DIFFERENTIAL INDUCTION OF RAT HEPATIC CYTOCHROMES P450 3A1, 3A2, 2B1, 2B2, AND 2E1 IN RESPONSE TO PYRIDINE TREATMENT

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ABSTRACT:

Pyridine (PY) effects on rat hepatic cytochromes P450 (CYP) 3A1 and 3A2 expression were examined at the levels of metabolic activity, protein, and mRNA and were compared with those of CYP2B1/2 and CYP2E1. CYP3A metabolic activity as well as CYP3A protein and mRNA levels increased following treatment of rats with PY. CYP3A1 and CYP3A2 were differentially affected by PY treatment in terms of induction levels, dose dependence, and stability of mRNA. CYP3A1 mRNA levels maximally increased ~42-fold after PY treatment, whereas CYP3A2 mRNA level increased ~4-fold. Moreover, CYP3A1 mRNA levels decreased more rapidly than those of CYP3A2 as determined following inhibition of transcription with actinomycin D or cordycepin. Treatment of rats with PY resulted in a dose-dependent increase in CYP3A1, CYP3A2, and CYP2B1/2B2 protein levels. In contrast to the effects of PY treatment on CYP3A1 and 2B, CYP2E1 protein levels increased in the absence of a concomitant increase in CYP2E1 mRNA levels. Treatment of rats with PY at 200 mg/kg/day for 3 days increased both protein and mRNA levels of CYP3A2, whereas treatment with higher than 200 mg/kg/day for 3 days increased CYP3A2 protein levels without an increase in CYP3A2 mRNA levels. These data demonstrated that PY regulates the various CYPs examined in this study at different levels of expression and that PY regulates CYP3A1 expression through transcriptional activation and CYP3A2 expression through transcriptional and post-transcriptional activation at a low- and high-dose PY treatment, respectively.

Pyridine (PY) is a widely used industrial solvent and a constituent of tobacco and tobacco smoke. Research in this laboratory has shown that PY is a pleotropic modulator of hepatic drug-metabolizing enzymes and that the molecular mechanisms by which PY induces these enzymes vary between different cytochrome P450 (CYP) subfamilies and, in some cases, are cell type- and organ-specific. For example, PY enhances hepatic CYP2E1 protein levels and enzymatic activity without altering mRNA levels by increasing the translational efficiency of CYP2E1 mRNA (Kim and Novak, 1990; Kim et al., 1990). In contrast, PY-mediated increases in renal CYP2E1 protein levels were associated with increased CYP2E1 mRNA levels (Kim et al., 1992). PY also transiently elevated hepatic (Kim et al., 1991) and renal (Kim et al., 1995) CYP1A1 and 1A2 mRNA levels. PY treatment increased CYP2B1/2B2 levels in rat liver (Kim et al., 1993), and these changes were associated with ~70- and 30-fold increases in CYP2B1 and 2B2 mRNA levels, respectively.

The CYP3A subfamily is involved in the metabolism of steroids and numerous xenobiotics (Shimada and Guengerich, 1989; Gonzalez, 1990; Murray and Reidy, 1990). These enzymes appear to be highly conserved in mammals and possibly across vertebrate species (Nelson et al., 1996). In rat liver, both CYP3A1 and 3A2 are expressed (Gonzalez et al., 1985, 1986). Although the sequences are ~90% identical and the proteins appear to have similar substrate specificities, the regulation of these forms in rat liver varies markedly during development, with gender, and in response to xenobiotic treatment (Gonzalez et al., 1986). Xenobiotics have been shown to alter CYP3A expression at many different levels. Many inducing agents act by transcriptionally activating CYP3A genes (Gonzalez et al., 1986). However, Talhada et al. (1992) reported that in the adult rat, dexamethasone (DEX)-mediated increases in CYP3A1 mRNA levels were also dependent on post-transcriptional mechanisms. Macrolide antibiotics have been shown to stabilize CYP3A protein in a process that is associated with tight binding of a bioactivated metabolite to the CYP3A protein and subsequent loss of enzymatic activity (Watkins et al., 1986). Recent reports have also shown that dimethyl sulfoxide and ethylbenzene increase CYP3A protein in the absence of increased mRNA levels (Yuan et al., 1994; Zangar et al., 1997), suggesting that certain solvents may also alter CYP3A protein turn-
over. Since these solvents also increased CYP3A metabolic activity, it may be that inhibition of CYP3A activity is not essential for decreased degradation of CYP3A protein.

