Enhanced Expression and Glucocorticoid-Inducibility of Hepatic Cytochrome P450 3A Involve Recruitment of the Pregnane-X-Receptor to Promoter Elements in Rats Fed Soy Protein Isolate¹–³

Martin J. J. Ronis, Ying Chen, Xioli Liu, Michael L. Blackburn, Kartik Shankar, Reid D. Landes, Nianbai Fang and Thomas M. Badger

¹Department of Pharmacology and Toxicology, ²Department of Physiology and Biophysics, ³Department of Pediatrics, and ⁴Department of Biostatistics, University of Arkansas for Medical Sciences, Little Rock, AR 72205; and ⁵Arkansas Children’s Nutrition Center, Little Rock, AR 72202

Abstract

Previous studies and Expt. 1 of the current study demonstrate that diets made with soy protein isolate (SPI) enhance the glucocorticoid-inducibility of hepatic cytochrome P450 (CYP)3A-dependent monooxygenase activities (P < 0.05) compared with diets made with casein (CAS). To determine the underlying molecular mechanism, in a second experiment, we analyzed the time course of dexamethasone (DEX)-induction of hepatic CYP3A mRNA expression on postnatal d (PND) 25 and PND60 in male and female rats fed SPI- or CAS-based diets. After 50 mg/kg DEX, CYP3A1 mRNA expression increased 200-fold in SPI-fed males and females at PND25 compared with a 100-fold increase in CAS-fed rats (P < 0.05). The DEX-induced increase in CYP3A1 mRNA in SPI-fed rats on PND60 was also greater than that in CAS-fed rats. The induction by DEX of CYP3A2 mRNA was 1- to 3-fold greater in rats fed SPI compared with those fed CAS on PND25 (P < 0.05). Quantitation of newly synthesized CYP3A1 RNA transcripts by nuclear run-on analysis demonstrated a greater rate of basal transcription in SPI-fed compared with CAS-fed rats on PND60 accompanied by greater binding of the pregnane X receptor (PXR) to a response element on the CYP3A1 promoter in SPI-fed compared with CAS-fed rats (P < 0.05). These data suggest that increased hepatic CYP3A expression and inducibility following SPI feeding involves recruitment of PXR to its response element and suggests that soy consumption has potential effects on metabolism and transport of a wide variety of drugs and on bile acid homeostasis via proteins regulated by this transcription factor.  J. Nutr. 141: 10–16, 2011.

Introduction

Drug metabolizing enzymes, such as the cytochrome P450 (CYP)⁹-dependent microsomal monooxygenases, are quite variably expressed in human liver (1). Inter-individual differences may be explained in part by differences in diet. Diet has been shown to alter CYP expression and activity in both humans and animal models (2,3). We have previously demonstrated that feeding rats diets containing soy protein isolate (SPI), the sole protein source in soy infant formulas and a major form of soy in processed foods, results in alterations in expression and inducibility of a number of different CYP enzymes, including CYP1A1, CYP1A2, CYP2B1, and CYP2C11 (4–10). In addition, we and others have reported a significant elevation in expression and glucocorticoid-inducibility of hepatic CYP3As after feeding SPI-containing diets to rats and mice and after feeding soy infant formula to neonatal piglets (4,7,8,11,12).

CYP3A enzymes catalyze the rate-limiting step in metabolism and clearance of a large percentage of clinical medications. In addition, they have been implicated in bioactivation of procarcinogens, such as aflatoxin B1, and in the metabolism of endogenous estrogens and bile acids (4,13). The extent of the effect of SPI on hepatic CYP3A appeared to be influenced by age, sex, and CYP3A type (4,7,8) and to be a direct diet effect as...
opposed to metabolic imprinting (8). CYP3A expression is regulated via the nuclear hormone receptor, pregnane X receptor (PXR; NR11), which is activated by a wide variety of endogenous and exogenous chemicals, including bile acids, steroids, antibiotics, and herbal phytochemicals (13). However, although Li et al. (12) demonstrated that the soy-associated isoflavones genistein, daidzein, and equal can transactivate both mouse and human PXR, induce the recruitment of the steroid receptor co-activator 1 (SRC-1) to PXR, and induce mouse CYP3A11 in a PXR-dependent manner in primary hepatocytes in vitro, we have not observed significant increases in CYP3A1 mRNA or apoprotein in male and female rats weaned onto AIN-93G diets supplemented with pure genistein or pure daidzein at levels similar to those found in SPI for 2 wk (8). Moreover, we have previously reported that although basal expression and CYP3A mRNA inducibility were consistently elevated after feeding SPI, no effect was observed on steady-state expression of heterologous nuclear RNA (HnRNA) using RT-PCR with intron-specific primers (7).

