Cytochrome P450 mRNA Expression in the Rodent Brain: Species-, Sex- and Region-Dependent Differences

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ABBREVIATIONS: CO: corn oil; Ct: fractional amplification (cycle number at which fluorescence exceeds a user-defined threshold); CYP: cytochrome P450; DEX: dexamethasone; qPCR: quantitative (real-time) polymerase chain reaction; OPs: organophosphorus pesticides; PB: phenobarbital; PBDEs: polybrominated diphenyl ethers; PCBs: polychlorinated biphenyls
ABSTRACT

Cytochrome P450 (CYP) enzymes play a critical role in the activation and detoxication of many neurotoxic chemicals. While research has largely focused on CYP-mediated metabolism in the liver, emerging evidence suggests that brain CYPs influence neurotoxicity by modulating local metabolite levels. As a first step towards better understanding the relative role of brain CYPs in determining neurotoxic outcome, we characterized mRNA expression of specific CYP isoforms in the rodent brain. Adult mice (male and female) and rats (male) were treated with vehicle, phenobarbital or dexamethasone. Transcripts for CYP2B, CYP3A, CYP1A2 and the orphan CYP4X1 and 2S1 were quantified in the liver, hippocampus, cortex and cerebellum by qPCR. These CYPs were all detected in the liver with the exception of CYP4X1, which was detected in rat but not mouse liver. CYP expression profiles in the brain varied regionally. With the exception of the hippocampus, there were no sex differences in regional brain CYP expression profiles in mice; however, there were marked species differences. In the liver, phenobarbital induced CYP2B expression in both species. Dexamethasone induced hepatic CYP2B and CYP3A in mice but not rats. In contrast, brain CYPs did not respond to these classic hepatic CYP inducers. Our findings demonstrate that CYP mRNA expression in the brain varies by region, that regional brain CYP profiles vary between species and their induction varies from that of hepatic CYPs. These novel data will be useful for designing mechanistic studies to examine the relative role of CYP-mediated brain metabolism in neurotoxicity.
INTRODUCTION

The cytochrome P450 (CYP) superfamily is a diverse group of enzymes that catalyze the oxidative metabolism of not only endogenous substrates but also xenobiotics, including environmental contaminants of significant public health concern that target the nervous system, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organophosphorus pesticides (OPs) (Ariyoshi et al., 1995; Foxenberg et al., 2007; Erratico et al., 2013; Feo et al., 2013). Biotransformation of these compounds by CYPs can result in bioactivation or detoxication, and the balance between these activities influences the bio-effective dose, and thus, the neurotoxic outcome, following environmental exposures, as shown in studies of humans and animal models (Foxenberg et al., 2007; Curran et al., 2011; Kim et al., 2011; Crane et al., 2012; Khokhar and Tyndale, 2012).

Much of the research effort to characterize CYP-mediated metabolism of neurotoxic compounds has focused on the liver. However, it is now evident that CYPs are expressed in a number of extrahepatic tissues, including brain (Ding and Kaminsky, 2003; Ferguson and Tyndale, 2011). While total CYP content in the human and rodent brain is generally significantly lower than that in liver (Warner et al., 1988; Bhamre et al., 1992; Volk et al., 1995), recent evidence from rat studies demonstrates that CYP-mediated metabolism in the brain can contribute significantly to neurotoxicity (Khokhar and Tyndale, 2012; Zhou et al., 2013). These data coupled with reports that CYP expression in the brain may vary between anatomic regions of the brain (Warner et al., 1988; Dutheil et al., 2009) have led to growing interest in the putative role of brain CYPs in determining sensitivity and response to neurotoxic compounds via modulation of local metabolite levels (Meyer et al., 2007; Ferguson and Tyndale, 2011; Ravindranath and Strobel, 2013).