In this study, we examined effects of varying doses of PY on the expression of CYP3A1 and CYP3A2 and compared these effects with those observed for CYP2B1, CYP2B2, and CYP2E1. We observed that PY is an efficacious inducer of CYP3A and that higher doses of PY are required to enhance the expression of CYP3A1 than are required for CYP3A2. PY appears to enhance CYP3A expression by transcriptional (CYP3A1 and CYP3A2 with lower dose treatment) and post-transcriptional (CYP3A2 with a higher dose treatment) activation.

Experimental Procedures

Materials. Alkaline phosphatase-conjugated donkey anti-rabbit or anti-goat IgG and horseradish peroxidase-conjugated donkey anti-mouse IgG were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Streptavidine-horseradish peroxidase conjugate and 4-chloro-1-naphthol were obtained from Life Technologies, Inc. (Gaithersburg, MD). A mixture of prestained molecular mass standards was obtained from Bio-Rad Co. (Hercules, CA). Oligonucleotide probes were synthesized by Research Genetics (Huntsville, AL). Other reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals and Treatments. Male Sprague-Dawley rats (160–200 g) were treated i.p. with PY (100–400 mg/kg/day for 1–3 days), phenobarbital (PB) (100 mg/kg/day for 3 days), DEX (5 mg/kg/day for 4 days), 3-methylcholanthrene (25 mg/kg, single injection), clofibrate (200 mg/kg/day for 3 days), or corn oil (2 ml/day for 4 days). DEX, 3-methylcholanthrene, and clofibrate were dissolved in corn oil. Animals were treated with actinomycin D (7 mg/kg), cordycepin (20 mg/kg), or cycloheximide (10 mg/kg) following a single treatment of 25 mg of 3-methylcholanthrene/kg or following 3 consecutive days of treatment with 200 mg of PY/kg/day or 100 mg of PB/kg/day.

Preparation of Microsomes. Microsomes were prepared from rat livers as described previously (Kaul and Novak, 1987; Kim et al., 1993). Microsomes were stored at −80°C in 50 mM Tris acetate buffer, pH 7.4, containing 1 M EDTA and 20% glycerol until used. Protein was assayed by the method of Lowry et al. (1951). Total P450 content in microsomal suspensions was determined according to the procedure of Omura and Sato (1964).

Enzyme Assays. Erythromycin N-demethylation activity was measured by the spectrophotometric detection of formaldehyde formation (Nash, 1953), pentoxyresorufin O-dealkylation activity was determined by the direct fluorometric detection of resorufin formation (Lubet et al., 1985), and p-nitrophenol hydroxylolation activity was determined by the spectrophotometric detection of 4-nitrophenolate formation (Koop, 1986). Statistical differences between treatment groups were determined by one way analysis of variance and Fisher’s protected least significant difference according to the procedure of Omura and Sato (1964).

Electrophoresis and Electroblotting. SDS-PAGE was carried out on 7.5 or 10% acrylamide gels (Laemmli, 1970; Kim et al., 1993). Molecular mass standards used in SDS-PAGE gels were a mixture of myosin (205 kDa), Escherichia coli b-galactosidase (116 kDa), rabbit phosphorylase B (97 kDa), bovine serum albumin (66 kDa) (Sigma Chemical Co.), or a mixture of prestained phosphorylase B (106 kDa), bovine serum albumin (80 kDa), and ovalbumin (49.5 kDa) (Bio-Rad Co.). Electroblotting of proteins from the SDS-PAGE onto a nitrocellulose membrane was carried out according to the manufacturer’s protocol (Bio-Rad Co.).

