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Higher gene expression of CYP1A2, 2B1 and 2D2 in the brain of female compared with male rats

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Cytochrome P450 (CYP) in the brain plays an essential role in the local metabolism of various compounds, including clinically used drugs, toxins, and endogenous substances. In the present study, we compared the expression profiles of mRNAs for several CYP subtypes in the brain between male and female rats. The expression of CYP1A2, CYP2B1, and CYP2D2 in females was significantly higher than that in males. On the other hand, the expression level of the other CYP subtypes examined in the male brain was similar to that in the female brain. These results strongly suggest that marked gender differences exist in the expression profiles of some CYP subtypes in rat brain.

1. Introduction

Cytochrome P450 enzymes (CYPs) are found throughout the animal and plant kingdom. CYP consists of many isoforms, and these are classified into 18 families and 57 subfamilies based on their amino acid identity (Nelson 2011). Members of families 1, 2, and 3 are primarily responsible for the oxidative metabolism of a wide variety of both exogenous and endogenous compounds (McDonnell and Dang 2013). CYPs are expressed not only in the liver but also in other organs, including the brain, where they can contribute significantly to local metabolism (Hedlund et al. 2001). In several CYP isoforms constitutively expressed in the liver, direct relationships have been found between the expression level and sex hormonal status. The male-predominant expression of CYP2C11 in the rat liver was reported (Kato and Yamazoe 1992). A previous study demonstrated that hepatic CYP3A2 was expressed at a low level in female rats, while CYP3A1 expression levels were shown to be 40-50% higher than that in the liver of male rats (Ribeiro and Lechner 1992; Dhir and Shapiro 2003; Aiba et al. 2005; Kusuda et al. 2012). However, in contrast to hepatic CYPs, the presence or absence of gender difference in the expression profiles of CYPs in the brain has not been confirmed.

2. Investigations, results and discussion

In the present study, we compared the expression profile of mRNAs for major CYP subtypes of families 1, 2, and 3 (CYP1A2, CYP2B1, CYP2C6, CYP2C11, CYP2D1, CYP2D2, CYP2E1, CYP3A1, and CYP3A2) in the brain between male and female rats. The gene expression of CYP1A2, CYP2B1 and CYP2D2 in the brain of male rats was significantly lower than that of female rats among cerebral CYPs examined here, while no significant differences were observed in the expression of the other CYPs between males and females (Table 1). These results provide experimental evidence for gender differences in the cerebral expression profiles of mRNAs for several CYPs in rat brain.

Responses to clinical drugs acting on the central nervous system (CNS) are not always directly related to the drug and its metabolite levels circulating in the plasma (Michels and Marzuk 1993). A previous study using rats demonstrated that the sedation by administration of propofol, a commonly used anesthetic, correlated more strongly with the brain level than with the plasma level of this drug (Shyr et al. 1995; Khokhar and Tyndale 2011). Propofol undergoes metabolic conversion to inactive 4-hydroxide by CYP2B1 (Tai et al. 2015). CYP2B in the brain, but not in the liver, was induced by treatment with nicotine (Miksys et al. 2000). The propofol level in the brain of nicotine-treated rats was lower than that of saline-treated rats regardless of change in its systemic concentration, which resulted in sleep for 60% less time (Khokhar and Tyndale 2011). These findings suggested that the central actions of propofol could be influenced by its local metabolism mediated by CYP2B1 in the brain. In contrast to lipophilic propofol, application of hydrophilic agents to the treatment of cerebral lesion is practically limited because of their poor uptake into the brain. However, increased delivery to the brain can be achieved by creating a prodrug that easily penetrates the blood brain barrier, which is then metabolized to the active compound by enzymes present in the brain. Cyclophosphamide is a chemotherapeutic prodrug that is used to treat CNS tumors, and this drug is metabolically activated by brain CYP2B1 (Lenglar et al. 2006; Mercapide et al. 2010). The enhancement of metabolic activation of prodrugs by enzymes such as CYP2B1 in the brain has been considered as a rational therapeutic approach to achieving successful reduction in size of CNS tumors that are extremely difficult to treat. In light of a difference in CYP2B1 expression level in the brain between male and female rats, pharmacological effects may differ depending upon the gender when treated with CYP2B1-metabolizing drugs.