Thus, the molecular mechanisms underlying the effects of soy feeding on CYP3A mRNA expression remain unclear. In the current study, we confirmed enhanced metabolism of the model CYP3A substrate midazolam after treatment with the glucocorticoid dexamethasone (DEX) in rats fed SPI during development compared with rats fed casein (CAS)-based diets and determined the time course of hepatic induction of CYP3A1 (CYP3A23) and CYP3A2 mRNA by DEX in male and female rats immediately postweaning on postnatal d (PND) 25 or postpuberty on PND60. In addition, we examined the effects of SPI on basal CYP3A1 gene transcription in female rats on PND60 by nuclear run-on analysis of CYP3A1 transcriptional rate and examined the nuclear expression and the binding of PXR to its response element on the CYP3A1 promoter in vivo using chromatin immunoprecipitation (ChIP).

Methods

Animal care and experiment design. The experiments received prior approval from the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences. Time-impregnated female Sprague-Dawley rats were purchased from Harlan Industries and housed separately in an AAALAC-approved animal facility at the Arkansas Children’s Hospital Research Institute at 22°C, constant humidity, and with lights on from 0600 to 1800 h. The rats were given free access to water and isocaloric and isonitrogenous diets formulated exactly as described by Reeves et al. (14) for AIN-93G, except that soybean oil was replaced with corn oil and included CAS or SPI as the sole protein source. The diets were supplemented with amino acids to meet NRC recommendations (5). At birth, litters were culled to 6 male and female pups per litter. Dams continued to be fed their respective diets throughout lactation and offspring were fed the same diet postweaning. In Expt. 1 at PND 65, 1 male pup from each of 3–7 litters per diet group was administered 50 mg/kg DEX or corn oil vehicle by oral gavage. The rats were killed by decapitation after anesthesia with 100 mg/kg Nembutal intraperitoneally 17 h later. In Expt. 2 at PND25 and PND60, 1 male and 1 female pup from 10 litters fed each diet were given 50 mg/kg DEX dissolved in corn oil (oral gavage). Rats were killed at 0, 8, 17, 24, 48, and 96 h following treatment.

Liquid chromatography-tandem MS analysis of midazolam metabolites. Livers from PND65 rats in Expt. 1 were used to prepare microsomes by differential ultracentrifugation (15). Midazolam metabolism was carried out using 5.5 mmol/L midazolam in a total volume of 100 μL with 0.3 mg microsomal protein and 1 mmol/L NADPH at 37°C for 40 min. The reaction was terminated with 200 μL of 100 mmol/L sodium bicarbonate, pH 11.0. Substrate and products were extracted using Supelclean LC-18 SPE-tubes (Supelco). Samples were eluted with methanol, evaporated, and resuspended in 64% sodium acetate, pH 4.7, 31% acetonitrile, 3% methanol, 2% tetrahydrofuran. Extracts were analyzed directly by LC/MS/MS using a Bruker Model Esquire-LC multiple ion trap mass spectrometer equipped with an Agilent 1100 series liquid chromatograph. An Eclipse XDB-C8 column (150 × 4.6-mm i.d., Agilent Technologies) was used at a flow rate of 0.8 mL/min. The HPLC gradient was acetonitrile (solvent B) in 0.1% formic acid/H2O (solvent A) 5–100 for 55 min and finally returned to initial concentrations from 55 to 60 min. Midazolam eluted at 17 min. In addition, 3 major metabolites were detected and quantitated: 4-OH-midazolam (15.9 min); 1’-OH-midazolam (14.8 min), and 4,1’-dihydroxymidazolam (15.7 min). In addition, trace amounts of 4 other minor metabolites were observed below the limits of quantitation: 3,4-dihydroxymidazolam and 1’,3-dihydroxymidazolam (13.5 and 10.8 min), N-hydroxymidazolam (19.9 min), and N-hydroxydihydroxymidazolam (20.4 min) (16).

