4 Sex Differences in Drug Metabolism

THOMAS K. H. CHANG

Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, British Columbia, Canada

DAVID J. WAXMAN

Department of Biology, Boston University, Boston, MA, USA

4.1 Summary 1
4.2 Introduction 2
4.3 Sex differences in hepatic drug metabolism 2
4.4 Sex-dependent expression of hepatic drug-metabolizing enzymes 4
4.5 Hormonal determinants of sex-dependent expression of hepatic cytochrome P450s 7
4.6 Molecular determinants of sex-dependent expression of hepatic cytochrome P450s 10
4.7 Concluding remarks 12
Acknowledgment 12
References 13

4.1 SUMMARY

Sex differences in drug metabolism are recognized as a major determinant of sex differences in pharmacokinetics and an important contributor to interindividual differences in drug metabolism and, in some cases, drug action. Historically, sex differences in drug metabolism were widely studied in rat and mouse models in the 1960s and 1970s. Sex differences in human drug metabolism have also been observed with a variety of drugs; however, the quantitative differences in rates of metabolism between men and women are typically much smaller than those seen in rats. The biochemical basis of sex differences in hepatic drug metabolism was largely unknown until the 1980s when it was discovered that expression of some of the drug-metabolizing enzymes, including those in the cytochrome P450 (CYP) family, is sex-dependent in mouse and rat liver. Thus, the expression of certain rat CYP enzymes is male specific (e.g., CYP2C11), whereas others are female specific (e.g., CYP2C12) or female predominant (e.g., CYP2A1). The major gonadal hormones, testosterone and estradiol, regulate the sex-dependent expression of rat hepatic CYP enzymes indirectly through their effects on the hypothalamus and its regulation of the sexually dimorphic, ultradian

Encyclopedia of Drug Metabolism and Interactions, 6-Volume Set, First Edition.
Edited by Alexander V. Lyubimov.
© 2012 John Wiley & Sons, Inc. Published 2012 by John Wiley & Sons, Inc.

rhythm of pituitary growth hormone (GH) secretion, which ultimately dictates the sex-dependent patterns of expression of select CYPs and certain other drug-metabolizing enzymes. In male rats, the intermittent, pulsatile pattern of GH secretion stimulates the expression of male-specific liver enzymes, whereas the more continuous pattern of GH release in females suppresses the expression of male-specific enzymes while inducing female-specific enzymes. Studies conducted in the past decade have identified essential molecular mediators of the effects of GH on sex-dependent liver CYP enzymes and their genes, in particular, the transcription factors—signal transducer and activator of transcription (STAT) 5b and hepatocyte nuclear factors (i.e., HNF4α). The current view is that STAT5b and HNF4α coordinately regulate the action of GH and its resultant effect on the sex-dependent expression of hepatic *CYP* genes and thus provide a molecular basis for sex differences in drug metabolism.

4.2 INTRODUCTION

Individual variability in drug metabolism and drug response is well documented in the biomedical literature [1–3]. This variability is manifested as qualitative and/or quantitative differences in drug response within a group of patients (i.e., interindividual variability) or in the same patient (i.e., intraindividual variability). Within a population, patient-specific characteristics such as sex [4], age [5], and genetics [6,7] have been identified as determinants of interindividual variability in drug action. Intraindividual differences in drug action can be induced by administration of a second drug leading to drug–drug interactions, may be caused by deterioration of organ function, or may be by progression in the severity of a disease state, among others [8]. The pharmacological basis for variability in drug response can be attributed to interindividual differences in pharmacokinetics (i.e., drug absorption, distribution, metabolism, and excretion) and/or pharmacodynamics (i.e., the physiological, biochemical, cellular, and molecular actions of a drug). Indeed, patient-specific characteristics are known to influence the biological processes that govern drug absorption, distribution, metabolism, excretion, and functionality of therapeutic targets (e.g., receptors, enzymes) and their associated signal transduction pathways.

Sex differences in drug action were first observed in the 1930s, where adult female rats were found to require only half the dosage of amobarbital (Amytal), as compared to adult male rats, to elicit an anesthetic effect [9], and the duration of action of certain barbiturates (e.g., amobarbital and pentobarbital) was found to be longer in female than in male rats [10]. Subsequent animal and human studies have shown sex differences in the pharmacokinetics and pharmacodynamics of various other drugs [11–15]. Considerably more is known about sex differences in pharmacokinetics, especially sex differences in drug metabolism in the liver than about sex differences in pharmacodynamics. The overall aim of this chapter is to provide an overview of our current understanding of sex differences in hepatic drug metabolism.

4.3 SEX DIFFERENCES IN HEPATIC DRUG METABOLISM

4.3.1 Rat Hepatic Drug Metabolism

Sex differences in hepatic drug metabolism were widely studied in the 1960s and 1970s in experiments involving the incubation of rat hepatic microsomes (liver membrane

TABLE 4.1 Metabolism of Drugs and Other Substrates Catalyzed by Hepatic Microsomes Isolated from Male and Female Rats

Drug/ Substrate	Reaction	Strain of Rat	Sex Difference (Male/Female Ratio)	References
5α-androstane-3α,17β-diol-3,17-disulfate	15β-Hydroxylation	Long-Evans	<0.05	21
Aminopyrine	N-demethylation	Sprague-Dawley[a]	3.5	22
			4.0	23
Aniline	Hydroxylation	Sprague-Dawley[a]	1.1	22
Diazepam	3-Hydroxylation	Wistar[a]	5.2	16
		Wistar[b]	0.7	16
		BD[a]	10	16
	N-desmethylation	Wistar[a]	0.9	16
		Wistar[b]	0.9	16
		BD[a]	2.6	16
Diphenhydramine	N-demethylation	Sprague-Dawley[a]	2.4	23
Hexobarbital	Hydroxylation	Sprague-Dawley[a]	4.2	22
			3.0	23
Imipramine	N-demethylation	Sprague-Dawley[a]	2.5	24
	2-Hydroxylation	Sprague-Dawley[a]	0.7	24
	N-oxidation	Sprague-Dawley[a]	2.9	24
Indinavir	Oxidation[c]	Sprague-Dawley[a]	3.3	25
Lidocaine	N-demethylation	Sprague-Dawley[a]	8.5	24
	3-Hydroxylation	Sprague-Dawley[a]	0.7	24
Meperidine	N-demethylation	Sprague-Dawley[a]	2.4	23
3,4-Methylenedioxy-methamphetamine	N-demethylation	Sprague-Dawley[a]	3.3	18
Morphine	N-demethylation	Sprague-Dawley[a]	2.6	23
Pentobarbital	Oxidation	Sprague-Dawley[a]	2.6	23

[a]Postpubertal rats.
[b]Neonatal rats.
[c]Total oxidation products.

preparations enriched in endoplasmic reticulum) with drugs, other foreign chemicals, or endogenous lipophilic substrates (e.g., endogenous steroids and fatty acids). As illustrated in Table 4.1, drugs such as diazepam, lidocaine, and indinavir are metabolized more rapidly by liver microsomes isolated from postpubertal male rats than by those from postpubertal female rats. In contrast, other substrates (e.g., the steroid 5α-androstane-3α,17β-diol-3,17-disulfate) are more rapidly metabolized by hepatic microsomes from female rats. However, not all substrates (e.g., aniline) exhibit sex difference in hepatic metabolism. Table 4.1 also shows that the sex dependence of drug metabolism can be pathway specific; for example, lidocaine N-demethylation, but not lidocaine 3-hydroxylation, is preferentially catalyzed by hepatic microsomes from male rats. With some drugs, sex differences in hepatic metabolism are strain specific; for example, liver microsomes prepared from BD rats but not those from Wistar rats exhibit sex differences in diazepam N-desmethylation. Sex differences in hepatic drug metabolism may also vary as a function of age (Table 4.1), typically being

manifested during the pubertal and adult periods but not prepubertally [16] and present at a reduced level during senescence [17]. Sex differences in hepatic drug bioactivation can lead to deleterious consequences, as exemplified by the finding that the enhanced N-demethylation of 3,4-methylenedioxymethamphetamine (MDMA, ectasy) by hepatic microsomes from male rats is associated with an increased susceptibility to the acute toxicity of MDMA, when compared to female rats [18]. In addition to phase I oxidative drug metabolism, sex differences in phase II metabolism affecting glucuronidation, sulfation, and glutathione conjugation, have also been reported in animal studies [19,20].

