

Sex Differences of Drug-metabolizing Enzyme: Female Predominant Expression of Human and Mouse Cytochrome P450 3A Isoforms

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Sex differences have been found in the pharmacokinetics of many drugs, and sex differences of drug-metabolizing enzymes have been considered one of the major factors of this issue. Cytochrome P450, a Phase I drug-metabolizing enzyme, consists of many isoforms having divergent substrate specificities. Some isoforms in rodents show sex-specific expression, and some in humans also show moderate differences, *e.g.*, the activity of CYP2E1 and CYP1A2 is slightly higher in men than women. CYP3A4, the most clinically relevant isoform in humans, appears to have greater expression in women, as determined by mRNA and protein levels as well as the activities for more than ten clinically employed drugs, and the difference is increased by induction of the gene expression during pregnancy, implying the need to adjust the dose of a variety of drugs ingested by pregnant women. Regarding the mechanism of the sexually dimorphic expression of *CYP3A* genes, at least two hypotheses have been suggested. The first is that pregnane X receptor (PXR), activated by a higher concentration of female sex hormones, enhances the expression of PXR-target genes, including *CYP3A*. The second is that the different secretion patterns of growth hormone between men and women activate divergent sets of the signal transduction cascade discriminately, resulting in the sexually dimorphic expression of subsets of genes. In this review, we mainly introduce studies of female-specific *CYP3A* genes due to the recent progress of analysis.

Key words — sex difference, drug-metabolizing enzymes, cytochrome P450 3A4, growth hormone, female sex hormone, pregnane X receptor

INTRODUCTION

Sex differences in the pharmacological response have been analyzed from both pharmacodynamic and pharmacokinetic aspects; however, sex-related pharmacodynamic data are limited. For example, evidence suggests that women are more prone to developing *torsade de pointes* from proarrhythmic drugs, such as quinidine or d-sotalol. There is no doubt that chlorpromazine, fluspirilene and various antipsychotics appear more effective in women than men at the same dosage and plasma concentration. While the detailed mechanisms are un-

known, it is inferred that these differences might, at least in part, be the consequence of modulation by sex hormones.^{1,2)} In contrast, sex differences in drug pharmacokinetics have been recognized many times. Some factors have been revealed to account for sex-related pharmacokinetic differences, *i.e.*, lower body weight and organ size, a higher percentage of body fat, a lower glomerular filtration rate in women than men, *etc.* Of other factors, sex differences of drug-metabolizing enzymes and/or transporter proteins have been considered important factors. Drug metabolism can be demonstrated by Phase I and Phase II reactions. Phase I reactions are mediated by oxidation, reduction and hydrolysis. Phase II reactions involve glucuronidation, methylation, sulfation and acetylation reactions of the parent drug or Phase I metabolites and produce polar conjugates to aid excretion. The cytochrome

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P450 (P450 or CYP) family is the primary system for Phase I hepatic and intestinal drug metabolism, and consists of many isoforms having divergent substrate specificities. For two decades, it has been well known that many isoforms in rodents show sex differences in their expression, and subsets of isoforms show sex-specific expressions.³⁾ In addition, recent studies have suggested that CYP2E1 and CYP1A2 activity is slightly higher in men than women, while CYP2B6, CYP2A6 and CYP3A, the most clinically relevant P450 isoform, appears to have greater activity in women.^{1,2,4-7)} Thus, this review first focuses on sex differences of CYP3A4 due to its high clinical relevance. There has been speculation that sex hormones may be responsible for sex differences in drug metabolism and an increase of CYP3A4 activity has been observed during pregnancy, which is also described. The mechanisms of female-dominant expression of the *CYP3A4* gene have not been clarified; however, some promising observations of human the *CYP3A4* gene as well as murine female-specific *Cyp3a* genes, namely, *Cyp3a41* and *Cyp3a44*, have been reported. Finally, we discuss two possible mechanisms of the sex-dimorphic expression of *CYP3A* genes.

SEX DIFFERENCE OF CYP3A4

Of the P450 enzymes, the CYP3A subfamily is the most abundantly expressed, comprising approximately 30% of hepatic P450⁸⁾ and 70% of intestinal P450,⁹⁾ and is involved in the metabolism of approximately 50% of drugs currently on the market.¹⁰⁾ This is of particular concern in the development of new drugs, because many are used in combination therapies and coadministration with other drugs can lead to drug-drug interactions. Therefore, if there is large sex difference in CYP3A activity, it may modulate the pharmacokinetics of the substrate drugs, and may influence the efficiency and safety of the drugs. Additionally, CYP3A isoforms are involved in the metabolism of other compounds, such as steroid hormones, toxins and procarcinogens;^{8,11,12)} for example, human CYP3A activates polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene (B[*a*]P) and other procarcinogens present in tobacco smoke. Hepatocarcinogenic mycotoxins, aflatoxin B₁, aflatoxin G₁ and sterigmatocystin, are also targets of metabolic activation by CYP3A enzymes. It has therefore been postulated that the expression of CYP3A could af-

fect the predisposition of an individual to cancers caused by environmental procarcinogens bioactivated by CYP3A enzymes.¹³⁻¹⁵⁾ Four CYP3A isoforms have been identified in humans: CYP3A4, CYP3A5, CYP3A7 and CYP3A43 (Table 1).¹⁶⁾ Of these, CYP3A4 has the greatest importance, as it exhibits the highest expression, *i.e.*, 95% of total CYP3A mRNA pools in the liver¹⁷⁾ and broad substrate specificity.^{8,11,12)} Regarding substrate specificity, CYP3A4 and CYP3A5 show extensive overlap.¹⁸⁾ CYP3A7 exhibits similar, albeit usually lower, activities towards many CYP3A4 substrates. CYP3A43 is a recently identified isoform, and its substrate specificity is not well characterized.¹⁹⁾ Therefore, it is difficult to determine the relative contribution of each isoform to total enzyme activities, which were examined in human microsomes or calculated by *in vivo* studies. Thus, the enzyme activity described as "CYP3A"-mediated metabolism in this review means total activity of the respective enzymes which were involved in the metabolism of the drugs investigated, unless otherwise noted.

In vitro studies conducted on human liver microsomes prepared from men and women suggest that CYP3A content and activity is higher in women. For example, Wolbold and coworkers examined 39 surgical liver samples which had not been exposed to chemicals which can alter the expression of CYP3A (CYP3A modulators), and found 2-fold higher CYP3A4 levels of protein and mRNA in female compared with male samples and a corresponding 50% increase in the CYP3A-dependent *N*-dealkylation of verapamil (Fig. 1, model 1).²⁰⁾ A similar tendency observed in *in vitro* studies using human liver microsomes has been reported by other groups,²¹⁻²⁴⁾ while studies that failed to detect female dominance have also been reported.²⁵⁻²⁸⁾

Assessment of CYP3A activity *in vivo* has been carried out by measuring the clearance of various CYP3A substrates. Although there are some conflicting results, the majority of studies suggest that women exhibit 20-50% faster clearance of CYP3A-metabolized drugs. Those drugs include triazolam, diazepam, midazolam, methylprednisolone, nifedipine, diltiazem, verapamil, ifosfamide, cyclosporine, erythromycin, tirilazad, quinine and alprazolam;^{1,4,29)} however, the observed sex differences of certain substrates are dependent on the administration route. For example, clearance of intravenously administered verapamil is greater in