Rodents are important models for studying the relative influence of brain versus liver CYPs on neurotoxicity; however, most of our knowledge of CYP expression in the rodent brain
is derived from studies of whole brain homogenates, and there is a paucity of data on regional CYP expression in the rodent brain. Additional questions include whether the well-known sex- and species-specific differences in hepatic CYP expression extend to the brain and whether CYPs in the brain respond to classic inducers of hepatic CYP expression. Here, we address these questions by comparing CYP transcript levels in three distinct regions of the rodent brain relative to expression levels in the liver under basal conditions and following treatment with phenobarbital and dexamethasone, which are classic inducers of hepatic CYP2B and CYP3A expression [reviewed by (Corcos, 2008; Greenblatt et al., 2008)]. We also assessed the influence of sex and species on CYP expression profiles in the brain using the male mouse as the reference. We studied CYP2B (mouse 2B10/rat 2B1/2), CYP3A (mouse 3A11/rat 3A2) and CYP1A2 because these isoforms have been implicated in the metabolism of PCBs (Kania-Korwel et al., 2008; Curran et al., 2011; Kania-Korwel et al., 2012), PBDEs (Erratico et al., 2013; Feo et al., 2013) and OPs (Tang et al., 2001; Foxenberg et al., 2007). Two orphan CYPs, CYP4X1 and CYP2S1 (Guengerich et al., 2010), were also included in this study because CYP2S1 is abundantly expressed in many extrahepatic tissues (Choudhary et al., 2003) and CYP4X1 is predominantly expressed in the rodent brain (Bylund et al., 2002; Al-Anizy et al., 2006). The data reported herein demonstrate brain region-specific expression of CYPs in the rodent brain that is sex and species-dependent and generally not altered by the classical inducers phenobarbital and dexamethasone under conditions that significantly induce CYP expression in the liver.
MATERIALS AND METHODS

Animals and treatments: Experiments involving animals were approved by the Institutional Animal Care and Use Committee at the University of Iowa. Male and female C57BL/6 mice (7-8 weeks) were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA) and randomly assigned to one of four groups: (1) phenobarbital (PB, Sigma-Aldrich, St. Louis, MO) at 102 mg/kg/d in saline or (2) saline at 20 mL/kg/d, i.p. for three consecutive days; (3) dexamethasone (DEX, Sigma-Aldrich) at 50 mg/kg/d in corn oil (CO) or (4) CO (Fisher Scientific, Pittsburg, PA) at 10 mL/kg/d, i.p. for four consecutive days (Kania-Korwel et al., 2008). Male Sprague-Dawley rats (8 weeks) were purchased from Harlan, Inc. (Indianapolis, IN, USA), acclimated for one week then randomly assigned to one of four groups: (1) PB at 102 mg/kg/d in saline or (2) saline at 5 mL/kg/d, i.p. for three consecutive days; (3) DEX at 50 mg/kg/d in CO or (4) CO vehicle control at 5 mL/kg/d, i.p. for four consecutive days (Kania-Korwel et al., 2008). Animals were euthanized 24 h after the last treatment by CO2 asphyxiation followed by cervical dislocation. Brain regions and livers were immediately collected on ice, weighed, placed in RNALater overnight and then stored at -80°C. The effects of treatments on liver and total body weight are summarized in the Supplemental Information (Tables S1-S3).

Assessment of mRNA levels by quantitative polymerase chain reaction (qPCR): Tissue levels of isoform-specific CYP transcripts were quantified using a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). CYP mRNA levels were normalized to the reference gene phosphoglycerate kinase 1 (Pgk1), and relative expression ratios between treated and vehicle control animals were calculated by the Pfaffl method (Pfaffl, 2001) using REST 2009 software (Qiagen, Valencia, CA). Statistical analysis was performed using the built-in randomization techniques of REST 2009 (detailed descriptions of RNA isolation and mRNA quantification and analyses are provided in the Supplemental Information. Supplemental Tables S6 and S7 list primer sequences and amplification efficiencies of primers sets, respectively).
RESULTS AND DISCUSSION

Adult male C57BL/6 mice were used as the reference for comparison of sex- and species-dependent differences in CYP gene expression. In male mice, tissue-specific CYP expression patterns were similar between saline (Figure 1A) and CO (Figure 1C) vehicle controls. The liver expressed CYP2B10, 3A11, 1A2 and 2S1, but not CYP4X1, which is consistent with previous reports of brain-specific CYP4X1 expression in mice (Al-Anizy et al., 2006; Renaud et al., 2011). CYP expression profiles in the brain were region-specific: all five CYP isoforms were expressed in the hippocampus; the cerebellum expressed transcripts for all but CYP1A2 and the cortex expressed only CYP2S1 and CYP4X1 mRNA (Figures 1A, 1C).

Treatment with either PB or DEX induced CYP expression in the liver (Figure 1B, 1D; see also Supplemental Information, Tables S4 and S5): hepatic CYP2B10 was induced by PB (mean of 35.1; 68% CI: 14.8-75.7) and by DEX (mean of 58.8; 68%CI: 30.6-137.5). DEX also induced hepatic CYP3A11 (mean of 6.9; 68%CI: 3.8 -12.2). However, neither PB nor DEX induced expression of any target CYP in the hippocampus, cerebellum or cortex (Figure 1B, 1D).