Exposure to chlopyrifos, which is widely used as organophosphate pesticide, causes cognitive defects and other neurologic disorders (Steenland et al. 2000). Chlopyrifos is converted to chlopyrifos oxon primarily by CYP2B1 (D'Agostino et al. 2015), and this oxon metabolite contributes to neurotoxicity through the inhibition acetylcholinesterase (Sultatos 1994; Tang et al. 2001). The previous study demonstrated that the inhibition of brain CYP2B activity was a useful strategy for attenuating the neurotoxicity of chlopyrifos such as abnormal gait, incline plane slippage and righting reflex latency (Khokhar and Tyndale 2012). In this regard, development of CYP2B inhibitors will be required in consideration of the gender difference in expression level of the brain CYP subtype, as shown in our study.

Most brain CYPs including CYP1A are capable of metabolizing chemical substances as with hepatic CYPs, and they share similarity in substrate specificity and inhibitor selectivity (Miksys and Tyndale 2013). CYP1A2 is the major enzyme involved in the *N*-demethylation and 8-hydroxylation of theophylline, a xanthine derivative. Theophylline was reported to easily permeate across the blood brain barrier and to induce convulsion and acute cerebral injury (Ramzan 1990), and these adverse events were thought to be associated with the cerebral theophylline metabolites produced by CYP1A2. Based on our present findings showing a significantly higher expression level of CYP1A2 in the female brain compared

with male, confirmation of gender difference in the emergence of theophylline-induced convulsion and cerebral damage will be desired for appropriate use of this drug.

Parkinson's disease (PD) is a progressive neurodegenerative disease that is characterized by the loss of dopamine-producing pigmented neurons in the substantia nigra, but the etiology of PD is not entirely understood. The prevalence of PD was previously reported to be lower in females (Elbaz et al. 2002). An *in vitro* study showed that rat CYP2D2 was capable of forming dopamine from tyramine similarly to human CYP2D6 (Bromek et al. 2010). There is evidence that individuals with a CYP2D6 poor metabolizer genotype are at higher risk for development of PD and that this risk becomes even greater with neurotoxic pesticides (McCann et al. 1997; Elbaz et al. 2004). In the present study, the expression level of CYP2D2 in the female brain was higher than that in the males. This difference in the expression of CYP2D2 may be, at least partly, responsible for the disparity in incidence of PD in males and females.

In conclusion, the present study demonstrated that gender differences exist in the expression profiles of CYP1A2, 2B1 and 2D2 in rat brain, providing a more detailed insight into the pharmacological and pathological roles of CNS-related compounds.

Table 1: Gene expression of CYPs in rat brain

Subtypes	Male	Female
CYP1A2	1.000 ± 0.017	1.565 ± 0.053 *
CYP2B1	1.000 ± 0.031	1.302 ± 0.002 *
CYP2C6	1.000 ± 0.093	0.971 ± 0.105
CYP2C11	1.000 ± 0.212	0.873 ± 0.135
CYP2D1	1.000 ± 0.039	1.037 ± 0.089
CYP2D2	1.000 ± 0.154	1.465 ± 0.055 *
CYP2E1	1.000 ± 0.225	1.140 ± 0.115
CYP3A1	1.000 ± 0.015	0.993 ± 0.019
CYP3A2	1.000 ± 0.050	0.925 ± 0.137

Data were normalized to GAPDH mRNA, which was used as an internal control. Results were expressed relative to the respective male group, which were arbitrarily assigned a value of 1, and were shown as the means ± SD of three rats per group. Significant differences between male and female groups were evaluated by the Student's unpaired *t*-test (*: $p<0.05$, *ver* the respective male group).

3. Experimental

3.1. Animals

Male and female Wistar rats aged 7 weeks were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The animals were acclimatized for 7 days before the experiment, and were housed in a clean room maintained at 23±2 °C with a relative humidity of 55±10 % and 12-h light/dark cycle. Experimental protocols and animal care methods in the present study were approved by the Animal Experiment Committee at Osaka Ohtani University.

