TOXICOLOGY AND METABOLISM

Michael M. Iba · Jacqueline Fung
Paul E. Thomas · Yangwon Park

Constitutive and induced expression by pyridine and β-naphthoflavone of rat CYP1A is sexually dimorphic

Received: 20 January 1999 / Accepted: 13 April 1999

Abstract Adult male and female Sprague-Dawley rats were compared in terms of the constitutive levels and inducibility of CYP1A1 and CYP1A2 (CYP1A) in lung, kidney, and liver. CYP1A were induced by i.p. treatment with pyridine (75 mg/kg per day) or β-naphthoflavone (βNF; 25 mg/kg per day) for two consecutive days and analyzed catalytically (via O-dealkylation of resorufin ethers), at the protein level (by Western blot analysis) and at the mRNA level (by Northern blot analysis). In untreated rats, CYP1A1 protein and its mRNA were detectable only in the lung and kidney of females but not males, whereas CYP1A2 protein and its mRNA were detectable only in the liver in either gender. Pyridine treatment upregulated CYP1A1 mRNA and its protein in the lung, kidney and liver in female rats, and upregulated the mRNA but not the protein in the lung and liver in male rats. Conversely, pyridine induced both CYP1A2 mRNA and protein in the liver in female rats, whereas it induced the protein but not its mRNA in the liver in male rats. No gender difference was observed in the plasma elimination rate of administered pyridine. βNF, in contrast to pyridine, induced CYP1A proteins, activities, and mRNA to higher levels in male than in female rats. The results show that the constitutive as well as inducible expression of CYP1A is sexually dimorphic in the Sprague-Dawley rat, with females being more responsive than males to induction by pyridine but with males being more responsive than females to induction by βNF. The findings support the involvement of different mechanisms in CYP1A induction by pyridine and βNF.

Key words CYP1A induction · Sexual dimorphism · Pyridine · β-Naphthoflavone

Introduction

Cytochromes P450 (CYP) catalyze the bioinactivation of xenobiotics and endogenous compounds as well as the biotransformation of inert compounds to active derivatives (Nelson et al. 1996). These reactions may be sexually dimorphic in many species, being more or less pronounced in one gender than in the other (Kato 1974; Skett 1988). Similarly, sensitivity to the pharmacological or toxicological effects resulting from these biotransformation reactions may be sexually dimorphic in many species (Reynolds and Burger 1994; Skett 1987). Sexual dimorphism in CYP-catalyzed biotransformation is reported to be most pronounced in the rat (Kato 1974; Skett 1988). In this species, the constitutive level and inducibility of several forms of CYP, notably members of the 2C (Guengerich 1987; Sundseth et al. 1992), 3A (Cooper et al. 1993), and 4A (Sundseth and Waxman 1992) subfamilies, are typically higher in the adult of one gender than in the other.

CYP1A1 and CYP1A2, the two members of the CYP1A subfamily, are among the P450 enzymes for which the existence of sexual dimorphism has not been well studied. CYP1A1 and CYP1A2 play pivotal roles in xenobiotic toxicity, such as bioactivation of polyaromatic hydrocarbons (PAHs) and aryl/heterocyclic amines, respectively, to toxic derivatives (Shimada et al. 1989, 1992). The two enzymes are inducible by the simple heterocyclic amine pyridine (Kim et al. 1991; Iba et al. 1993), a major constituent of tobacco smoke (International Agency for Research on Cancer 1986), and a high volume industrial chemical (Agency for Toxic Substances and Disease Registry 1991). CYP1A1 and CYP1A2 are
also inducible by a variety of compounds, of which PAHs are the most effective and most extensively studied and, therefore, the prototypes (Nebert 1989; Okey et al. 1994). PAHs induce CYP1A by binding to the ligand-activated transcription factor, aryl hydrocarbon receptor (AhR), in the cytosol. Following nuclear translocation and binding of the ligand-receptor complex to a second protein, aryl hydrocarbon nuclear translocator (ARNT), the resulting heteromeric complex binds to its specific cognate DNA sequences known as xenobiotic response elements (XREs), with resulting increased transcription of CYP1A1 and other responsive genes, including CYP1A2 (Nebert 1989; Okey et al. 1994).

Several non-PAH compounds other than pyridine also induce CYP1A. These include the tobacco alkaloid nicotine (Iba et al. 1998), the endogenous heme-derived pigments bilirubin (Phelan et al. 1998; Sinal and Bend 1997) and biliverdin (Phelan et al. 1998), and the xenobiotics carbaryl (Denison et al. 1998), omeprazole (Kikuchi et al. 1998; Lesca et al. 1995), and imidazole (Ding et al. 1992). While some of these non-PAHs can bind to the AhR (Denison et al. 1998; Iba et al. 1998; Phelan et al. 1998; Sinal and Bend 1997), evidence has been presented that some non-PAH inducers of CYP1A1 may effect the induction by mechanisms possibly distinct from those involving PAHs (Kikuchi et al. 1998; Lesca et al. 1995).