Western Blot Analyses. Anti-rabbit polyclonal antibodies against rat CYP2B1 (Shen et al., 1991), anti-goat polyclonal antibodies against rabbit CYP2E1 IgG (Kim and Novak, 1990; Kim et al., 1992), and antipeptide antibodies against CYP3A2 (Debri et al., 1995) were previously described. Monoclonal antibody (Mab) 6B was produced using CYP3A1 isolated from hepatic microsomes obtained from rats after treatment with DEX. Polyclonal antibodies produced using the immunogens were characterized in Forrester et al. (1992).

Western blot analyses were carried out using a horseradish peroxidase system or an alkaline phosphatase system with a colorimetric substrate or the AttoPhos substrate (JBL Scientific, San Luis Obispo, CA) as previously described (Kim et al., 1992, 1993; Zangar et al., 1999). The intensities of immunoblot bands were determined using a laser densitometer or a Vistra Fluorimagre with ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Northern Blot Analyses. A 32- or 31-mer oligonucleotide sequence complementary to CYP3A1 or 32, respectively, and which was reported to be specific for each CYP (Gonzalez et al., 1986), was truncated to 20-mer. The 20-mer oligonucleotide sequences for probes specific for CYP3A1 and 32 were 5'-dTGAAGCCTTATCCAGAGCAGT-3' and 5'-dACCCATGCATCAAGGACAGCT-3', respectively. The sequences of 20-mer oligonucleotide probes were missing 11 and 10 nucleotide sequences from 5'-ends and 1 each from 3'-ends of the previously used 32- and 31-mer probes of CYP3A1 and 32, respectively. Recently, several novel forms of CYP3A subfamily have been reported to be expressed in rat liver, including CYP3A9, 3A18, and 3A23 (Kirita and Matsubara, 1993; Komori and Oda, 1994). Therefore, we cannot eliminate the possibility that some of the results reported here for CYP3A1 may actually reflect changes in CYP3A2 levels as well2. Nucleotide sequences from 3'-untranslated region of CYP3A1 or CYP3A2 cDNAs against which the nucleotide probes were produced are shown in Fig. 1. The closest match to CYP3A1/3A2 among CYP3A subfamily members is CYP3A2. Similarity with other cDNA sequences reported in GenBank was investigated using the National Center for Biotechnology Information (NCBI) BLAST sequence analysis program against over 600,000 sequences or ~2 billion nucleotides to ensure specificity of each probe. No significant homology was found among rat CYP3A subfamily or other rat sequences reported to GenBank3.

We used an oligonucleotide probe, previously shown to specifically detect CYP2E1, having the sequence of 5'-d(CAAAGCCCAACTGTGACAGG) (Song et al., 1986; Umeno et al., 1988; Kim et al., 1992, 1993). Oligonucleotide

2 The NCBI BLAST search result was as of May 25, 2000.
probes previously shown to hybridize specifically to CYP2B1 or -2B2 mRNA were 5'-d(GGTTGGTAGCCGGTGTGA) and 5'-d(GGATGGTGGCCTGTGAGA), respectively (Mizukami et al., 1983; Yuan et al., 1983; Omiecinski et al., 1985; Kim et al., 1992). The oligonucleotide probes were obtained from Research Genetics.

Total RNA was prepared using a single-step acid guanidinium thiocyanate-phenol-chloroform extraction procedure, as described previously (Chomczynski and Sacchi, 1987; Kim et al., 1995). Poly(A)⁺ RNA was isolated from total RNA using oligo(dT)-cellulose according to the method of Jacobson (1987). Aliquots of poly(A)⁺ RNA were subjected to denaturing electrophoresis on agarose-formaldehyde gels (Sambrook et al., 1989) and transferred to nitrocellulose filters (Sigma Chemical Co., St. Louis, MO). Hybridization analyses with CYP2B1, 2B2, or 2E1 oligonucleotide probes or a dT16 probe were carried out as previously described (Kim et al., 1991, 1992, 1993). Hybridization analyses with CYP3A1 or 3A2 oligonucleotide probes were carried out as described for the other oligomeric probes except that the probes were hybridized at 51 and 55°C, respectively. Following hybridization analysis of CYP3A1, CYP3A2, CYP2B1, or CYP2B2, the membrane was stripped and rehybridized with an oligonucleotide probe specific for CYP2E1 or a dT16 probe. We have previously shown that CYP2E1 mRNA levels remain unchanged after treatment of rats with 100 to 400 mg of PY/kg/day for 1 to 3 days (Kim et al., 1993). Therefore, normalization for the amounts of poly(A)⁺ RNA loaded on the membrane was carried out as described above using a dT16 probe as well as oligonucleotide probes specific for CYP2E1.