Real-time RT-PCR quantitation of CYP3A mRNA. Total RNA was extracted from the livers in Expt. 2. CYP3A1 and CYP3A2 mRNA expression was quantitated by real-time RT-PCR. CYP3A1 specific primer set for: forward: GCCATACGCGCAGACAGAATA starting at 328 bp, reverse: (GAAGCGTGGG-TGCAGTAAGGCT) ending at 747 bp. CYP3A2-specific primer set for: forward: (CTCTCAACGAT-TGGAACCCATTAG) starting at 131 bp, reverse: (TTGTTAGAATTTACACCGATG CCTAA) ending at 232 bp. Probe specificity was verified by assessment of a single PCR product on agarose gels and a single temperature dissociation peak. Real-time RT-PCR was quantitated as a ratio with the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (forward primer TGGATGTCACCTGTTCTG; reverse primer TGGATACCGCGTCCGATA) within the linear range of product amplification (8).

Nuclear run-on analysis of CYP3A1 transcription rate. We conducted a nuclear run-on analysis on livers from PND60 female rats from Expt. 2. Nuclei were isolated from 1 g of frozen liver tissue following the procedure for solid tissues in the Nuclei PURE Prep kit (Sigma). Full-length CYP3A1 was cloned into pCRII-TOPO (Invitrogen) using primers (forward) 5’-AGG AGT AAT TTG CTG ACA GTC GTG C-3’ and (reverse) 5’-GAA GAA TTC TCG AGA AGA ACT CCT TGA GGG-3’. Nuclear run-on assays were performed as described by Greenberg and Bender (17) using an empty vector control to ensure specificity of hybridization and the resulting blots were analyzed by autoradiography.

ChIP analysis of PXR binding to the DRE Element on the CYP3A1 promoter. The ChIP-It Express kit (ActiveMotif) protocol was followed using a PXR antibody (A-20) (SantaCruz) for immunoprecipitation. The following primers were used for PCR analysis of the DR3 of rat CYP3A1 promoter (forward) 5’-AGA CAG TTC TTG AGA TGC AAC TCT T3’ and (reverse) 5’-AGA TGA ACT TCA TGA ACT GTC T3’.

Statistical analysis. Data are expressed as mean ± SEM. Statistical analysis was performed using SAS software (version 9.2; SAS Institute). Data from Expt. 1 were analyzed by 2-factor ANOVA followed by Student-Keuls post hoc multiple pairwise comparisons. In Expt. 2, we analyzed the DEX-induction time course data for the 2 sexes independently. To examine the effects of age, diet, and time on DEX-induction, we analyzed the CYP3A1 and CYP3A2 data in 3-factor ANOVA, which included all interactions of the 3 factors. In the presence of interactions, we first examined mean profiles over the levels of the interacting factors to determine whether upstream main effects (or lesser interactions) were still interpretable. In the event the interaction precluded direct interpretation of the upstream effects, we examined simple effects. To compare the integrated response to DEX treatment among different diet and sex groups, we computed areas under the curve (AUC) for the CYP3A mRNA expression-time course. An AUC value was calculated using 1 observation per time point at each time point; this was done until all the observations from a particular age-sex-diet group were exhausted. These AUC were then analyzed in 2-factor ANOVA with sex, diet, and their interaction as factors. We adjusted the P-values within sets of multiple comparisons with Holm’s step-down procedure.
Results