4.3.2 Human Hepatic Drug Metabolism

Sex differences in human hepatic drug metabolism have been investigated in clinical pharmacokinetic studies that compare the clearance of a drug in men and women. Sex differences characterize the clearance of certain drugs, including cyclosporine [26], erythromycin [27], nifedipine [28], triazolam [29], midazolam [30,31], alprazolam [32], and verapamil [33]. However, in some cases, conflicting data exists with regard to the effect of sex on drug clearance [34–36]. Possible reasons for the conflicting data include (i) the small sample size employed in some of the studies resulting in a lack of statistical power to detect a sex-based difference and (ii) the role of confounding variables, such as age [12,35], ethnicity [37], and other biological determinants of drug clearance, which can include the level of expression of uptake and efflux transporters, although it is debatable whether human hepatic P-glycoprotein expression differs between males and females [38], given the conflicting data in the literature [39,40]. With the increased availability of human hepatic microsomes for research, the *in vitro* metabolism of drugs and other substrates can readily be assessed using microsomes isolated from liver tissue samples obtained from male and female donors. As shown in Table 4.2, in general, sex differences are not apparent or are relatively modest in human hepatic microsomal drug metabolism. An exception is *S*-mephenytoin N-demethylation, whose metabolism is substantively greater in hepatic microsomes from females than in those from males, but this occurs only in samples from Hispanic donors [37]. Overall, sex differences occur in hepatic phase I and phase II drug metabolism, and the differences are generally larger and hence more apparent in rats than in humans.

4.4 SEX-DEPENDENT EXPRESSION OF HEPATIC DRUG-METABOLIZING ENZYMES

The underlying biochemical basis for sex differences in drug metabolism came to light when it was discovered in the 1980s that many of the rat hepatic CYP enzymes (Table 4.3) are expressed in a sex-dependent manner [47]. Since then, sex-dependent expression of other rodent drug-metabolizing enzymes, including UDP-glucuronosyltransferases [48–50], sulfotransferases [49,51,52], and glutathione *S*-transferases [49,53,54], has also been reported. The available experimental evidence suggests that certain human hepatic CYP enzymes may also be expressed differentially in men and women. Overall, among the drug-metabolizing enzymes, the hepatic CYPs are the best characterized with respect to their sex-dependent expression. Thus, the

TABLE 4.2 Effect of Sex on Metabolism of Drugs and Other Substrates Catalyzed by Human Hepatic Microsomes

Drug	Reaction	Effect	References
Aminopyrine	N-demethylation	M = F	41
Chlorzoxazone	6-Hydroxylation	M = F	42
Coumarin	7-Hydroxylation	M = F	42
Dextromethorphan	O-demethylation	M = F	42
Diclofenac	4′-Hydroxylation	M = F	42
Erythromycin	N-demethylation	F > M (1.2-fold)	43
7-Ethoxyresorufin	O-demethylation	M = F	42
Ifosfamide	N-dechloroethylation	F > M (2.2-fold)	44
Lauric acid	12-Hydroxylation	M = F	42
S-mephenytoin	N-demethylation	F > M (7.8-fold)[a]	37
		M = F[b,c]	37
		M = F[d]	42
	4′-Hydroxylation	M = F	42
Nicotine	C-oxidation	M = F	45
S-oxazepam	Glucuronidation	M > F (1.7-fold)	46
Paclitaxel	6α-Hydroxylation	M = F	42
Testosterone	6β-Hydroxylation	M = F	42

[a] Hispanic.
[b] African-American.
[c] Caucasian.
[d] Samples from Hispanics, African-Americans, and Caucasians.

remainder of this chapter focuses on hepatic CYP enzymes and the various factors that regulate their sex-dependent expression.

4.4.1 Rat Cytochrome P450s

As summarized in Table 4.3, multiple CYP enzymes are expressed constitutively in a sex-dependent manner in rat liver, and their expression is developmentally regulated. Interestingly, a catalytic function of many of these enzymes is the oxidative metabolism of sex steroid hormones such as testosterone. The prototype male-specific P450 enzyme of rat liver is CYP2C11 [55–57, 75]. CYP2C11 is essentially undetectable in both male and female rat liver before puberty, but its expression increases rapidly at puberty (at approximately day 35 of age) in males only and reaches a maximum during early adulthood [58,59]. A similar pattern of postnatal developmental expression has been described for several other male-specific rat hepatic CYP enzymes, that is, CYP2A2 [60,61], CYP2C13 [62], and CYP3A18 [63,64]. In contrast, hepatic expression of the male-specific CYP3A2 is detectable during the prepubertal period in both sexes, whereas at the onset of puberty, the level of CYP3A2 is increased in male liver, but at about the same time it decreases to barely quantifiable levels in females [58]. Another sex-dependent rat hepatic CYP is the female-specific CYP2C12 (Table 4.3). Although CYP2C12 is expressed at low levels in the livers of both sexes prepubertally, its expression is increased at puberty in female rat liver but is suppressed in male rats [58,65]. The female-predominant enzymes CYP2A1, CYP2A7, and CYP3A9 (Table 4.3) comprise a third group of sex-dependent liver P450s. Expression is either similar (CYP2A1

TABLE 4.3 Sex-Dependent Hepatic Expression of Rat Cytochrome P450 Enzymes[a]

Enzyme	Catalytic Reaction	Developmental Expression	
		Prepuberty	Puberty
Male Specific			
CYP2A2	Testosterone 15α-hydroxylation	None	↑ in male
CYP2C11	Testosterone 2α-hydroxylation	None	↑ in male
CYP2C13	Testosterone 15α-hydroxylation	Minimal	↑ in male
CYP3A2	Testosterone 6β-hydroxylation	Male ∼ female	↑ in male, ↓ in female
CYP3A18	Testosterone 6β-hydroxylation	Minimal	↑ in male
CYP4A2	Lauric acid ω-hydroxylation	None[b]	↑ in male[b]
Female Specific			
CYP2C12	5α-Androstane-3α,17β-diol-3,17-disulfate15β-hydroxylation	Minimal/moderate	↑ in female None in male
Female Predominant			
CYP2A1	Testosterone 7α-hydroxylation	Male ∼ female	Female > male
CYP2C7	Retinoic acid 4-hydroxylation	Male ∼ female	Female > male
CYP3A9	Testosterone 6β-hydroxylation	↓	Female > male

[a]Based on Refs 21,55–73.
[b]Based on data for renal CYP4A2 [74].

and CYP2C7) or absent (CYP3A9) in both sexes during prepuberty, and the levels of these enzymes are greater in postpubertal female rats than in postpubertal male rats [58,62,64].