Earlier evidence indicated that neither hormonal status nor gender affected the induction of rat CYP1A (Guengerich 1987; Waxman et al. 1982; Ryan and Levin 1990). However these findings were based on studies conducted primarily in the liver, in which high doses of PAHs were the inducing agents (Guengerich 1987; Ryan and Levin 1990). Other studies with other classes of inducers, e.g. heterocyclic amines (Degawa et al. 1987, 1989; Kleman et al. 1990) and nicotine (Iba et al. 1998), indicated the existence of sexual dimorphism in CYP1A induction in rats. In the present communication, we present evidence that the constitutive level of pulmonary and renal CYP1A1 is higher in female than in male rats, and that the female rat is more responsive than the male to pyridine-induced upregulation of the enzyme, at the level of catalytic activity, protein, and mRNA. We also show that CYP1A1 induction by the polyaromatic hydrocarbon βNF is also sexually dimorphic, but with male rats being more sensitive than females to the induction.

Materials and methods

Animals and pretreatment

Male and female Sprague-Dawley rats (from Taconic Farm, Germantown, N.Y.), 8–10 weeks of age, were housed at our animal care facility for at least 1 week prior to experiments, under a temperature and relative humidity of 20 °C and 45 (±5)%, respectively. The animals were fed water and Purina rat chow ad libitum and were housed in 225–285 g body wt at the time of experiments. Pyridine (75 mg/ml of saline) or βNF (50 mg/ml of corn oil) was administered i.p. at a dose of 75 mg/kg or 25 mg/kg, respectively, once a day for two consecutive days. Control rats received saline or corn oil only. These doses have been determined in studies in our laboratory (Scholl et al. 1997) to be lower than the optimum (near-threshold) i.p. doses for hepatic CYP1A1 induction. The doses are still larger than those likely to be encountered in real-life exposures, but lower than those commonly used in CYP induction studies. The animals were decapitated 18 h (between 8.00 and 10.00 a.m.) after the last dose of either compound; pieces of the lung, liver, and kidney were removed and quick-frozen in liquid nitrogen for the isolation of total RNA. Each remaining tissue was excised and stored at −80 °C for the isolation of microsomes. In studies of plasma pyridine elimination, animals were injected with a single i.p. dose of the chemical (75 mg/kg) and tail blood samples (0.10–0.16 ml) obtained at varying time intervals for pyridine determination by gas chromatography-mass spectrometry as described previously (Scholl and Iba 1997).

Assay of ethoxyresorufin O-deethylase or methoxyresorufin O-demethylase activity

Microsomal O-dealkylation of methoxyresorufin (MROD) or ethoxyresorufin (EROD) 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 concentration used (50 μg protein for liver microsomes and 100 μg protein for lung or kidney microsomes per ml incubation).

Electrophoresis and western blot analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis of microsomes were performed as described previously (Iba et al. 1999a). Briefly, microsomes were electrophoresed and the separated proteins transferred onto nitrocellulose membranes and probed with CD5, a monoclonal antibody raised in the mouse against rat CYP1A1, which also recognizes CYP1A2 (Thomas et al. 1984). Immunoreactive bands on the membranes were detected by enhanced chemiluminescence and quantified by densitometry, using a Bio Image IQ scanner. Each densitometric area under the curve, AUC value was linear with respect to the protein concentration analyzed.

Northern blot analysis

Northern blot analysis of total RNA for CYP1A1 and CYP1A2 transcripts was performed as described previously (Iba et al. 1999a). Briefly, 20 μg total RNA was fractionated on a denaturing formaldehyde/agarose (1%) gel, transferred to Zeta-Probe nylon membrane, and fixed to the filters by UV-irradiation. The filters were then incubated for 1 h at 45 °C with 10 ml of hybridization solution comprising: 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 32P-labeled DNA probe (specific activity, 1–2 × 106 cpm/μg) for 20 h at 45 °C. The rat CYP1A1- and mouse CYP1A2-specific probes were prepared by random priming (Sambrook et al. 1989) of the inserts from plasmid p210 (Fagan et al. 1985) and pMP3450-3′ [obtained from American Type Culture Collection (ATCC), Rockville, Md.], respectively, using [α-32P]ATP. After hybridization, the filters were washed with a solution of 0.15 M NaCl, 15 mM sodium citrate, pH 7.4 (1 × SSC), containing 0.5% SDS, at room temperature for 15 min and twice with 0.1 × SSC, containing 0.1% SDS, at 60 °C for 15 min each. The blots were exposed to X-ray film (Kodak X-Omat AR) at −70 °C.

Other assays and analysis

Protein was determined by the method of Lowry et al. (1951). Total RNA was isolated by the method of Chomczynski and Sacchi (1981). Pyridine concentrations and half-lives in plasma were