Table 1. Human and Mouse *CYP3A* Genes ^{8,9,16,19,51–53)}

| Species | Gene | Expression in liver | Expression in extrahepatic organs |
|---------|----------------|--|--|
| Human | <i>CYP3A4</i> | High (dominant isoform in adult) | dominant isoform in intestine, brain; minor isoform in lung |
| | <i>CYP3A5</i> | Medium (polymorphic in adult) | dominant isoform in stomach, oesophagus, kidney, lung, prostate; minor isoform in intestine, brain |
| | <i>CYP3A7</i> | Medium (dominant isoform in fetus; polymorphic in adult) | intestine, kidney |
| | <i>CYP3A43</i> | Low | dominant isoform in brain; minor isoform in kidney, lung, heart |
| Mouse | <i>Cyp3a11</i> | High (both sexes; dominant isoform in adult male) | kidney, intestine, stomach, lung, brain, testis, ovary |
| | <i>Cyp3a13</i> | Low (both sexes) | dominant isoform in intestine, stomach, lung, brain |
| | <i>Cyp3a16</i> | Low (fetus and adult female) | |
| | <i>Cyp3a25</i> | Medium (both sexes) | colon, intestine, lung, kidney, brain |
| | <i>Cyp3a41</i> | High (female specific; dominant isoform in adult female) | intestine, stomach, kidney, lung, brain, ovary, testis |
| | <i>Cyp3a44</i> | Medium (female specific in adult) | intestine, stomach, ovary |
| | <i>Cyp3a57</i> | — | — |
| | <i>Cyp3a59</i> | — | — |

High, Medium and Low indicate relative expression level within respective species. —: no information is available.

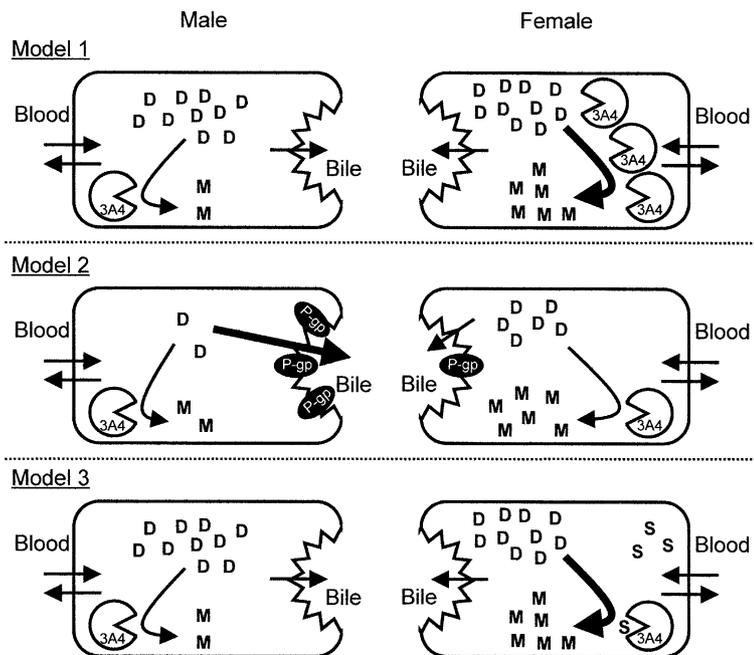


Fig. 1. Schematic Diagram of Three Proposed Models Causing Sex Difference of Clearance of CYP3A Substrates *In Vivo*^{20–24,32,34–38)}
 Model 1: higher expression of CYP3A4 enzyme in women causes the sex difference. Model 2: Drug in the blood enters the hepatocyte from the basolateral membrane. The lower expression of P-gp in women compared with men could result in higher intracellular drug levels and consequently higher metabolite levels, even though there is no sex-related difference in the CYP3A4 enzyme expression. Model 3: higher level of intracellular female steroid hormone enhance CYP3A4 activity by binding one or more of the multiple domains of the CYP3A active site. Abbreviations: D, drug; M, metabolite; S, female steroid hormone.

women than in men. In contrast, the clearance of orally administered verapamil is smaller in women than in men, suggesting that intestinal processes

are a factor in the sex differences of drug clearance.^{30,31)} One possible explanation for this contradiction is higher CYP3A activity in the intestinal

mucosa, but a recent study by Paine and coworkers found no significant difference between the sexes.³²⁾

In terms of substrates, inducers and tissue specificity, P-glycoprotein (P-gp) has extensive overlap with CYP3A.³³⁾ This overlap facilitates P-gp/CYP3A interplay, implying clinical relevance. To explain conflicting *in vivo* studies, one plausible mechanism has been proposed that sex differences of the pharmacokinetics of CYP3A substrates may actually be caused by P-gp.^{32,34)} Namely, if the P-gp level in the hepatocytes of women is lower than in men, then it causes higher intracellular hepatic levels of drugs, thereby exposing the drug to greater metabolism and leading to higher clearance (Fig. 1, model 2). Supporting this possibility, Schuetz and coworkers demonstrated that liver samples from men exhibited 2.4-fold higher levels of P-gp than from women.³⁵⁾

There are studies showing that certain steroids may inhibit and/or activate CYP3A function,^{36–38)} possibly by binding one or more of the multiple domains in the CYP3A active site, therefore, modulation of CYP3A activity by steroid hormone is another possible mechanism for the sex differences of CYP3A activity *in vivo* (Fig. 1, model 3).

FLUCTUATION OF CYP3A ACTIVITY DURING PREGNANCY

Pregnancy could alter the pharmacokinetic profile of a drug due to the changes of many physiological factors, *e.g.*, plasma protein-binding, plasma volume, body water, cardiac output, hepatic blood flow, and glomerular filtration.³⁹⁾ Another factor is the alteration (induction or suppression) of drug-metabolizing enzymes during pregnancy. In other words, pregnancy extends or alters the impact of sex differences of drug metabolism. Tracy and coworkers analyzed CYP3A activity in 25 pregnant women by measuring *N*-demethylation of dextromethorphan.⁴⁰⁾ In that study, all subjects were examined in 4 periods, *i.e.*, 14 to 18 weeks of gestation, 24 to 28 weeks and 36 to 40 weeks and again at 6 to 8 weeks after delivery, and the group found that CYP3A activity was consistently and significantly increased (35–38%) during all stages of pregnancy. This was in contrast to CYP1A2, another clinically important P450 isoform, in which activity was significantly reduced in all periods of pregnancy as compared the postpartum period during the first (–32.8%), sec-

ond (–48.1%), and third periods (–65.2%), respectively. Because CYP1A2 activity in women is lower than in men, pregnancy also enhances the impact of these differences. Similar observations in *in vivo* studies have been reported for many CYP3A substrates, including nifedipine, carbamazepine, midazolam, indinavir, lopinavir, ritonavir and an endogenous substrate, cortisol.^{39,41)} Regarding the mechanism of these alterations during pregnancy, the induction of CYP3A isoforms at the level of both protein and mRNA has been demonstrated in rodents, *i.e.*, female-specific *Cyp3a41*, *Cyp3a44* and *Cyp3a16* genes in mice⁴²⁾ and *CYP3A9* gene in rats.⁴³⁾ Using human *CYP3A4*-promoter-luciferase transgenic mice, Zhang and coworkers indicated that the pregnancy-related factors that transcriptionally activated mouse *Cyp3a* genes can activate human *CYP3A4* gene promoter during pregnancy.⁴²⁾ This study strongly supports the possibility of *CYP3A4* induction during pregnancy in the human liver. Thus, such an increase has significant ramifications for designing appropriate dosing regimens for pregnant women receiving narrow-therapeutic-window drugs cleared extensively by CYP3A4.