4.4.2 Human Cytochrome P450s

In contrast to the rat CYP enzymes, relatively little is known about the sex-dependent expression of human CYPs. With the available information in the literature, many of the conclusions have been drawn from *in vivo* pharmacokinetic studies that measured the plasma clearance of an enzyme-selective substrate (e.g., midazolam for CYP3A enzymes [31]) or urinary formation of a biomarker (e.g., 6β-hydroxycortisol for CYP3A enzymes [76]). In other cases, conclusions regarding the sex dependence of human enzyme levels have been drawn from *in vitro* metabolism studies (Table 4.2) based on the conversion of an enzyme-selective substrate to its metabolite (e.g., microsomal formation of 6β-hydroxytestosterone as a catalytic marker for CYP3A [42]). More limited information is available on the immunoreactive protein levels of human CYP enzymes in livers from both sexes. In a panel of 94 liver samples (48 from males and 46 from females), the median level of CYP3A4 was approximately twofold greater in liver samples from females than in samples from males [40]. By comparison, CYP2B6 protein levels were found to be approximately twofold higher in livers from Caucasian women ($N = 15$) than from Caucasian men ($N = 28$), whereas it was approximately fourfold higher in liver samples from Hispanic women ($N = 3$) than in those from Hispanic men ($N = 4$) [37]. In another study of human livers from donors of unknown ethnic origin, multiple linear regression analysis indicated that sex was a

predictor of hepatic CYP2B6 protein levels, but only accounted for 14% of the variance [77]. Overall, it appears that sex differences occur in hepatic expression of human CYP, namely, CYP3A4 and CYP2B6, although the effects are rather modest and considerably less pronounced when compared to the sex differences in the expression of rat hepatic CYP enzymes (Table 4.3).

4.5 HORMONAL DETERMINANTS OF SEX-DEPENDENT EXPRESSION OF HEPATIC CYTOCHROME P450s

A hormonal basis for sex differences in drug metabolism was apparent from early studies showing that (i) castration of postpubertal male rats decreased hepatic microsomal CYP-mediated metabolism of aminopyrine and hexobarbital and (ii) exogenous administration of an androgen (methyltestosterone) fully reversed the suppressive effects of castration [22,78]. These observations paved the way for subsequent studies by various research groups elucidating the hormonal factors that determine the sex-dependent expression of CYP enzymes in rat liver. Collectively, the results of those studies indicate that in addition to gonadal hormones, other hormones also regulate the sex-dependent expression of rat hepatic CYPs. Among these, GH is the most important and the best characterized [79].

4.5.1 Gonadal Hormones

4.5.1.1 Androgen. Androgen has distinct effects on the hepatic expression of the male-specific and female-specific rat CYP enzymes. In male rats, androgen is required for the pubertal expression of the male-specific forms, such as CYP2A2 [60], CYP2C11 [55,58,59,80], CYP2C13 [66], CYP3A2 [58,81], CYP3A18 [82], and CYP4A2 [67], as shown in experiments involving castration of neonatal male rats and exogenous administration of testosterone. Thus, exposure to androgen early in life (e.g., neonatal period) is sufficient to ensure enzyme expression later in adult life in a process known as *neonatal programming* [83]. Full adult expression levels of the male-specific enzymes are achieved in rats exposed to androgen during both the neonatal and peripubertal periods [47]. In contrast to the positive regulation of the male-specific rat hepatic CYP enzymes by androgen, this hormone negatively regulates the hepatic expression of the female-specific CYP2C12, as shown by the decreased enzyme levels in intact, adult female rats administered with exogenous androgen [65,84]. An important conclusion from the available experimental evidence [47,85] is that androgen controls hepatic expression of the sex-dependent rat hepatic CYP enzymes in an indirect manner through its action on the hypothalamic pituitary axis, which results in a sex-specific pattern of pituitary GH secretion (Section 4.5.2).

4.5.1.2 Estrogen. Estrogen also has distinct effects on the expression of sex-dependent rat liver CYP enzymes. In female rats, ovariectomy and exogenous estrogen replacement experiments show that estrogen is required for liver expression of the female-specific CYP2C12 [58,65] and the female-predominant CYP2C7 [80] and CYP3A9 [86]. However, estrogen is not absolutely required for hepatic expression of these enzymes, as these enzymes are still expressed, albeit at low levels, in ovariectomized rats. In intact and castrated male rats, estrogen suppresses the hepatic

expression of the male-specific CYP2C11 [59,80,84]. The effects of estrogen on these sex-dependent CYP enzymes also involves its effects on the sex-specific pattern of pituitary GH secretion [47,85].

4.5.2 Growth Hormone

GH is a 191-amino-acid protein hormone that is secreted by the anterior pituitary gland. Its secretion is rhythmic and episodic over a 24-h period in mammals, including rats [87] and humans [88]. The ultradian rhythm of pituitary GH secretion can be influenced by various factors, including age [89], prolonged food deprivation [90], and sex steroids, particularly, estrogen [91]. There is a distinct sexual dimorphic pattern of GH secretion, which becomes apparent at the onset of puberty [92]. In adult male rats, the plasma GH profile is characterized by well-defined peaks (up to 250 ng GH/mL) and troughs where the levels are very low or undetectable (Fig. 4.1). The period between peaks typically lasts 3–4 h. By comparison, in female rats, GH is secreted in a more continuous manner such as the plasma GH peaks are lower (75 ng/mL), but circulating hormone levels are nearly always detectable (Fig. 4.1).

An initial clue that GH plays a role in regulating hepatic CYP expression was provided by the findings that GH administration decreased total CYP content and hepatic microsomal metabolism of CYP substrates [94]. It is now widely recognized that the sexual dimorphic pattern of GH secretion is the major hormonal determinant

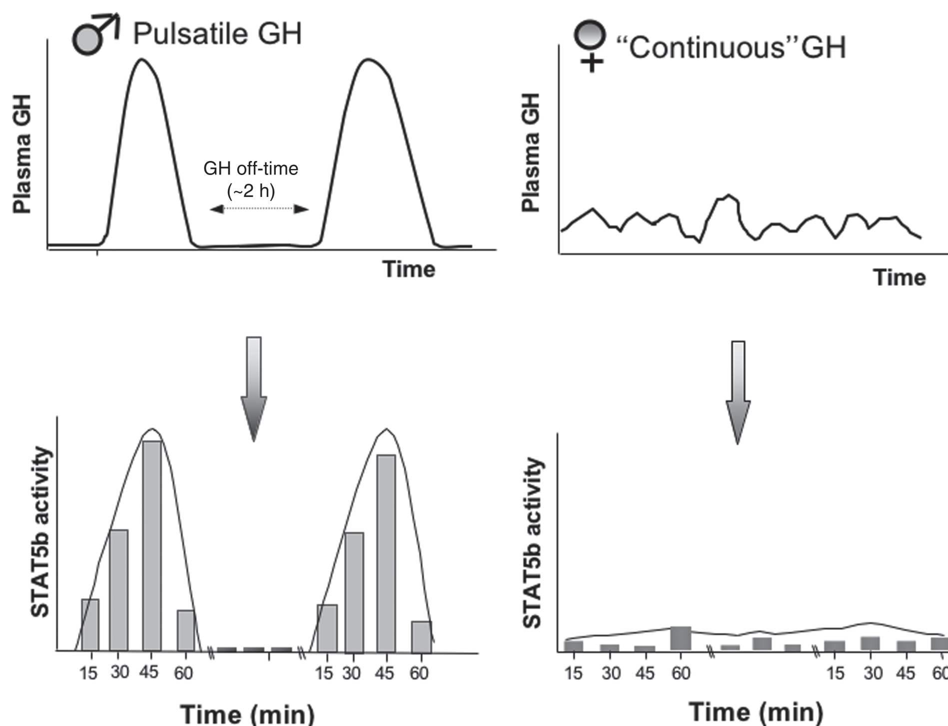

Figure 4.1 Sex dependence of the temporal pattern of plasma GH secretion and hepatic STAT5b activity in male and female rats [93]