To explore the influence of sex, we measured CYP transcript levels in female C57BL/6 mice. Baseline CYP expression in the liver and cortex were similar between the saline (Figure 2A) and CO (Figure 2C) vehicle controls, and comparable to profiles observed in male vehicle controls (Figure 1A, 1C). Similar to males, all five CYP isoforms were expressed in the hippocampus of CO-treated females (Figure 2C); however, in contrast to males, only CYP4X1 and CYP2S1 were detected in the hippocampus of saline-treated females (Figure 2A). CO has been previously reported to influence P450 expression in the rat liver (Yoo et al., 1990), but it is unclear whether our findings reflect CO-mediated increase in CYP levels in the female mouse hippocampus. While differences between saline- and CO-treated female mice could be experimental artifact, this seems unlikely because hippocampal expression levels of CYP4X1 and CYP2S1 were comparable between the two vehicle treatments and between sexes.
As observed in male mice, PB induced hepatic CYP2B10 expression in female mice by a mean factor of 13.2 (68%CI: 6.6-23.7), while DEX induced hepatic expression of CYP2B10 and CYP3A11 by 48.6 (68%CI: 30.6-91.1) and 8.21 (68%CI: 5.6-11.0), respectively (Figure 2B and 2D). Also consistent with male mice, PB or DEX did not change CYP expression in the cortex of female mice. Similarly, DEX had no effect on CYP expression in the female hippocampus. However, in contrast to males, PB significantly induced expression in the hippocampus of females of CYP2B10 (mean of 164; 68%CI: 56-529), CYP3A11 (mean of 279; 68%CI: 113-724) and CYP1A2 (mean of 36; 68% CI:14-94) relative to saline vehicle controls (Figure 2B). Data for these three transcripts are shown for individual female mice in Figure 2E.

To investigate species-dependent differences, CYP transcripts were quantified in male rats. Tissue-specific CYP expression profiles were similar between saline (Figure 3A) and CO (Figure 3C) vehicle controls but varied from those observed in the comparable male mouse treatment groups (Figure 1A, 1C). Specifically, in the male rat, all five CYP isoforms of interest were expressed in the liver, including CYP4X1 (Figure 3A, 3C). Also in contrast to male mice, rat brain expressed CYP3A2, CYP4X1 and CYP2S1 in the hippocampus, cortex and cerebellum, but neither CYP2B1/2 nor CYP1A2 were detected in any of these three brain regions (Figure 3A, 3C). These findings are consistent with previous studies of regional CYP expression in rat brain, with the exception that others have reported the presence of CYP2B in rat brain (Schilter and Omiecinski, 1993). Similar to male mice, PB (Figure 3B) and DEX (Figure 3D) induced CYP expression in the male rat liver but not in any of the three brain regions. However, the profile of hepatic CYP isoforms induced by these chemical treatments showed species variation. In the rat, PB induced the expression of not only CYP2B1/2 (by a mean factor of 504; 68%CI: 298-1044) but also CYP3A2 (by a mean factor of 3.4; 68%CI: 1.8-7.1). Surprisingly, DEX did not significantly alter hepatic CYP2B1/2 or CYP3A2 expression, but instead significantly upregulated CYP4X1 expression (by a mean factor of 6.5; 68%CI: 1.5-
Although the latter is a novel finding, given the low fold-induction and the lack of protein expression data the functional significance of this upregulation is not clear.

To further investigate sex- and species-specific differences in hepatic CYP induction, we compared relative CYP2B and CYP3A induction in the liver of male versus female mice and between male mice and male rats. We found no significant sex differences in CYP induction patterns in mice (Figure 4A). Conversely, there were significant differences in hepatic CYP induction between mouse and rat (Figure 4B). Hepatic CYP2B expression was induced by DEX in mice but not rats, and CYP3A expression was induced by PB in mice but not rats and by DEX in rats but not mice.

