kg nicotine, was more prominent in the lung than in the kidney or liver, as was the induction by the intermediate (60 mg/kg) dietary dose of the compound. Only at the intermediate to high dietary dose of nicotine was CYP1A1 induced in the liver or was CYP1A2 detected in the kidney. The observed induction of CYP1A2 in the kidney supports our previous report (Iba et al., 1993b) and the report by other investigators (Beebe et al., 1995; Kim et al., 1995) that CYP1A2 is inducible in the kidney.

CYP1A1 mRNA was up-regulated by nicotine in the three tissues that we examined. This effect of the alkaloid, together with its ability to activate the aryl hydrocarbon receptor, albeit weakly (Iba et al., 1998), constitutes evidence that the nicotine-mediated induction of the enzyme was transcriptional. The higher nicotine-mediated up-regulation of the enzyme in the lung than in the kidney or liver suggests tissue selectivity of the induction. The selectivity appears to be independent of the route of nicotine administration, as it was also observed after s.c. administration of the alkaloid (Iba et al., 1998). The basis of the tissue preference of the induction is unknown at this time but may be related to the reported selective accumulation of injected nicotine in respiratory tissues (Lindquist and Ullberg, 1974) and the possible metabolic formation of CYP1A1-inducing product(s) therein (see further discussion below). The lung selectivity of the induction is noteworthy given the fact that, in contrast to the liver or kidney, is a highly heterogenous tissue in terms of cell-type composition, with only a small subset of its total cells expressing CYP1A1 (Baron and Voigt, 1993). It should be pointed out that we estimated the pulmonary induction on the basis of total tissue RNA or microsomal protein rather than on the RNA or microsomal protein from the CYP1A1-expressing cells, a factor that grossly underestimates the magnitude and tissue selectivity of the induction.

Although the magnitude of CYP1A1 induction increased with dietary nicotine dose, a linear correlation of the induction with nicotine dose was prominent only in the lung. The lack of significant induction of the enzyme in the liver at dietary nicotine doses lower than 200 mg/kg, coupled with the exponential increase in the induction in the kidney with nicotine dose (Fig. 1), constitutes additional evidence of tissue differences in the induction. Hepatic CYP1A2 induction was also exponentially related to dietary nicotine dose, implicating also multiple mechanisms in the induction. It is noteworthy that hepatic CYP1A1 induction by nicotine was most pronounced in animals on the high dietary level of the alkaloid, which had the lowest body weight gains. However, the low body weight gain is unlikely to be a factor in the induction, because tissue CYP contents and activities usually correlate positively rather than inversely with body weight gains. The cause of the low body weight gains in the high-nicotine dose animals is unknown at present but most likely resulted from nicotine-induced hypermetabolic states not unlike those reported in human smokers (Perkins, 1992).

Although it remains to be established whether nicotine induces CYP1A1 in humans, it is noteworthy that the plasma concentrations of nicotine or cotinine at which induction of CYP1A1 and CYP1A2 occurred in the current study are comparable to those reported in

**Fig. 1.** Western blot analysis of lung, kidney, and liver microsomes from control or nicotine-treated rats.

Rats were fed a control liquid diet either alone (I) or supplemented with 20 mg (II), 60 mg (III), or 200 mg (IV) of nicotine per kilogram of diet. Top, each lane represents pooled microsomes (75 μg of protein for lung or kidney and 15 μg for liver) from three individual rats. Liver microsomes (0.5 μg of protein) from β-naphthoflavone-treated rats are shown in the end lanes (b) as positive controls for CYP1A1 and CYP1A2. Bottom, densitometric data for the immunoreactive bands shown at top. AUC, area under the curve.

**Fig. 2.** Northern blot analysis of CYP1A1 mRNA from control rats or rats on the high-nicotine diet.

A, total RNA (20 μg) pooled from three control rats (CON) or three rats fed the high-nicotine diet (NIC) was electrophoresed and the separated RNAs were transferred to Zeta-Probe membranes. The blot was probed for CYP1A1 or GAPDH transcripts with a 32P-labeled CYP1A1- or GAPDH-specific cDNA and autoradiographed as described in Experimental Procedures. B, densitometric analysis of the Northern blot data shown in A. *, The densitometric value of each CYP1A1 mRNA has been normalized to that of the corresponding GAPDH mRNA.
Each value represents the mean (±S.D.) of determinations in eight rats. *Significantly different from control animals (p < .05). ○, cotinine; ■, nicotine; ▲, nicotine + cotinine.

human smokers or subjects on therapy with various nicotine formulations (Wall et al., 1988; Gourlay et al., 1997). Thus, the potential for contribution of nicotine to CYP1A induction in nicotine- and tobacco-exposed humans exists and deserves investigation.

The plasma concentrations of nicotine and cotinine did not correlate with total nicotine intake, especially at the high nicotine dose. This is explainable by the fact that cotinine undergoes further biotransformation (Gorrod and Jenner, 1976; Jacob et al., 1988; Benowitz, 1996), although plasma concentration of the compound has been considered a sufficiently stable and useful marker of nicotine exposure (Benowitz and Jacob, 1984). The metabolites resulting directly from cotinine biotransformation, along with others from other pathways of nicotine metabolism, account for the many biotransformation products reported for nicotine (Testa and Jenner, 1976). However, we did not attempt to determine the metabolites of nicotine other than cotinine in the current study. The failure of plasma nicotine and cotinine levels to parallel the total intake of nicotine at the high-nicotine diet in the current study could have been contributed by enhanced elimination of the metabolites, parent compound, or both (including conjugates). We speculate that enhanced biotransformation was a major factor in the enhanced elimination of nicotine at the high dose of the compound and that the enhanced biotransformation resulted from induction of nicotine-metabolizing enzymes [e.g., a rat equivalent of the major nicotine-metabolizing enzyme CYP2A6 (Nakajima et al., 1996)]. Alternatively, enzymes capable of significant metabolism of nicotine, cotinine, or both, only at high substrate concentrations of the substrate (i.e., high capacity, low-affinity enzymes) could have played the predominant metabolic role at the high nicotine dose.

The exponential induction of CYP1A1 at the high nicotine dose suggests the involvement of indirect mechanisms, most probably metabolites of the compound. The exponential induction also suggests that such metabolites may be more potent than the parent compound in the induction. We argued previously (Iba et al., 1998) that CYP1A1 induction by nicotine was due primarily to the parent compound, based primarily on our observation that the metabolite cotinine, unlike nicotine, neither induced CYP1A1 in vivo nor activated the aryl hydrocarbon receptor in vitro. However, in the current study, maximal CYP1A1 induction was observed with the high nicotine dose, yet the resulting plasma concentration of nicotine was similar to that from the low or intermediate nicotine dose. Thus, although nicotine may be directly involved in CYP1A1 up-regulation, it is unlikely to play an exclusive role in the induction, as metabolites are expected to contribute. With the exception of cotinine, which does not induce CYP1A1 (Iba et al., 1998), we do not as yet know which of the pyridine metabolites are active or inactive as inducers, but we expect the metabolites to differ in their CYP1A1-induction potencies, with the most potent not necessarily being the most abundant. Further support of the speculated role of metabolites in CYP1A1 induction by nicotine is our observation that the pyridine metabolites pyridine N-oxide and pyridine methonium ion are effective inducers of pulmonary and hepatic CYP1A1 in the rat (Iba et al., 1999). It remains to be determined whether nicotine N-oxide, which is a major metabolite of nicotine in animals and humans (Gorrod and Jenner, 1976), is an inducer of CYP1A1.

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References