TABLE 4.4 Responsiveness of Sex-Dependent Rodent Hepatic Cytochrome P450 Genes to Hypophysectomy and Exogenous Administration of GH[a]

Rodent Hepatic Cytochrome P450 Genes	Hypophysectomy		Exogenous Administration of GH	
	Male	Female	Intermittent[b]	Continuous[c]
	Effect on Gene Expression		Effect on Gene Expression	
Rat, male-specific (class I) *CYP2C11*	↓	↑	↑	↓
Rat, male-specific (class II) *CYP2A2, CYP2C13,* and *CYP3A2*	No effect	↑	↓	↓
Rat, female-specific (class I) *CYP2C12*	↓	↓	↓	↑
Mouse, male-specific (class IA) *Cyp2d9*	↓	No effect	↑	↓
Mouse, male-specific (class IB) *Cyp7b1*	↓	↓	↑	↓
Mouse, male-specific (class IC) *Cyp4a12*	↓	↑	↑	↓
Mouse, female-specific (class II) *Cyp2a4* and *Cyp2b9*	↑	No effect	↓	↑

[a] As summarized in Ref. 79.
[b] Intermittent GH administration to hypophysectomized male rats.
[c] Continuous GH administration of intact male rats.

of the sex-dependent expression of rat hepatic CYPs [47]. On the basis of the available evidence, it appears that the interpulse period devoid of GH is a key signal in the masculinization of CYP expression in rat liver [95,96]. As shown in Table 4.4, the GH-regulated CYP enzymes can be categorized into different classes, based on their response to hypophysectomy [79,127,128]. The expression of a male class I gene (i.e., *CYP2C11*) is decreased in hypophysectomized male rats, whereas it is increased in hypophysectomized female rats but only to a level comparable to that in hypophysectomized male rats. CYP2C11 levels are increased by intermittent administration of GH and decreased by continuous GH treatment. A corresponding pattern of response is seen for a female class I gene (e.g., *CYP2C12*), except that decreased expression occurs in hypophysectomized female rats and by intermittent GH administration. By comparison, hypophysectomy of female rats increases the expression of class II male-specific genes (i.e., *CYP2A2, CYP2C13*, and *CYP3A2*), whereas GH, in particular, continuous infusion of GH in male rats, represses the expression of these genes.

GH regulates the clearance of certain drugs that undergo CYP-mediated clearance [97–99], and also it appears that the sex-dependent pattern of GH secretion controls the expression of human CYP enzymes such as CYP3A4, which appears to be expressed at a somewhat greater level in livers from women than in livers from men (Section 4.4.2). As demonstrated in primary cultures of human hepatocytes, continuous GH treatment (to reflect the female pattern of GH secretion) increases CYP3A4 expression [100,101], whereas intermittent GH administration (i.e., two daily doses, to mimic the pulsatile, male pattern of GH secretion) decreases CYP3A4 expression, although the effect was

greater in hepatocytes isolated from males than in those from females [101]. The suppressive effect of intermittent GH has also been reported *in vivo*. In a human study conducted in male and female subjects with GH deficiency, GH infusion was associated with an increase in CYP3A activity, whereas GH administered as three daily doses was associated with a decrease in CYP3A activity, as assessed by the erythromycin breath test [102]. In another study conducted in GH-deficient boys and girls, administration of GH once daily for 30 days decreased CYP3A activity, as assessed by the urinary ratio of 6β-hydroxycortisol/free cortisol, but this response was only observed in boys [103]. Interestingly, the administration of GH by constant infusion over a seven-day period increases CYP3A4 mRNA and protein levels in transgenic male mice [104]. Overall, it appears that the mode of GH administration (intermittent vs continuous) exerts differential effects on human CYP expression in a manner similar to that documented for rat CYP enzymes.

4.6 MOLECULAR DETERMINANTS OF SEX-DEPENDENT EXPRESSION OF HEPATIC CYTOCHROME P450s

Studies in the past decade have increased our understanding of the underlying molecular mechanisms through which GH controls the sex-dependent expression of hepatic CYP enzymes. GH exerts its action by binding to the growth hormone receptor (GHR), which is a member of the superfamily of cytokine receptors [105]. This leads to a conformational change of GHR and activation of a tyrosine kinase known as Janus kinase 2 (JAK2), which catalyzes the tyrosine phosphorylation of various intracellular proteins, including GHR and those belonging to the STAT family, such as STAT5b [106]. The GHR-bound, tyrosine phosphorylated STAT5b dimerizes and translocates to the nucleus where it binds to DNA response elements of target genes and stimulates gene transcription [105,107].

Whereas STAT5b is a direct transcriptional mediator of GH action, GH may also exert indirect effects on gene expression, through various HNFs, including HNF4α [108], a member of the nuclear receptor superfamily [109]. The available evidence to date indicates that STAT5b and HNF4α are molecular determinants of the sex-dependent expression of hepatic CYP enzymes [79]. These factors are likely to regulate hepatic CYPs and other sex-specific genes both directly and indirectly, via a complex transcriptional regulatory network, as indicated by the complex time course that characterizes the feminization of these sex-specific genes in male mice infused with GH continuously over a 14-day period [110]. Several other liver-expressed transcription factors show strong sex differences in their expression, including the female-specific factor Cux2 (Cutl2) [111] and the male-specific factor Bcl6 [112]. These factors are both dependent on GH and STAT5b for their sex-specific expression, suggesting that they may play a key role in this transcriptional regulatory network by regulating downstream targets of GH and STAT5b.

4.6.1 STAT5b

A hormonal basis for the tyrosine phosphorylation of STAT5b is provided by the experimental findings indicating that hepatic STAT5b activity is (i) greater in adult

TABLE 4.5 STAT5b and HNF4α Regulate Sex-Dependent Mouse Hepatic Cytochrome P450 Gene Expression[a]

Hepatic Cytochrome P450 Genes	Male		Female	
	STAT5b Deficiency	HNF4α Deficiency	STAT5b Deficiency	HNF4α Deficiency
	Effect on Gene Expression			
Male-Specific Genes				
Cyp2d9	↓	↓	↑	↓
Cyp7b1	↓	↓	↓	↓
Cyp4a12	↓	↓	No effect	No effect
Female-Specific Genes				
Cyp2a4	↑	↑	No effect	No effect
Cyp2b9	↑	↑	No effect	No effect

[a]Based on Refs 110,118, and 119.

male rats than in adult female rats, (ii) abolished by hypophysectomy, (iii) stimulated by intermittent administration of GH but decreased by continuous administration of GH in hypophysectomized male rats, and (iv) stimulated by continuous GH infusion in hypophysectomized female rats [113,114]. Furthermore, there is a direct temporal relationship between the occurrence of a plasma GH pulse and the activation of hepatic STAT5b activity [115]. As illustrated in Fig. 4.1, in adult male rats, STAT5b is activated by each pulsatile secretion of GH, such that the time for peak STAT5b activity corresponds to the time for peak plasma GH levels and little or no STAT5b activity is detected during the interpulse, GH-free period. In contrast, in adult female rats, there is a very low but persistent level of STAT5b activity over time, similar to the temporal pattern of the low level, continuous release of plasma GH.