Expt. 1
In Expt. 1, CYP3A-dependent midazolam metabolism was greater at 17 h after DEX treatment compared with vehicle treatment (P < 0.05; 2-way ANOVA). However, DEX-induced increases in metabolism only reached significance compared with vehicle in the SPI-fed group (Table 1). There was a significant diet × DEX interaction in the rate of formation of 1,4-dihydroxymidazolam (P < 0.05) and a similar trend for overall midazolam metabolism (P = 0.085) and formation of 4-hydroxymidazolam (P = 0.064). The rate of formation of 1,4’-dihydroxymidazolam was greater in DEX-treated, SPI-fed rats relative to this activity in DEX-treated CAS-fed rats (P < 0.05) (Table 1).

Expt. 2
CYP3A1. In male rats, diet comparisons after DEX treatment depended on both time and age (age-diet-time interaction, P < 0.0001). For both age groups, SPI-fed rats elicited a higher CYP3A1 mRNA response to DEX treatment than did the CAS-fed rats at all time points (Fig. 1). An aspect of the age dependence was that the CYP3A1 mRNA response to DEX in the PND25 group peaked at 17 h and peaked at 24 h in the PND60 rats; the other aspect was that the difference in CYP3A1 mRNA expression between the 2 diets at peak DEX response for PND25 male rats was greater than that for CYP3A1 mRNA expression at peak DEX response in PND60 male rats (Fig. 1). Despite the dependence on the other 2 factors, it was clear that, overall, male rats fed the SPI diet had higher levels of CYP3A1 mRNA expression than male rats fed CAS diets (diet main effect, P < 0.0001). CYP3A1 mRNA levels for males fed both diets steadily rose to a peak after DEX treatment and declined thereafter. The timing of the peak depended on the age of the male rats (age-time interaction, P < 0.0001). Regardless of age or timing of the peak, the 2 time points adjacent to the peak were lower for both PND25 and PND60 male rats (all P < 0.0001, adjusted for 2 comparisons per age). We compared CYP3A1 mRNA expression in time 0, CAS-fed male rats to CYP3A1 mRNA levels following DEX treatment rose steadily until 17 h and decreased steadily thereafter. The peak at 17 h was greater than the 2 adjacent time points (both P < 0.0001). Within each age and diet group, we compared CYP3A1 mRNA expression at each time point to the CYP3A1 mRNA expression in CAS-fed animals at time 0. At age PND25, CAS-fed female rats had increased levels of CYP3A1 mRNA at 8 and 17 h after DEX treatment (P ≤ 0.016, adjusted for 5 comparisons); their SPI-fed counterparts had higher levels of CYP3A1 mRNA at 8, 17, and 24 h after DEX treatment (P < 0.0008, adjusted for 6 comparisons) (Fig. 1). At age PND60, female rats fed CAS diets had increased CYP3A1 mRNA levels from baseline at 17 h after DEX treatment (P ≤ 0.0001, adjusted for 5 comparisons); those fed SPI diets had increased CYP3A1 mRNA levels from baseline at 8, 17, and 24 h after DEX treatment (P ≤ 0.0001, adjusted for 6 comparisons) (Fig. 1).

We compared integrated changes in CYP3A1 mRNA expression in response to DEX treatment over time using AUC. In PND25 rats, the AUC was greater in SPI-fed animals than in their counterparts fed CAS diets (P < 0.0001) and was greater in females compared with males (P = 0.008). There was no compelling evidence of a sex-diet interaction (P = 0.26). Similar patterns were observed in the PND60 rats; females tended to be have greater AUC than males (P < 0.0001) and SPI-fed rats had greater AUC than CAS-fed rats (P < 0.0001). However, the diet comparisons did depend on gender (sex-diet interaction: P = 0.0007); in females, the increase in CYP3A1 mRNA response to DEX in SPI-fed rats compared with CAS-fed rats was clear (P < 0.0001), whereas in males it was marginal (P = 0.077) (Table 2).