Results

Induction of Erythromycin N-Demethylation Activity, CYP3A Protein, and mRNA in Rat Hepatic Tissue Following Treatment. Erythromycin N-demethylation, a reaction catalyzed by members of the CYP3A subfamily, was increased ∼2-fold by 100 mg of PY/kg/day after 2 or 3 days of treatment (Fig. 2A). Treatment with 200, 300, or 400 mg of PY/kg/day for 3 days further increased rates of erythromycin oxidation ∼3- to 4-fold relative to control values (Fig. 2B).

CYP3A1 and CYP3A2 protein levels also increased after PY treatment ∼6- and 39-fold, respectively, as detected by Western blot analyses using monoclonal antibodies against CYP3A1 (Mab p6) and antipeptide antibodies against CYP3A2, polyclonal antibodies against CYP2B1/2B2, and polyclonal antibodies against CYP2E1, respectively.

Ten micrograms of microsomal proteins were loaded to each lane and separated by SDS-PAGE with 10% gels. The proteins were then electroblotted onto nitrocellulose membranes. Western blot analyses were carried out using a horseradish peroxidase or an alkaline phosphatase system. The intensities of immunoblot bands were determined using a laser densitometer or a Vistra FluorImager with ImageQuant software (Molecular Dynamics). Lane 1, untreated control; lanes 2, 3, and 4, 1-, 2-, or 3-day treatment with 100 mg of PY/kg/day, respectively; lanes 5, 6, and 7, 3-day treatment with 200, 300, or 400 mg of PY/kg/day, respectively; and lane 8, 3-day treatment with 100 mg of phenobarbital/kg/day.

These results suggest that there is a greater increase in CYP3A2 than 3A1 protein following PY treatment. The CYP2E1 and 2B1/2 protein levels increased ∼4- and 9-fold, respectively, following PY treatment in a dose-dependent manner (Fig. 3). The CYP3A1 and CYP3A2 proteins migrated more rapidly than did CYP2E1, 2B1, or 2B2.

Northern blot analyses showed that 100 mg of PY/kg/day, for 1 to 3 days, failed to increase CYP3A1 mRNA levels, although an ∼2-fold increase in CYP3A2 mRNA levels was observed (Fig. 4). The slight increase in CYP3A1 mRNA band intensity observed after a 2-day...
treatment of 100 mg of PY/kg/day (Fig. 4, 3A1, lane 3) was due to greater loading of mRNA compared with that of the untreated as evidenced by a similar increase in CYP2E1 mRNA band density (Fig. 4). CYP3A1 mRNA levels, however, were increased ~42-fold and CYP3A2 mRNA levels were increased ~4-fold following 3 days of treatment with 200 or 400 mg of PY/kg/day (Fig. 4, lanes 5 and 6).

**Induction and Decrease of a Protein Immunologically Related to CYP3A1 and CYP2E1, Respectively, in Microsomes Obtained from Rats following DEX Treatment.** In addition to detecting the CYP3A1 band (51 kDa), the Mab p6 also cross-reacted with a 50-kDa species. Levels of this 50-kDa species failed to increase substantially following treatment with PY, PB, 3-methylcholanthrene, or clofibrate (Fig. 5). In contrast, the 50-kDa species band intensity increased ~5-fold following DEX treatment (Fig. 6).