According to these studies conducted in rodents, the major mechanism for increased CYP3A4 activity during pregnancy is suggested to be the induction of gene expression; however, other attractive evidence has been observed. Zhang and coworkers found a positive correlation between progesterone serum concentration and activity.⁴⁴⁾ Taken together with the reports that CYP3A4 exhibits positive cooperative characteristics with substrates such as carbamazepine and 17 β -estradiol in the presence of progesterone in an *in vitro* incubation system,^{36–38)} they proposed the hypothesis that the increased activity observed in pregnant women is due to the enhancement of CYP3A4 activity by exposure to higher levels of progesterone. To date, it is unclear whether changes in the pharmacokinetics of CYP3A substrates during pregnancy are due to the induction of *CYP3A* genes, cooperative enhancement by steroids, or both, and further studies are required.

It has been demonstrated that human immunodeficiency virus (HIV)-infected women have reduced exposure [area under the curve (AUC)] to anti-HIV protease inhibitors, *e.g.*, nelfinavir, during pregnancy. Although the mechanism has not been completely elucidated to date, the induction of intestinal and/or hepatic activity of CYP3A dur-

ing pregnancy is seen as the major cause of this issue.^{45,46)} In clinical use it was observed that ritonavir is a potent, irreversible inhibitor of CYP3A and that the use of subtherapeutic doses of ritonavir can lead to a substantial increase in circulating concentrations of other drugs subject to metabolism by CYP3A *in vivo*. Thus, ritonavir-mediated “boosting” of protease inhibitor-containing regimens has become a standard of care for nonpregnant adults.⁴⁷⁾ Kosel and coworkers found that the induction of CYP3A4 during pregnancy was offset by this regimen, implying the usefulness of this prescription for pregnant women.⁴⁸⁾

MECHANISMS FOR SEX DIFFERENCES OF CYP3A GENE EXPRESSION

Female Sex Hormone-mediated Mechanism

In this review, we present two possible mechanisms for the female-dominant expression of CYP3A genes. The first is the mechanism that pregnant X receptor (PXR) activated by female sex hormones is a key determinant of female-dominant expression. A characteristic of a subset of the CYP superfamily able to metabolize xenobiotic compounds is their highly elevated expression in the presence of their own substrates or other inducer compounds. The human CYP3A4 gene is inducible by some of its substrates through the activation of PXR.⁴⁹⁾ PXR, a member of the nuclear receptor superfamily of transcription factors (NR1I2), is activated by various CYP3A-inducing drugs, such as rifampicin, dexamethasone, clotrimazole troglitazone, spironolactone, RU486 and pregnenolone 16 α -carbonitril (PCN). In addition, endogenous compounds including pregnane derivatives, progesterone and some of its hydroxylated metabolites, cortisol, cortisone, 17 β -estradiol, dihydrotestosterone, dehydroepiandrosterone, and other steroids have been shown to affect PXR to various extents, raising speculation about the putative physiological role of this receptor.⁴⁹⁾ It is therefore expected that CYP3A4 activity is higher in women than in men because of their high female sex hormone level. One *in vitro* result supporting this hypothesis is that the reporter construct containing three copies of the PXR responsive element of the CYP3A4 gene was PXR-dependently activated by 17 β -estradiol.⁵⁰⁾

Mice have eight complete *Cyp3a* genes,

namely *Cyp3a11*, *Cyp3a13*, *Cyp3a16*, *Cyp3a25*,⁵¹⁾ *Cyp3a41*,⁵²⁾ *Cyp3a44*,⁵³⁾ *Cyp3a57* and *Cyp3a59*, illustrating the complexity of CYP gene families between species (Table 1). Of these eight genes, at least two genes, namely *Cyp3a41* and *Cyp3a44*, show female specificity in adult livers, and similar expression profiles to human CYP3A4, *i.e.*, tissue specificity and postnatal developmental change. Ovariectomy reduced the mRNA expression of both genes and replacement with 17 β -estradiol recovered it, indicating that both genes are regulated under the control of female sex hormones.⁵³⁾ In a study conducted in pregnant mice and cultured cells, Masuyama and coworkers demonstrated that progesterone concentration increased as a function of gestation, and that progesterone significantly stimulated PXR-mediated transcription at a relatively high concentration, comparable with that at term pregnancy.⁵⁴⁾ The expressions of both PXR and CYP3A mRNA in the liver increased towards term about fifty-fold and twenty-fold, respectively, compared with in non-pregnancy, and decreased postpartum.

However, some findings are inconsistent with this hypothesis. The concentrations of 17 β -estradiol required to activate PXR are superphysiological.⁵⁰⁾ Additionally, sexually dimorphic expression of the *Cyp3a44* gene did not change in PXR- or constitutive androstane receptor (CAR)-deficient mice.⁵⁵⁾ CAR is evolutionally the closest member to PXR in xenobiotic-sensing nuclear receptors. Between PXR and CAR, considerable redundancy exists with regard to the overlapping ligand and activator spectrum and the binding of both receptors to DNA-response elements of each other with overlapping affinity.⁴⁹⁾ PXR and CAR might thus compensate for the loss or malfunction of each other to a certain degree, which might explain the lack of an obvious phenotype in PXR- and CAR-knockout animals; however, while the mouse *Cyp2b10* gene, a representative CAR target, can be induced by 17 β -estradiol via the activation of CAR,^{56,57)} the concentrations of 17 β -estradiol required to activate mouse CAR are also superphysiological. In addition, progesterone suppresses mouse CAR.⁵⁶⁾ Human CAR did not respond well to either 17 β -estradiol or progesterone;⁵⁶⁾ therefore, the hypothesis that higher activation of PXR or CAR by high levels of estrogen in the female liver leads to female-predominant expression of CYP3A genes is still nebulous, although the contribution of progesterone-activated PXR to a higher expression

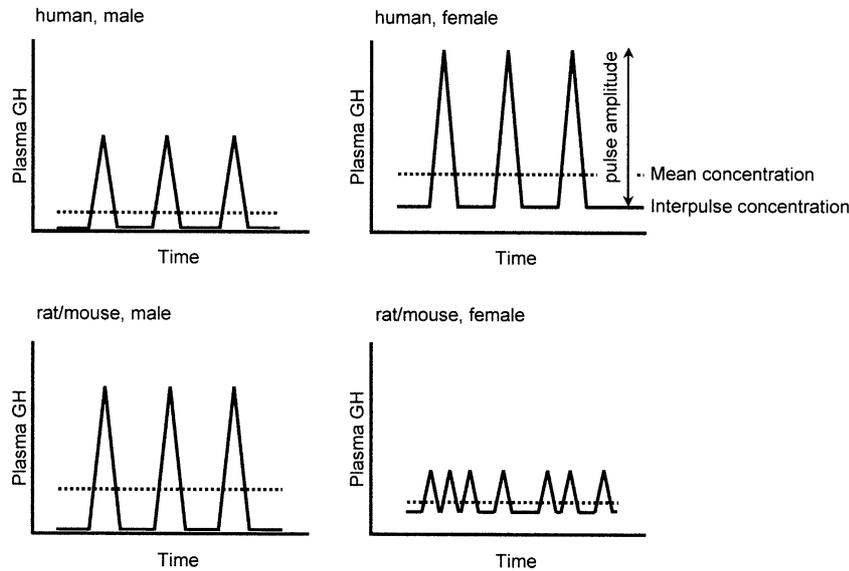


Fig. 2. Temporal Profile of Circulating GH Concentrations^{58–62)}

In humans, mean plasma GH concentrations, interpulse plasma GH concentrations and GH pulse amplitude are greater in women than in men. Plasma GH pulse frequency does not differ between sexes in humans. In mice and rats, mean plasma GH concentrations and GH pulse amplitude in blood are greater in males than in females, whereas interpulse plasma GH concentrations are opposite. Plasma GH pulses are less frequent in males than in females in rodents. Pulsatile GH secretion is less regular in female rats than in males.

at term is possible.