STAT5b is essential for the sex-dependent expression of hepatic CYP enzymes and many other sex-dependent genes, as determined in mice genetically deficient in STAT5b. Thus, the sexual dimorphism of hepatic CYP genes is abolished in the absence of STAT5b [116]. As shown in Table 4.5, when compared to the wild-type control male mice, STAT5b-deficient mice have decreased hepatic gene expression of male-specific CYPs (i.e., *Cyp2d9, Cyp4a12,* and *Cyp7b1*), but increased levels of female-specific CYPs (i.e., *Cyp2a4, Cyp2b9,* and *Cyp2b13*) [110,117]. In female mice, STAT5b deficiency leads to increased hepatic expression of the male-specific *Cyp2d9,* leads to decreases of the male-specific *Cyp7b1,* and has little or no effect on the female-specific *Cyp2a4* or *Cyp2b9* [118]. However, not all sex-dependent CYP genes are regulated by STAT5b because STAT5b deficiency does not influence the hepatic expression of the female-specific *Cyp3a16, Cyp3a41,* and *Cyp3a44* [118]. Moreover, hepatic *Cyp2d9* and *Cyp4a12* levels are increased in response to intermittent GH administration in hypophysectomized, STAT5b-expressing male mice but not in hypophysectomized, STAT5b-deficient male mice [110,117]. Collectively, these findings indicate positive regulation of male-specific CYP genes and negative regulation of a subset of female-specific CYP genes by GH pulse-activated STAT5b.

4.6.2 HNF4α

HNF4α (gene designation *NR2A1*) is a transcription factor initially known for its role in liver-specific gene expression [120]. It is expressed at high levels in liver and several tissues, and it is constitutively localized in the nucleus [121]. Among its many functions, HNF4α has been shown to regulate the expression of CYP genes by interacting with various nuclear receptors including constitutive androstane receptor and pregnane X receptor [122,123]. HNF4α also play a role in hepatic expression of sex-dependent CYP genes [110,124]. As summarized in Table 4.5, HNF4α deficiency in male mice is associated with a decrease in hepatic levels of the male-specific genes *Cyp2d9, Cyp7b1,* and *Cyp4a12,* and an increase in that of the female-specific *Cyp2a4*. By comparison, HNF4α deficiency in female mice decreases the low level of expression of *Cyp2d9,* whereas it has no effect on the male-specific *Cyp7b1* and *Cyp4a12* and the female-specific *Cyp2a4* and *Cyp2b9*. This pattern of response to HNF4α deficiency is similar to that of STAT5b deficiency (Table 4.5) and is consistent with the idea that STAT5b is not the sole regulator of the sex-dependent hepatic *Cyp* genes [125,126]. The current view is that HNF4α functions cooperatively with GH pulse-activated STAT5b to achieve the full expression of male-specific CYP genes [79], a view that is supported by microarray analysis showing global similarities in the dependence of sex-specific genes on STAT5b and HNF4α in mouse liver [119].

4.7 CONCLUDING REMARKS

Sex differences in hepatic drug metabolism are well documented in rat and mouse models and are increasingly recognized as making a key contribution to sex differences in pharmacokinetics. The underlying mechanisms responsible for the sex differences in the hepatic expression of drug-metabolizing enzymes, in particular, CYPs, are not fully understood but involve complex patterns of hormonal control and intracellular signaling pathways. It is now well established that the sexually dimorphic pattern of plasma GH secretion is a major regulator that controls the sex-dependent expression of CYPs. Studies over the past few years have revealed that sex differences in the liver extend well beyond enzymes of drug metabolism to include more than 1000 genes [127,128], and the transcription factors STAT5b and HNF4α have been identified as essential determinants of the sex-dependent expression of a large fraction of these genes, including many CYPs. Several other GH-regulated liver transcription factors that show strong sex differences in their expression, as well as a dependence on STAT5b, have also been identified and seem likely to be part of a complex transcriptional regulatory network that regulates the sex-dependent expression of CYPs and many other liver-expressed genes. Much has been learned but much more remains to be elucidated regarding the complex signaling pathways involved in the cellular and molecular actions of GH and how they contribute to sex differences in drug metabolism.

ACKNOWLEDGMENT

This work was supported by the Canadian Institutes of Health Research (Grant MOP-84581 to T. K. H. C.), National Institutes of Health (Grant DK33765 to D. J. W.), and Michael Smith Foundation for Health Research (Senior Scholar Award to T. K. H. C.).

REFERENCES

1. El Desoky ES, Derendorf H, Klotz U. Variability in response to cardiovascular drugs. Curr Clin Pharmacol 2006;1:35–46.
2. Materson BJ. Variability in response to antihypertensive drugs. Am J Med 2007;120: S10–S20.
3. Wiechec E, Hansen LL. The effect of genetic variability on drug response in conventional breast cancer treatment. Eur J Pharmacol 2009;625:122–130.
4. Dahan A, Kest B, Waxman AR, *et al*. Sex-specific responses to opiates: animal and human studies. Anesth Analg 2008;107:83–95.
5. McLachlan AJ, Hilmer SN, Le Couteur DG. Variability in response to medicines in older people: phenotypic and genotypic factors. Clin Pharm Ther 2009;85:431–433.
6. Bhatnagar P, Guleria R, Kukreti R. Pharmacogenomics of beta2-agonists: key focus on signaling pathways. Pharmacogenomics 2006;7:919–933.
7. Romaine SP, Bailey KM, Hall AS, *et al*. The influence of SLCO1B1 (OATP1B1) gene polymorphisms on response to statin therapy. Pharmacogenomics J 2010;10:1–11.
8. Levy G. Predicting effective drug concentrations for individual patients. Determinants of pharmacodynamic variability. Clin Pharmacokinet 1998;34:323–333.
9. Nicholas JS, Barron DH. The use of sodium amytal in the production of anesthesia in the rat. J Pharmacol Exp Ther 1932;46:125–129.
10. Holck HG, Kanan MA, Mills LM, *et al*. Studies upon the sex-difference in rats in tolerance to certain barbiturates and to nicotine. J Pharmacol Exp Ther 1937;60:323–346.
11. Gandhi M, Aweeka F, Greenblatt RM, *et al*. Sex differences in pharmacokinetics and pharmacodynamics. Annu Rev Pharmacol Toxicol 2004;44:499–523.
12. Schwartz JB. The current state of knowledge on age, sex, and their interactions on clinical pharmacology. Clin Pharm Ther 2007;82:87–96.
13. Franconi F, Brunelleschi S, Steardo L, *et al*. Gender differences in drug responses. Pharmacol Res 2007;55:81–95.
14. Soldin OP, Mattison DR. Sex differences in pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 2009;48:143–157.
15. Nicolas JM, Espie P, Molimard M. Gender and interindividual variability in pharmacokinetics. Drug Metab Rev 2009;41:408–421.
16. Nau H, Liddiard C. Postnatal development of sex-dependent differences in the metabolism of diazepam by rat liver. Biochem Pharmacol 1980;29:447–449.
17. Fujita S, Morimoto R, Chiba M, *et al*. Evaluation of the involvement of a male specific cytochrome P-450 isozyme in senescence-associated decline of hepatic drug metabolism in male rats. Biochem Pharmacol 1989;38:3925–3931.
18. Fonsart J, Menet MC, Decleves X, *et al*. Sprague-Dawley rats display metabolism-mediated sex differences in the acute toxicity of 3,4-methylenedioxymethamphetamine (MDMA, ectasy). Toxicol Appl Pharmacol 2008;230:117–125.
19. Mulder GJ. Sex differences in drug conjugation and their consequences for drug toxicity. Sulfation, glucuronidation and glutathione conjugation. Chem-Biol Interact 1986;57:1–15.
20. Mugford CA, Kedderis GL. Sex-dependent metabolism of xenobiotics. Drug Metab Rev 1998;30:441–498.
21. Ryan DE, Dixon R, Evans RH, *et al*. Rat hepatic cytochrome P-450 isozyme specificity for the metabolism of the steroid sulfate, 5α-androstane-3α,17β-diol-3,17-disulfate. Arch Biochem Biophys 1984;233:636–642.
22. Kato R, Gillette JR. Sex differences in the effects of abnormal physiological states on the metabolism of drugs by rat liver microsomes. J Pharmacol Exp Ther 1965;150:285–291.
23. Kato R, Takayanaghi M. Differences among the action of phenobarbital, methylcholanthrene and male sex hormone on microsomal drug-metabolizing enzyme systems of rat liver. Jpn J Pharmacol 1966;16:380–390.