CYP2E1 levels decreased after treatment of rats with 5 mg of DEX/kg/day for 4 days (Fig. 6) and 10 mg of DEX/kg/day for 4 days (data not shown). A band migrating faster than CYP2E1 on SDS-PAGE was immunoreactive to polyclonal antibodies produced against native rat CYP2E1 as shown in Fig. 6. This band was induced after PY and DEX treatment (Fig. 6).

**Differential Dose Response of Hepatic CYP3A1/2, 2B1/2, and 2E1 following PY Treatment: Metabolic Activity.** The dose response of erythromycin N-demethylation activity, a metabolic activity associated with CYP3A1/2, was compared with those for pentoxyresorufin O-dealkylation and p-nitrophenol hydroxylation activities, which are primarily catalyzed by CYP2B1/2 and 2E1, respectively (Fig. 7). Erythromycin N-demethylation activity and p-nitrophenol hydroxylation activity were increased by PY treatment of 100 mg/kg/day, whereas pentoxyresorufin O-dealkylation activity increased following PY treatment of 200 mg/kg/day or greater (Fig. 7). Doses of PY required to increase erythromycin N-demethylation, p-nitrophenol hydroxylation, or pentoxyresorufin O-dealkylation activity to 50% of maximum levels were calculated to be 145, 130, and 210 mg/kg/day for 3 days, respectively (Fig. 7).

**Differential Dose Response of Hepatic CYP3A1, 3A2, 2B1/2, and 2E1 following PY Treatment: Protein.** Microsomal levels of CYP3A1, 3A2, 2B1/2, and 2E1 in PY-treated rats were determined using Western blot analyses. CYP2E1, CYP2B1/2B2, and CYP3A2 protein levels increased to ~40, 15, and 2% of maximally induced levels, respectively, after treatment with 100 mg/kg/day for 3 days, whereas those of CYP3A1 failed to increase (Fig. 8). The 2% of maximally induced levels of CYP3A2 after PY treatment was equivalent to ~2-fold increase of the CYP3A2 protein levels. The PY dose required to increase CYP3A1, 3A2, and 2B1/2 protein levels to 50% of maximum was calculated to be ~330, 260, and 180 mg/kg/day for 3 days, respectively (Fig. 8). A PY dose that would comparably increase CYP2E1 protein levels was calculated to be 130 mg/kg/day for 3 days (Fig. 8).
Western blot analyses were carried out using a horseradish peroxidase or an alkaline phosphatase system. For each lane, 5 μg of microsomal proteins was loaded. Lane 1, microsomes obtained following treatment of rats for 3 days with 400 mg of PY/kg/day; lane 2, microsomes obtained from untreated rats; lane 3, microsomes obtained following treatment of rats for 4 consecutive days with 2 ml of corn oil; lane 4, microsomes obtained following treatment of rats for 4 consecutive days with 5 mg of DEX/kg/day.

**Fig. 7.** Comparison of dose-dependent changes for the induction of the catalytic activities of hepatic CYP3A, 2B, and 2E1. Measurement of erythromycin (EM) N-demethylation activity of CYP3A was carried out in reaction mixtures (1.0 ml) that contained 0.5 mg of microsomal protein, 1.0 mM EM, and 1.0 mM NADPH in 50 mM potassium phosphate buffer, pH 7.4. Measurement of pentoxyresorufin (PR) O-dealkylation activity of CYP2B was carried out in a reaction mixture (1.0 ml) containing 0.5 mg of microsomal protein, 100 μM PNP, 1.0 mM ascorbate, and 1.0 mM NADPH in 100 mM potassium phosphate buffer, pH 6.8. Reaction mixtures for both EM N-demethylation activity and PNP hydroxylation activity were incubated for 10 min at 37°C. Measurement of pentoxyresorufin (PR) O-dealkylation activity of CYP2B was carried out in a reaction mixture (1.0 ml) containing 0.5 mg of microsomal protein, 5 μM PR, and 1 mM NADPH in 50 mM potassium phosphate buffer, pH 7.4. The reaction mixture was incubated for 2 min at 37°C.