Growth Hormone (GH)-mediated Expression of Human and Mouse CYP3A Genes

The second mechanism is that sex-dependent GH secretion is the key determinant for the sexually dimorphic expression of *CYP* genes. Whereas males and females secrete the same daily amount of GH, the secretory pattern in rats is sexually dimorphic, characterized as “continuous” for females and “episodic” for males (Fig. 2).⁵⁸⁾ In adult male rats, GH is secreted in a highly pulsatile manner, with peak plasma GH levels of approximately 200 ng/ml observed about every 3.5 hr, followed by GH-free intervals typically lasting about 2 hr. In contrast, GH release in adult female rats is characterized by a more frequent and near-continuous presence in plasma at an average level of approximately 30–60 ng/ml. A key difference between male and female GH profiles in both rats and mice is the sustained interpulse interval of little or no detectable circulating GH that is characteristic of adult males.⁵⁹⁾ Essentially the same differences are observed in both mice and humans,^{60–62)} and the difference has been noted to affect *CYP3A* activity and expression in these species. In fact, our observations indicate that the sex difference of GH secretion is a determinant of the female-specific expression of both *Cyp3a41* and *Cyp3a44*

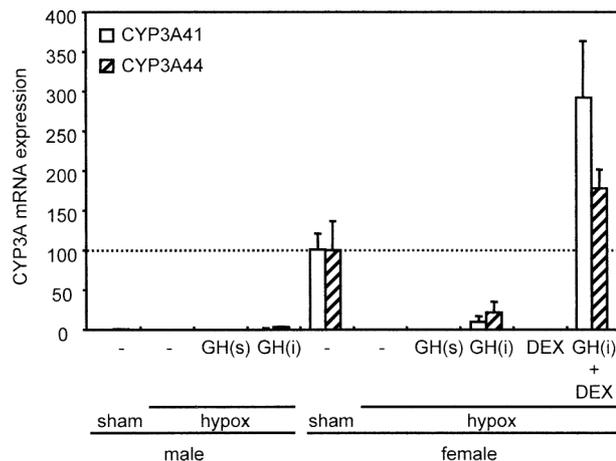


Fig. 3. Effects of GH Replacements and Combined Treatment with GH and DEX on the Expression of CYP3A41 and CYP3A44 mRNAs in the Livers of C57BL/6 Mice

Amount of each mRNA was determined by semi-quantitative RT-PCR. Each CYP3A mRNA level was normalized to those in untreated sham-operated (Sham/-) female mice (= 100). Each column represents the mean \pm S.D. ($n = 3-5$). Abbreviations: —, nontreated; sham, sham-operated; hypox, hypophysectomized; GH(s), twice-daily subcutaneous injection of recombinant human GH; GH(i), continuous infusion of human GH using an osmotic minipump; DEX, subcutaneous injection of dexamethasone.

genes in mouse livers (Fig. 3). Hypophysectomy for adult⁵³⁾ or neonatal monosodium L-glutamate (MSG) treatment,⁶³⁾ which results in rather selective GH deficiency through irreversible hypothala-

mic lesions, completely abolished the expressions of both *Cyp3a41* and *Cyp3a44* genes in the livers of adult female mice. Although GH administration with twice daily subcutaneous injection, which mimics pulsatile masculine secretion, did not show any effect, continuous delivery using an infusion mini-pump, which mimics female-type secretion, caused the slight but significant expression of both genes. These findings suggest that female-specific expression of mouse *Cyp3a41* and *Cyp3a44* genes is driven by the inductive effect of continuous GH. In addition, concomitant treatment with a synthetic glucocorticoid hormone, dexamethasone, and continuous delivery of GH recovered the expression levels of both genes over the level seen in intact female mice. Taken together with the finding that low expression levels of both genes persisted after adrenalectomy, these findings suggest that female-specific expression of both murine *Cyp3a* genes is dictated by cooperative control with GH and glucocorticoid hormone.^{64, 65)} The same responses of both genes to the respective GH exposure patterns were also confirmed in mouse primary hepatocytes in culture.⁶⁵⁾ *In vivo* observations suggesting GH-dependent expression of human CYP3A4 have been reported. In GH-deficient adults, it was demonstrated that the type of GH pulse is an important factor of GH action on the *CYP3A4* gene, with an increase in CYP3A activity when GH is administered continuously (mimicking the female profile) and a tendency to decrease when pulses mimic the GH male profile.⁶⁶⁾ A significant decrease after exposure with one daily subcutaneous administration was observed in GH-deficient boys.⁶⁷⁾ Higher CYP3A activity was found in acromegaly patients,⁶⁸⁾ and is explained as the result of a higher GH level and lack of physiological low nadir GH concentrations in acromegaly.⁶⁹⁾ The GH secretory pattern dependency of *CYP3A4* gene expression has also been confirmed in *in vitro* experiments. As seen in mouse *Cyp3a* genes, human CYP3A4 expression in primary culture showed GH exposure-pattern dependency.^{70, 71)} Additionally, GH secretory pattern-dependent regulation of the *CYP3A4* gene was investigated in a line of transgenic mice.⁷²⁾ Transgenic mice generated from a BAC clone with both *CYP3A4* and *CYP3A7* genes revealed constitutive CYP3A4 expression in the livers of both sexes of immature mice and adult female mice, indicating that the transgene is developmentally regulated in a sex-specific manner. This alteration of CYP3A4 mRNA expression during de-

velopment is parallel with that of the endogenous GH-dependent *Cyp3a44* gene in transgenic mice and intact mice.⁵³⁾ Furthermore, continuous infusion of GH by implanting GH-releasing pellets into male transgenic mice, which was shown to convert the male GH secretory pattern to the female pattern, increased the expressions of both *CYP3A4* and *Cyp3a44* genes in the liver. These observations indicate that the human *CYP3A4* gene contains all of the gene regulatory sequences required for it to respond to endogenous hormonal regulators of developmental expression and sexual dimorphism, in particular GH, and imply that the female-dependent expression of *CYP3A* genes in the livers of both mice and humans shares a fundamentally common mechanism. The absence of CYP3A4 protein in transgenic adult male mice is a contrast to the stable expression of CYP3A4 in humans, suggesting potential influences of the hormonal environment, inducers, or other regulatory mechanisms in human subjects that are absent in the mouse model.

Molecular Mechanisms Mediating GH-dependent Female-dominant Expression of CYP3A Genes

Recent studies identified the signal transducer and activator of transcription 5b (STAT5b) as a factor which can be activated preferentially by masculine pulsatile GH secretion, and demonstrated several lines of evidence that the factor might be directly or indirectly involved in the suppression of a subset of female-specific genes as well as the activation of a subset of male-specific genes.^{73–76)} However, the possibility that STAT5b participates in the expression of *CYP3A* genes as a suppressive factor in males, resulting in female-specific expression in the liver, is negligible and was deduced from the following observations. The expressions of both mouse *Cyp3a41* and *Cyp3a44* genes have not appeared in the liver of either hypophysectomized or neonatally MSG-treated male mice.^{53, 63, 65)} STAT5b-deficient mouse lines did not show any alteration of the expression of either gene in comparison with wild-type control mice.⁷⁴⁾ It would therefore be of interest to determine the key factor, which can be activated by near-continuous feminine-type GH secretion, resulting in the induction of *CYP3A* genes in female livers.