In Expt. 2, we also observed increased expression of CYP3A1 apoprotein in SPI-fed female rat liver compared with CAS-fed female rat liver on PND60 prior to inducer treatment (7.1 ± 0.5 vs. 10.2 ± 0.6 densitometric units; CAS vs. SPI (P < 0.05) (Fig. 2). We measured CYP3A1 gene transcription rates directly in female rat livers at PND60 prior to inducer treatment using nuclear run-on analysis. Dot blots using pools of 5 livers/diet group had greater CYP3A1 transcription rates in livers of SPI-fed rats compared with CAS-fed rats (fold-change: 1.0 ± 0.5 vs. 2.3 ± 0.2; CAS vs. SPI) (Supplemental Fig. 1). Because basal expression and induction of CYP3A3s is transcriptionally regulated through the nuclear receptor transcription factor PXR (18–20), we measured PXR mRNA expression using real-time RT-

TABLE 1

<table>
<thead>
<tr>
<th>CAS</th>
<th>DEX</th>
<th>Vehicle</th>
<th>DEX</th>
<th>P diet</th>
<th>P diet</th>
</tr>
</thead>
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<tr>
<td>Vehicle</td>
<td>59.7 ± 4.2</td>
<td>37.5 ± 12.7</td>
<td>136.4 ± 28.7</td>
<td>0.486</td>
<td>0.006</td>
</tr>
<tr>
<td>4-OH</td>
<td>46.8 ± 1.7</td>
<td>32.0 ± 11.4</td>
<td>94.9 ± 17.0</td>
<td>0.356</td>
<td>0.041</td>
</tr>
<tr>
<td>1'-OH</td>
<td>9.8 ± 3.4</td>
<td>3.7 ± 2.9</td>
<td>30.4 ± 10.0</td>
<td>0.771</td>
<td>0.004</td>
</tr>
<tr>
<td>1',4-(OH)2</td>
<td>0.8 ± 0.4</td>
<td>0.2 ± 0.2</td>
<td>9.3 ± 2.5</td>
<td>0.054</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Data are mean ± SEM, n = 3–7, CAS DEX vs. SPI DEX: a > b, P < 0.05, vehicle vs. DEX: c > d, P < 0.05.
2 Total = Overall midazolam metabolism.
PCR and PXR protein expression in nuclear extracts from the same livers in which we observed increased basal CYP3A1 gene transcription. There was no effect of SPI feeding on PXR expression (Supplemental Fig. 2). However, ChIP analysis of PXR binding to its DRE response element on the CYP3A1 promoter in pooled livers from PND60 female rats at 0, 8, and 17 h following treatment with DEX demonstrated increased PXR promoter binding in SPI-fed rats compared with CAS-fed rats at all 3 time points (Fig. 3).

CYP3A2. For male rats, differences in CYP3A2 mRNA expression between the diets depended on the age and time elapsed since DEX treatment (age-diet-time interaction, \( P = 0.04 \)) (Fig. 4). However, diet differences in CYP3A2 mRNA levels tended to be greater for the older males than for the younger ones (age-diet interaction, \( P = 0.01 \)). For both age groups, CYP3A2 mRNA levels were consistently lower in male rats fed CAS diets than in male rats fed SPI diets (both \( P \leq 0.002 \), adjusted for 2 comparisons, 1 for each age), a difference not found in females on PND60. Comparing the time 0, CAS-fed rats to each of the other diet-time groups showed the effects of DEX treatment on CYP3A2. DEX increased CYP3A2 mRNA levels in PND25 male rats fed CAS diets at 8 and 17 h after administration (both \( P \leq 0.01 \), adjusted for 5 comparisons), whereas DEX increased CYP3A2 mRNA levels in PND25 male rats fed SPI diets at all time points after time 0 (\( P = 0.036 \), adjusted for 6 comparisons) (Fig. 4). For male rats at PND60, no differences were found from baseline for CYP3A2 mRNA levels in rats fed CAS diets (among 5 comparisons, smallest observed unadjusted, \( P = 0.9 \)) (Fig. 4).