**Fig. 6.** Western blot analyses of hepatic microsomal CYP3A1, 2E1, and 2B1/2 following treatment with PY and DEX.

Western blot analyses were carried out using a horseradish peroxidase or an alkaline phosphatase system. For each lane, 5 μg of microsomal proteins was loaded. Lane 1, microsomes obtained following treatment of rats for 3 days with 400 mg of PY/kg/day; lane 2, microsomes obtained from untreated rats; lane 3, microsomes obtained following treatment of rats for 4 consecutive days with 2 ml of corn oil; lane 4, microsomes obtained following treatment of rats for 4 consecutive days with 5 mg of DEX/kg/day.

**Differential Induction Response of Hepatic CYP3A1, 3A2, 2B1, and 2B2 mRNA following PY Treatment: mRNA.** CYP3A1 mRNA levels increased ~42-fold, which is less than that of CYP2B1 (~80-fold) but about the same as 2B2 following a high-dose PY treatment. CYP3A2 mRNA levels maximally increased ~4-fold after PY treatment. After low-dose PY treatment (100 mg/kg/day for 3 days), CYP3A1 mRNA levels failed to increase (Fig. 9). Therefore, slight increases in erythromycin N-demethylation activity (Fig. 7) after treatment of rats with PY at the low dose were a result of increases in CYP3A2 protein (Fig. 8) and mRNA levels (Fig. 9).

Previous studies in this laboratory showed that expression of CYP2E1 is regulated by post-transcriptional mechanisms (Kim and Novak, 1990; Kim et al., 1992). CYP3A is the most abundant human hepatic CYP3A1 and 3A2 proteins and mRNAs.

**Loss of Hepatic CYP3A1 and 3A2 mRNA following Inhibition of Transcription.** Transcription was inhibited by treatment of rats with actinomycin D following pretreatment of rats with PY, PB, or 3-methylcholanthrene. Actinomycin D treatment of PY- or PB-pretreated rats decreased CYP3A1 mRNA in 6 h by ~60% relative to levels in the respective induced rats (Fig. 10A). In untreated or PB-treated rats, actinomycin D treatment decreased hepatic CYP3A2 mRNA levels 8% or less (Fig. 10A). However, in the presence of PY or 3-methylcholanthrene, CYP3A2 levels were decreased to ~50% of control levels. These results suggest that PY treatment does not increase CYP3A2 mRNA levels by decreasing CYP3A2 mRNA turnover and, in fact, may increase CYP3A2 mRNA degradation. In a separate study, treatment with a structurally distinct transcription inhibitor, cordycepin, decreased CYP3A1 and 3A2 mRNA levels in rats pretreated with PY ~66 and 42%, respectively, in 6 h. These values are comparable with those manifested with actinomycin D (63 ± 7 and 41 ± 8% decreases in CYP3A1 and 3A2 mRNA levels, respectively) (Fig. 10).

Treatment with cycloheximide decreased CYP3A1 and 3A2 mRNA levels to values comparable with those observed with the inhibitors of transcription (Fig. 10B). This result suggests that continued transcription of CYP3A genes may be dependent on the continued synthesis of labile protein(s), which possibly function as transcription factors or regulators of transcription factors.