In summary, these data suggest that CYP mRNA expression in the brain: (1) differs significantly from hepatic CYP transcript profiles in rodent models; (2) varies between brain regions; (3) exhibits subtle sex-dependent differences in the C57BL/6 mouse, but significant species-specific differences between mouse and rat; and (4) with the possible exception of CYPs in the hippocampus of the female mouse, is not induced by PB or DEX regimens that induce hepatic orthologs. With respect to the last finding, previous studies of whole brain homogenates have reported either no CYP induction by these classical inducers (Schilter et al., 2000; Hedlund et al., 2001; Upadhya et al., 2002; Woodland et al., 2008) or CYP2B induction by PB (Schilter and Omiecinski, 1993; Schilter et al., 2000; Upadhya et al., 2002). Discrepancies between studies likely reflect differences in dose and duration of treatment, species and/or strain, whole brain versus isolated brain regions, primer specificity and methods of mRNA quantification. While it will be necessary to confirm protein levels and activity of these CYP isoforms to corroborate the significance of these findings, emerging evidence of brain CYP-mediated xenobiotic activation strongly suggests that differences in regional expression of brain CYPs may be an important mechanism contributing to region-selective neurotoxicity (Spencer and Lein, 2013).
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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Stamou, Wu, Kania-Korwel, Lehmler, Lein
Conducted experiments: Stamou, Wu, Kania-Korwel
Performed data analysis: Stamou, Wu
Wrote or contributed to the writing of the manuscript: Stamou, Wu, Korwel, Lehmler, Lein
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FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1: CYP expression profiles in the brain of adult male C57BL/6 mice. Mice were treated for 3 consecutive days with either (A) saline (20 mL/kg/d, i.p.) or (B) an equal volume of PB in saline (102 mg/kg/d, i.p.); or for 4 consecutive days with either (C) CO (10 mL/kg/d, i.p.) or (D) an equal volume of DEX in CO (50 mg/kg/d, i.p.). Tissues were harvested 24 h after the last injection and CYP mRNA quantified by qPCR. (A and C) Baseline CYP expression determined by normalizing Ct values for CYP transcripts in control samples to Ct values for the reference gene (Pgk1) in the same sample. (B and D) Change in expression of the target gene in PB- or DEX-treated animals relative to vehicle control (saline for PB and CO for DEX). All data are expressed as the mean relative expression ± SE (n = 4). *p < 0.05; **p < 0.01; ***p < 0.001 significantly different from vehicle control as determined by automated randomization and bootstrapping tests (REST 2009 software). BD, below detection limit.

Figure 2: CYP expression profiles in the brain of adult female C57BL/6 mice. Mice were treated for 3 consecutive days with either (A) saline (20 mL/kg/d, i.p.) or (B) an equal volume of PB in saline (102 mg/kg/d, i.p.); or for 4 consecutive days with either (C) CO (10 mL/kg/d, i.p.) or (D) an equal volume of DEX in CO (50 mg/kg/d, i.p.). Tissues were harvested 24 h after the last injection and CYP mRNA measured by qPCR. (A and C) Baseline CYP expression determined by normalizing Ct values for CYP transcripts in control samples to Ct values for Pgk1 in the same sample. (B and D) Change in expression of the target gene in PB- or DEX-treated animals relative to vehicle control (saline for PB and CO for DEX). (E) Ct values for hippocampal CYP2B10, CYP3A11 and CYP1A2 in the PB group (a,b,c) and the DEX group (d,e,f) of individual mice. Data in A-D expressed as the mean ± SE (n = 4-5). *p < 0.05; **p < 0.01; ***p < 0.001 significantly different from vehicle controls as determined using the REST 2009 software. BD, below detection limit.
Figure 3: CYP expression profiles in the brain of adult male Sprague Dawley rats. Rats were treated for 3 consecutive days with either (A) saline (5 mL/kg/d, i.p.) or (B) an equal volume of PB in saline (102 mg/kg/d, i.p.); or for 4 consecutive days with either (C) CO (5 mL/kg/d, i.p.) or (D) an equal volume of DEX in CO (50 mg/kg/d, i.p.). Tissues were harvested 24 h after the last injection and CYP mRNA quantified by qPCR. (A and C) Baseline CYP expression determined by normalizing Ct values for CYP transcripts in vehicle control tissues to Ct values for Pgk1 in the same sample. (B and D) Change in expression of the target gene in PB- or DEX-treated animals relative to control (saline for PB and CO for DEX). Data expressed as the mean ± SE (n = 3 except for PB-treated cerebellum, in which n = 2). *p < 0.05; **p < 0.01; ***p < 0.001 significantly different from vehicle controls as determined by automated randomization and bootstrapping tests (REST 2009 software). BD, below detection limit; NA, not available because of low amplification efficiency.

Figure 4. CYP induction in liver is species-dependent but not sex-dependent. (A) In both male and female mice, hepatic CYP2B10 is significantly induced by both PB and DEX whereas hepatic CYP3A11 is upregulated by DEX but not PB. Differences in induction between sexes are not statistically significant as determined by Student’s t-test (p < 0.05). (B) Hepatic CYP2B10 is induced by both PB and DEX in male mice, but the rat orthologue, CYP2B1/2, is induced only by PB in male rats. CYP3A11 is upregulated in male mice by DEX but not by PB. Conversely, the rat orthologue, CYP3A2 is induced by PB but not by DEX. CYP transcript levels in animals treated with either PB or DEX are presented relative to species-specific vehicle controls. *p < 0.05; **p < 0.01; ***p < 0.001 significantly different from vehicle controls as determined by automated randomization and bootstrapping tests (REST 2009 software).
Figure 4