Comparing the effects of CAS and SPI diets on CYP3A2 mRNA levels in DEX-treated female rats depended somewhat on the age of the rats and when they were treated with DEX (age-diet-time interaction, \( P = 0.06 \)). Diet differences tended to be greater in female rats at PND25 compared with rats at PND60 and CYP3A2 mRNA tended to peak earlier in the younger rats. The younger SPI-fed females had higher levels of CYP3A2 mRNA than their counterparts fed CAS diets (among 5 comparisons, smallest observed, \( P = 0.064 \)). But for the PND60 males, CYP3A2 mRNA levels were greater in rats fed SPI diets than in rats fed CAS diets at all time points, except for 96 h after DEX administration (\( P = 0.04 \), adjusted for 6 comparisons) (Fig. 4).

Comparing the effects of CAS and SPI diets on CYP3A2 mRNA levels in DEX-treated female rats depended somewhat on the age of the rats and when they were treated with DEX (age-diet-time interaction, \( P = 0.06 \)). Diet differences tended to be greater in female rats at PND25 compared with rats at PND60 and CYP3A2 mRNA tended to peak earlier in the younger rats. The younger SPI-fed females had higher levels of CYP3A2 mRNA than their counterparts fed CAS diets at 8, 24, 48, and 96 h after DEX treatment (all \( P \leq 0.01 \), adjusted for 6 comparisons) (Fig. 4), but their CYP3A2 peaks, both at 17 h, did not differ (\( P = 0.27 \), adjusted for 6 comparisons). In the PND60 female rats, CYP3A2 levels differed little between the 2 diets, regardless of time after DEX treatment (among 6 time points, smallest observed unadjusted, \( P = 0.9 \)) (Fig. 4). To evaluate whether DEX treatment alters CYP3A2 mRNA compared with no DEX treatment, we compared all DEX-treated groups to CAS-fed females at time 0, grouping the comparisons by age and sex.

### TABLE 2

<table>
<thead>
<tr>
<th>CAS</th>
<th>PND25</th>
<th>PND60</th>
<th>PND25</th>
<th>PND60</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A1 mRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2147 ± 140&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1104 ± 84&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4079 ± 242&lt;sup&gt;e,f&lt;/sup&gt;</td>
<td>1688 ± 188&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Female</td>
<td>2529 ± 136&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1602 ± 128&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4946 ± 231&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4101 ± 615&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYP3A2 mRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>152 ± 13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9 ± 1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>278 ± 24&lt;sup&gt;e&lt;/sup&gt;</td>
<td>18 ± 4&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Female</td>
<td>138 ± 11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>73 ± 14&lt;sup&gt;e&lt;/sup&gt;</td>
<td>292 ± 18&lt;sup&gt;f&lt;/sup&gt;</td>
<td>123 ± 25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Data are mean ± SEM, \( n = 10 \), for area under the mRNA expression-time courses shown in Figures 1 and 4. \( a > b, P < 0.05, \) PND25 vs. PND60; \( c > d, P < 0.05, \) CAS vs. SPI; \( e > f, P < 0.05, \) male vs. female.

**FIGURE 1** Time course of CYP3A1 mRNA expression in liver of male and female rats fed CAS or SPI following challenge with the glucocorticoid DEX on PND25 (A, B) or PND60 (C, D) (Expt. 2). Data are mean ± SEM, \( n = 9–10 \). Ratios of target mRNA:GAPDH housekeeping gene were determined by real-time RT-PCR. \( a\) Different from basal expression at time 0 h in CAS-fed rats, \( P < 0.05 \); \( b\) different from basal expression at time 0 h in SPI-fed rats, \( P < 0.05 \); \( c\) different in SPI-fed compared with CAS-fed rats that at that time point, \( P < 0.05 \).
diet. Treating CAS-fed females at PND25 with DEX increased CYP3A2 over baseline at 8, 17, and 24 h (all \( P \leq 0.0001 \), adjusted for 5 comparisons) (Fig. 4). The same aged rats fed SPI diets had increases in CYP3A2 mRNA expression after DEX treatment at all observed time points beyond time 0 (all \( P \leq 0.0001 \), adjusted for 6 comparisons) (Fig. 4).