**Discussion**

PY is a pleotropic modulator of drug-metabolizing enzymes that has previously been shown to enhance the expression of hepatic CYP1A (Kim et al., 1991), CYP2B (Kim et al., 1993), CYP2E1 (Kim et al., 1988, 1990, 1993; Kim and Novak, 1990), and nasal carboxylesterase (Nikula et al., 1995) and is elevated in response to many xenobiotics by both transcriptional and post-transcriptional mechanisms. Therefore, we examined whether PY altered the expression of CYP3A. We found that PY treatment increased erythromycin N-demethylation (which is primarily catalyzed by CYP3A) and the levels of immunodetectable CYP3A1 and 3A2 proteins and mRNAs.
Xenobiotics have been reported to regulate CYP3A at the levels of transcription, mRNA turnover, and protein degradation (Gonzalez et al., 1986; Watkins et al., 1986; Talhada et al., 1992; Yuan et al., 1994; Zangar et al., 1997). CYP3A1 protein levels increased with concomitant increase of CYP3A1 mRNA as shown in Figs. 8 and 9. This result suggested that PY treatment altered CYP3A1 expression by pretranslational mechanisms. CYP3A2 mRNA levels also increased with a concomitant increase of CYP3A2 protein levels in the rat liver tissue obtained after a low-dose PY treatment. However, at high-dose PY treatment, CYP3A2 protein levels increased without a corresponding increase in CYP3A2 mRNA levels. These effects of PY on CYP3A2 at a high dose of PY treatment are in agreement with those of certain other solvents such as ethylbenzene and dimethyl sulfoxide, which increase CYP3A protein and catalytic activity but do not alter CYP3A mRNA levels (Yuan et al., 1994; Zangar et al., 1997). PY increases hepatic CYP2E1 levels without increased CYP2E1 mRNA levels (Kim and Novak, 1990; Kim et al., 1990). Whether the increase of CYP3A2 proteins is a result of altered CYP3A2 protein turnover or enhanced translational efficiency of CYP3A2 mRNA is unknown.

Studies examining the stability of the CYP mRNAs in the presence of transcriptional inhibitors showed that PY at a dose of 200 mg/kg/day for 3 days did not decrease CYP3A1 and 3A2 mRNA turnover. These results suggest that PY, at the doses examined, likely increases hepatic CYP3A1 and 3A2 mRNA by transcriptional activation.

PY treatment appears to result in an induction profile distinct from that observed with prototypical P450 inducers such as PB or DEX. Like PB, PY increases CYP3A1, 3A2, 2B1, and 2B2. PY induces CYP1A1 and 1A2 (Kim et al., 1991, 1995) and 2E1, which are not normally increased following PB or DEX treatment. Furthermore, PY suppresses glutathione S-transferase activity (Primiano et al., 1992), whereas the agents PB (Igarashi et al., 1987) and DEX (Wrighton et al., 1985) induce these enzymes in the rat. Therefore, changes in drug metabolism associated with PY exposure are distinct from those observed with prototypical inducers such as PB or DEX.

Erythromycin N-demethylation activity increased ~2-fold in the hepatic microsomes obtained from rats treated with 100 mg of PY/kg/day for 3 days with a slight increase in CYP3A2 proteins and an increase in CYP3A2 mRNA, but not CYP3A1 mRNA. Western blot analysis using a Mab p6 that primarily cross-reacts with CYP3A1 as well as Northern blot analysis using synthetic oligo probes specific for CYP3A1 showed that CYP3A1 expression levels increased only after high doses of PY treatment (Figs. 8 and 9). Therefore, we concluded that the 2-fold increase in erythromycin N-demethylation activity in response to 100 mg of PY/kg/day for 3 days was a result of an increase in CYP3A2 expression.

CYP3A1 mRNA level maximally increased ~42-fold, whereas CYP3A2 mRNA level increased ~4-fold. However, loss of CYP3A1 mRNA was greater than that of CYP3A2 as determined in rats 6 h after treatment with transcriptional inhibitors, actinomycin D, and cordycepin. CYP3A1 protein level maximally increased ~6-fold, whereas CYP3A2 protein level increased ~39-fold. These results showed that PY differentially regulates CYP3A1 and CYP3A2 expression.

The dose-response curve of CYP3A1 protein expression differed from that observed for CYP3A2 protein, which also markedly differed from those of CYP2B1/2B2 and CYP2E1 (Fig. 8). Doses of PY required to increase CYP3A1, 3A2, 2B1/2B2, and 2E1 protein levels to 50% of maximum were ~330, 260, 180, and 130 mg/kg/day for 3 days, respectively (Fig. 8). CYP2E1 expression was rapidly increased at the lowest PY dose examined in this study, 100 mg/kg/day for 3 days. Furthermore, previous studies have shown that CYP2E1 was increased following a single i.p. dose of 10 mg/kg (Kim and Novak, 1990) and that nose-only inhalation exposure of rats to PY at the threshold limit value of 5 ppm resulted in increased hepatic CYP2E1 levels (Hotchkins et al., 1993).
levels without inducing the diverse response in drug-metabolizing enzymes observed at higher doses of PY.