GH actions are mediated at the cellular level by recruiting and/or activating a variety of signaling molecules, including mitogen-activated protein kinase (MAPK) [*i.e.*, p42/p44 MAPK, ex-

tracellular signal-regulated kinase 1 (ERK1), and ERK2], insulin receptor substrates, phosphatidylinositol 3'-phosphate kinase, hepatocyte nuclear factor (HNF) proteins, diacylglycerol, protein kinase C, intracellular calcium and Stat proteins.^{73,77}) Of these molecules, HNF4 α might be a candidate for a key determinant of female-specific expression of *CYP3A* genes from the following observations. HNF4 α is shown to be associated with basal *CYP3A4* expression in the liver.⁷⁸⁻⁸¹) In a study of the *Cyp3a41* gene using a series of reporter constructs, the region responsible for conferring constitutive expression of the gene was determined, and a putative HNF4 α binding site was identified in that region. Binding of HNF4 α to that site was confirmed by Chromatin Immunoprecipitation (ChIP) assay and electrophoretic mobility shift assay, and the amount of HNF4 α bound was higher (*ca.*, 5-fold) in female than in male hepatocytes (our unpublished data). Moreover, the expression of *Cyp3a41* or *Cyp3a44* genes was completely abolished by liver-specific deletion of the HNF4 α gene, suggesting that the nuclear factor contributes, at least in part, to female-specific expression of both genes.⁸²)

CYP2C12 is female-specific, expressing most abundant P450 in the liver of female rats, and is regulated by continuous GH exposure.^{83,84}) HNF4 α is predominantly expressed in female rat liver and has been implicated in continuous GH regulation of *CYP2C12* gene expression.^{85,86}) Thus, studies of this gene provided various suggestions. With respect to the signaling cascade activated by feminine continuous GH secretion, Thangavel and Shapiro recently proposed elements in that pathway,⁸⁷) growth hormone receptor (GHR), extracellular signal-regulated kinase (Erk), the cAMP-response element-binding protein (CBP), and hepatocyte nuclear factors 4 α and 6, in their likely order of activation. In other words, continuous GH induces *CYP2C12* expression by activating Erk1 and Erk2 via GHR, which in turn activates nuclear CBP, which acetylates HNF4 α and HNF6, which then bind to the *CYP2C12* promoter, contributing to the gene's transcription. There is an intrinsic, irreversible sexually dimorphic response to continuous GH exposure; female hepatocytes were considerably (4- to 5-fold) more responsive to continuous GH induction of *CYP2C12* than male-derived hepatocytes. Recruitment and/or activation levels of all component factors in the pathway were highly suppressed in male hepatocytes, possibly explaining this difference.⁸⁷) Consistent with this finding, the

induction of *Cyp3a41* and *Cyp3a44* gene expression after exposure to continuous GH is prominent in female-derived hepatocytes and not observed in male-derived cells.⁶⁵) In contrast, although female-derived hepatocytes will respond to male episodic GH, the response is inherently limited, *i.e.*, responses are more prominent in male-derived than female-derived hepatocytes.⁸⁸) These findings may have some clinical relevance. GH replacement therapy has clearly demonstrated an intrinsic, irreversible, sexually dimorphic response in which the effectiveness of the same GH treatment differs in men and women.⁸⁸) One possible reason for these difference is that daily injections, the most feasible and efficacious therapeutic approach, may evoke a male-like episodic GH profile, leading to a higher response in men.

Finally, we introduce one intriguing mechanism of female-specific *CYP* gene expression proposed by Endo and coworkers.⁸⁶) This group proposed that the mechanism that sex differences in the chromatin structure of the *CYP2C12* gene in rat hepatocytes may contribute to the sex specificity of *CYP* expression by controlling access of the DNA to liver-specific and GH-regulated transcriptional factors. This hypothesis was led by the observation that transcription of the *CYP2C12* reporter constructs has been observed not only in female but also in male livers in an *in vivo* reporter gene assay. The same phenomenon was also observed in the study of the *Cyp3a41* gene, implying the participation of a mechanism for female-dominant *CYP3A* gene expression (our unpublished data).

While the female circulating GH profile is the sole endogenous regulator of hepatic *CYP2C12* expression in the rat, higher expression of *Cyp3a41* and *Cyp3a44* comparable to those in intact female mice require synergistic interaction with glucocorticoid hormones, indicating detailed differences in the regulation mechanism between rat *CYP2C12* and mouse *Cyp3a* genes.

CONCLUSIONS

Sex differences in drug-metabolizing enzyme activity in humans are not so clear as seen in rodents; however, despite such a small difference, it might have significant ramifications for designing appropriate dosing regimens for women, especially pregnant women, receiving narrow-therapeutic-window drugs cleared extensively by *CYP3A4*. Be-

cause of the possible mechanisms described above, alteration of the hormonal environment caused by hormone replacement therapy may significantly alter the pharmacokinetics and pharmacodynamics of drugs metabolized by CYP3A4, leading to a change in drug efficiency and possibly adverse effects from interactions between certain hormones and CYP3A4-metabolizing drugs. Further insight will be provided by studies of the hormonal regulation of human *CYP3A4* as well as rodent *CYP3A* genes.