We compared integrated changes in CYP3A2 mRNA expression in response to DEX treatment over time using AUC. For rats on PND25, the AUC of CYP3A2 time courses for SPI-fed animals was greater than that for CAS-fed animals (\( P < 0.0001 \), adjusted for 5 comparisons) (Fig. 4). The same aged rats fed SPI diets had increases in CYP3A2 mRNA expression after DEX treatment relative to those fed CAS immediately after weaning (8). Increases in DEX-induced metabolism of other CYP3A substrates (erythromycin, ethylmorphine, and corticosterone and testosterone 6\( \beta \)-hydroxylation) have been reported in adult male rats fed SPI throughout development (4). These data are also consistent with recent findings demonstrating increased CYP3A2 expression but was unable to increase expression in prepubertal rats, we demonstrated that SPI processing to contain over 100 phytochemicals in addition to protein and peptide components (27). In previous studies of CYP3A induction of the human CYP3A ortholog CYP3A4 in HepG2 hepatoma cells (12). These effects were linked to PXR transactivation and increased interaction of PXR with the coactivator SRC-1 in in vitro assays using luciferase constructs, presumably as a result of the isoflavones acting as PXR ligands (12). However, the importance of isoflavone-mediated PXR transactivation in induction of hepatic CYP3A expression after soy consumption in vivo is unclear. Soy products and SPI may reduce phytochemical content to negligible concentrations (25).

Discussion

The current data extend previous studies demonstrating increased CYP3A-dependent midazolam hydroxylation after DEX treatment in SPI-fed male rats relative to those fed CAS immediately after weaning (8). Increases in DEX-induced metabolism of other CYP3A substrates (erythromycin, ethylmorphine, and corticosterone and testosterone 6\( \beta \)-hydroxylation) have been reported in adult male rats fed SPI throughout development (4). These data are also consistent with recent findings demonstrating increased expression of hepatic CYP3A proteins and mRNA in rats and CYP3A11 expression in mice following feeding of soy protein-containing diets (4,7,12) and with increased expression of hepatic CYP3A enzymes in neonatal piglets following feeding of soy infant formula (11). They suggest that clearance of many drugs that are both CYP3A substrates and inducers such as glucocorticoids and antibiotics is likely to be enhanced in soy consumers (4,13). Our data also confirm the previously reported relative lack of glucocorticoid inducibility of CYP3A2 relative to CYP3A1 (21,22) and the previous studies of Pereira and Lechner (23) demonstrating that in older male rats CYP3A1 induction is reduced and delayed relative to responses prior to puberty. Interestingly, this was not observed in older female rats.

Previous studies in our laboratory have demonstrated a significant interaction between SPI and DEX on CYP3A mRNA and apoprotein expression and enzyme activity, but the underlying molecular mechanisms are unclear (4,7,8). We were previously unable to observe effects of SPI feeding on hepatic CYP3A1 HnRNA expression using RT-PCR with intron-specific primers (8). This suggested potential effects on mRNA stability and half life. However, lack of an effect on the time course of CYP3A1 mRNA induction in the current study suggested a true transcriptional effect that might have been missed in steady-state HnRNA measurements. Consistent with the hypothesis that increased inducibility of CYP3A enzymes after SPI feeding was independent of sex differences following DEX treatment, basal CYP3A1 apoprotein expression and basal CYP3A1 gene transcription rate measured directly by nuclear run-on increased after rats were fed SPI relative to rats fed CAS in the current study.

Interestingly, the increased CYP3A1 transcription was not associated with increased expression of the PXR transcription factor but was linked to increased binding of PXR to its response element on the CYP3A1 promoter as determined by ChIP analysis. A substantial body of data demonstrates that binding of PXR/RXR heterodimers is required for both basal expression and induction of CYP3A enzymes in rodents and humans (18–20,23,24). Furthermore, glucocorticoid treatment can upregulate PXR expression as well as CYP3A expression (24).