Protein levels after treatment of 100 mg of PY/kg/day for 3 days were ~40 and 20% of maximum expression levels of CYP2E1 and CYP2B1/2B2, respectively, whereas levels of catalytic activities of the CYPs were ~35 and 2% of the maximum levels for the subfamily. While CYP2E1 showed catalytic activity levels comparable with their protein levels, the catalytic activity of CYP2B1/2B2 was much lower than the protein level. This discrepancy was also previously observed by Kim et al. (1993) in microsomes obtained from rats after PY treatment: pentoxyresorufin O-dealkylation activity of microsomes obtained from rats after PY treatment was about half of that of phenobarbital-induced microsomes. A subsequent experiment showed that PY inhibited pentoxyresorufin O-dealkylation activity of CYP2B1 in a dose-dependent manner with a Ki of 0.9 mM, suggesting that PY inhibits CYP2B1/2B2 (Kim et al., 1993).

A minor species (molecular mass of ~50 kDa), which migrates more rapidly than CYP3A1 or 3A2 on SDS-PAGE, cross-reacted with Mab p6, a monoclonal antibody raised against CYP3A1. The levels of this 50-kDa species increased ~5-fold following 5 mg of DEX/kg/day for 4 days of treatment, whereas it was unaffected by PY, PB, clofibrate, or 3-methylcholanthrene treatment (Figs. 5 and 6). Recently, several novel forms of CYP3A subfamily have been reported to be expressed in rat liver, including CYP3A9, 3A18, and 3A23 (Kirita and Matsubara, 1993; Nagata et al., 1996; Wang et al., 1996). A recent study using a polyclonal antibody raised against a peptide corresponding to a unique region of the CYP3A23 protein showed that PY, as well as PB and DEX, also induces the CYP3A23 protein (Kim et al., 1997). The 50-kDa band detected in the immunoblots may be CYP3A18. Among the reported CYP3A subfamily, CYP3A18 is the lowest molecular mass protein (i.e., it is six amino acids shorter than either CYP3A1, 3A2, or 3A23). CYP3A18 has also been reported to be induced by DEX, but not by PB (Nagata et al., 1996; Mahnke et al., 1997). If so, these data would indicate that not all hepatic CYP3A subfamily members are responsive to PY treatment.

In conclusion, PY treatment showed differential behavior between CYP3A1 and CYP3A2 with regard to induction levels, dose dependence of expression, and stability of mRNA. Our data suggest that PY regulates CYP3A1 expression by transcriptional activation and CYP3A2 expression by transcriptional activation at a low dose and by post-transcriptional activation at a high dose of PY.

References


Animals were sacrificed 24 h after the final treatment with chemical inducer and 6 h after treatment with actinomycin D, cordycepin, or cycloheximide. A, lane 1, untreated rats; lane 2, rats treated with actinomycin D alone; lanes 3 and 4, rats treated for 3 days with 200 mg of PY/kg/day in the absence or presence, respectively, of actinomycin D; lane 5 or 6, rats treated for 3 consecutive days with 100 mg of PB/kg/day in the absence or presence, respectively, of actinomycin D; lane 7 or 8, rats treated with 25 mg of 3-methylcholanthrene/kg in the absence or presence, respectively, of actinomycin D. B, lane 1, untreated rats; lane 3, rats treated with PY for 3 days; lanes 2, 4, and 5, rats treated with PY for 3 days before treatment with actinomycin D, cordycepin, or cycloheximide, respectively, at 6 h before sacrifice.

Fig. 10. Effects of transcription or translation inhibitors on hepatic CYP3A1 and 3A2 mRNA expression following treatment with PY, PB, and 3-methylcholanthrene.