REFERENCES

- 1) Harris, R. Z., Benet, L. Z. and Schwartz, J. B. (1995) Gender effects in pharmacokinetics and pharmacodynamics. *Drugs*, **50**, 222–239.
- 2) Anderson, G. D. (2005a) Sex and racial differences in pharmacological response: where is the evidence? pharmacogenetics, pharmacokinetics, and pharmacodynamics. *J. Women's Health*, **14**, 19–29.
- 3) Kato, R. and Yamazoe, Y. (1993) Hormonal regulation of cytochrome P450 in rat liver. In *Handbook of Experimental Pharmacology, volume 105, Cytochrome P450* (Schenkman, J. B. and Greim, H., Eds.), Springer-Verlag, Berlin, pp. 447–459.
- 4) Cotreau, M. M., von Moltke, L. L. and Greenblatt, D. J. (2005) The influence of age and sex on the clearance of cytochrome P450 3A substrates. *Clin. Pharmacokinet.*, **44**, 33–60.
- 5) Scandlyn, M. J., Stuart, E. C. and Rosengren, R. J. (2008) Sex-specific differences in CYP450 isoforms in humans. *Expert opinion on drug Metabolism & toxicology*, **4**, 413–424.
- 6) Lamba, V., Lamba, J., Yasuda, K., Strom, S., Davila, J., Hancock, M. L., Fackenthal, J. D., Rogan, P. K., Ring, B., Wrighton, S. A. and Schuetz, E. G. (2003) Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. *J. Pharmacol. Exp. Ther.*, **307**, 906–922.
- 7) Nakajima, M., Fukami, T., Yamanaka, H., Higashi, E., Sakai, H., Yoshida, R., Kwon, J. T., McLeod, H. L. and Yokoi, T. (2006) Comprehensive evaluation of variability in nicotine metabolism and CYP2A6 polymorphic alleles in four ethnic populations. *Clin. Pharmacol Ther.*, **80**, 282–297.
- 8) Guengerich, F. P. (1999) Cytochrome P-450 3A4: regulation and role in drug metabolism. *Annu. Rev. Pharmacol. Toxicol.*, **39**, 1–17.
- 9) Watkins, P. B., Wrighton, S. A., Schuetz, E. G., Molowa, D.T. and Guzelian, P. S. (1987) Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats and man. *J. Clin. Invest.*, **80**, 1029–1036.
- 10) Williams, J. A., Hyland, R., Jones, B. C., Smith, D. A., Hurst, S., Goosen, T. C., Peterkin, V., Koup, J. R. and Ball, S. E. (2004) Drug-drug interactions for UDP-glucuronosyltransferase substrates: A pharmacokinetic explanation for typically observed low exposure AUCi/AUC ratios. *Drug Metab. Dispos.*, **32**, 1201–1208.
- 11) Guengerich, F. P., Shimada, T., Yun, C. H., Yamazaki, H., Raney, K. D., Thier, R., Coles, B. and Harris, T. M. (1994) Interactions of ingested food, beverage, and tobacco components involving human cytochrome P4501A2, 2A6, 2E1, and 3A4 enzymes. *Environ. Health Perspect.*, **102 Suppl 9**, 49–53.
- 12) Li, A. P., Kaminski, D. L. and Rasmussen, A. (1995) Substrates of human hepatic cytochrome P450 3A4. *Toxicology*, **104**, 1–8.
- 13) Kirby, G. M., Wolf, C. R., Neal, G. E., Judah, D. J., Henderson, C. J., Srivatanakul, P. and Wild, C. P. (1993) *In vitro* metabolism of aflatoxin B1 by normal and tumorous liver tissue from Thailand. *Carcinogenesis*, **14**, 2613–2620.
- 14) Dally, H., Edler, L., Jäger, B., Schmezer, P., Spiegelhalder, B., Dienemann, H., Drings, P., Schulz, V., Kayser, K., Bartsch, H. and Risch, A. (2003) The *CYP3A4*1B* allele increases risk for small cell lung cancer: effect of gender and smoking dose. *Pharmacogenetics*, **13**, 607–618.
- 15) Keshava, C., McCanlies, E. C. and Weston, A. (2004) CYP3A4 polymorphisms-potential risk factors for breast and prostate cancer: a HuGE review. *Am. J. Epidemiol.*, **160**, 825–841.
- 16) Burk, O. and Wojnowski, L. (2004) Cytochrome P450 3A and their regulation. *Naunyn-Schmiedeberg's archives of pharmacology*, **369**, 105–124.
- 17) Koch, I., Weil, R., Wolbold, R., Brockmüller, J., Hustert, E., Burk, O., Nuessler, A., Neuhaus, P., Eichelbaum, M., Zanger, U. and Wojnowski, L. (2002) Interindividual variability and tissue-specificity in the expression of cytochrome P450 3A mRNA. *Drug Metab. Dispos.*, **30**, 1108–1114.
- 18) Niwa, T., Murayama, N., Emoto, C. and Yamazaki, H. (2008) Comparison of kinetic parameters for drug oxidation rates and substrate inhibition potential mediated by cytochrome P450 3A4 and 3A5. *Curr. Drug Metab.*, **9**, 20–33.
- 19) Agarwal, V., Kommaddi, R. P., Valli, K., Ryder, D., Hyde, T. M., Kleinman, J. E., Strobel, H. W. and Ravindranath, V. (2008) Drug metabolism in human brain: high levels of cytochrome P4503A43 in brain

- and metabolism of anti-anxiety drug alprazolam to its active metabolite. *PLoS ONE*, **3**, e2337.
- 20) Wolbold, R., Klein, K., Burk, O., Nussler, A. K., Neuhaus, P., Eichelbaum, M., Schwab, M. and Zanger, U. M. (2003) Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology*, **38**, 978–988.
 - 21) Hunt, C. M., Westerkam, W. R. and Stave, G. M. (1992) Effect of age and gender on the activity of human hepatic CYP3A. *Biochem. Pharmacol.*, **44**, 275–283.
 - 22) Schmidt, R., Baumann, F., Hanschmann, H., Geissler, F. and Preiss, R. (2001) Gender difference in ifosfamide metabolism by human liver microsomes. *Eur. J. Drug Metab. Pharmacokinet.*, **26**, 193–200.
 - 23) Parkinson, A., Mudra, D. R., Johnson, C., Dwyer, A. and Carroll, K. M. (2004) 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.*, **199**, 193–209.
 - 24) Schirmer, M., Rosenberger, A., Klein, K., Kulle, B., Toliat, M. R., Nürnberg, P., Zanger, U. M. and Wojnowski, L. (2007) Sex-dependent genetic markers of CYP3A4 expression and activity in human liver microsomes. *Pharmacogenomics*, **8**, 443–453.
 - 25) Schmucker, D. L., Woodhouse, K. W., Wang, R. K., Wynne, H., James, O. F., McManus, M. and Kremers, P. (1990) Effects of age and gender on in vitro properties of human liver microsomal monooxygenases. *Clin. Pharmacol. Ther.*, **48**, 365–374.
 - 26) Shimada, T., Yamazaki, H., Mimura, M., Inui, Y. and Guengerich, F. P. (1994) Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. Exp. Ther.*, **270**, 414–423.
 - 27) George, J., Byth, K. and Farrell, G. C. (1995) Age but not gender selectively affects expression of individual cytochrome P450 proteins in human liver. *Biochem. Pharmacol.*, **50**, 727–730.
 - 28) Williams, E. T., Leyk, M., Wrighton, S. A., Davies, P. J., Loose, D. S., Shipley, G. L. and Strobel, H. W. (2004) Estrogen regulation of the cytochrome P450 3A subfamily in humans. *J. Pharmacol. Exp. Ther.*, **311**, 728–735.
 - 29) Chen, M., Ma, L., Drusano, G. L., Bertino, J. S., Jr. and Nafziger, A. N. (2006) Sex differences in CYP3A activity using intravenous and oral midazolam. *Clin. Pharmacol. Ther.*, **80**, 531–538.
 - 30) Dilger, K., Eckhardt, K., Hofmann, U., Kucher, K., Mikus, G. and Eichelbaum, M. (1999) Chronopharmacology of intravenous and oral modified release verapamil. *Br. J. Clin. Pharmacol.*, **47**, 413–419.
 - 31) Krecic-Shepard, M. E., Barnas, C. R., Slimko, J. and Schwartz, J. B. (2000) Faster clearance of sustained release verapamil in men versus women: continuing observations on sex-specific differences after oral administration of verapamil. *Clin. Pharmacol. Ther.*, **68**, 286–292.
 - 32) Paine, M. F., Ludington, S. S., Chen, M. L., Stewart, P. W., Huang, S. M. and Watkins, P. B. (2005) Do men and women differ in proximal small intestinal CYP3A or P-glycoprotein expression? *Drug Metab. Dispos.*, **33**, 426–433.
 - 33) Wachter, V. J., Wu, C. Y. and Benet, L. Z. (1995) Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol. Carcinog.*, **13**, 129–134.
 - 34) Cummins, C. L., Wu, C.-Y. and Benet, L. Z. (2002) Sex-related differences in the clearance of cytochrome P450 3A substrates may be caused by P-glycoprotein. *Clin. Pharmacol. Ther.*, **72**, 474–489.
 - 35) Schuetz, E. G., Furuya, K. N. and Schuetz, J. D. (1995) Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms. *J. Pharmacol. Exp. Ther.*, **275**, 1011–1018.
 - 36) Kerlan, V., Dreano, Y., Bercovici, J. P., Beaune, P. H., Floch, H. H. and Berthou, F. (1992) Nature of cytochromes P450 involved in the 2-/4-hydroxylations of estradiol in human liver microsomes. *Biochem. Pharmacol.*, **44**, 1745–1756.
 - 37) Kerr, B. M., Thummel, K. E., Wurden, C. J., Klein, S. M., Kroetz, D. L., Gonzalez, F. J. and Levy, R. H. (1994) Human liver carbamazepine metabolism. Role of CYP3A4 and CYP2C8 in 10,11-epoxide formation. *Biochem. Pharmacol.*, **47**, 1969–1979.
 - 38) Nakamura, H., Nakasa, H., Ishii, I., Ariyoshi, N., Igarashi, T., Ohmori, S. and Kitada, M. (2002) Effects of endogenous steroids on CYP3A4-mediated drug metabolism by human liver microsomes. *Drug Metab. Dispos.*, **30**, 534–540.
 - 39) Mattison, D. and Zajicek, A. (2006) Gaps in knowledge in treating pregnant women. *Gender Medicine: official journal of the Partnership for Gender-Specific Medicine at Columbia University*, **3**, 169–182.
 - 40) Tracy, T. S., Venkataramanan, R., Glover, D. D. and Caritis, S. N. (2005) Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A4 activity) during pregnancy. *Am. J. Obstet. Gynecol.*, **192**, 633–639.