However, the relative importance of the level of PXR/RXR binding and binding of coactivators such as SRC-1 to response elements on the CYP3A promoter compared with ligand activation of PXR already bound to chromatin in the control of CYP3A gene transcription is less clear. Several studies have demonstrated PXR binding to the promoter of CYP3A and other PXR-regulated genes in the absence of inducer, which may be related to levels of basal transcription (23,26). Moreover, the level of additional recruitment of PXR to promoter regions of genes inducible by PXR ligands following inducer treatment appears to vary between genes and inducers (24). Our data are consistent with increased basal expression of CYP3A1 after feeding SPI as a result of increased PXR promoter recruitment followed by additional increases in gene transcription related to ligand activation of PXR by DEX binding.

Recent in vitro studies with the soy-associated isoflavones genistein, daidzein, and equol in primary mouse hepatocytes have demonstrated PXR-dependent increases in CYP3A11 and similar upregulation of the human CYP3A ortholog CYP3A4 in HepG2 hepatoma cells (12). These effects were linked to PXR transactivation and increased interaction of PXR with the coactivator SRC-1 in in vitro assays using luciferase constructs, presumably as a result of the isoflavones acting as PXR ligands (12). However, the importance of isoflavone-mediated PXR transactivation in induction of hepatic CYP3A expression after soy consumption in vivo is unclear. Soy products and SPI may contain over 100 phytochemicals in addition to protein and peptide components (27). In previous studies of CYP3A induction in prepubertal rats, we demonstrated that SPI processes to reduce phytochemical content to negligible concentrations increased CYP3A2 expression but was unable to increase expression of CYP3A1 relative to feeding CAS (8). Moreover, although small but significant increases were observed in CYP3A2.
expression in male and female rats weaned onto CAS diets supplemented with pure daidzein at levels similar to those found in SPI, we did not observe significant increases in CYP3A1 mRNA or apoprotein expression after feeding CAS diets supplemented with either daidzein or genistein (8). These data suggest that nonisoflavone components of SPI are primarily responsible for increased CYP3A1 expression. The effects of SPI feeding on CYP3A expression are unlikely to be due to differences in amino acid composition between the 2 diets, because we supplemented the SPI diet with methionine and other essential amino acids to meet NRC recommendations and make the diets isonitrogenous (5). We suspect that the lack of isoflavone effects on CYP3A1 expression in our studies is a dose-response issue, because the lowest concentration of isoflavones required to significantly transactivate PXR in vitro was reported to be 5 μmol/L (12). Data from our laboratory indicate that tissue concentrations of the bioactive isoflavone aglycones in liver following consumption of SPI diets is in the range of only 0.2–0.3 μmol/L (28). Thus, although isoflavone-mediated transactivation of PXR may occur after consumption of isoflavone supplements, it is unlikely to account for effects on PXR-mediated gene transcription after consumption of soy foods.

In summary, we have demonstrated increased basal expression of multiple rat liver CYP3A enzymes after feeding diets high in SPI relative to expression in rats fed CAS and greater induction of CYP3A expression after DEX treatment in SPI-fed rats compared with CAS-fed rats. Our data suggest that increased hepatic CYP3A expression following SPI feeding involves recruitment of PXR to its response element and increased basal transcription rates. This suggests that soy consumption has potential effects on metabolism and transport of a wide variety of drugs and on bile acid homeostasis regulated via this transcription factor. Identification of the soy component(s) responsible for PXR promoter recruitment and the molecular mechanisms underlying this effect require additional studies.

Acknowledgments
M.J.J.R., K.S., and T.M.B. designed research; Y.C., X.L., M.L.B., and N.F. conducted research; M.J.J.R. M.L.B., N.F., K.S., and R.D.L. analyzed data; M.J.J.R. and T.M.B. wrote the paper; and M.J.J.R. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