24. Skett P, Mode A, Rafter J, *et al*. The effects of gonadectomy and hypophysectomy on the metabolism of imipramine and lidocaine by the liver of male and female rats. Biochem Pharmacol 1980;29:2759–2762.
25. Lin JH, Chiba M, Chen IW, *et al*. Sex-dependent pharmacokinetics of indinavir. *in vivo* and *in vitro* evidence. Drug Metab Dispos 1996;24:1298–1306.
26. Kahan BD, Kramer WG, Wideman C, *et al*. Demographic factors affecting the pharmacokinetics of cyclosporine estimated by radioimmunoassay. Transplantation 1986;41:459–464.
27. Austin KL, Mather LE, Philpot CR, *et al*. Intersubject and dose-related variability after intravenous administration of erythromycin. Brit J Clin Pharmacol 1980;10:273–279.
28. Krecic-Shepard ME, Park K, Barnas C, *et al*. Race and sex influence clearance of nifedipine: results of a population study. Clin Pharm Ther 2000;68:130–142.
29. Greenblatt DJ, Harmatz JS, von Moltke LL, *et al*. Age and gender effects on the pharmacokinetics and pharmacodynamics of triazolam, a cytochrome P450 3A substrate. Clin Pharm Ther 2004;76:467–479.
30. Chen M, Ma L, Drusano GK, *et al*. Sex differences in CYP3A activity using intravenous and oral midazolam. Clin Pharm Ther 2006;80:531–538.
31. Tsunoda SM, Velez RL, von Moltke LL, *et al*. Differentiation of intestinal and hepatic cytochrome P450 3A activity with use of midazolam as an *in vivo* probe: effect of ketoconazole. Clin Pharm Ther 1999;66:461–471.
32. Kristjansson F, Thorsteinsson SB. Disposition of alprazolam in human volunteers. Differences between genders. Acta Pharm Nord 1991;3:249–250.
33. Krecic-Shepard ME, Barnas CR, Slimko J, *et al*. Gender-specific effects on verapamil pharmacokinetics and pharmacodynamics in humans. J Clin Pharmacol 2000;40:219–230.
34. Anderson GD. Sex differences in drug metabolism: cytochrome P-450 and uridine diphosphate glucuronosyltransferase. J Gend Specif Med 2002;5:25–33.
35. Cotreau MM, von Moltke LL, Greenblatt DJ. The influence of age and sex on the clearance of cytochrome P450 3A substrates. Clin Pharmacokinet 2005;44:33–60.
36. Scandlyn MJ, Stuart EC, Rosengren RJ. Sex-specific differences in CYP450 isoforms in humans. Expert Opin Drug Metab Toxicol 2008;4:413–424.
37. Lamba V, Lamba J, Yasuda K, *et al*. Hepatic CYP2B6 expression: Gender and ethnic differences and relationship to *CYP2B6* genotype and CAR (constitutive androstane receptor) expression. J Pharmacol Exp Ther 2003;307:906–922.
38. Bebawy M, Chetty M. Gender differences in P-glycoprotein expression and function: effects on drug disposition and outcome. Curr Drug Metab 2009;10:322–328.
39. Schuetz EG, Furuya KN, Schuetz JD. Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms. J Pharmacol Exp Ther 1995;275:1011–1018.
40. Wolbold R, Klein K, Burk O, *et al*. Sex is a major determinant of CYP3A4 expression in human liver. Hepatology 2003;38:978–988.
41. Garcia-Agundez JA, Luengo A, Benitez J. Aminopryine *N*-demethylase activity in human liver microsomes. Clin Pharm Ther 1990;48:490–495.
42. Parkinson A, Mudra DR, Johnson C, *et al*. The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes. Toxicol Appl Pharmacol 2004;199:193–209.
43. Hunt CM, Westerkam WR, Stave GM. Effect of age and gender on the activity of human hepatic CYP3A. Biochem Pharmacol 1992;44:275–283.
44. Schmidt R, Baumann F, Hanschmann H, *et al*. Gender differences in ifosfamide metabolism by human liver microsomes. Eur J Drug Metab Pharmacokinet 2001;26: 193–200.
45. Al Koudsi N, Hoffmann EB, Assadzadeh A, *et al*. Hepatic CYP2A6 levels and nicotine metabolism: impact of genetic, physiological, environmental, and epigenetic factors. Eur J Clin Pharmacol 2010;66:239–251.

46. Court MH, Hao Q, Krishnaswamy S, *et al*. UDP-glucuronosyltransferase (UGT) 2B15 pharmacogenetics: UGT2B15 D85Y genotype and gender are major determinants of oxazepam glucuronidation by human liver. J Pharmacol Exp Ther 2004;310:656–665.
47. Waxman DJ, Chang TKH. Hormonal regulation of liver cytochrome P450 enzymes. In: Ortiz de Montellano PR, editor. Cytochrome P450. Structure, mechanism, and biochemistry. New York: Kluwer Academic/Plenum Publishers; 2005. pp. 347–376.
48. Takeuchi T, Tsutsumi O, Nakamura N, *et al*. Gender difference in serum biphenol A levels may be caused by liver UDP-glucuronosyltransferase activity in rats. Biochem Biophys Res Commun 2004;325:549–554.
49. Clodfelter KH, Holloway MG, Hodor P, *et al*. Sex-dependent liver gene expression is extensive and largely dependent upon signal transducer and activator of transcription 5b (Stat5b): STAT5b-dependent activation of male genes and repression of female genes revealed by microarray analysis. Mol Endocrinol 2006;20:1333–1351.
50. Buckley DB, Klassen CD. Tissue- and gender-specific mRNA expression of UDP-glucuronosyltransferases (UGTs) in mice. Drug Metab Dispos 2007;35:121–127.
51. Iwasaki K, Tokuma Y, Noda K, *et al*. Age- and sex-related changes of sulfotransferase activities in the rat. Chem Biol Interact 1994;92:209–217.
52. Kocarek TA, Duanmu Z, Fang HL, *et al*. Age- and sex-dependent expression of multiple murine hepatic hydroxysteroid sulfotransferase (SULT2A) genes. Biochem Pharmacol 2008;76:1036–1046.
53. Srivastava PK, Waxman DJ. Sex-dependent expression and growth hormone regulation of class Alpha and class Mu glutathione *S*-transferase mRNAs in rat liver. Biochem J 1993;294:159–165.
54. Knight TR, Choudhuri S, Klaassen CD. Constitutive mRNA expression of various glutathione *S*-transferase isoforms in different tissues of mice. Toxicol Sci 2007;100:513–524.
55. Kamataki T, Maeda K, Yamazoe Y, *et al*. Sex difference of cytochrome P-450 in the rat: Purification, characterization, and quantitation of constitutive forms of cytochrome P-450 from liver microsomes of male and female rats. Arch Biochem Biophys 1983;225:758–770.
56. Waxman DJ. Rat hepatic cytochrome P-450 isoenzyme 2c. Identification as a male-specific, developmentally induced steroid 16α-hydroxylase and comparison to a female-specific cytochrome P-450 isoenzyme. J Biol Chem 1984;259:15481–15490.
57. Ryan DE, Iida S, Wood AE, *et al*. Characterization of three highly purified cytochromes P-450 from hepatic microsomes of adult male rats. J Biol Chem 1984;259:1239–1250.
58. Waxman DJ, Dannan GA, Guengerich FP. Regulation of rat hepatic cytochrome P-450: Age-dependent expression, hormonal imprinting, and xenobiotic inducibility of sex-specific isoenzymes. Biochemistry 1985;24:4409–4417.
59. Morgan ET, MacGeoch C, Gustafsson JA. Hormonal and developmental regulation of expression of the hepatic microsomal steroid 16α-hydroxylase cytochrome P-450 apoprotein in the rat. J Biol Chem 1985;260:11895–11898.
60. Waxman DJ, LeBlanc GA, Morrissey JJ, *et al*. Adult male-specific and neonatally programmed rat hepatic P-450 forms RLM2 and 2a are not dependent on pulsatile plasma growth hormone for expression. J Biol Chem 1988;263:11396–11406.
61. Thummel KE, Favreau LV, Mole JE, *et al*. Further characterization of RLM2 and comparison with a related form of cytochrome P450, RLM2b. Arch Biochem Biophys 1988;266:319–333.
62. Bandiera S, Ryan DE, Levin W, *et al*. Age- and sex-related expression of cytochrome P450f and P450g in rat liver. Arch Biochem Biophys 1986;248:658–676.
63. Nagata K, Murayama N, Miyata M, *et al*. Isolation and characterization of a new rat P450 (CYP3A18) cDNA encoding $P4506_{\beta\text{-}2}$ catalyzing testosterone 6β- and 16α-hydroxylations. Pharmacogenetics 1996;6:103–111.