- 41) Anderson, G. D. (2005b) Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. *Clin. Pharmacokinet.*, **44**, 989–1008.
- 42) Zhang, H., Wu, X., Wang, H., Mikheev, A. M., Mao, Q. and Unadkat, J. D. (2008) Effect of pregnancy on cytochrome P450 3a and P-glycoprotein expression and activity in the mouse: mechanisms, tissue specificity, and time course. *Mol. Pharmacol.*, **74**, 714–723.
- 43) Anakk, S., Ku, C. Y., Vore, M. and Strobel, H. W. (2003) Insights into gender bias: rat cytochrome P450 3A9. *J. Pharmacol. Exp. Ther.*, **305**, 703–709.
- 44) Zhang, Y., Kim, M. J., Bertino, J. S. Jr., Nafziger, A. N. and Sellers, E. M. (2006) Use of omeprazole as a CYP3A probe drug: effect of sex and menstrual cycle phase on CYP3A activity in healthy Caucasian adults. *J. Clin. Pharmacol.*, **46**, 345–352.
- 45) Mathias, A. A., Maggio-Price, L., Lai, Y., Gupta, A. and Unadkat, J. D. (2006) Changes in pharmacokinetics of anti-HIV protease inhibitors during pregnancy: the role of CYP3A and P-glycoprotein. *J. Pharmacol. Exp. Ther.*, **316**, 1202–1209.
- 46) Unadkat, J. D., Wara, D. W., Hughes, M. D., Mathias, A. A., Holland, D. T., Paul, M. E., Connor, J., Huang, S., Nguyen, B. Y., Watts, D. H., Mofenson, L. M., Smith, E., Deutsch, P., Kaiser, K. A. and Tuomala, R. E. (2007) Pharmacokinetics and safety of indinavir in human immunodeficiency virus-infected pregnant women. *Antimicrob. Agents Chemother.*, **51**, 783–786.
- 47) Mathias, A. A., West, S., Hui, J. and Kearney, B. P. (2009) Dose-response of ritonavir on hepatic CYP3A activity and elvitegravir oral exposure. *Clin. Pharmacol. Ther.*, **85**, 64–70.
- 48) Kosel, B. W., Beckerman, K. P., Hayashi, S., Homma, M. and Aweeka, F. T. (2003) Pharmacokinetic of nelfinavir and indinavir in HIV-1-infected pregnant women. *AIDS*, **17**, 1195–1199.
- 49) Handschin, C. and Meyer, U. A. (2003) Induction of drug metabolism: the role of nuclear receptors. *Pharmacol. Rev.*, **55**, 649–673.
- 50) Masuyama, H., Suwaki, N., Tateishi, Y., Nakatsukasa, H., Segawa, T. and Hiramatsu, Y. (2005) The pregnane X receptor regulates gene expression in a ligand- and promoter-selective fashion. *Mol. Endocrinol.*, **19**, 1170–1180.
- 51) Dai, D., Bai, R., Hodgson, E. and Rose, R. L. (2001) Cloning, sequencing, heterologous expression, and characterization of murine cytochrome P450 3a25* (Cyp3a25), a testosterone 6beta-hydroxylase. *J. Biochem. Mol. Toxicol.*, **15**, 90–99.
- 52) Sakuma, T., Takai, M., Endo, Y., Kuroiwa, M., Ohara, A., Jarukamjorn, K., Honma, R. and Nemoto, N. (2000) A novel female-specific member of the CYP3A gene subfamily in the mouse liver. *Arch. Biochem. Biophys.*, **377**, 153–162.
- 53) Sakuma, T., Endo, Y., Mashino, M., Kuroiwa, M., Ohara, A., Jarukamjorn, K. and Nemoto, N. (2002) Regulation of the expression of two female-predominant CYP3A mRNAs (CYP3A41 and CYP3A44) in mouse liver by sex and growth hormones. *Arch. Biochem. Biophys.*, **404**, 234–242.
- 54) Masuyama, H., Hiramatsu, Y., Mizutani, Y., Inoshita, H. and Kudo, T. (2001) The expression of pregnane X receptor and its target gene, cytochrome P450 3A1, in perinatal mouse. *Mol. Cell. Endocrinol.*, **172**, 47–56.
- 55) Anakk, S., Huang, W., Staudinger, J. L., Tan, K., Cole, T. J., Moore, D. D. and Strobel, H. W. (2007) Gender dictates the nuclear receptor-mediated regulation of CYP3A44. *Drug Metab. Dispos.*, **35**, 36–42.
- 56) Kawamoto, T., Kakizaki, S., Yoshinari, K. and Negishi, M. (2000) Estrogen activation of the nuclear orphan receptor CAR (constitutive active receptor) in induction of the mouse *Cyp2b10* gene. *Mol. Endocrinol.*, **14**, 1897–1905.
- 57) Yamamoto, M., Sakuma, T., Ichimaru, H. and Nemoto, N. (2001) Localization of estradiol-responsive region in the phenobarbital-responsive enhancer module of mouse *Cyp2b-10* gene. *J. Biochem. Mol. Toxicol.*, **15**, 76–82.
- 58) Jansson, J. O., Eden, S. and Isaksson, O. (1985) Sexual dimorphism in the control of growth hormone secretion. *Endocr. Rev.*, **6**, 128–150.
- 59) Waxman, D. J., Pampori, N. A., Ram, P. A., Agrawal, A. K. and Shapiro, B. H. (1991) Interpulse interval in circulating growth hormone patterns regulates sexually dimorphic expression of hepatic cytochrome P450. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 6868–6872.
- 60) MacLeod, J. N., Pampori, N. A. and Shapiro, B. H. (1991) Sex differences in the ultradian pattern of plasma growth hormone concentrations in mice. *J. Endocrinol.*, **131**, 395–399.
- 61) Veldhuis, J. D. and Bowers, C. Y. (2003) Human GH pulsatility: an ensemble property regulated by age and gender. *J. Endocrinol. Invest.*, **26**, 799–813.
- 62) Gatford, K. L., Egan, A. R., Clarke, I. J. and Owens, P. C. (1998) Sexual dimorphism of the somatotrophic axis. *J. Endocrinol.*, **157**, 373–389.
- 63) Jarukamjorn, K., Sakuma, T., Jaruchotikamol, A., Ishino, Y., Oguro, M. and Nemoto, N. (2006) Modified expression of cytochrome P450 mRNAs by growth hormone in mouse liver. *Toxicology*, **219**, 97–105.