3.2. Real-time quantitative PCR

Total RNA in the brain of rats was extracted with ISOGEN solution (Nippon Gene Co., Ltd., Tokyo, Japan) and purified with a GenElute Mammalian Total RNA kit (Sigma-Aldrich Co., St. Louis, MO, USA) according to the manufacturer's instructions. Total RNA was reverse-transcribed into cDNA by means of Oligo-T priming and Moloney murine leukemia virus reverse transcriptase (GE Healthcare, Seattle, WA, USA). The expression levels of targeted genes were determined by real-time quantitative PCR with a MyiQ analyzer (Bio-Rad Lab., Inc., Hercules, CA, USA), using SYBR green as the fluorescence dye (Toyobo Co., Ltd., Osaka, Japan). cDNA was PCR-amplified at 95 °C for 10 s, at 60 °C (CYP2D1 and CYP3A1) or 55 °C (others) for 10 s, and at 72 °C for 30 s. In the initial experiments, a melting curve analysis was performed to monitor PCR product purity. The relative quantitation of the mRNA expression of targeted genes was calculated using the comparative threshold cycle number for each sample. To adjust for variations in the amount of DNA, the gene expression of the target sequence was normalized in relation to the expression of an endogenous control, GAPDH. The synthetic oligonucleotide primers (Hokkaido System Science Co., Ltd., Sapporo, Japan) used to investigate the expression levels of targeted genes were designed by Beacon designer 8 (Bio-Rad Lab., Inc.), and are listed in Table 2. mRNA levels were quantified based on standard curves. Results were expressed relative to each male group, which were arbitrarily assigned a value of 1.

Table 2: Forward and reverse oligonucleotide primer sequences of CYPs

Target	Direction	Sequence
CYP1A2	Forward	GCCACGCTTCTCCAAGTG
	Reverse	CGAAACAGGGTAGGAGGAATAGT
CYP2B1	Forward	AACCCTTGATGACCGCAGTAAA
	Reverse	TGTGGTACTCCAATAGGGACAAGATC
CYP2C6	Forward	CTTCAAGATTCAAGAAATATCCA
	Reverse	GTGAATTACCAGTGCTACA
CYP2C11	Forward	TCCAGTGAACATCAAACCTCAT
	Reverse	ATGGGAATGTCACAAGATATAGGG
CYP2D1	Forward	CCACGCATCACGAGTTGT
	Reverse	CGGTCTCATCCTTCAGCAC
CYP2D2	Forward	TCTGTTGTAAGAGGATCTTTCC
	Reverse	GTCCACCAGAAGCAAGAA
CYP2E1	Forward	TCTGCTCCTGTCTGCTATTCTG
	Reverse	GGATACTGCCAAAGCCAACTG
CYP3A1	Forward	TGCCATCACGGACACAGA
	Reverse	ATCTCTTCCACTCCTCATCCTTAG
CYP3A2	Forward	GGACTTAATTGACTGCTCTTGATG
	Reverse	GGACGAGGACATGGTTACTATC
GAPDH	Forward	CCCTTCATTGACCTCAACTACAATGGT
	Reverse	GAGGGGCCATCCACAGTCTTCTG

3.3. Statistical analysis

Data were represented as means±S.D. Differences in expression levels between males and females were examined using the Student's unpaired *t*-test, and differences with a *p* value of 0.05 or less were considered significant.

References

Aiba T, Yoshinaga M, Ishida K, Takehara Y, Hashimoto Y (2005) Intestinal expression and metabolic activity of the CPY3A subfamily in female rats. Biol Pharm Bull 28: 311-315.

Bromek E, Haduch A, Daniel WA (2010) The ability of cytochrome P450 2D isoforms to synthesize dopamine in the brain: An *in vitro* study. Eur J Pharmacol 626: 171-178.

D'Agostino J, Zhang H, Kenaan C, Hollenberg PF (2015) Mechanism-based inactivation of human cytochrome P450 2B6 by chlorpyrifos. Chem Res Toxicol 28: 1484-1495.

Dhir RN, Shapiro BH (2003) Interpulse growth hormone secretion in the episodic plasma profile causes the sex reversal of cytochrome P450s in senescent male rats. Proc Natl Acad Sci USA 100: 15224-15228.

Elbaz A, Bower JH, Maraganore DM, McDonnell SK, Peterson BJ, Ahlskog JE, Schaid DJ, Rocca WA (2002) Risk tables for parkinsonism and Parkinson's disease. J Clin Epidemiol 55: 25-31.

Elbaz A, Levecque C, Clavel J, Vidal JS, Richard F, Amouyel P, Alperovitch A, Chart-Harlin MC, Tzourio C (2004) CYP2D6 polymorphism, pesticide exposure, and Parkinson's disease. Ann Neurol 55: 430-434.