64. Mahnke A, Strotkamp D, Roos PH, *et al*. Expression and inducibility of cytochrome P450 3A9 (CYP3A9) and other members of the CYP3A subfamily in rat liver. Arch Biochem Biophys 1997;337:62–68.
65. MacGeoch C, Morgan ET, Gustafsson JA. Hypothalamo-pituitary regulation of cytochrome $P\text{-}45015_{5\beta}$ apoprotein levels in rat liver. Endocrinology 1985;117:2085–2092.
66. McClellan-Green PD, Linko P, Yeowell HN, *et al*. Hormonal regulation of male-specific rat hepatic cytochrome P-450g (P-450IIC13) by androgens and the pituitary. J Biol Chem 1989;264:18960–18965.
67. Sundseth SS, Waxman DJ. Sex-dependent expression and clofibrate inducibility of cytochrome P450 4A fatty acid ω-hydroxylases. Male specificity of liver and kidney CYP4A2 mRNA and tissue-specific regulation by growth hormone and testosterone. J Biol Chem 1992;267:3915–3921.
68. Kawai M, Bandiera SM, Chang TKH, *et al*. Growth hormone regulation and developmental expression of rat hepatic CYP3A18, CYP3A9, and CYP3A2. Biochem Pharmacol 2000;59:1277–1287.
69. Arlotto MP, Parkinson A. Identification of cytochrome P450a (P450IIA1) as the principal testosterone 7α-hydroxylase in rat liver microsomes and its regulation by thyroid hormones. Arch Biochem Biophys 1989;270:458–471.
70. Waxman DJ. Interactions of hepatic cytochromes P-450 with steroid hormones. Regioselectivity and stereospecificity of steroid metabolism and hormonal regulation of rat P-450 enzyme expression. Biochem Pharmacol 1988;37:71–84.
71. Leo MA, Iida S, Lieber CS. Retinoic acid metabolism by a system reconstituted with cytochrome P450. Arch Biochem Biophys 1984;234:305–312.
72. Wang H, Napoli KL, Strobel HW. Cytochrome P450 3A9 catalyzes the metabolism of progesterone and other steroid hormones. Mol Cell Biochem 2000;213:127–135.
73. Hoch U, Zhang Z, Kroetz DL, *et al*. Structural determination of the substrate specificities and regioselectivities of the rat and human fatty acid omega-hydroxylases. Arch Biochem Biophys 2000;373:63–71.
74. Kroetz DL, Huse LM, Thuresson A, *et al*. Developmentally regulated expression of the *CYP4A* genes in the spontaneous hypertensive rat kidney. Mol Pharmacol 1997;52:362–372.
75. Morgan ET, MacGeoch C, Gustafsson JA. Sexual differentiation of cytochrome P-450 in rat liver. Evidence for a constitutive isozyme as the male-specific 16α-hydroxylase. Mol Pharmacol 1985;27:471–479.
76. Inagaki K, Inagaki M, Kataoka T, *et al*. A wide interindividual variability of urinary 6β-hydroxycortisol to free cortisol in 487 healthy Japanese subjects in near basal conditions. Ther Drug Monit 2002;24:722–727.
77. Al Koudsi N, Tyndale RF. Hepatic CYP2B6 is altered by genetic, physiologic, and environmental factors but plays little role in nicotine metabolism. Xenobiotica 2010;40:381–392.
78. Kato R, Kinichi O. Studies on the regulation of the activity of drug oxidation in rat liver microsomes by androgen and estrogen. Biochem Pharmacol 1970;19:1649–1660.
79. Waxman DJ, Holloway MG. Sex differences in the expression of hepatic drug metabolizing enzymes. Mol Pharmacol 2009;76:215–228.
80. Bandiera S, Dworschak C. Effects of testosterone and estrogen on hepatic levels of cytochromes P450 2C7 and P450 2C11 in the rat. Arch Biochem Biophys 1992;296:286–295.
81. Shimada M, Murayama N, Yamazoe Y, *et al*. Further studies on the persistence of neonatal androgen imprinting on sex-specific cytochrome P-450, testosterone and drug oxidations. Jpn J Pharmacol 1987;45:467–478.
82. Robertson GR, Farrell GC, Liddle C. Sexually dimorphic expression of rat CYP3A9 and CYP3A18 genes is regulated by growth hormone. Biochem Biophys Res Commun 1998;242:57–60.