- 64) Sakuma, T., Kitajima, K., Nishiyama, M., Endo, Y., Miyauchi, K., Jarukamjorn, K. and Nemoto, N. (2004) Collaborated regulation of female-specific murine *Cyp3a41* gene expression by growth and glucocorticoid hormones. *Biochem. Biophys. Res. Commun.*, **314**, 495–500.
- 65) Sakuma, T., Bhadhprasit, W., Hashita, T. and Nemoto, N. (2008) Synergism of glucocorticoid hormone with growth hormone for female-specific mouse *Cyp3a44* gene expression. *Drug Metab. Dispos.*, **36**, 878–884.
- 66) Jaffe, C. A., Turgeon, D. K., Lown, K., Demott-Friberg, R. and Watkins, P. B. (2002) Growth hormone secretion pattern is an independent regulator of growth hormone actions in humans. *Am. J. Physiol. Endocrinol. Metab.*, **283**, E1008–E1015.
- 67) Sinués, B., Mayayo, E., Fanlo, A., Mayayo, E. Jr., Bernal, M. L., Bocos, P., Bello, E., Labarta, J. I. and Ferrández-Longás, A. (2004) Effects of growth hormone deficiency and rhGH replacement therapy on the 6beta-hydroxycortisol/free cortisol ratio, a marker of CYP3A activity, in growth hormone-deficient children. *Eur. J. Clin. Pharmacol.*, **60**, 559–564.
- 68) Watkins, P. B., Turgenon, D. K., Jaffe, C. A., Ho, P. J. and Barkan, A. L. (1993) Pulsation frequency of growth hormone may mediate gender difference in CYP3A activity in man (Abstract). *Clin. Res.*, **41**, 132.
- 69) Matthews, D. R., Hindmarsh, P. C., Pringle, P. J. and Brook, C. G. (1991) A distribution method for analysing the baseline of pulsatile endocrine signals as exemplified by 24-hour growth hormone profiles. *Clin. Endocrinol. (Oxf)*, **35**, 245–252.
- 70) Dhir, R. N., Dworakowski, W., Tangavel, C. and Shapiro, B. H. (2006) Sexual dimorphic regulation of hepatic isoforms of human cytochrome P450 by growth hormone. *J. Pharmacol. Exp. Ther.*, **316**, 87–94.
- 71) Liddle, C., Goodwin, B. J., George, J., Tapner, M. and Farrell, G. C. (1998) Separate and interactive regulation of cytochrome P450 3A4 by triiodothyronine, dexamethasone, and growth hormone in cultured hepatocytes. *J. Clin. Endocrinol. Metab.*, **83**, 2411–2416.
- 72) Cheung, C., Yu, A.-M., Chen, C.-S., Krausz, K. W., Byrd, L. G., Feigenbaum, L., Edwards, R. J., Waxman, D. J. and Gonzalez, F. J. (2006) Growth hormone determines sexual dimorphism of hepatic cytochrome P450 3A4 expression in transgenic mice. *J. Pharmacol. Exp. Ther.*, **316**, 1328–1334.
- 73) Waxman, D. J. and O'Connor, C. (2006) Growth hormone regulation of sex-dependent liver gene expression. *Mol. Endocrinol.*, **20**, 2613–2629.
- 74) Holloway, M. G., Cui, Y., Laz, E. V., Hosui, A., Hennighausen, L. and Waxman, D. J. (2007) Loss of sexually dimorphic liver gene expression upon hepatocyte-specific deletion of *Stat5a-Stat5b* locus. *Endocrinology*, **148**, 1977–1986.
- 75) Sakuma, T., Kitajima, K., Nishiyama, M., Mashino, M., Hashita, T. and Nemoto, N. (2004) Suppression of female-specific murine *Cyp2b9* gene expression by growth or glucocorticoid hormones. *Biochem. Biophys. Res. Commun.*, **323**, 776–781.
- 76) Hashita, T., Sakuma, T., Akada, M., Nakajima, A., Yamahara, H., Ito, S., Takesako, H. and Nemoto, N. (2008) Forkhead box A2-mediated regulation of female-predominant expression of the mouse *Cyp2b9* gene. *Drug Metab. Dispos.*, **36**, 1080–1087.
- 77) Carter-Su, C., Schwartz, J. and Smit, L. (1996) Molecular mechanism of growth hormone action. *Annu. Rev. Physiol.*, **58**, 187–207.
- 78) Jover, R., Bort, R., Gomez-Lechon, M. J. and Castell, J. V. (2001) Cytochrome P450 regulation by hepatocytes nuclear factor 4 in human hepatocyte: A study using adenovirus-mediated antisense targeting. *Hepatology*, **33**, 668–675.
- 79) Tirona, R. G., Lee, W., Leake, B. F., Lan, L. B., Cline, C. B., Lamba, V., Parviz, F., Duncan, S. A., Inoue, Y., Gonzalez, F. J., Schuetz, E. G. and Kim, R. (2003) The orphan nuclear receptor HNF4alpha determines PXR- and CAR-mediated xenobiotic induction of CYP3A4. *Nat. Med.*, **9**, 220–224.
- 80) Matsumura, K., Saito, T., Takahashi, Y., Ozeki, T., Kiyotani, K., Fujieda, M., Yamazaki, H., Kunitoh, H. and Kamataki, T. (2004) Identification of a novel polymorphic enhancer of the human *CYP3A4* gene. *Mol. Pharmacol.*, **65**, 326–334.
- 81) Kamiyama, Y., Matsubara, T., Yoshinari, K., Nagata, K., Kamimura, H. and Yamazoe, Y. (2007) Role of human hepatocyte nuclear factor 4 α in the expression of drug-metabolizing enzymes and transporters in human hepatocytes assessed by use of small interfering RNA. *Drug Metab. Pharmacokinet.*, **22**, 287–298.
- 82) Wiwi, C. A., Gupte, M. and Waxman, D. J. (2004) Sexually dimorphic P450 gene expression in liver-specific hepatocyte nuclear factor 4-deficient mice. *Mol. Endocrinol.*, **18**, 1975–1987.
- 83) Kamataki, T., Shimada, M., Maeda, K. and Kato, R. (1985) Pituitary regulation of sex-specific forms of cytochrome P-450 in liver microsomes of rats. *Biochem. Biophys. Res. Commun.*, **130**, 1247–1253.
- 84) MacGeoch, C., Morgan, E. T. and Gustafsson, J.-Å. (1985) Hypothalamo-pituitary regulation of cytochrome P-450 15 apoprotein levels in rat liver.

- Endocrinology*, **117**, 2085–2092.
- 85) Sasaki, Y., Takahashi, Y., Nakayama, K. and Kamataki, T. (1999) Cooperative regulation of *CYP2C12* gene expression by STAT5 and liver-specific factors in female rats. *J. Biol. Chem.*, **274**, 37117–37124.
- 86) Endo, M., Takahashi, Y., Sasaki, Y., Saito, T. and Kamataki, T. (2005) Novel gender-related regulation of *CYP2C12* gene expression in rats. *Mol. Endocrinol.*, **19**, 1181–1190.
- 87) Thangavel, C. and Shapiro, B. H. (2008) Inherent sexually dimorphic expression of hepatic *CYP2C12* correlated with repressed activation of growth hormone-regulated signal transduction in male rats. *Drug Metab. Dispos.*, **36**, 1884–1895.
- 88) Thangavel, C. and Shapiro, B. H. (2007) A molecular basis for the sexually dimorphic response to growth hormone. *Endocrinology*, **148**, 2894–2903.