Hedlund E, Gustafsson JA, Warner M (2001) Cytochrome P450 in the brain; a review. Curr Drug Metab 2: 245-263.

Kato R, Yamazoe Y (1992) Sex-specific cytochrome P450 as a cause of sex- and species-related differences in drug toxicity. Toxicol Lett 64-65.

Khokhar JY, Tyndale RF Drug metabolism within the brain changes drug response: selective manipulation of brain CYP2B alters propofol effects. Neuropsychopharmacology 36: 692-700.

Khokhar JY, Tyndale RF (2011) Rat brain CYP2B-enzymatic activation of chlorpyrifos to the oxon mediates cholinergic neurotoxicity. Toxicol Sci (2012) 126: 325-335.

Kusuda J, Kajikawa N, Kawasaki H, Kurosaki Y, Hashimoto Y (2012) Intestinal expression and metabolic activity of the CYP3A subfamily in female rats. Biopharm Drug Dispos 33: 22-29.

Lenglar J, Omann M, Duvier D, Holzmuller H, Gregor W, Salmons B, Gunzburg WH, Renner M (2006) Cytochrome P450 reductase dependent inhibition of cytochrome P450 2B1 activity: Implications for gene directed enzyme prodrug therapy. Biochem Pharmacol 72: 893-901.

McCann SJ, Pond SM, James KM, Le Couteur DG (1997) The association between polymorphisms in the cytochrome P-450 2D6 gene and Parkinson's disease: a case-control study and meta-analysis. J Neurol Sci 153: 50-53.

McDonnell AM, Dang CH (2013) Basic review of the cytochrome p450 system. J Adv Pract Oncol 4: 263-268.

Mercapide J, Rappa G, Anzanello F, King J, Fodstad O, Lorico A (2010) Primary gene-engineered neural stem/progenitor cells demonstrate tumor-selective migration and antitumor effects in glioma. Int J Cancer 126: 1206-1215.

Michels R, Marzuk PM (1993) Progress in psychiatry (1). N. Engl. J. Med. 329: 552-560.

Miksys S, Hoffmann E, Tyndale RF (2000) Regional and cellular induction of nicotine-metabolizing CYP2B1 in rat brain by chronic nicotine treatment. Biochem Pharmacol 59: 1501-1511.

Miksys S, Tyndale RF (2013) Cytochrome P450-mediated drug metabolism in the brain. J Psychiatry Neurosci 38: 152-163.

Nelson DR (2011) Progress in tracing the evolutionary paths of cytochrome P450. Biochem Biophys Acta 1814: 14-8.

Ramzan I (1990) Theophylline neurotoxicity is unaffected by glycerol-induced renal failure. Pharmacol Biochem Behav 37: 583-585.

Ribeiro V, Lechner MC (1992) Cloning and characterization of a novel CYP3A1 allelic variant: analysis of CYP3A1 and CYP3A2 sex-hormone-dependent expression reveals that the CYP3A2 gene is regulated by testosterone. Arch Biochem Biophys 293: 147-152.

Shyr MH, Tsai TH, Tan PP, Chen CF, Chan SH (1995) Concentration and regional distribution of propofol in brain and spinal cord during propofol anesthesia in the rat. Neurosci Lett 184: 212-215.

Steenland K, Dick RB, Howell RJ, Chrislip DW, Hines CJ, Reid TM, Lehman E, Laber P, Krieg EF Jr, Knott C (2000) Neurologic function among termiticide applicators exposed to chlorpyrifos. Environ Health Perspect 108: 293-300.

Sultatos LG (1994) Mammalian toxicology of organophosphorus pesticides. J Toxicol Environ Health 43: 271-289.

Tai YT, Lin YL, Chang CC, Cherng YG, Don MJ, Chen RM Ring-oxidative biotransformation and drug interactions of propofol in the liver of rats. Biomed Res Int doi: 10.1155/2015/658928.

Tang J, Cao Y, Rose RL, Brimfield AA, Dai D, Goldstein JA, Hodgson E (2001) Metabolism of chlorpyrifos by human cytochrome P450 isoforms and human, mouse, and rat liver microsomes. Drug Metab Dispos 29: 1201-1204.