83. Einarsson K, Gustafsson JA, Stenberg A. Neonatal imprinting of liver microsomal hydroxylation and reduction of steroids. J Biol Chem 1973;248:4987–4997.
84. Dannan GA, Guengerich FP, Waxman DJ. Hormonal regulation of rat liver microsomal enzymes. Role of gonadal steroids in programming, maintenance, and suppression of delta^4-steroid 5α-reductase, flavin-containing monooxygenase, and sex-specific cytochromes P-450. J Biol Chem 1986;261:10728–10735.
85. Mode A, Gustafsson J-Å. Sex and the liver - a journey through five decades. Drug Metab Rev 2006;38:197–207.
86. Anakk S, Ku CY, Vore M, *et al*. Insights into gender bias: rat cytochrome P450 3A9. J Pharmacol Exp Ther 2003;305:703–709.
87. Tannenbaum GS, Martin JB. Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. Endocrinology 1976;98:562–570.
88. Weitzman ED. Circadian rhythms and episodic hormone secretion. Annu Rev Med 1976;27:225–243.
89. Eden S, Albertsson-Wikland K, Isaksson O. Plasma levels of growth hormone in female rats of different ages. Acta Endocrinol Bucharest 1978;88:676–690.
90. Tannenbaum GS, Rorstad O, Brazeau P. Effects of prolonged food deprivation on the ultradian growth hormone rhythm and immunoreactive somatostatin tissue levels in the rat. Endocrinology 1979;104:1733–1738.
91. Mode A, Gustafsson JA, Jansson JO, *et al*. Association between plasma level of growth hormone and sex differentiation of hepatic steroid metabolism in the rat. Endocrinology 1982;111:1692–1697.
92. Eden S. Age- and sex-related differences in episodic growth hormone secretion in the rat. Endocrinology 1979;105:555–560.
93. Waxman DJ, O'Connor C. Growth hormone regulation of sex-dependent liver gene expression. Mol Endocrinol 2006;20:2613–2629.
94. Wilson JT. Growth hormone modulation of liver drug metabolic enzyme activity in the rat. I. Effect of the hormone on the content and rate of reduction of microsomal cytochrome P-450. Biochem Pharmacol 1973;22:1717–1728.
95. Waxman DJ, Pampori NA, Ram PA, *et al*. Interpulse interval in circulating growth hormone patterns regulates sexually dimorphic expression of hepatic cytochrome P450. Proc Natl Acad Sci U S A 1991;88:6868–6872.
96. Dhir RN, Shapiro BH. Interpulse growth hormone secretion in the episodic plasma profile causes the sex reversal of cytochromes P450 in senescent male rats. Proc Natl Acad Sci U S A 2003;100:15224–15228.
97. Redmond GP, Bell JJ, Perel JM. Effect of human growth hormone on amobarbital metabolism in children. Clin Pharm Ther 1978;24:213–218.
98. Cheung NW, Liddle C, Coverdale S, *et al*. Growth hormone treatment increases cytochrome P450-mediated antipyrine clearance in man. J Clin Endocrinol Metab 1996;81:1999–2001.
99. Berglund EG, Johannsson G, Beck O, *et al*. Growth hormone replacement therapy induces codeine clearance. Eur J Clin Invest 2002;32:507–512.
100. Liddle C, Goodwin BJ, George J, *et al*. Separate and interactive regulation of cytochrome P450 3A4 by triiodothyronine, dexamethasone, and growth hormone in cultured hepatocytes. J Clin Endocrinol Metab 1998;83:2411–2416.
101. Dhir RN, Dworakowski W, Thangavel C, *et al*. Sexually dimorphic regulation of hepatic isoforms of human cytochrome P450 by growth hormone. J Pharmacol Exp Ther 2006;316:87–94.
102. Jaffe CA, Turgeon DK, Lown K, *et al*. Growth hormone secretion pattern is an independent regulator of growth hormone actions in humans. Am J Physiol Endocrinol Metab 2002;283:E1008–E1015.

103. Sinues B, Mayayo E, Fanlo A, *et al*. Effects of growth hormone deficiency and rhGH replacement therapy on the 6β-hydroxycortisol/free cortisol ratio, a marker of CYP3A activity, in growth hormone-deficient children. Eur J Clin Pharmacol 2004;60:559–564.
104. Cheung C, Yu AM, Chen CS, *et al*. Growth hormone determines sexual dimorphism of hepatic cytochrome P450 3A4 expression in transgenic mice. J Pharmacol Exp Ther 2006;316:1328–1334.
105. Herrington J, Carter-Su C. Signaling pathways activated by the growth hormone receptor. Trends Endocrinol Metab 2001;12:252–257.
106. Lanning NJ, Carter-Su C. Recent advances in growth hormone signaling. Rev Endocr Metab Disord 2006;7:225–235.
107. Levy DE, Darnell JE Jr. STATS: transcriptional control and biological impact. Nat Rev Mol Cell Biol 2002;3:651–662.
108. Wiwi CA, Waxman DJ. Role of hepatocyte nuclear factors in growth hormone-regulated, sexually dimorphic expression of liver cytochromes P450. Growth Factors 2004;22:79–88.
109. Germain P, Staels B, Dacquet C, *et al*. Overview of nomenclature of nuclear receptors. Pharmacol Rev 2006;58:685–704.
110. Holloway MG, Laz EV, Waxman DJ. Codependence of growth hormone-responsive, sexually dimorphic hepatic gene expression on signal transducer and activator of transcription 5b and hepatic nuclear factor 4α. Mol Endocrinol 2006;20:647–660.
111. Laz EV, Holloway MG, Chen CS, *et al*. Characterization of three growth hormone-responsive transcription factors preferentially expressed in adult female liver. Endocrinology 2007;148:3327–3337.
112. Meyer RD, Laz EV, Su T, *et al*. Male-specific hepatic Bcl6: growth hormone-induced block of transcription elongation in females and binding to target genes inversely coordinated with STAT5. Mol Endocrinol 2009;23:1914–1926.
113. Waxman DJ, Ram PA, Park SH, *et al*. Intermittent plasma growth hormone triggers tyrosine phosphorylation and nuclear translocation of a liver-expressed, Stat5-related DNA binding protein. Proposed role as an intracellular regulator of male-specific liver gene transcription. J Biol Chem 1995;270:13262–13270.
114. Choi HK, Waxman DJ. Growth hormone, but not prolactin, maintains low-level activation of STAT5a and STAT5b in female rat liver. Endocrinology 1999;140:5126–5135.
115. Tannenbaum GS, Choi HK, Gurd W, *et al*. Temporal relationship between the sexually dimorphic spontaneous GH secretory profiles and hepatic STAT5 activity. Endocrinology 2001;142:4599–4606.
116. Udy GB, Towers RP, Snell RG, *et al*. Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. Proc Natl Acad Sci U S A 1997;94:7239–7244.
117. Davey HW, Park SH, Grattan DR, *et al*. STAT5b-deficient mice are growth hormone pulse-resistant. Role of STAT5b in sex-specific liver P450 expression. J Biol Chem 1999;274:35331–35336.
118. Holloway MG, Cui Y, Laz EV, *et al*. Loss of sexually dimorphic liver gene expression upon hepatocyte-specific deletion of *Stat5a- Stat5b* locus. Endocrinology 2007;148:1977–1986.
119. Holloway MG, Miles GD, Dombkowski AA, *et al*. Liver-specific hepatocyte nuclear factor-4α deficiency: greater impact on gene expression in male than in female mouse liver. Mol Endocrinol 2008;22:1274–1286.
120. Sladek FM, Zhong WM, Lai E, *et al*. Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. Gene Dev 1990;4:2353–2365.
121. Giguere V. Orphan nuclear receptors: from gene to function. Endocr Rev 1999;20:689–725.
122. Gonzalez FJ. Regulation of hepatocyte nuclear factor 4α-mediated transcription. Drug Metab Pharmacokinet 2008;23:2–7.

123. Jover R, Moya M, Gomez-Lechon MJ. Transcriptional regulation of cytochrome P-450 genes by the nuclear receptor hepatocyte nuclear factor 4-alpha. Curr Drug Metab 2009;10:508–519.
124. Wiwi CA, Gupte M, Waxman DJ. Sexually dimorphic P450 gene expression in liver-specific hepatocyte nuclear factor 4α-deficient mice. Mol Endocrinol 2004;18:1975–1987.
125. Choi HK, Waxman DJ. Plasma growth hormone pulse activation of hepatic JAK-STAT5 signaling: developmental regulation and role in male-specific liver gene expression. Endocrinology 2000;141:3245–3255.
126. Verma AS, Dhir RN, Shapiro BH. Inadequacy of the Janus kinase 2/signal transducer and activator of transcription signal transduction pathway to mediate episodic growth hormone-dependent regulation of hepatic CYP2C11. Mol Pharmacol 2005;67:891–901.
127. Wauthier V, Waxman DJ. Sex-specific early growth hormone response genes in rat liver. Mol Endocrinol 2008;22:1962–1974.
128. Wauthier V, Sugathan A, Meyer RD, *et al*. Intrinsic sex differences in the early growth hormone responsiveness of sex-specific genes in mouse liver. Mol Endocrinol 2010;24:667–678.